CHEMICAL SPECIATION AND BIOAVAILABILITY OF HEAVY METALS DURING WATER HYACINTH COMPOSTING

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By

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Candidate's Declaration

I hereby declare that the work presented in this thesis is to the best of my knowledge, original, except as acknowledged in the text. This material has not been submitted, either in whole or in part, for degree at any University.

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This is to certify that the thesis entitled **"Chemical Speciation and Bioavailability of Heavy Metals during Water Hyacinth Composting"** submitted by **Jiwan Singh** (Registration No. 10610405) to the Indian Institute of Technology Guwahati for the degree of Doctor of Philosophy is a record of bonafide research work carried out by him under my supervision and guidance. The thesis work, in my opinion has reached the requisite standard fulfilling the requirement for award of the degree of Doctor of Philosophy. This work has not been submitted earlier for the award of any degree or diploma to the best of my knowledge and belief.

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ABSTRACT

Water hyacinth (*Eichhornia crassipes)* is the noxious weed in the whole world and it is most commonly used plant in constructed wetlands due to its fast growth rate and large uptake of nutrients and contaminants. The final disposal of water hyacinth used in wastewater treatment is still an unresolved problem. Composting and vermicomposting are the best alternative for management of this weed but final product may have high content of heavy metals due to accumulation of heavy metals in body parts of water hyacinth. The presence of non-biodegradable and high level of toxic heavy metals in the compost frequently hinders agricultural land application. Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animal and human health. The toxicity of heavy metals for plants and animals depend on the different forms of heavy metals rather than the total concentration. Thus the bioavailability and leachability of heavy metals play an important role in the toxicity of heavy metals during composting followed by land application. Therefore, studies were carried out on bioavailability, leachability and speciation of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during agitated pile composting, rotary drum composting and vermicomposting of water hyacinth mixed with cattle manure and sawdust. Five different proportions (Trials 1, 2, 3, 4 and 5) of water hyacinth, cattle manure and sawdust were prepared for the composting process. *Eisenia fetida* earthworm was used for the vermicomposting process. The best proportion of waste materials during agitated pile (trial 4) and rotary drum composting (trial 4) was selected on the basis of bioavailability of heavy metals reduction. The effects of lime (1, 2 and 3%) and zeolite (5, 10 and 15%) on bioavailability, leachability and speciation of heavy metals were studied with best trial (trial 4).

Total heavy metal concentration obtained after strong acid digestion of compost sample was useful as an overall pollution indicator. The bioavailability of heavy metals was investigated in the form of water soluble and diethylene triamine penta-acetic acid (DTPA) extractable. Tessier sequential extraction method was employed to investigate the changes in heavy metals speciation. Toxicity characteristics leaching procedure (TCLP) test was performed for leachability of heavy metals. This study investigated the influence of parameters such as pH, temperature and organic matter content on distribution of heavy metal bioavailability and leachability during composting of water hyacinth. Total concentrations of heavy metals were increased during agitated pile and rotary drum composting, however total concentrations of few metals (Cu, Mn, Fe, Ni and Cd) were reduced during the vermicomposting process. Water solubility, DTPA extractability, leachability and most bioavailable fractions were reduced during agitated pile, rotary drum composting and vermicomposting process. The *Eisenia fetida* was very effective for reduction of bioavailability of heavy metals during vermicomposting of water hyacinth.

The maximum reduction of water soluble, plant available, leachable and most bioavailable fractions (exchangeable and carbonate) of heavy metals were observed in lime 2 treatment (2%) of agitated pile composting and reduction of these fractions was observed in lime 1 and 2 treatments during rotary drum composting, which indicated optimum percentage of lime can enhance organic matter degradation and humification process; consequently it reduced the toxicity of the metals. Addition of the natural zeolite (clinoptilolite) during water hyacinth composting process led to the increase in Na, Ca and K concentrations, and reduced significantly water solubility, DTPA and TCLP extractability of heavy metals. The maximum reduction of bioavailability and leachability of heavy metals were observed in zeolite 1 and 2 treatments (5 and 10%) during both agitated and rotary drum composting, which indicated optimum percentage of zeolite could enhance organic matter degradation; therefore it decreased the toxicity of the heavy metals during water hyacinth composting mixed with cattle manure and sawdust. The water soluble Ni, Cd and Pb were not detected, and DTPA extractable Cd and Pb were also not detected during the composting and vermicomposting process. Reducible and oxidizable fractions of Ni, Pb and Cd were not found during water hyacinth composting.

From this study it can be concluded that the appropriate proportion of water hyacinth, cattle manure and sawdust (trial 4), significantly reduced the water solubility, DTPA extractability, leachability and most bioavailable fractions (exchangeable and carbonate) of heavy metals during the pile and rotary drum composting process. The reduction of bioavailability and leachability of heavy metals was not correlated with proportion of water hyacinth, cattle manure and sawdust during vermicomposting process. Appropriate proportion of lime and natural zeolite addition during the composting process enhanced the organic matter degradation consequently converted into most stabilize organic matter, and reduced the bioavailability and leachability of heavy metals. Addition of natural zeolite was much effective for reduction of bioavailability, leachability and most bioavailable fractions in comparison to lime addition.

Keywords: Water hyacinth, composting, vermicomposting, heavy metals, bioavailability, leachability, speciation, bioavailability factor, natural zeolite, lime

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NOTATIONS

MSW	Municipal Solid Waste
L	Liter
Mg	Milligram
g	Gram
Kg	Kilogram
EC	Electrical Conductivity
TN	Total Nitrogen
C/N	Carbon/Nitrogen
NH_4-N	Ammonical Nitrogen
APHA	American Public Health Association
ANOVA	Analysis of Variances
NH_3	Ammonia
P	Provability
TCLP	Toxicity Characteristic Leaching Procedure
DTPA	Diethylene Triamine Penta Acetic acid
F1	Exchangeable fraction
F2	Carbonate fraction
F3	Reducible fraction
F4	Oxidizable fraction
F5	Residual fraction
BF	Bioavailability Factor
SSM	Separated Swine Manure
US EPA	United State Environmental Protection Agency
MT	Metallothioneins
SSL	Sodium Sulfide and Lime
BC	Bamboo Charcoal
BV	Bamboo Vinegar
SD	Standard Deviation
ND	Not Detected
SEM	Scanning Electron Microscopy
h	Hour
BIS	Bureau of Indian Standard

VS	Volatile Solid
K_b	Biodegradability Coefficient
AAS	Atomic Absorption Spectrometer
M	Molar
kcal	Kilo calories
SOD	Super oxide dismutase
RPM	Round per minute

Chapter 1

INTRODUCTION

This chapter consists of brief discussion of water hyacinth problems, treatment through different composting techniques, heavy metals in the final compost, effects of heavy metals on the environment, bioavailability, leachability and speciation of heavy metals, and metal reduction approaches during the composting process.

1.1 OVERVIEW

Macrophytes are aquatic plants, growing in or near water that are emergent, submerged or floating. Water hyacinth *(Eichhornia crassipes)* is a free floating aquatic macrophyte originated in the Amazon in South America where it was kept under control by natural predators (Gunnarsson and Petersen, 2007). It is considered to be the most troublesome plant species in aquatic systems in the world (Zirbes et al., 2011). There is no effort to control or destroy it by chemical, biological, mechanical, or hybrid means has ever achieved total success (Gajalakshmi et al., 2002). The nutrients levels in water body increases rapidly due to its decaying body, which eventually generates the problem of eutrophication in aquatic system (Gupta et al., 2007). It can grow rapidly to very high densities (over 60 kg/m^2); thereby completely blocking water bodies, destroying natural wetlands and reducing water quality from decomposing plants, which may have negative effects on the environment, human health and economic development (Malik, 2007; Rai, 2009). However it has been used for treating heavily polluted wastewaters due to its capacity for heavy metal accumulation (Malik, 2007). Water hyacinth is most commonly used plant in constructed wetlands due to its fast growth rate and large uptake of nutrients and contaminants (Rai, 2009). The final disposal of water hyacinth used in wastewater treatment is still an unresolved problem (Gupta et al., 2007). Composting followed by land application represents one of the most economical ways for the treatment and final disposal of water hyacinth because it combines material recycling and biomass disposal at the same time (Villasenor et al., 2011). Composting and vermicomposting are the best-known processes for biological stabilization of green waste by transforming them into a safer and more stabilized material (compost), that can be used as a soil conditioner in agricultural applications (Deka et al., 2011; Gabhane et al., 2012). Composting can be defined as the process in which of organic waste treatment by aerobic microorganisms; as such, it comprises of three major phases such as mesophilic and thermophilic stages and cooling (the compost stabilization stage) (Neklyudov et al., 2008).

Several successful studies were conducted in agitated pile composting, rotary drum composting and vermicomposting of water hyacinth (Gajalakshmi et al., 2001; Gajalakshmi et al., 2002; Mohee and Mudhoo, 2005; Gupta et al., 2007; Das and Kalamdhad, 2011; Dhal et al., 2012). Agitated pile or windrow is simple and economic method for management of all degradable wastes such as sewage sludge, municipal solid waste and water hyacinth. During agitated pile composting suitable turning and moisture is required for maintaining proper aeration and moisture content for microbial survival (Kalamdhad et al., 2009). Das and Kalamdhad (2011) studied stability parameters during agitated pile composting of water hyacinth and found that proper combination of water hyacinth, cattle manure and sawdust/rice straw formed highly stabilized compost, indicated a good alternative for management of this noxious weed.

Rotary drum composting is also highly efficient technique for management of all type of organic wastes. It reduces the composting time in comparison to agitated pile composting. It provides favourable conditions for microbes due to proper mixing and turning. Singh et al. (2012) has done composting of water hyacinth in rotary drum and found good reduction in stability parameters such as oxygen uptake rate and CO_2 evolution rate confirmed that rotary drum is highly efficient for making water hyacinth compost mixed with cattle manure and rice straw.

During vermicomposting process, earthworms can hasten the composting process to a significant extent with production of a better quality of compost as compared with those prepared through traditional composting methods (Gupta and Garg, 2008). Vermicomposting has been considered as a sustainable, potential and cost effective alternative for the treatment of this noxious weed. Gajalakshmi et al. (2001 and 2002) studied vermicomposting of water hyacinth using earthworm *Eudrilus eugeniae* and reported that parent earthworm grew in size, produced offspring and no mortality was observed. This study concluded that water hyacinth can be vermicomposted in any form with *E. eugeniae*. According to Gupta et al. (2007), water hyacinth is competent of accumulating large quantities of heavy metals from the water. Hence, the composts made from water hyacinth might contain higher concentrations of heavy metals. Although some literatures are available on bioavailability and speciation of heavy metals during sewage sludge composting; suggested that toxicity of heavy metals depends on their different bioavailable fractions rather that total concentration (Wong and Selvam, 2006; Nomeda et al., 2008; Kang et al., 2011).

Therefore aim of this thesis was to evaluate bioavailability, leachability and speciation of heavy metals in agitated pile, rotary drum composting and vermicomposting of water hyacinth mixed with different proportions of cattle manure and sawdust.

1.2 BACKGROUND OF PROBLEM

Hydrophytes (water hyacinth) can absorb heavy metals through roots, stems or leaves, and accumulate them in body organs. Accumulation of heavy metals in the plant depends on the plant species, bioavailability, pH, cation exchange capacity, dissolved oxygen, temperature and secretion of roots (Buta et al., 2011). Water hyacinth is capable of accumulating high quantities of heavy metals from the water. Hence, the composts/vermicompost prepared from water hyacinth might contain higher concentrations of heavy metals (Gupta et al., 2007). The presence of non-biodegradable and high level of toxic heavy metals in the compost frequently hinders its agricultural land application. Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animal and human health (Wong and Selvam, 2006; Iwegbue et al., 2007; He et al., 2009a). Excessive accumulation of heavy metals in soil and other media may eventually contaminate both human and animal food chain (Iwegbue et al., 2007). Heavy metals can affect the growth, morphology and metabolism of the soil microorganisms, and reduce both the size and activity of microbial pools, and therefore decrease soil fertility (Bragato et al., 1998). Heavy metals uptake by plants and successive accumulation in human tissues, and biomagnification through the food chain causes both human health and environmental concerns (Wong and Selvam, 2006).

The total metal concentration obtained after strong acid digestion of compost sample is useful as an overall pollution indicator, but it does not provide useful information about the risk of bioavailability, which depends on their chemical form (Walter et al., 2006; Venkateswaran et al., 2007). The term bioavailability of any element is used to indicate that part of total concentration of the element eagerly soluble in water and considered as easily available to plants and soil microorganisms. The bioavailability of metals in soil is a self-motivated process that depends on explicit combinations of chemical, biological and environmental parameters. These include soil properties such as pH, organic matter (OM) content, redox potential, cation exchange capacity, sulphate, carbonate, hydroxide, soil texture and clay content (Prabpai et al., 2009; Guala et al., 2010). The pH and OM content are the major critical factors for heavy metal accumulation by both plants and animals (Li et al., 2010). Persistence of soluble organo-metal complexes in compost added to soil could increase potential toxicity since soluble material will tend to be more bioavailable to receptor organisms (Zheng et al., 2007).

The water soluble fraction of metal is most readily bioavailable in compost when applied to soils. Water soluble metals are biologically more dynamic, consequently highest potential of contaminating food chain, surface water and ground water (Hsu and Lo, 2001). The DTPA-extractable fraction of metals might represent a supplemental approach to check the bioavailability of heavy metals in the soil and sludge amended soil for plant uptake (Bragato et al., 1998; Fang and Wong, 1999; Fuentes et al., 2006). The toxicity characteristic leaching procedure (TCLP) is used to determine the mobility of toxic contaminants present in waste materials. Heavy metals can be leached from soil and find a way to underground water in the case that compost contains high amounts of heavy metals have been applied to soil (Bridget et al., 1995). Estimation of the chemical speciation of heavy metals in compost helps to evaluate their bioavailability and suitability for land application (Wong and Selvam, 2006). Leachability of metals can be defined as the ratio of the amount of a heavy metal released from TCLP to its total concentration; it is commonly used to assess the leachability of heavy metals in the compost and amended soil (Chiang et al., 2007).

The mobility of trace metals, their bioavailability and related eco-toxicity to plants, depends strongly on their specific chemical forms or ways of binding (Fuentes et al., 2006; Gupta and Sinha, 2007). Sequential extraction techniques afford a useful tool for understanding the different chemical forms of heavy metals in soils or contaminated organic matter. This technique has been applied to organic soils, which are very similar to composts, for studying metal mobility and bioavailability (Yuan et al., 2011). In the sequential chemical extraction methods various chemical extractants are applied successively to a sample, dissolving the components of the sample matrix in sequential order. Preferably, a reagent should liberate all the metals from a particular matrix's component (i.e. exchangeable, carbonate fraction), without affecting other components (Li et al., 2001). The sequential chemical extraction procedure could provide an understanding of the heavy metal speciation with different natures and allows the prediction of metal mobility, bioavailability and leaching rates (He et al., 2009b). Chemical speciation or sequential extraction of heavy metals from compost is a useful technique for determining the chemical forms in which these are present (Walter et al., 2006).

Evaluation of the chemical speciation of heavy metals in composts permits to assess their bioavailability and suitability for land application (Wong and Selvam, 2006). The mobility and bioavailability of the metals are decreased approximately in the order of extraction sequence (Nair et al., 2008). According to Tessier et al. (1979) sequential extraction procedure heavy metals are bound with five fractions:

- Exchangeable fraction (F1): It is likely to be affected by changes in water ionic composition as well as sorption - desorption process.
- Carbonate fraction or acid extractable (F2): It is susceptible to changes in pH and this can become soluble and mobilized in acidic condition.
- Reducible fraction (F3): It is thermodynamically unstable under anoxic conditions.
- Oxidizable fraction or fraction bound to organic matter (F4): It can be released and solubilize under oxidizing conditions.
- Residual fraction (F5): It is permanently bound in crystal lattice of the mineral components of the compost and soil. This fraction will never be available for plants under normal natural conditions.

Therefore reduction of heavy metals or their various fractions from the compost produced from different waste materials such as municipal solid waste (Ciba et al., 1999), sewage sludge (Liu et at., 2007), tannery waste (Shukla et al., 2009) and are considerable steps for reducing metal toxicity for soil, plants and human health.

Earthworms are capable of reducing possible toxic effects of heavy metals by utilizing them for physiological metabolism (Jain et al., 2004). The earthworm can reduce toxicity of heavy metals by accumulation in their body tissues (Suthar, 2009). Hait and Tare (2012) have demonstrated that vermicomposting caused considerable reduction in concentration of water soluble metals like Cu, Zn and Cr during vermicomposting of sewage sludge. When the organic matter passes through the gut of earthworm some part of it's digested, and pH and microbial activity of the gut increased. As a consequence, the possibilities of binding of metals to ions and carbonates (i.e. more soluble fractions) increased in ingested material (Suthar, 2009).

Lime is the familiar alkaline material used for the reduction of heavy metal mobility during the composting process (Chiang et al., 2007). Liming, as a general means for raising soil pH, could also minimize uptake of metals by plants on acid soils (Wong and Fang, 2000). Lime is an additive which enhances the composting process by providing a buffering against the decrease in pH and an appropriate amount of Ca, which would improve the metabolic activity resulting increase in temperature and CO_2 evolution without any negative effects on microbial community (Fang and Wong, 1999; Gabhane et al., 2012). Lime amendment was very efficient in reducing heavy metal bioavailability of heavy metals during water hyacinth composting by forming less soluble carbonate salts (Fang and Wong, 1999).

Natural zeolite is a naturally occurring hydrated aluminosilicate mineral. It belongs to the class of minerals known as “tectosilicates” (Erdem et al., 2004). It has been used widely for improvement of the physical and chemical properties of the compost and immobilization of heavy metals during the sewage sludge composting (Zorpas et al., 2000; Sprynskyy et al., 2007; Villasenor et al., 2011). Zeolite improves the composting process due to its ability to increase the porosity of the substrate (Zorpas et al., 2000). It has the ability to readily uptake of almost all heavy metals that are bound to exchangeable and carbonate fractions (Zorpas et al., 2000). It can increase sodium and potassium in compost through exchange with toxic metals.

The above mentioned investigations generally dealt with the bioavailability, leachability and speciation of heavy metals in compost of different waste materials (sewage sludge, municipal solid waste, industrial wastes) with lime and natural zeolite. However, the information on bioavailability, leachability speciation of heavy metals during composting and vermicomposting of water hyacinth is rather limited. In addition reduction of bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate) of heavy metals during composting of water hyacinth mixed with cattle manure, sawdust, lime and natural zeolite is very limited.

Hence the present study focused on total concentration, bioavailability, leachability and speciation of heavy metals during agitated pile, rotary drum composting and vermicomposting of water hyacinth mixed with cattle manure and sawdust. In the best selected trials from agitated pile and rotary drum, lime and natural zeolite was used for reduction of bioavailability of heavy metals during the composting process.

1.3 OBJECTIVES OF THE STUDY

The main objective of the study was to reduce bioavailable and leachable fractions of heavy metals during water hyacinth composting. The purpose was also to find best way for reducing bioavailability of heavy metals during different type composting process. The scope of the present study is limited to:

1) Assessment of bioavailability, leachability and speciation of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during agitated pile and rotary drum composting of water hyacinth mixed with cattle manure and sawdust.

2) Assessment of bioavailability, leachability and speciation of heavy metals during vermicomposting of water hyacinth mixed with cattle manure and sawdust.
3) Effects of lime on bioavailability, leachability and speciation of heavy metals during agitated pile and rotary drum composting of water hyacinth.
4) Effects of natural zeolite on bioavailability, leachability and speciation of heavy metals during agitated pile and rotary drum composting of water hyacinth.

1.4 NEED OF THE STUDY

The production of compost from water hyacinth is an important means of recovering organic matter and minerals for plants. However, the presence of heavy metal in composts may cause undesirable effects on environment, which limits the compost application due to mobility and availability of heavy metals. The toxicity of metals depends on their mobility and bioavailability rather than its total concentration. The compost with background concentration of heavy metals used as a soil conditioner, consequently metals may uptake by plants, pollute surface water through runoff and contaminate ground water in the course of leaching through porous media. Therefore, assessment of bioavailability and leachability of metals, and its reduction solution is needed. Application of earthworms and some selected chemicals such as lime and natural zeolite during the water hyacinth composting may be beneficial for reducing bioavailable and leachable fractions of heavy metals. Before application of compost for land application, it should be tested for bioavailability and leachability of heavy metals. A guideline should be prepared for bioavailable fractions of metals instead of total metal concentration obtained after strong acid digestion.

1.5 THE SCOPE OF THE THESIS

To achieve the above mentioned objectives, laboratory experiments as well as actual on-site plant studies were performed alternatively under different conditions. In the first attempt, performances of the batch operated agitated pile, rotary drum composter and vermireactors on the basis of different proportion of water hyacinth, cattle manure and sawdust were studied. Second attempt was to set-up experimental conditions for sequential extraction procedure such as water bath shaker for maintaining required temperature and shaking, and Pelican digestion equipment for digestion of sample for determination of total heavy metal and residual fraction of metals. A large part of the work was collection of water hyacinth, sawdust and cattle manure, cutting of water hyacinth in proper size, handling and analysis of data including daily visual observations of the feedstock materials followed by learning of chemical speciation and atomic absorption spectrometer.

1.6 THESIS ORGANIZATION

The thesis was organized in following chapters:

- Chapter 1 gives brief introduction of water hyacinth problem, composting and vermicomposting, heavy metals problems, bioavailability, leachability and speciation of heavy metals and objectives of thesis.
- Chapter 2 gives detail literature review of water hyacinth problem, utilization of water hyacinth, different types of composting process, heavy metals problems, bioavailability, leachability, speciation and reduction of heavy metals during composting process.
- Chapter 3 deals with collection and initial characterizations of raw materials such as water hyacinth, cattle manure, sawdust, lime and natural zeolite. Experimental design of phase 1, 2, 3 and 4 given in flow charts. The detail procedures for physico-chemical and heavy metal analysis (total heavy metal, water soluble, diethylene triamine pentacetic acid (DTPA) extractable, leachable and speciation of heavy metals.
- Chapter 4 gives detail results and discussion of bioavailability, leachability and speciation of heavy metals during agitated pile, rotary drum composting and vermicomposting.
- Chapter 5 gives detail results and discussion about effects of lime and natural zeolite addition on bioavailability, leachability and speciation of heavy metals during agitated pile and rotary drum composting process.
- Chapter 6 lists the conclusions and recommendations of the thesis.

Chapter 2

LITERATURE REVIEW

This chapter covering detail literature on water hyacinth and its problems, management of water hyacinth through different composting methods. Chapter also dealt with detail composting and vermicomposting process, heavy metals in the final compost, effects of heavy metals on soil, plants and human health, bioavailability, leachability and speciation of heavy metals, their reduction approaches during composting process and concluding remarks.

2.1 WATER HYACINTH

The water hyacinth is a tropical species belonging to the pickerelweed family (*Pontederiaceae*). A native of Brazil and possibly other central South American countries, now it occurs in lakes, slowly moving rivers and swamps in most countries of the world lying between 40°N and 40°S includes India, South Africa and the USA (Malik, 2007). Water hyacinth grows very fast in nutrient-enriched waters; however it can tolerate in considerable variation of nutrients, temperature and pH levels (Wilson et al., 2005). The optimum pH for growth of water hyacinth is 6-8 and it can grow in a wide range of temperature from 1 to 40°C (optimum growth at 25-27.5°C) but it is highly cold-sensitive (Wilson et al., 2005). Its growth rates increase with the increase in water nitrogen concentration while salinity is a major limitation. In coastal regions, salinity levels of 6 and 8% may be fatal for water hyacinth (Malik, 2007). Water hyacinth reproduces sexually by seeds and vegetative propagation by budding and stolen production (Verma et al., 2005).

It has been considered that water hyacinth is one of the most proliferative plants species on earth and world's worst aquatic weed (Gajalakshmi et al., 2002). It formed dense mats in aquatic system which interferes in navigation, recreation, irrigation, blockage of canals and rivers can cause flooding. Moreover, the fish population is also affected due to reduction in dissolved oxygen levels, and beneath the mats creates good breeding conditions for mosquito vectors of malaria, encephalitis and filariasis (Malik, 2007).

Thomson et al. (2002) suggested some weed management strategy such as utilization of the weed for mushroom cultivation and vegetable production. Water hyacinth contain appropriate amount of hemicellulose (30-55% of dry weight) which could be utilized as a good source of energy as resulting bioconversion of hemicellulose in to alcohol production (Nigam, 2002). Water hyacinth has ability to grow in heavily polluted water and capacity to uptake toxic metals as a result it can be used in phytoremediation. Natural wetland can be protected from incoming polluted effluent by colonization of water hyacinth which could be serving as 'nature's kidneys' and it has been used for decontaminating inorganic nutrients, toxic metals as well as persistent organic pollutants (Malik, 2007).

The accumulation of metals in the water hyacinth occurs by intracellular component through energy dependent process and passive adsorption onto the body surface. Generally two mechanisms are accountable for the survival of plant in heavy metal polluted site: (a) their ability to decrease metal uptake, e.g., by inducing pH change around root; and (b) their ability to accumulate and translocate metal in to plant body (Verma et al., 2005). Accumulation and distribution of heavy metals in the plant depends on the plant species, bioavailability, pH, cation exchange capacity, dissolved oxygen, temperature and secretion of roots (Buta et al., 2011; Rascio and Izzo, 2011). The body of water hyacinth contains many polyfunctional metal-binding sites for both cationic and anionic metal complexes (Mahamadi, 2011). The final disposal of water hyacinth used in wastewater treatment and natural/constructed wetland is still an unresolved problem (Gupta et al., 2007). Unscientific disposal of biomass holding unwanted substances such as heavy metals may be hazardous for human heath through polluting soil, ground water and surface water.

Hence protection of aquatic ecosystem from high proliferation of noxious weed (water hyacinth) and solving disposal problem after treating polluted effluent are needed in the present situation. Therefore composting and vermicomposting are contemptible and cost effective alternative for solving above problems. This plant has been successfully used for vermicomposting and composting (pile and drum) by many researchers (Gajalakshmi et al., 2001; Gajalakshmi et al., 2002; Gupta et al., 2007; Das and Kalamdhad, 2011; Singh et al., 2012). The application of water hyacinth as compost and vermicompost to the land has become an attractive substitute. This practice could solve three problems: (i) uncontrolled growth in water body (ii) unscientific disposal of water hyacinth after treated heavily polluted water and (iii) enhanced soil productivity and reduces required synthetic fertilizers (which have intrinsic economic benefits).

2.2 COMPOSTING

Composting is the biological decomposition and stabilization of organic substrates, under conditions that allow development of thermophilic temperatures as a result of biologically produced heat, to produce a final product that is stable, free of pathogens and plant seeds, and can be beneficially applied to land (Haug, 1993). Aerobic composting is the decomposition of organic substrates in the presence of oxygen air (Fig. 2.1). The main products of biological metabolism are carbon dioxide, water and heat.

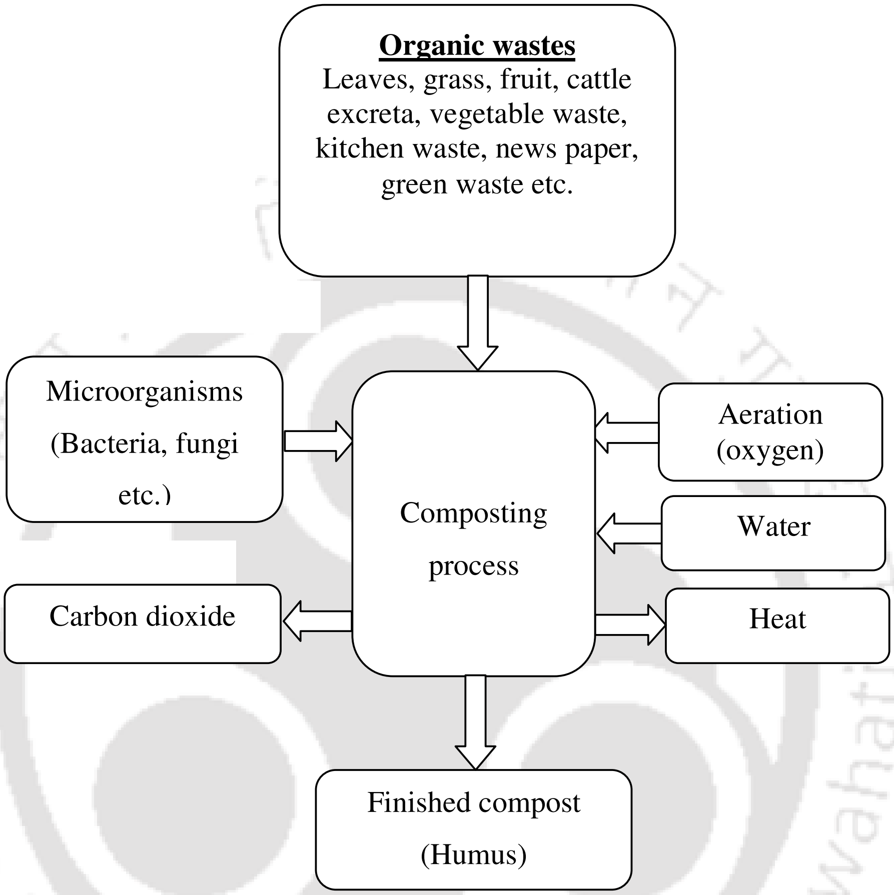


Fig. 2.1 Mechanism of composting process

2.2.1 FACTORS AFFECTING COMPOSTING PROCESS

- **Nutrient balance**

The most important nutrient parameter is the carbon/nitrogen or C/N ratio. Phosphorus is next in importance, and sulfur, calcium, and trace quantities of several other elements all play a part in cell metabolism. The organisms involved in stabilization of organic matter utilize about 30 parts of carbon for each part of nitrogen and hence an initial C/N ratio of 30 is most favorable for composting. Research workers have reported the optimum value to range 26-31 depending upon other environmental conditions. Whenever the C/N ratio is less than the optimum, carbon source such as straw, sawdust, paper are added while if the ratio is too high, sewage sludge, slaughter house waste, blood etc. are added as a source of nitrogen.

- **Particle size**

The particle size of composting materials should be as small as possible so as to allow for efficient aeration and to be easily decomposed by the bacteria, fungi and actinomycetes. Therefore MSW and agricultural residues, such as aquatic weeds and straw, should be shredded into small pieces prior to composting. Night soil, sludge and animal manure usually contain fine solid particles suitable for microbial decomposition.

- **Moisture control**

The moisture tends to occupy the free air space between the particles. Higher moisture content may create anaerobic conditions in the compost mass. However, the composting mass should have certain minimum moisture content in it for the organisms to survive. The optimum moisture content is known to be between 50 to 60%. Higher moisture content may be required while composting straw and strong fibrous material which soften the fibre and fills the large pore spaces.

- **Aeration requirement**

It is necessary to ensure that oxygen is supplied throughout the mass and aerobic activity is maintained. During the decomposition, the oxygen gets depleted and has to be continuously replenished. This can be achieved either by turning of windrows or by supplying compressed air. During the turning, it is necessary to bring inner mass to the outer surface and to transfer the outer waste to the inner portion. In case of artificial air supply the quantity of air supply is normally maintained at 1-2 m^3/day/kg of volatile solids.

- **Temperature**

The aerobic decomposition of a gram mole of glucose releases 484 to 674 kilo calories (kcal) energy under controlled conditions, while only 26 kcal are released when it is decomposed anaerobically. Municipal solid waste is known to have good insulation properties and hence the released heat results in increase in temperature of the decomposing mass. As some of the heat loss occurs from the exposed surface, the actual rise in temperature will be slightly less. When the decomposing mass is disturbed, the resultant heat loss results in drop in temperatures. Under properly controlled conditions temperatures are known to rise beyond 70^{o}C in aerobic composting. As the compost heats up above 40°C, thermophilic bacteria take over.

The microbial populations during this phase are dominated by members of the genus Bacillus. The diversity of bacilli species is fairly high at temperatures from 50-55°C but decreases dramatically at 60°C or above. When conditions become unfavorable, bacilli survive by forming endospores, thick-walled spores that are highly resistant to heat, cold, dryness, or lack of food. They are ubiquitous in nature and become active whenever environmental conditions are favorable. Once the compost cools down, mesophilic bacteria again predominate. The numbers and types of mesophilic microbes that recolonize compost as it matures depend on what spores and organisms are present in the compost as well as in the immediate environment. In general, the longer the curing or maturation phase, the more diverse the microbial community it supports. Various studies have shown that the activity of cellulose enzyme reduces at more than 70°C and the optimum temperature range for nitrification is 30° to 50°C beyond which nitrogen loss is known to occur. The high temperature also helps in destruction of some common pathogens and parasites. Heat death of a cell means thermal inactivation of its enzymes. Without enzymatic activity a cell cannot function and will die. Pathogens are also destroyed or controlled by: competition with other microbes, antagonistic relationship, antibiotic of inhibiting substances produced by microbes and time of survival (Haug, 1993). But temperature is the only factor that operator can measure and control during composting. Hence, US Environmental Protection Agency recommended 53°C for 5 days, 55°C for 2 days and 70°C for 30 minutes for destruction of pathogens.

2.2.2 TYPES OF COMPOSTING PROCESS

Fig. 2.2 shows the different types of reactor and non-reactor type composting processes (Haug, 1993).

- **Nonreactor processes**

Windrow Process

The windrow system is the most popular example of a nonreactor, agitated solids bed system. Mixed feedstocks are placed in rows and turned periodically, usually by mechanical equipment. Height, width, and shape of the windrows vary depending on the nature of the feed material and the type of equipment used for turning. Oxygen is supplied primarily by natural ventilation resulting from the buoyancy of hot gases in the windrow, and to a lesser extent, by gas exchange during turning. Periodic agitation by turning is used to restructure the windrow. As a result, considerable mixing can be expected along the height and width of the row but little mixing will occur along the length.

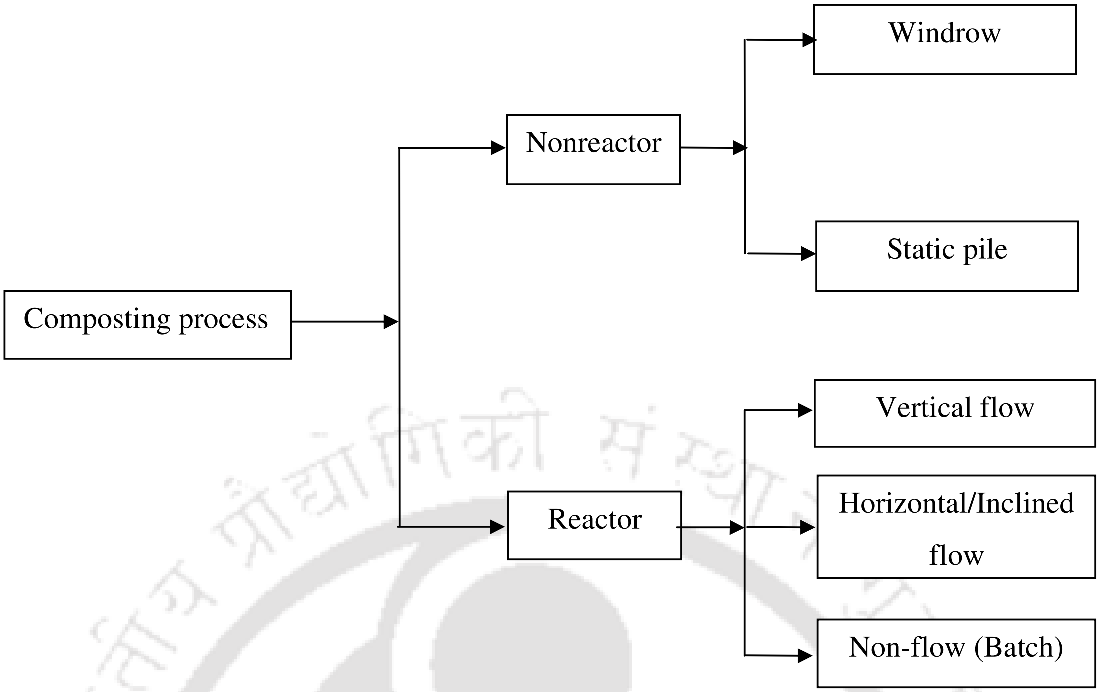


Fig. 2.2 Different types of compositing processes

Static Pile Process

The aerated, static pile process is the premiere example of a nonreactor static solid bed system. The substrate is mixed with a bulking agent, such as wood chips, and formed into a large pile. The bulking agent provides structural stability to the material and maintains air voids without the need for periodic agitation. A distribution system is used to allow either forced or induced draft aeration. No agitation or turning of the static bed occurs during the compost cycle, and the piles are formed on a batch basis. Because there is no bed agitation, no mixing occurs once the pile is formed.

- **Reactor processes**

Vertical Flow Processes

Vertical flow reactors are further defined according to bed conditions in the reactor. Some systems allow for agitation of solids during their transit down the reactor and are termed moving agitated bed reactors. These are usually fed on either continuous or intermittent basis. In other systems, the composting mixture occupies the entire bed volume and is not agitated during any single pass in the bed. These are termed moving packed bed reactors and can be fed on either a continuous, intermittent, or batch basis. The moving packed bed systems often allow for periodic transfer of solids from the bottom to the top of the reactor. Agitation of solids occurs as a result of this transfer, but on any single pass the bed solids remain static until they are again withdrawn from the bottom for transfer.

Horizontal and inclined flow processes

Horizontal flow reactors are divided into those that employ a rotating or rotary drum (tumbling solids bed reactor), those that use a bin structure of varying geometry and method of agitation (agitated solids bed reactors), and those that use a bin type structure but with a static solids bed (static solids bed reactor). Such systems have been applied to a wide variety of composting substrates including MSW, agricultural wastes and sewage sludges. Rotary (Rotating) drums can be distinguished based on the solids flow pattern within the reactor. In the dispersed flow case, material inlet and outlet are located on opposite ends of the drum. Plug flow conditions exist within the vessel except for some dispersion of material resulting from the tumbling action. This is probably the most commonly used of the drum systems and has been widely applied to MSW composting.

A well-organized and promising technique in decentralized composting is the rotary drum composter, which provides agitation, aeration and mixing of the compost, to produce a consistent and homogeneous end product without any odor or leachate related problems (Kalamdhad et al., 2009). Warm and moist environments with the ample amount of oxygen and organic material available within the rotary drum, allow aerobic microbes to flourish and decompose the waste more rapidly; resulting the composting time significantly reduced to 2-3 weeks. Rotary drum composter has been used to compost diverse organic wastes such as cattle manure, swine manure, municipal biosolids, brewery sludge, chicken litter, animal mortalities, olive mill waste and food residuals (Kalamdhad et al., 2009; Fernandez et al., 2010; Rodriguez et al., 2012; Villasenor et al., 2011).

Non-flow Processes

A number of composting technologies that use batch operated, compost ‘boxes’ are available. Materials are loaded at the start of the cycle and typically remain in the box reactor for 7 to 14 days. Aeration is usually controlled and may alternate between positive and negative modes. Curing is usually conducted in windrows for several months.

2.3 VERMICOMPOSTING

Fig. 2.3 shows the mechanism of vermicomposting process. Vermicomposting has been arising as a pioneering eco-technology for the conversion of various types of wastes into vermicompost. Vermicomposting is a biotechnological process in which earthworms are employed to convert the organic wastes into humus like material known as vermicompost (Yadav and Garg, 2011). Vermicomposting is a new and useful technology that recently used for recycling the organic part of these wastes (Ghyasvand et al., 2008). Vermicomposting is a better way of waste conversion since it makes quality manure from primarily organic waste mixtures quickly and gives a nutritionally rich and biologically more active product at reasonable cost. During vermicomposting, earthworms maintain aerobic conditions in waste pile through burrowing and inverting, resulting biochemical processes are enhanced by microbial decomposition of the substrate in the earthworm intestine (Majumdar et al., 2006). During vermicomposting earthworms eat, grind and digest organic wastes with the help of aerobic and some anaerobic microflora, converting it into a much finer, humified and microbially active material (Maboeta et al., 2003).

Earthworm requires an optimum temperature (12-28^{o}C), pH (5.5-8.5), moisture (60-80%) and sufficient air for their growth. During vermicomposting process earthworms ingest organic waste materials and egest a homogeneous humus-like substance (Yadav and Garg, 2011). Specific earthworm species are proficient for consuming a wide range of organic wastes from sewage sludge, animal wastes, agricultural residues, domestic wastes and industrial wastes. The earthworm's actions on organic matter proceeded through physical and biochemical process. The physical process completed by fragmentation, turnover and aeration; while biochemical actions include enzymatic digestion, nitrogen enrichment, transport of inorganic and organic materials (Yadav and Garg, 2011).

During vermicomposting process, the important plant nutrients such as nitrogen, potassium, phosphorus and calcium present in the feeding material are converted through microbial action into more soluble and available forms that plant can assimilate (Kaushik and Garg, 2003). Vermicomposting is a relatively new approach employs the combined activity of earthworms and microorganisms, and involves the bioaccumulation of high concentrations of metals in earthworm tissue through their ingestion of large amounts of substrate (Wang et al., 2013). Although microbes are reliable for biochemical degradation of organic matter, earthworms are the important drivers of the process by conditioning the substrate and altering the biological activity (Gupta and Garg, 2008; Khwairakpam and Bhargava, 2009; Vig et al., 2011; Zirbes et al., 2011).

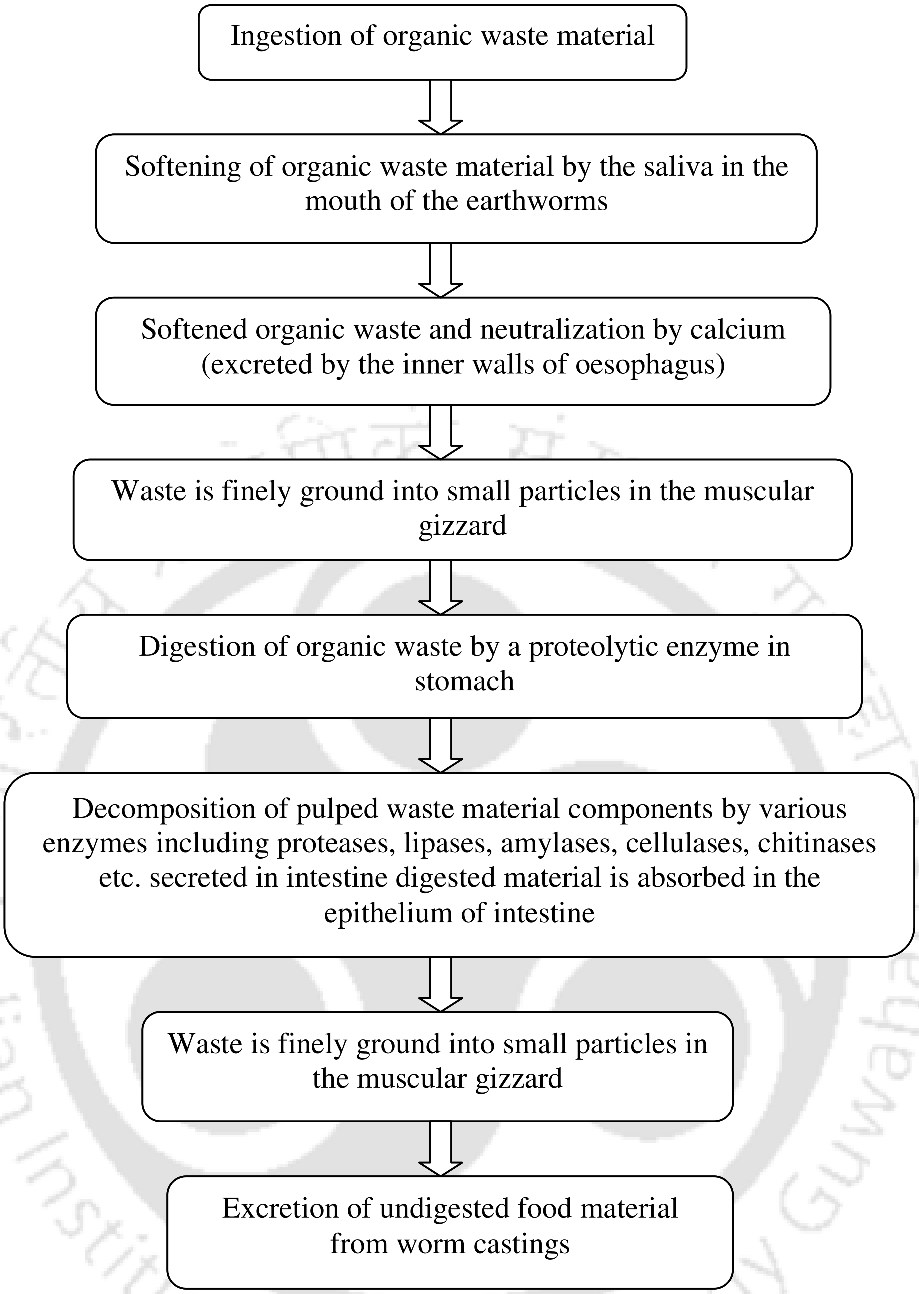


Fig. 2.3 Mechanism of vermicomposting process (Yadav and Garg, 2011)

Earthworms can accumulate heavy metals (Cu, Zn, Pb and Cd) when they are exposed to heavy metal-contaminated soils; however the accumulation of heavy metal in earthworms depends strongly on the metal that is bioavailable for uptake rather than the total (Li et al., 2010). Heavy metals can be detoxifying by inoculation of microorganisms or the addition of a earthworms to initial organic substrates. Earthworms adjust and change the physico-chemical property of organic substrates through the mechanism of adsorption, complexation, precipitation, ion exchange and redox reactions, resulting they reduce heavy metals bioavailability and mobility in the substrate environment (Wang et al., 2013).

2.4 HEAVY METALS DURING COMPOSTING

The application of sewage sludge and its compost or other wastes compost contains high concentration of heavy metals may be dangerous for human health through uptake by plants from soil medium. Heavy metals are natural non-degradable inorganic materials in the environment. It commonly defined as being those metallic elements with a density > 5000 kg/ m^3 (Talbot, 2006). Heavy metals are considered one of the major sources of soil pollution. Heavy metal pollution of the soil is caused by various metals, especially Cu, Ni, Cd, Zn, Cr and Pb (Karaca et al., 2010; Chiroma et al., 2012). Some heavy metals (Fe, Zn, Ca and Mg) have been reported to be of bio-importance to man and their daily medicinal and dietary allowances had been recommended. However, some others (like As, Cd, Pb, and methylated forms of Hg) have been reported to have no known bio-importance in human biochemistry and physiology, and consumption even at very low concentrations can be toxic (Duruibe et al., 2007).

2.4.1 EFFECTS OF HEAVY METALS ON ENVIRONMENT

- **Effects of heavy metals on soil**

Soil contamination by heavy metals is most important apprehension throughout the industrialized world (Hinojosa et al., 2004). Heavy metals are considered as one of the major sources of soil pollution. Heavy metal pollution of the soil is caused by various metals especially Cu, Ni, Cd, Zn, Cr, and Pb (Hinojosa et al., 2004). Heavy metals indirectly affect soil enzymatic activities by shifting the microbial community which synthesizes enzymes (Shun-hong et al., 2009). Heavy metals exhibit toxic effects towards soil biota by affecting key microbial processes and decrease the number and activity of soil microorganisms. Chen et al. (2010a) suggested that heavy metals caused a decrease in bacterial species richness and a relative increase in soil actinomycetes or even decreases in both the biomass and diversity of the bacterial communities in contaminated soils. Cu inhibits b-glucosidase activity more than cellulose activity. Pb decreases the activities of urease, catalase, invertase and acid phosphatase significantly. Phosphatase and sulfatase are inhibits by As (V) but that urease was unaffected. Cd contamination has a negative effect on the activities of protease, urease, alkaline phosphatase and arylsulfatase but no significant effect on invertase. Each soil enzyme exhibits a different sensitivity to heavy metals.

Cr (VI) has been reported to cause shifts in the composition of soil microbial populations, and known to cause detrimental effects on microbial cell metabolism at high concentrations (Shun-hong et al., 2009). Ashraf and Ali (2007) also reported that the heavy metals exert toxic effects on soil microorganism hence results in the change of the diversity, population size and overall activity of the soil microbial communities and observed that the heavy metal (Cr, Zn and Cd) pollution influenced the metabolism of soil microbes in all cases. In general, an increase of metal concentration adversely affects soil microbial properties e.g. respiration rate, enzyme activity, which appears to be very useful indicators of soil pollutions. In case of soil contaminated with lead (Pb) slight change was observed in the soil microbial profile.

- **Effects of heavy metals on plants**

Some of these heavy metals i.e. As, Cd, Hg, Pb or Se are not essential for plants growth, since they do not perform any known physiological function in plants. Others i.e. Co, Cu, Fe, Mn, Mo, Ni and Zn are essential elements required for normal growth and metabolism of plants, but these elements can easily lead to poisoning when their concentration greater than optimal values (Garrido et al., 2002; Rascio et al., 2011). Uptake of heavy metals by plants and subsequent accumulation along the food chain is a potential threat to animal and human health (Sprynskyy et al., 2007).

The absorption by plant roots is one of the main routes of entrance of heavy metals in the food chain (Jordao et al., 2006). Heavy metal accumulation in plants depends upon plant species and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil to plant transfer factors of the metals (Khan et al., 2008). Elevated Pb in soils may decrease soil productivity, and a very low Pb concentration may inhibit some vital plant processes, such as photosynthesis, mitosis and water absorption with toxic symptoms of dark green leaves, wilting of older leaves, stunted foliage and brown short roots (Bhattacharyya et al., 2008). Heavy metals are potentially toxic and phytotoxicity for plants resulting in chlorosis, weak plant growth, yield depression, and may even be accompanied by reduced nutrient uptake, disorders in plant metabolism and reduced ability to fixate molecular nitrogen in leguminous plants (Guala et al., 2010). Seed germination was gradually delayed in the presence of increasing concentration of lead (Pb), it may be due to prolong incubation of the seeds that must have resulted in the neutralization of the toxic effects of lead by some mechanisms e. g. leaching, chelation, metal binding or/and accumulation by microorganisms (Ashraf and Ali, 2007).

- **Effects of heavy metals on human health**

The plant uptake of heavy metals from soils at high concentrations may result in a great health risk taking into consideration food-chain implications. Utilization of food crops contaminated with heavy metals is a major food chain route for human exposure. The food plants whose examination system is based on exhaustive and continuous cultivation have great capacity of extracting elements from soils. The cultivation of such plants in contaminated soil represents a potential risk since the vegetal tissues can accumulate heavy metals (Jordao et al., 2006).

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (Sobha et al., 2007). Chronic level ingestion of toxic metals has undesirable impacts on humans and the associated harmful impacts become perceptible only after several years of exposure (Khan et al., 2008). The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones (Sobha et al., 2007). The Itai-itai disease in Japan brought the dangers of environmental Cd to world attention. Cd has been associated to a lesser or greater extent with many clinical conditions including anosmia, cardiac failure cancers, cerebrovascular infarction, emphysema, osteoporosis, proteinuria cataract formation in the eyes (Lalor, 2008).

Zn is considered to be relatively non-toxic, especially if taken orally. However, excess amount can cause system dysfunctions that result in impairment of growth and reproduction. The clinical signs of zinc toxicosis have been reported as vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anemia (Duruibe et al., 2007). Cu is an essential element in mammalian nutrition as a component of metalloenzymes in which it acts as an electron donor or acceptor. Excessive human intake of Cu may leads to severe mucosal irritation and corrosion, widespread capillary damage, hepatic and renal damage and central nervous system irritation followed by depression. The effects of Ni exposure vary from skin irritation to damage to the lungs, nervous system, and mucous membranes (Argun et al., 2007). Pb is physiological and neurological toxic to humans. Acute Pb poisoning may results in a dysfunction in the kidney, reproduction system, liver and brain resulting in sickness and death (Odum, 2000). Cr (VI) is toxic to plants and animals, being a strong oxidizing agent, corrosive, soluble in alkaline and mildly acidic water, toxic and potential carcinogens (Kazemipour et al., 2008). The toxicity of Cr (VI) derives from its ability to diffuse through cell membranes and oxidize biological molecules (Shaffer et al., 2001).

2.4.2 BIOAVAILABILITY OF HEAVY METALS

The mobility and phytoavailability of heavy metals depends on different extractable forms rather than to the total metal contents. The study of bioavailability and leachability of heavy metals gives more significant information about toxicity (Nair et al., 2008). Although, the heavy metal concentrations in sewage sludge or compost were far below the regulation standard but the long-term land application of compost with background heavy metal concentration can increase content and accumulation of the heavy metals in the soil (Chiang et al., 2007). Metal in soils can be divided into two fractions: (i) inert fraction assumed as the non-toxic fraction and (ii) the labile fraction, assumed to be potentially toxic (Yobouet et al., 2010). To assess the availability of heavy metals, only the soil labile fraction is taken into account because this fraction is often called as bioavailable fraction. However, the bioavailable fraction can differ from one metal to another and from one receptor to another. The availability of metals for plants and micro-organisms in soil depends on the composition of the different component of soil such as carbonates, (oxy) metal hydroxides, organic matter and silica (Yobouet et al., 2010).

The trace elements bioavailability has been considered as one of the most crucial problem in agricultural and environmental studies (Gupta and Sinha, 2007). The mobilization of pollutants depends on several factors: their mobility, their concentration in the soil and their solubility (Yobouet et al., 2010). The solubility depends on the chemical composition of leachate in equilibrium with the material; this chemical composition is influenced by the variation of pH that moves the redox equilibrium to predominant forms (Yobouet et al., 2010). At high pH, the predominant forms are hydroxides with low solubility and at low pH, free metallic ions which are highly soluble and predominated. There are two types of complexes in metal complexation reactions with soil particles: the soluble and the insoluble complexes. At pH 9 the solubility of Cu is increases due to the formation of soluble complexes (Yobouet et al., 2010). Fig. 2.4 shows the correlation in pH, bioavailability and solubility of heavy metals.

- **Water-soluble metals**

The extraction of metals by deionized water is the simplest method for determination of bioavailability of heavy metals in the compost and vermicompost. This fraction signifies most available to plant, resulting potential toxic to the plant followed by human health. The water soluble fraction is positively the most biologically active. The water soluble fraction has highest potential of contamination of food chain, surface water and ground water (Iwegbue et al., 2007).

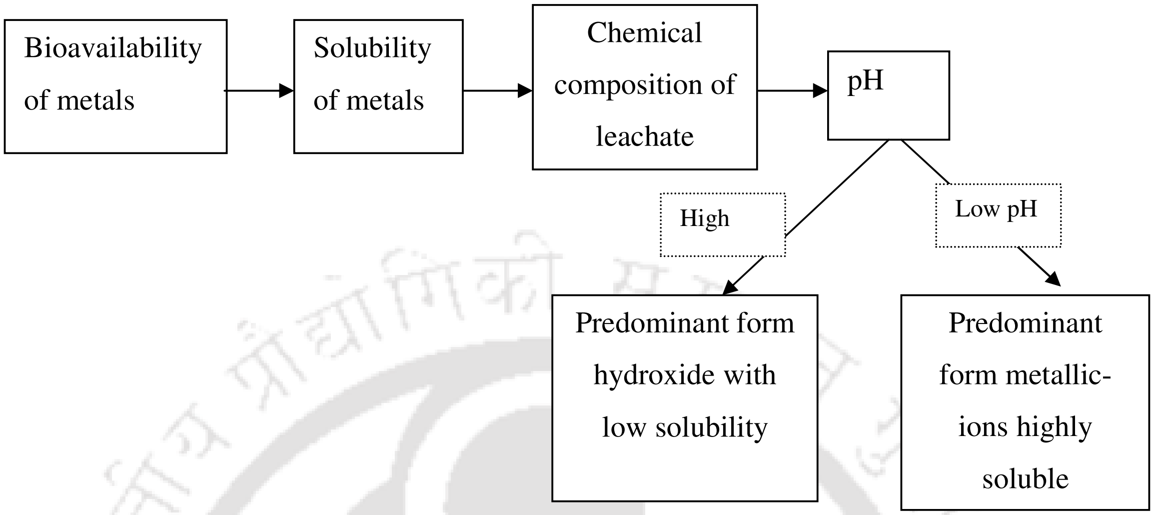


Fig. 2.4 Correlation in pH, bioavailability and solubility of heavy metals

The pH is considered 'master variable' controlling ion exchange, reduction/oxidation, adsorption and complexation reactions. Cations are adsorbed on organic matter at high pH (Samuel et al., 2013). The effect of organic matter amendments on heavy metal solubility also depend greatly upon the degree of humification of their organic matter and their effect upon soil pH (Gupta and Sinha, 2007). In a risk assessment, the pH is one of the factors that most affect the mobility and bioavailability of metals.

Ni is recognized as a potentially mobile and water-soluble element and the labile pool in compost is relatively easily solubilized by mild extractants due to the weaker binding to the matrix compared with other key metals which is also consistent with its behaviour in sewage sludge for example (Smith, 2009). The oxidation process and the formation of organo-metallic complexes taking place during the composting could reduce the soluble contents of Ni (Fang and Wong, 1999). The water-soluble fraction of Zn, Pb, Cu and Cd were found to decrease and stabilize after the thermophilic stage of composting. The pH of compost just before the thermophilic stage was found to be acidic causing higher available Pb and Zn, which have higher water-extractable concentrations at low pH (Hargreaves et al., 2008). The increases in total metal concentrations during composting was not accompanied increase in the amount of water soluble Cu, Mn and Zn. Sequential changes in the water soluble fractions of Cu, Mn and Zn were reflected by changes in water soluble organic carbon concentrations, which increased to a maximum at day 18 of composting and then declined (Hsu and Lo, 2001).

Fang and Wong (1999) studied the changes in water-soluble Cu, Mn, Ni and Zn contents in sewage sludge co-composted with lime at day 0, 7, 21, 49 and 100 expressed as mg/kg dry sludge-sawdust mixture and reported that water-soluble Pb, Cr and Cd contents in all treatments were below the detection limits of 0.1, 0.1 and 0.02 mg/L for these elements. Fuentes et al. (2004) studied different types of sludges (aerobic, anaerobic, unstabilised and sludge from a waste stabilization pond) for water soluble heavy metals and reported that the water-soluble Pb was in all cases below 0.2 mg/kg. However, the quantity of water-soluble Ni was lower than 1 mg/kg for all sludges. Hsu and Lo (2001) studied concentrations and water solubility of Cu, Mn and Zn in the composting of two swine manure was performed in two piles for 122 days, and reported that the water soluble fraction of Cu (expressed as percent of the total concentration in the sample) increased from 3% in the raw separated swine manure (SSM) to 5% at day 12, sharply increased to about 16% at day 18 and gradually decreased to 3% in the compost. The water extractable fractions of Mn or Zn increased from 1% in the raw material to 2% at day 18 and then gradually decreased to 0.5% at the end of the process. These results indicate that composting substantially modifies Cu leachability in SSM compost and appears to have little influence on Mn or Zn leachability.

The metallic form of Cr is quite stable and can resist attacks from environmental processes. However, in either the trivalent or hexavalent forms, chromium exhibits higher chemical activity (including amphotericity, i.e., the ability to react chemically as an acid or a base), as well as solubility and hence potential mobility and subsequent removal from the solid fraction (Haroun et al., 2009). Ciavatta et al. (1993) reported that Cr appears to be the heavy metal that tends to insolubilize during composting and thus to decrease in solubility in the absence of soil. Cr released from organic matter in neutral or alkaline soils precipitates as insoluble forms; therefore it is not adsorbed by plants. Due to its low solubility, only a little amount of chromium is bioavailable, meaning that even when crops are grown in soils treated with sludge relatively high in Cr, phytotoxicity is rarely observed.

- **Diethylene triamine pentracetic acid (DTPA) extractable heavy metals**

It has been considered that DTPA is a chelating agent and widely used for diagnosis of plant availability of the metals in soil at regular or even higher concentration (Guan et al., 2011). The DTPA-extractable fraction of metals might represent a supplemental approach to check the bioavailability of heavy metals in the soil and sludge amended soil for plant uptake (Fang and Wong, 1999; Fuentes et al., 2006). DTPA solution is assumed to extract both carbonate-bound and organically bound metal fractions in calcareous soils, and indicates the amount of metals potentially available for plant uptake (Walter et al., 2006). The mobility of trace metals, their bioavailability and related eco-toxicity to plants, depend strongly on their specific chemical forms or ways of binding (Walter et al., 2006; Gupta and Sinha, 2007). The bioavailability of heavy metals in the soil also depends on their distribution between solid and solution phases, dependent on the soil processes like cation exchange, specific adsorption, precipitation and complexation. The process of metal uptake and accumulation by different plants depend on the concentration of available metals, solubility progressions and the plant species growing on these soils (Gupta and Sinha, 2007). The fate of toxic metals largely depends on their interactions with inorganic and organic soil surfaces (Bragato et al., 1998).

Bragato et al. (1998) studied DTPA extractable heavy metals in soil with 0, 7.5 and 15 t/ha sewage sludge addition and reported that in the control soil, DTPA solution extracted about 6% of total Zn, 3% of total Ni, 29% of total Cu and 24% of total Pb. DTPA-extractable Cu and Pb did not change significantly between the treatments, while DTPA-extractable Zn and Ni gradually increased in direct proportion to the amount of sewage sludge added to the soil. In the 7.5 t/ha sludge treatments, DTPA-extractable Zn represented about 9% and DTPA-extractable Ni about 4% of their total content in the soil. Larger amounts of total Zn and Ni, 11 and 5%, respectively, were extracted by DTPA in the soil treated with 15 t/ha of sludge.

Wong and Selvam (2006) reported that after composting, the Cu, Mn, Ni and Pb and Zn contents decreased with an increase in lime amendment rate. The difference in Ni and Zn contents between control and lime treated composts was not significant. In the sludge, the order of metal contents in DTPA extracts was: Zn > Cu > Mn > Pb > Ni, but after composting the order was: Zn > Mn > Cu > Pb > Ni. Chiang et al. (2007) reported that in case of added lime, the amount of DTPA-extractable Pb, Cu and Zn significantly decreased in the composting because of the higher ion exchange capacity and alkalinity. Fang and Wong (1999) reported that a significant decrease in the initial extractable Ni content was only obtained at the high lime- amendment levels (51%). Chen et al. (2010b) investigated the effects of bamboo charcoal (BC) on nitrogen conservation and immobility of Cu and Zn during pile composting of pig manure and concluded that the extraction efficiency of DTPA extractable Cu and Zn also decreased with increasing amount of BC this means that BC was effective for reduction of bioavailability of Cu and Zn. It adsorb heavy metals due its large specific surface area, high surface density of functional groups and metal oxides, in addition, similar to other carbonaceous materials, BC has a large quantity of surface negative charge and a high charge density, which can also improve the adsorbing capacity of BC for positively charged heavy metal ions. Hua et al. (2009) concluded that mobility of heavy metals in the sludge composting could also be reduced by the addition of BC. However, the stabilization effect of BC was different for Cu and Zn. DTPA-extractable contents of Cu and Zn in sludge composting material with 9% BC amendment dropped 27.5 and 8.2%, respectively, at the end of composting as compared with control.

2.4.3 LEACHABILITY OF HEAVY METALS

The heavy metal leached fraction concept, defined as the ratio of the amount of a heavy metal released from toxicity characteristics leaching procedure (TCLP) test to its total content, is commonly used to assess the leachability of heavy metals in the compost (Chiang et al., 2007). The TCLP is designed to determine the mobility of both organic and inorganic analytes present in liquid, solid and multiphasic wastes. If an analysis of any one of the liquid fractions of the TCLP extract indicates that regulated heavy metals are present at such high concentrations that, even after accounting for dilution from the other fractions of the extract, the concentration would be above the regulatory level for those metals, then the waste is hazardous (US EPA, 1992). TCLP is used to determine the suitability of compost for land application or should be considered a hazardous waste. The procedure is designed to simulate the leaching potential of the compost material when it is applied to soil. The regulatory limits for leached toxic heavy metals are based on avoiding groundwater contamination that would create a risk to human and environmental health (Chiroma et al., 2013). The threshold limit for heavy metals contamination in mg/kg is given in table 2.1.

The mobility, bioavailability and eco-toxicity of the metals depend on the specific chemical forms or bindings in which the metals exist in different waste materials. It is very much necessary to examine leachability of heavy metals to determine the suitability of decontaminated sludge for land application (Pathak et al., 2009). Wang et al. (2010) studied leachability of Cu in chicken manure compost during the 110 days of composting period and reported that the amount of leached Cu increased initially but at the end of composting process it was reduced significantly. The amount of Cu leached from manure throughout the composting process averaged 20% of the total Cu in the compost (Villasenor et al., 2011). The Ni was present at much higher water soluble levels in municipal solid waste (MSW) compost and considered as leachable (Hargreaves et al., 2008). An acid neutralization capacity test in order to estimate the metal leachability in the compost produced by using clinoptilolite as additive and reported that by increasing the leachate pH, the heavy metal concentration decreased (Villasenor et al., 2011).

Table 2.1 Threshold limits for leachable heavy metals (US EPA, 1992)

Contaminants	Threshold (mg/kg)
Arsenic	100
Barium	2000
Cadmium	20
Chromium	100
Lead	100
Mercury	4
Selenium	20
Silver	100

Note: Only applicable to samples which are 100% solid

Ciba et al. (1999) reported that Zn can be leached in significant amounts, and only if pH is 2.5. As expected, leaching at pH 2.5 enables higher amounts of elements to be leached than at pH 4.5. This seems to be especially understandable in the case of metals chemically bound as carbonates. In the case of Zn, it can be seen that at lower pH a substantial percentage of this form can also be extracted. Chiang et al. (2007) revealed that the leached fraction of Cu, Zn and Ni decreased with increasing co-composting time. In all additive tests, the leached fraction of Zn was higher than that with other tested metals. Since the total Zn concentration was high in co-compost and most soluble Zn was leachable. Although the tested additives had higher alkalinity, larger surface sites and ion exchange ability for inhibiting the heavy metal mobility, the additives might not suppress the changes in Zn leaching.

2.4.4 SPECIATION OF HEAVY METALS

The determination of total heavy metals concentration does not provide useful information about risk of bioavailability, toxicity, capacity for remobilization of heavy metals in the environment and chemical forms of a metal in which they are accessible in the composting process (Liu et al., 2007). The bioavailability of metals in soil is a dynamic process that depends on specific combinations of chemical, biological, and environmental parameters. The mobility of trace metals, their bioavailability and related eco-toxicity to plants, depend strongly on their specific chemical forms or ways of binding (Fuentes et al., 2004). The action of microorganisms in composting may make the metals more available due to metal release from the decomposed organic matter in the sludge (Qiao and Ho, 1997).

Several extractants and extraction procedures have been proposed to determine the availability of elements, which are potentially toxic to plants (Nemeth et al., 1999). Single and sequential extraction methods are generally used in more fundamental studies such as: to elucidate the soil chemistry, to assess the structure and composition of soil components and to improve understanding of the processes in the soil. It can control the mobilization and retention of nutrient and toxic elements as well as to illuminate their transport mechanisms (Rao et al., 2008).

Sequential extraction procedures provide useful information for risk assessment, since the amount of metals mobilized under different environmental conditions can be estimated. The sequential chemical extraction procedure could provide an understanding of metal fractions with different natures, and allow the prediction of metal mobility, bioavailability and leaching rates (He et al., 2009a). The mobility and bioavailability of the metals decrease approximately in the order of the extraction sequence (Nair et al., 2008). The principle of sequential chemical extraction methods is that various chemical extractants are applied successively to a sample, dissolving the components of the sample matrix in sequential order. Preferably, a reagent should liberate all the metals from a particular matrix's component (i.e. exchangeable, carbonate, etc.), without affecting other components (Li et al., 2001). The principal advantage claimed for sequential extraction over the use of single extraction is that the phase specificity is improved. Of the total heavy metals, the water-extractable and exchangeable fractions are considered to be the most plant-available form (Pare et al., 1999).

Sequential chemical extraction can be used to investigate the speciation of heavy metals in various wastes and its compost since it can provide a more accurate measure of risk to the environment than that of total metal concentration obtained after strong acid digestion. It has been believed that water soluble and exchangeable fractions of metals are most available to the plants (Pare et al., 1999). Chemical speciation can be defined as the process of identifying and quantifying different species, forms or phases present in a material. These species can be defined: (1) functionally, for example those species that can be assimilated by plants, (2) operationally, according to the procedures or reagents used in their extraction and (3) specifically, as particular components or oxidation states of an element (Fuentes et al., 2004).

The speciation of heavy metals can play a major role in the toxicity of those metals in sewage sludge following land application (Zheng, et al., 2007). The evaluation of sewage sludge toxicity by chemical speciation is therefore very important when deciding on the suitability of sludge for land application, as well as for obtaining its optimum application rate (Walter et al., 2006). The chemical forms of a metal or speciation allows the estimation of heavy metal bioavailability and is related to the different natures of the metals, their bonding strength, either in free ionic form or complexed by organic matter or incorporated in the mineral fraction of the sample (Amir et al., 2005). The pH was decreased due to acid production during the composting process, which accelerated the mobilization of heavy metals; especially those in mobile fractions (Nomeda et al., 2008). Qiao and Ho (1997) reported that red mud affects the speciation of heavy metals through increasing the pH, solid-to-solution ratio and available adsorption sites. In general sewage sludge composting with red mud reduces the leachability and plant availability. The metal sulfides in the composted mixture were probably oxidized to metal sulphates. Therefore, metal bound with sulfides might have been released and then adsorbed on sulphate surfaces or precipitated as oxides (Nomeda et al., 2008). According to Tessier et al. (1979) heavy metals are associated with five fractions (Fig. 2.5).

Venkateswaran et al. (2007) reported that the F1 and F2 fractions contribute significant quantities of total heavy metals, which are easily bioavailable fractions and can be leached during the changes in environmental conditions and poses threat to groundwater quality also. Acidic environment enhances the mobility of metal in the environment; therefore the metals bound to F2 fraction are very sensitive to pH change and leached at lower pH (Zheng et al., 2007). Metals confined in the F5 fraction are usually not expected to release over short periods of time under the natural conditions (Gupta and Sinha, 2007). The mobility of element by its capacity to pass into soil compartments where it is less energetically retained. It depends on many factors which are related to the nature of the element, the soil properties (e.g. pH, Eh, clay content, organic matter rate), and to the type and quality of soil amendments. The mobility of heavy metals in the soils may be assessed on the basis of their absolute and relative content in fractions weakly bound to the soils (Achiba et al., 2009). Adsorptions on hydrous oxides of iron and cation exchange reactions are important modes of metal immobility (Khaled, 2004). The speciation of Zn, Cu, Mn, Ni, Pb, Cd and Cr in various waste compost and compost amended soil is given as follows:

- **Zinc (Zn)**

Zn is frequently the element where the largest increase in labile forms is reported when composted residuals or sewage sludge is applied to soil, particularly under acidic soil conditions (Smith, 2009). Based on the redox potential, the redox reaction with other metal ions, the Zn^{2+} can be expected to stay in ionic form in the solution. Zn was evenly distributed in F2, F3 and F4 fractions in the sludge as result of adsorption equilibrium relationships (Qiao and Ho, 1997). The stable F4 and F5 fractions of Zn were transformed into unstable F1 fraction due to oxidation of organic matter during the composting process (Chiang et al., 2007; Smith, 2009).

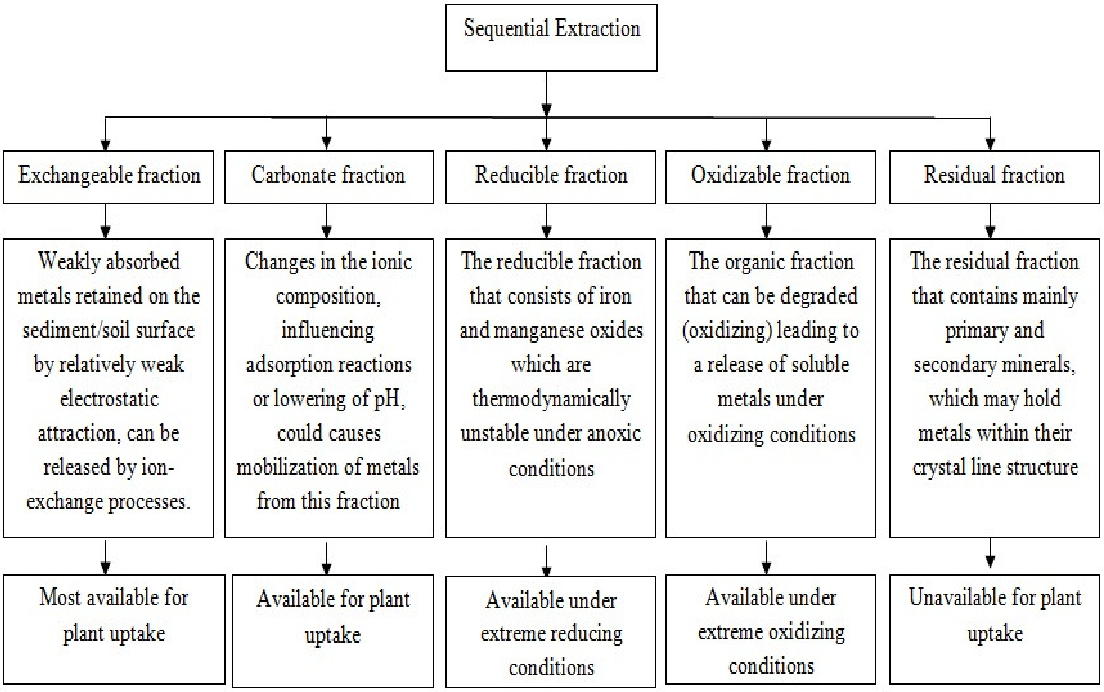


Fig. 2.5 Summary of the sequential extraction method for metals, as applied to the analysis of compost and compost- amended soil

Qiao and Ho (1997) reported that the composting process converted the F4 fraction of Zn into F1 fraction and red mud addition inhibited this conversion. Since the organic matter in the mixture was oxidized during the composting process, Zn was converted from F4 fraction into F1 fraction and tended to be more mobile as a result of the increase of the redox potential and decrease of the sulphides. Qiao and Ho (1997) concluded that change in redox potential more strongly affected the speciation of Zn than that of Cu and Pb. Factoring out the dilution effect, red mud addition converted the F1 fraction of Zn into F2 fraction due to the increase of pH and suppressed the changes of Zn speciation during the composting process. The F1 fraction of Zn during the composting was precipitated as zinc hydroxide and carbonate by red mud addition through raising pH from 5 to 7.2. Zn hydroxide dissolves under pH 7.2, so the F1 fraction was effectively controlled by pH in the sludge compost.

Cai et al. (2007) studied forced aerated composting of sludge and reported that F5 fraction of Zn was transformed into the F3 and F4 fractions in the sewage sludge composting. Wong and Selvam (2006) reported that the lime addition inhibited transformation of predominant F5 fraction of Zn into F4 fraction during the composting process. Venkateswaran et al. (2007) studied the distribution and speciation of toxic heavy metals in plating wastewater residues and sludge. This study revealed that the predominant form of Zn is the F3 fraction, followed by F2, F4, F5 and F1 fractions. Higher concentration of heavy metals in F3 fraction (bound to Fe and Mn oxides) can be attributed to diffusion mechanism.

Nomeda et al. (2008) found that the mobile fractions of Zn were increased during the composting process due to the oxidation of organic matter and its high oxidation-reduction potential. The formation of humic substances seems to transform Zn from sulfide fraction to organic fraction during the composting process. Gupta and Sinha (2007) reported that Zn was mostly associated with F3 fraction in most of the amendments of tannery sludge. Ciba et al. (1997) studied the contamination of waste with dust of metallic Zn and zinc sulfide, the composting process increases the mobility of Zn mainly due to the increase of the F2 form. However, the increase is relatively slight, especially in the case of zinc sulfide. Contamination of the waste with zinc sulfide, 70% of Zn remains bound with the not easily accessible to plants sulfide form. Ciba et al. (1999) reported that Zn mainly occurs in F4 fraction and considerably smaller amounts in F2 fraction during the composting of municipal solid waste.

He et al. (2009b) shows that the concentrations of F1 fraction of Zn in sewage sludge composting were 40–80 times higher than in swine manure composting, resulting bioavailability factor of Zn in sewage sludge composting 10–20 times larger than in swine manure composting. Significantly increased F1 and F2 fractions of Zn were observed but the concentrations tended to remain constant after composting for seven day. The F3 fraction of Zn in sewage sludge composting declined rapidly from 668.9 mg/kg to 302.3 mg/kg and kept steady at about 300 mg/kg during the cooling stage, but F3 fraction rose gradually. The F4 fraction fluctuated slightly throughout both composting processes and reached the lowest level in the final compost (357.5 mg/kg and 188.9 mg/kg in sewage sludge composting and swine manure composting, respectively). The F5 fraction decreased in the initial fourteen days and then leveled off. After sewage sludge composting, the dominant speciation for Zn transformed from F3 and F5 into more readily available fractions of F1 and F2, but predominant fractions of Zn in swine manure were turned from F4 fraction into F3 fraction by composting treatment.

Yang et al. (2011) concluded that Zn in the unpolluted oasis soil was mainly found in the F5 fraction; however, with the increasing contents of Zn in the oasis soil, the fractional distribution of Zn significantly changed. The fractionation distribution coefficient of Zn in the soil increased significantly in F1 and F2 fractions, changed small in F4 fraction and decreased significantly in F5. Fractionation distribution coefficient was usually defined as the one metal fractionation content accounting for the percentage by total amount of the same metal (Yang et al., 2011).

- **Copper (Cu)**

Cu is widely used as a mineral element in plant, derived from soil matrix; however, the potential behavior, bioavailability and the transfer dynamics of Cu in soil–plant system can be poorly predicted by total concentration. It has been realized that the toxicity of Cu is determined by cupric ion activity; as a result, the mobile Cu is more toxic to the plant growth than strongly complex forms (Guan et al., 2011). Cu strongly bound to F4 fraction and would be released slowly over time of the sludge is decomposed. The organic matter is one of the causes that may reduce the ability of metals for plant uptake in the soil due to metal-organic complexation (Yuan et al., 2011). The organic matter is frequently reported to have a dominant role in controlling the behaviour of Cu in the soil, due to it potentially important binding site for this element in compost and amended soil. However, organic matter may not necessarily represent the main binding site for Cu in soil, which may depend to an extent on the soil type (Smith, 2009). Cu dominated in the organic bound fraction in both sludge and sludge compost (> 80%) due to Cu forming very stable complexes with organic ligands. Furthermore Cu ion is directly bound to two or more organic functional groups mainly carboxylic, carbonyl and phenolic so that the ion is immobilized in a rigid inner-sphere complex (Qiao and Ho, 1997). Due to the high affinity for organic matter, Cu was not easily mobilized in the composting process. In addition, some parts of Cu were significantly transferred from mobile fractions (F1- F3) and F5 fraction to F4 fraction during the composting process (Nomeda et al., 2008). The largest increase of Cu in the soil treated with MSW compost has been reported to occur in the organic fraction, a fraction temporarily unavailable to the plants. It has been suggested that MSW compost increased F1 fraction of Cu, but its bioavailability was reduced because it formed complex compound with organic matter, which is often increased in soils amended with MSW compost (Hargreaves et al., 2008). Gupta and Sinha (2007) reported that Cu was bound with F4 and F5 fractions in the tannery sludge.

Hsu and Lo (2001) concluded that the greatest amount of Cu was in the F4 fraction, followed by the organically complexed, solid particulate and water soluble fractions. The small amount of Cu was associated with the F1 and F2 fractions in the compost. Approximately 70% of total Cu was associated with the F4 and organically complexed fractions, which may have been due to a high affinity of Cu for organics. Fuentes et al. (2004) studied different types of sludges (aerobic, anaerobic, unstabilized and sludge from a waste stabilization pond) for speciation of heavy metals. The result of this study shows that the Cu was mainly associated with the organic matter; the greatest extraction percentage was obtained in the F4 fraction of all the sludges, which was to be expected given the affinity of organic matter for this type of element and the formation of stable complexes. The sum of the last two fractions accounted for 95% of the total Cu, in all the sludges, which indicated that Cu was associated with strong organic ligands and probably occluded in minerals such as quartz, feldspars etc.

The mobile fraction of Cu declined with increasing content of humic substances and decrease in pH value (7.5–6.7) during composting, consistent with the known affinity of Cu to organic matter (Smith, 2009). Qiao and Ho (1997) reported that the amount of Cu adsorbed decreased with an increase in the amount of dissolved organic carbon in solution due to Cu forming stable complexes in solution which had a tendency to remain in solution. The Cu complexed with soluble fuivic or humic acids would be available to leaching and to presumably also plant.

He et al. (2009b) revealed that readily extractible fractions of Cu (F1 and F2 fractions) decreased during swine manure composting. Therefore, the bioavailability factor of Cu, which is defined as the ratio of the metal content in the F1 fraction to the total metal concentration, in percentage, significantly increased during sewage sludge composting but declined during swine manure composting. The F2 fraction of Cu was increased and maintained about 5mg/kg after the thermophilic phase. The F3 and F4 fractions dropped sharply in the initial stage of the sewage sludge composting process and then tended to remain constant after 21 days. But these two fractions decreased more steadily in the swine manure composting. The F5 fraction leveled off in both composting processes. The F4 and F5 fraction accounted for the most predominant speciation throughout the both composting processes, occupying more than 50 and 30% of the total Cu, respectively.

- **Manganese (Mn)**

Gupta and Sinha (2007) reported that Mn was mostly associated with F3 fraction in most of the amendments of tannery sludge. Venkateswaran et al. (2007) reported that the negligible amount of F1 fractions of Mn is found in all the samples, however; the F2 and F3 fractions occupy the highest proportions. Wong and Selvam (2006) revealed that the F1, F2 and F3 fraction of Mn increased after composting and when compared with control the contents of these fractions decreased marginally with the lime application. There was no significant difference in the transformation of F4 fraction of Mn between the control and other treatments. An extremely significant increase in F1 fraction was observed in all treatments, which suggest that the addition of lime could not prevent the mobility of Mn. This rise in pH can reduced bioavailabilty of Mn by precipitate formation, thereby increasing the number of adsorption sites and reducing the competition of H^+ for adsorption, followed by increase in metal stability with humic substances (Achiba et al., 2009).

- **Nickel (Ni)**

The Ni cation has the stability of complexes with organic ligands just less than Cu^{2+} in the transition metal cations, therefore Ni dominated in the F4 and F5 fractions in the sludge and compost mixture (Qiao and Ho, 1997). The F4 and F5 fractions of Ni to are not considered to be available to plants. During composting, the stabilization of Ni in sewage sludge offers the potential to reduce the risks of the heavy metals in the compost (Zheng et al., 2007). The mobility of Ni increased with declining pH and organic matter content (Smith, 2009). Gupta and Sinha (2007) reported that the maximum amount of Ni bound with F5 fraction in the tannery sludge, the significant increase in the level of Ni with F5 fraction may be due to alkaline stabilization process. Amir et al. (2005) considered that the high proportion of Ni in the sewage sludge that is present in an organic form that is readily biodegradable therefore releasing Ni in soluble forms during the composting process.

The majority of Ni in sewage sludge is present in F4 fractions, followed by F2 and F3 fractions, after composting, the F5 fraction contained the greatest amount of Ni concentration (Zheng et al., 2007). The proportions of F1, F2, F3 and F4 fraction of Ni decreased while the proportion of F5 fraction of Ni increased, it was due to F1, F2, F3 and F4 fraction of Ni were transformed to the F5 fraction during the composting process (Zheng et al., 2007; Wang et al., 2008). Wong and Selvam (2006) reported that the F1, F2, F3 and F4 fractions of Ni decreased, the F3 fraction of Ni was mainly transformed into F5 fraction after composting and lime application. Smith (2009) reported that the majority of Ni in MSW-compost (up to 50%) resides in the residual and low availability fractions (e.g. carbonate, phosphate and sulphide minerals), probably as metallic or alloy forms, silicate minerals, glass and inert plastics. Wang et al. (2008) concluded that the addition of SSL caused an increase in organic matter and sulfide fraction (27.8%) compared to that in the control, which might also be mainly because of the formation of nickel sulfide, by the sulfidation reaction. Qiao and Ho (1997) reported that Ni was mainly bound in F4 and F5 fraction in the sludge and the compost mixture. The stable fractions of Ni converted into more available when the sludge was mixed with the sawdust and recycled sludge compost, which might be due to the decline of pH from 8.3 to 5.2 after the sludge storage. The increase in total Ni was more than the reduction of dry matter during the composting. The percentage of F5 fraction of Ni appeared to decrease even though the concentration of this fraction remained the same to the 50th day of composting. The red mud was very effective for conversion of F1 fraction into F4 fraction. The Ni form complexes with the oxides surfaces in the red mud. It caused preventing the Ni to complex with the humic substance in the compost.

Fuentes et al. (2004) reported that Ni was mainly associated in the F5 fraction in sewage sludge. Venkateswaran et al. (2007) investigated that F3 fraction of Ni was predominant in all the samples. The order of different Ni fractions in the samples was: F3 > F2 > F4 > F5 > F1. The F1 and F2 fractions contributed about 15-30% which can cause environmental toxicity during mobility. Xu et al. (2012) reported that Ni was largely present in the F5 fraction in the soil without fly ash and sewage sludge. The F1, F2 and F3 fractions of Ni was increased, whereas the F5 fraction reduced drastically after the application of fly ash and sewage sludge.

- **Lead (Pb)**

The Pb has a stronger affinity to the adsorption sites on the clay materials such as silanol groups of silica and amorphous aluminium hydroxide. The Pb was dominated in the F5 and F4 fractions in the sludge, but it was more evenly distributed in the F2, F3 and F4 fractions in the compost mixture (Qiao and Ho, 1997). Pb occurs mainly in F2 and F4 fraction, in which F2 fraction is moderately mobile and F4 fraction is less mobile in the municipal solid waste compost (Ciba et al., 1999). Venkateswaran et al. (2007) reported that Pb was mainly existed in F4 fraction which can be leached only under extreme oxidizing conditions. Qiao and Ho (1997) revealed that the dissolved organic carbon make help in reduction of mobility and availability of Pb in the sewage sludge compost. Dissolved organic carbon adsorbed positively charged Pb-organic complexes, cation

Bridge, or hydrophobic interactions with the clay surface as a result of a lowering of the negative charge on the dissolved organic carbon in sewage sludge through complex formation, therefore composting of sludge decreased the Pb mobility and plant availability (Qiao and Ho, 1997).

He et al. (2009b) reported that the concentrations of five fractions in Pb were much lower in swine manure compost, but they had similar evolution trends as sewage sludge composting. The F1 fraction of Pb decreased about 50% in the first seven days and then declined smoothly. Therefore, the composting process significantly decreased BF of Pb in both sewage sludge and swine manure compost. The fraction of F2-Pb increased slightly and reached the maximum concentration at the end of composting. Similarly, Pb in F3 fraction declined gradually and reached the minimum content in the final compost. The F4 fraction of Pb was fluctuated during the thermophilic period, but the final amount was lower. The F5 fraction of Pb decreased slightly during two composting processes. Fuentes et al. (2004) was found that the high proportion of Pb was found in F3 fraction in the un-stabilized sludge (40%) and in the other sludges, the Pb was distributed between the F4 and F5 fraction, being the highest quantity mobilized (73%) in oxidizing conditions for the aerobic sludge. Qiao and Ho (1997) investigated that the composting process significantly stabilized the Pb in the mixture. The fifty days composting process converted all the Pb in the F1 and F2 forms into F4 fraction. Wong and Selvam (2006) found that the F1 fraction of Pb was almost absent till the end of composting and F2, F3 and F4 fractions increased after the composting process with simultaneous reduction in F5 fraction. The major transformation of different fractions of Pb were not found, however the F5 fraction of Pb increased in lime added compost.

- **Cadmium (Cd)**

In general Cd is regarded as a hard, high melting-point, unreactive substance, that exhibits a wide range of oxidation states and tends to form strong covalent bonds and one other characteristic of Cd is that its chemistry is similar to that of Zn (Whittle and Dyson, 2002). The Cd occurs mainly in the moderately mobile F2 fraction and in the less mobile, but for the plants potentially available, form of sulphides (Ciba et al., 1999). The Cd was found maximum in F3 fraction and not bound to F4 of the soil amended with sewage sludge (Gupta and Sinha, 2007). The Cd was mainly found in F4 fraction in waste stabilization pond and anaerobic sludge, while F1 fraction was about 15% of the total Cd in the anaerobic sludge (Fuentes et al., 2004). Relatively high percentages of easily available fractions (F1 and F3 faction) of Cd were obtained in the least stabilized sludge

(Fuentes et al., 2004). Yang et al. (2011) concluded that Cd in the unpolluted oasis soil was mainly found in the F5 fraction; however, with the increasing content of Cd in the oasis soil, the fractionation distribution of Cd was appreciably changed. The fractionation distribution coefficient of Cd in the soil increased significantly in F1 and F2 fractions, changed little in F4 fraction and decreased considerably in F5 fraction (Yang et al., 2011).

- **Chromium (Cr)**

Chromium is classed as a ‘hard’ metal, and as such, these metals are generally less mobile, in its metallic form it is quite stable, resisting attack from environmental processes (Whittle and Dyson, 2002). Cr is the 10th abundant element in the earth’s mantle and persists in the environment as either Cr (III) or Cr (VI). It is commonly present in soils as Cr(III) and Cr(VI), which are characterized by distinctive chemical properties and toxicities (Garnier et al., 2006). Cr (III) has an electron configuration closest to a noble gas with a high spherical symmetry and its polarisability is the lowest among the six tested metals, it has a valence of three and therefore it has a stronger electrostatic affinity for the sorption sites than divalent cations (Qiao and Ho, 1997). Cr (VI) is a strong oxidizing agent and is highly toxic; it is 10 to 100 times more toxic than Cr (III). Whereas Cr (III) is a micronutrient, insoluble in water and a non-hazardous species; (Shaffer et al., 2001). It is less toxic to mammalian, aquatic organisms and plants due to its low solubility, mobility and bioavailability (Zhou et al., 2006). However, in either the Cr(III) or Cr(VI) exhibits more chemical activity (including amphotericity) as well as solubility and hence potential mobility (Whittle and Dyson, 2002). The Cr (III) in particular, on the contrary, showed a decrease in solubility/extractability during the stabilization process, i.e. with increasing degree of humification (Ciavatta et al., 1993). The possibility that organic ligands and/or acid conditions increase Cr(III) mobility in soils and that MnO, allow its oxidation to the more toxic and mobile Cr(VI) forms, cannot be prohibited, therefore, removal of Cr from tannery sludge is essential for safe land application of sludge (Zhou et al., 2006). The composting process affected the speciation of Cr in the sludge compost though the changes were relatively small (Qiao and Ho, 1997).

The F1 fraction of Cr is probably related to toxicity more than the total Cr content, it is therefore essential to determine both the total amount of Cr in the soil, its solid speciation and oxidation state, in order to evaluate its potential impact on the biodiversity of the ecosystems (Garnier et al., 2006). The Cr was found maximum in F3 fraction and not bound to F4 fraction of the soil amended with sewage sludge (Gupta and Sinha, 2007). The Cr was present predominantly in F5 stable fraction and thus has extremely low bioavailability (Smith, 2009). Shukla et al. (2009) reported that F5 fraction of Cr in the composting mixture was very high at zero days. However, during maturity of compost it was reduced in contrast with Cr in carbonate fraction. Gupta and Sinha (2007) reported that Cr was mostly associated with F3 fraction in most of the amendments of tannery sludge. Zheng et al. (2007) reported that the F1, F2, F3 and F4 fraction of Cr decreased while the proportion of F5 fraction of Cr increased due to F1, F2, F3 and F4 fractions of Cr are transformed to the F5 fraction during composting process. The F4 and F5 fractions of Cr are not considered to be available to plants, therefore during composting; the stabilization of Cr in sewage sludge offers the potential to reduce the risks of the heavy metals in the compost (Zheng et al., 2007). The F2 and F3 fraction of Cr were converted into the F4 fraction during the composting process perhaps as a result of the competition of Cr with other metal cations for limited humic organic ligands which were produced during composting (Qiao and Ho, 1997).

The Cr was more likely to be found in the F4 fraction than in the F5 fraction, since it probably forms sulphides with this type of sludge (obtained by anaerobically and waste stabilization pond), while in the other types, Cr was mainly associated with the F5 fraction (Fuentes et al., 2004). Venkateswaran et al. (2007) found that the F1 fraction of Cr was not found in all the samples, resulting the leaching of Cr to environment from these samples may not occur readily. Qiao and Ho (1997) found that the F1 and F2 fraction of Cr in the initial mixture was about 20% of total Cr in the mixture may become leachable or available with a changed environmental condition such as a reduction in pH. Because of the high competitive nature of Cr for adsorption sites any released Cr will displace other adsorbed metals. Therefore there was no leachable and plant available Cr detected in the sludge compost even though it contained 29 mg/kg total Cr (Qiao and Ho, 1997). Xu et al. (2012) reported that Cr mainly distributed in F4 and F5 fractions with 75-97% of total Cr. This specified that Cr would not be easily released in the environment during utilization of fly ash-sludge. Application of coal fly ash-sludge resulted in an increase of Cr concentration in the F1 and F4 fractions consequently decreased the F5 fraction of Cr.

2.4.5 REDUCTION OF HEAVY METALS AND THEIR BIOAVAILABILITY IN COMPOST AND AMENDED SOIL

- **Physico-chemical methods**

The thermophilic phase of composting, which is the first step of composting, affects the exchangeable fraction. The oxic and anoxic conditions (produced by acetic acid and ammonia) at the first step of composting, affect the reducible and organic fractions

(Zorpas et al., 2000). He et al. (2009a) reported the content of F1 fraction of Pb increased during the later thermophilic stage, but dropped again in the cooling period. The relative percent of F5 fraction of Zn increased 3.38% during mesophilic and thermophilic phases but decreased 1.07% in the cooling stage. The F1 and F2 fractions of Cu only accounted for the small parts of total Cu. Their concentrations increased, although a decrease tendency appeared during thermophilic phase. Amir et al. (2005) studied heavy metals bioavailability or chemical forms of Cu, Zn, Pb and Ni in sewage sludge composting with straw, and concluded that the largest proportions of metals was found in the F5 fraction which is more stable forms and unavailable for plant uptake. The amount of potentially bioavailable metals was less than 2% of total metal. Physico-chemical and biological methods, reducing agent and target metals are given in Table 2.2.

Heavy metals contain compost quality can be improved by adding some chemical compounds. Many studied have been carried out by using natural zeolite (Villasenor et al., 2011; Stylianou et al., 2008; Zorpas et al., 2000; Zorpas et al., 2002; Sprynskyy et al., 2007; Zorpas et al., 2008), lime (Fang and Wong, 1999; Wong and Selvam, 2006) lime and sodium sulfide (Wang et al., 2008), bamboo charcoal (Chen et al., 2010b). These chemical compounds used as amendments, which can improve compost quality by removing or changing mobile and available form of metals to less mobile or residual or less available form. Mobile species of heavy metals cause more serious pollution problems since they can be easily uptake by plants and enter the trophic chain or pollute the ground waters (Sprynskyy et al., 2007). Heavy metals reduction mechanisms of physicochemical and biological methods are given in Table 2.3.

Lime and sodium sulfide treatment

Lime is considered as one of the most common amendment materials for sewage sludge stabilization, as it plays significant role in reducing the microbial content of sludge (pathogens), as well as the availability of heavy metals, enhancing the agricultural benefits and lowering the respective environmental risks (Samaras et al., 2008). Addition of lime during sewage sludge composting process is very useful amendment material for heavy metal immobilization (Wong and Selvam, 2006; Pardo et al., 2011).

A slower rise in temperature was observed in sludge with lime amendment as compared to that of the control with sludge only. The small amount of lime added provided a buffering against the decrease in pH and a suitable amount of Ca, which would improve the metabolic activity during composting (Fang and Wong, 1999). Addition of lime caused a significant reduction in water-soluble Cu, Mn and Zn contents during composting process. Cu, Mn and Zn contents in the control increased slightly initially, and then rose sharply after day 21 (Wong and Selvam, 2006).

Fang and Wong (1999) performed a batch composting study to evaluate the feasibility of co-composting sewage sludge with lime at 0, 0.63, 1.0 and 1.63% w/w, and composted for 100 days in laboratory batch reactors to reduce the availability of heavy metals in the sludge compost and stated that the lime amendment was effective in reducing heavy metal availability of sludge compost by forming less soluble carbonate salts. Ni demonstrated a different pattern of change with a decrease in Ni content according to the increase in time for all treatments as compared to other three metals (Fang and Wong, 1999). Chiang et al. (2007) investigated the effect of different amendments including lime, coal fly ash and natural zeolite on the potential for inhibition of heavy metal mobility during the sewage sludge composting process and reported that, the amounts of tested DTPA-metals decreased insignificantly using coal fly ash. In case of added zeolite and lime, because of the higher ion exchange capacity and alkalinity, the amount of DTPA-extractable Pb, Cu and Zn significantly decreased in the composting (Chiang et al., 2007). Addition of lime also resulted in a decrease in the extractable Pb contents throughout the composting period. At the end of composting, the percentage of reduction of Pb contents for lime-amended sludge compost higher than control (Fang and Wong, 1999). The lime amendment at 1% significantly reduced DTPA extractable metals. It is likely that the DTPA extractable heavy metals can form less soluble metal carbonates and hydroxides with lime and thereby reducing its availability (Wong and Selvam, 2006). Fang and Wong (1999) also reported that lime amendment significantly reduced DTPA-extractable metal contents. The maximum reductions were 60 and 40% for Cu, 80 and 40% for Mn, 55 and 10% for Zn, and 20 and 25% for Ni at the end of the composting period for the lime-amended sludge as compared to the control.

Wong and Selvam (2006) studied the effect of co-composting sewage sludge with lime on heavy metal speciation and reported that the F1, F2 and F3 fractions increased during the composting process and the addition of lime reduced this transformation. The alkaline lime can neutralize the organic acids released during composting, and thus reduced the formation of metal-organic matter complexes during lime sludge co-composting. Wang et al. (2008) reported that in sodium sulfide and lime treated compost at 3% (w/w, dw), the F4 fraction is higher than the F5 fraction, which is mainly because of the conversion of Zn to zinc sulfide by the sulfidation reaction.

Table 2.2 Physico-chemical and biological methods, reducing agents and target metals

Methods	Reducing agents	Target heavy metals	Authors
Physico-chemical methods	Natural zeolite	Zn, Cu, Ni, Cr, Cd, Pb, Fe, Mn	Zorpas et al. (2000)
		Cu, Ni, Cr, Cd, Pb	Sprynskyy et al. (2007)
		Zn, Cu, Ni, Cr, Pb, Mn	Stylianou et al. (2008)
		Zn , Cu, Ni, Cr, Cd, Pb, Hg	Villasenor et al. (2011)
		Cu, Zn, Mn, Co	Erdem et al. (2004)
		Zn , Cu, Ni, Cr, Cd, Pb	Chiang et al. (2007)
		Cu, Ni, Cr, Cd, Pb	Kosobucki et al. (2008)
	Lime and sodium sulfide	Cu, Mn, Ni, Zn	Fang and Wong (1999)
		Cu, Zn, Ni	Wang et al. (2008)
		Zn , Cu, Ni, Cr, Cd, Pb	Chiang et al. (2007)
	Bamboo charcoal and bamboo vinegar	Cu, Zn	Chen et al. (2010b)
		Cu, Zn	Hua et al. (2009)
	Red mud	Zn , Cu, Ni, Cr, Pb	Qiao and Ho (1997)
	Coal fly ash	Zn , Cu, Ni, Cr, Cd, Pb	Chiang et al. (2007)
Bilogical methods	Fungi	Pb	Zeng et al. (2007)
	Bacteria	Pb, Cd, Cr, Mn, Mg	Nair et al. (2008)
	Earthworm	Pb	Liu et al. (2009)
		Pb, Cd, Cr, Cu, Zn	Li et al. (2010)
		Zn , Cu, Cr, Cd, Pb, Fe	Gupta et al. (2007)

Table 2.3 Heavy metals reduction mechanism during composting process

Methods during composting		Reduction mechanisms	Authors
Physico-chemical methods	Themophilic phase	The oxidation process and the formation of organo-metallic complexes taking place during composting could reduce the soluble contents of metals. Humic substances bind with exchangeable and carbonate fractions of metals.	He et al. (2009a), Fang and Wong (1999)
	Natural zeolite	It has the ability to increase the porosity of the substrate and as a result, to improve the composting process and the biodegradability of the organic matter. It can increase Na and K in compost with exchange with toxic metals. It has the ability to readily take up almost all metals that are bound to the exchangeable and the carbonate fractions.	Zorpas et al. (2002)
	Lime and sodium sulfide	Lime provides a buffering against the decrease in pH and a suitable amount of Ca, which would improve the metabolic activity during composting. Alkaline lime could neutralize the organic acids released during composting, and thus reduce the formation of metal-organic matter complexes during lime-sludge co-composting. When Na_2S is added to sewage sludge, the heavy metals are converted to metal sulfides by the sulfidation reaction.	Fang and Wong (1999)
	Bamboo charcoal (BC) and	BC has a highly microporous physical structure. The porosity is about five times greater and the absorption efficiency ten	Chen et al. (2010b), Hua et al. (2009)

	bamboo vinegar (BV)	times higher than that of wood charcoal. BC has a high adsorption capacity for heavy metals and attributed this adsorption to the large specific surface area, surface negative charge and high surface density of functional groups and metal oxides of BC. BV played an important role in improving the immobilization of heavy metals; it could stimulate microbial activity during composting. Additionally, humus produced during composing was reported to reduce the mobility of HM successfully through metal– humus complex formation	
	Red mud	Red mud has a high pH, cation exchange capacity, AI and Fe oxides and clay minerals and can effectively adsorb free cations from solution. The addition of red mud and the composting process increased pH to over 7 in the red mud sludge compost. The soluble metals can precipitated as metal hydroxide. The increased pH also enhanced the precipitation of metal carbonates, thus reducing the exchangeable metal concentration. Red mud affects the speciation of heavy metals through increasing the pH, solid-to-solution ratio and available adsorption sites.	Qiao and Ho (1997)
Biological method	Fungi *(Phanerochaete chrysosporium)*	*Phanerochaete chrysosporium* capable of accumulating metal ions in their cells by intracellular uptake and can also chelate metal ions by the carboxyl, hydroxyl or other active functional groups on cell	Zeng et al. (2007), Liu et al. (2009)

	(including the dead cell) wall surface.	
Bacteria	Bacteria excrete a cheleting agent called Siderophore. Siderophores are specific Fe (III) ligands and are able to bind to other metals, such as magnesium, manganese, chromium (III), gallium (III) and radionuclides, such as plutonium(IV). Siderophores binding to metals, including toxic ones like lead and cadmium.	Nair et al. (2008)
Earthworm	They are capable of reducing possible toxic effects of superfluous heavy metals by utilizing them for physiological metabolism. Metals interact with many chemicals and participate in detoxification processes, as part of the enzymes of the antioxidant systems, such as superoxide dismutase (SOD), and in metallothioneins (MT). The availability of heavy metals decrease due to bioaccumulation of metals and organo-complexformation during this process	Ghyasvand et al. (2008), Li et al. (2010)

Wong and Selvam (2006) concluded that the F5 fraction of Zn was transformed mainly into F4 fraction and marginally to other forms of Zn during composting and lime addition at 1.63% could reduce this transformation.

Chemical forms of metals might be affected in general by the pH, the presence of organic matter, etc. The F5 fraction of Zn might be transformed mainly into the F4 fraction and marginally to other fractions of Zn during the stabilization and ageing process; however, increased residence time and lime addition may reduce this transformation (Samaras et al., 2008). Addition of lime caused a significant reduction in water-soluble Cu, Mn, and Zn contents at the beginning of composting and this effect became more obvious as composting proceeded (Fang and Wong, 1999). Wang et al. (2008) reported that the addition of sodium sulfide and lime (SSL) caused the F4 fraction of Cu increased in comparison to control compost; it may be due to the conversion of heavy metals to sulfides. Alkaline lime could neutralize the organic acids released during composting, and thus reduce the formation of metal-organic matter complexes during lime-sludge co-composting. Wong and Selvam (2006) reported that the addition of lime caused the F1, F2 and F3 fractions increased slightly after composting process and the addition of lime reduced this transformation. Since the stability constant of Cu complexes with organic matter is high, the F4 fraction was reported as the major fraction of Cu by many researchers.

Wang et al. (2008) also reported that the F5 fraction of Zn was the dominant fraction in the lime treated compost at 1 and 1.63%. The F1 and F2 fractions decrease from 10 and 27.5% in sewage sludge to 1.5 and 8.5% in SSL amendment compost, respectively, it can be concluded that the SSL amendment is significant in reducing the mobile and easily available fractions (F1 and F2) of the co-compost. It could also be concluded that the F2, F3 and F4 fractions of Ni were transformed mainly into F5 fraction of Ni during composting. The addition of SSL caused an increase in F4 fraction (27.8%) compared to that in the control, which might also be mainly because of the formation of NiS, by the sulfidation reaction expressed in Eq. (1).

$$Me^{2+} + S^{2-} = MeS \qquad (1)$$

Natural zeolite treatment

Zeolites are naturally occurring hydrated aluminosilicate minerals; they belong to the class of minerals known as “tectosilicates” (Erdem et al., 2004). It occurs as three-dimensional frame works of SiO_4 and AlO_4 tetrahedral (Villasenor et al., 2011). The aluminium ion is small enough to occupy the position in the centre of the tetrahedron of four oxygen atoms, while the isomorphous replacement of Si^{4+} by Al^{3+} produces a negative charge in the lattice. The exchangeable cations such as Na, K or Ca help in balancing net negative charge. These cations are exchangeable with certain cations in solutions, such as Pb, Th, Cd, Zn, Mn and NH_4^+ (Sprynskyy et al., 2007; Villasenor et al., 2011).

Natural zeolite has been used widely for reducing mobility and bioavailability heavy metals in sewage sludge composting due to its sorption and exchangeable properties towards the heavy metals (Zorpas et al., 2000; Sprynskyy et al., 2007). Natural zeolite has the ability to uptake of heavy metals which are in easily available fractions, and exchange of sodium and potassium (Zorpas et al., 2000).

Sprynskyy et al. (2007) reported natural zeolites (clinoptilolite) in particular seem to be appropriate amendment materials for sewage sludge taking into account their sorption and exchangeable properties towards the heavy metals. Addition of the clinoptilolite rock to sewage sludge might change chemical speciation of heavy metals in composts and decrease their mobility and bioavailability (Sprynskyy et al., 2007). Villasenor et al. (2011) reported that the zeolites retained 100% of the Ni, Cr and Pb that was present in the sludge. Zeocat was the most effective zeolite for the removal of Cu, Zn and Hg and also reported that clinoptilolite had the ability to uptake of metals associated with the mobile forms such as F1 and F2 fractions (Villasenor et al., 2011). Stylianou et al. (2008) determined that clinoptilolites displayed the following metal retention selectivity: $Zn^{+2} > Cr^{+3} > Ni^{+2} > Cu^{+2} > Mn^{+2}$.

Zorpas et al. (2000) observed that clinoptilolite has the ability to readily take up almost all metals that are bound to the F1 and F2 fractions. Clinoptilolite took up all metals bound in exchangeable and carbonate fractions according to the following selectivity series: Cu > Cr > Fe > Ni > Mn > Pb > Zn (Zorpas et al., 2000). Sprynskyy et al. (2007) concluded that the addition of the natural clinoptilolite to the sewage sludge led to the metals contents fall in all four fractions of the sequential procedure. Concentrations of mobile forms of Cd, Cr, Cu and Ni decreased by 87, 64, 35 and 24%, respectively, as a result of the addition of 9.09% of the clinoptilolite. The total concentration of metals decreases after 9.09% clinoptilolite addition to the sludge were around 11, 15, 25, 41 and 51% for Cu, Ni, Cr, Cd and Pb, respectively. The clinoptilolite rock may be considered as a suitable material to heavy metals immobilization by their bond into firmly sorbed forms of the pseudo residual fraction. The F1 fraction of Cr content was also decreased by 49% most after the zeolite addition (Sprynskyy et al., 2007).

Stylianou et al. (2008) reported that the zeolite has ability to trap metals by ion exchange and adsorption processes. The content of Zn is decreased by 94.1%, of Cu by 59.5%, of Cr by 82.2%, of Ni by 69%, while in the case of Mn the respective percentage is 48.1%. Through the composting process the break-up of organic matter increases the presence of metals in the F1 fraction and helps natural minerals, such as zeolite, to adsorb them, consequential the metals immobilized during the composting.

Zorpas et al. (2002) concluded that the 25% w/w addition of zeolite in compost is sufficiently removing heavy metals, 12% of Co, 27% of Cu, 14% of Cr, 30% of Fe, 40% of Zn, 55% of Pb, and 60% of Ni. The particle size of the clinoptilolite seems to affect the uptake of heavy metals. The metals concentration was uptake from the clinoptilolite increases with the particle size of clinoptilolite increases, it could be explained by the effect of surface dust, which clogs pores and causes structural damage in smaller particles, due to the grinding process.

Stylianou et al. (2008) revealed that hydration of the cation is also important during the process; for example, in clinoptilolite-water ion exchange systems the series found is: $Pb^{2+} > Cr^{3+} > Fe^{3+} > Cu^{2+}$ and $Pb^{2+} > Zn^{2+} > Cu^{2+}$. It should be noted that organic matter in soluble and insoluble forms plays contrasting roles in controlling total soluble metals. It promotes the dissolution of Cu, Zn and other metals by building organic complexes since the the addition of organic matter increased the solubility of metals by the formation of organometallic complexes (Stylianou et al., 2008). The percentage of Cr is higher in F4 fraction during the thermophilic phase than the other fractions but after the thermophilic phase, it seems to be transferred to the F5 fraction; while at the end of the maturity period (after 150 days) zeolite binds all of the readily available metal (metals which are associated with mobile forms) (Zorpas et al., 2008).

Bamboo charcoal and bamboo vinegar treatments

Bamboo is a renewable bioresource when the cycle of its plantation and use is properly managed. Pyrolyzing bamboo under oxygen- limited conditions produces bamboo charcoal (BC). BC has a large amount of micropores and an extremely large surface area, about 4 and 10 times greater than those in wood charcoal, respectively. Bamboo charcoal may be an ideal amendment for nutrient conservation and heavy metal stabilization due to its excellent adsorption capability (Hua et al., 2009).

Bamboo vinegar (BV) is a brown-red transparent liquid produced during pyrolysis of BC and contains more than 200 types of chemical components, in which acetic acid is the main component (Chen et al., 2010b). Chen et al. (2010b) reported that the addition of BC or BC + BV into pig manure (PM) composting materials influenced temperature, pH changes. It shortened the time needed to enter the thermophilic phase, decreased pH at the thermophilic phase. The addition of BC or BC + BV reduced total nitrogen loss and mobility of Cu and Zn in PM composting materials. The total nitrogen loss was reduced by 74% in the 3% BC + 0.4% BV treatment. The mobility of Cu and Zn was reduced about 35% and 39% respectively in the 9% BC treatment. Hua et al. (2009) concluded that mobility of heavy metals in the sewage sludge composting could also be reduced by the addition of BC. However, the stabilization effect of BC was different for Cu^{2+} and Zn^{2+}. DTPA-extractable contents of Cu and Zn in sludge composting material with 9% BC amendment dropped 27.5% and 8.2%, respectively, at the end of composting as compared with that of the non-BC control. There was no significant difference in the ability of fresh BC compared to composted BC to retain either Cu or Zn, indicating that composting had little effect on the adsorption capacity of BC for these heavy metals.

Red mud treatment

Red mud is an industrial waste by-product that could potentially to be used for remediation of heavy metals. It is produced during the refining of bauxite to alumina through the Bayer's process (Gadepalle et al., 2007). Red mud effectively adsorbs free cations from solution because it has a high pH, cation exchange capacity, Al-Fe oxides, and clay minerals Qiao and Ho 1997). It is composed of hematite (Fe_2O_3), boehmite (-AlOOH), quartz (SiO_2), sodalite ($Na_4Al_3Si_3O_{12}Cl$) and gypsum ($CaSO_4.2H_2O$) (Gadepalle et al., 2007). Red mud addition generally reduces metal leachability in sewage sludge compost through adsorption and complexation of the metals on to inorganic components to different extents for the different metals (Qiao and Ho, 1997).

Red mud affects the speciation of heavy metals through increasing the pH, solid-to-solution ratio, and available adsorption sites (Qiao and Ho, 1997). In general sludge composting with red mud addition reduces the leachability, plant availability and total metal content. Qiao and Ho (1997) reported that the effect of red mud was different for each metal; greater effect observed on Cr, Pb and Zn speciation than on Cu and Ni speciation. More than 80% of Cu was tightly bound to the F4 fraction, and red mud addition hardly affects the Cu speciation. The F4 fraction of Cu was converted into F2, F3 and F1 fractions. The red mud addition inhibited the increase of F1 fraction in red mud sludge compost through increasing pH to precipitate Cu and increasing the inorganic oxides surface to adsorb Cu, consequently reduced the metal mobility in the sewage sludge compost. Although the red mud increases total Cr in sludge compost however the leachable and plant available Cr were undetected in the red mud compost. The Cr remains in tightly bound with F5 fractions which unlikely to be released into the environment. Red mud amendment significantly reduced the mobility and plant availability of metals in MSW compost when the red mud was added at the beginning of the composting process (Qiao and Ho, 1997).

- **Biological method**

The way of removing heavy metals from compost is by chemical means, i.e., by leaching with solutions of sulfuric acid and nitric acid. Such techniques can be very expensive and may have several disadvantages, such as unpredictable metal ion removal, high reagent requirements, and the generation of toxic sludges, which are often difficult to dewater and may require extreme caution when disposing of them. Development of cost-effective alternatives, such as biosorption, bioaccumulation, and bioleaching has become an intensive area of exploitation over the past decade. Microbial biomass has been screened for its sorption capabilities (Siloniz et al., 2002). The property of nonliving microbial biomass to accumulate heavy metal ions, a non-metabolically driven process called biosorption, the term bioaccumulation described an active process where the removal of metals requires the metabolically activity of living organism (Philippis and Micheletti, 2009). Bioleaching process defined as the mobilization of metal cations from insoluble ores by biological oxidation and complexation processes (Rohwerder et al., 2003).

Mechanical treatment by grinding, mixing, and sieving out nondegradable or disturbing materials (metals, plastics, glass, stones) gives good conditions for biological treatment of compostable materials (Barker et al., 2002). Microorganisms like bacteria, fungi, algae and yeast are known to tolerate and accumulate heavy metals (Ahmad et al., 2005). The microorganisms able to adsorb and detoxify heavy metals have been applied to the treatment of metal-contaminated wastewater and could be expected to immobilize metals in solid waste (Zeng et al., 2007; Baker et al., 2011). Bioadsorption mechanism involved in the process may include ion exchange, coordination, complexation, chelation, adsorption, micro-precipitation, diffusion through cell walls and membrane which differs depending on the species used the origin and processing of the biomass and solution chemistry (Ahmad et al., 2005). Microbial communities play an important role in the detoxification, stabilization and transformation of Cr in the composting material to ensure the environmental sustainability (Shukla et al., 2009).

Using fungi

White-rot fungi have generally been found useful in absorbing heavy metal ions from dilute solutions or wastewater with their mycelium. White-rot fungi are capable of accumulating metal ions in their cells by intracellular uptake, as many researchers validated, and can also be chelated with metal ions by the carboxyl, hydroxyl or other active functional groups on cell (including the dead cell) wall surface (Zeng et al., 2007). White-rot fungi can concentrate metals taken up from substratein their mycelia (Baldrian, 2003). It has been reported that *Phanerochaete chrysosporium* is good at absorbing metal from dilute solutions by its mycelium and less Pb ion transferred, and it has ability to grow in both solid and liquid environment and degrade a wide range of xenobiotic effectively even in the nutrient-limited condition (Huang et al., 2006). Presence of heavy metals in the environment can directly interact with extracellular enzymes of fungi (Baldrian, 2003).

Preference of living biomass of metal binding ion depends on nutrient, environmental condition and cell age (Ahmad et al., 2005). Nonviable biomass has several advantages for metal biosorption from solution, especially as there is no requirement for maintenance and nutrition (Siloniz et al., 2002). Composting of Pb-polluted waste by fungi with the ability of immobilizing Pb as a promising method also needs further studies. *Phanerochaete chrysosporium* has been used to remove Pb ions in wastewater because it is capable of accumulating metal ions in their cells by intracellular uptake, as many researchers validated, and can also chelate metal ions by the carboxyl, hydroxyl or other active functional groups on cell (including the dead cell) wall surface (Liu et al., 2009).

Zeng et al. (2007) studied lead-contaminated solid waste composting with inoculant (white-rot fungus). The uncontaminated solid waste was put in reactor A, while the lead-contaminated solid waste was put into reactors B and C. Spore suspension was inoculated into the mixture in reactors A and C. This study reported that almost 70.5% of Pb was bound to the F5 fraction in reactor C and 58.7% in reactor B. After 80 days of composting, the content of the F1 fraction of Pb in reactor C even dropped to 0%, while that in reactor B remained 2.86%. The results showed that the bioavailability and transfer ability of Pb in reactor C were lower than those in reactor B. As a result, the potential hazards of compost in reactor C were lower than those in reactor B, which indicated that composting Pb-contaminated solid waste with white-rot fungus could control the phytotoxicity of Pb to some extent. Reasons for these results might be as follows: (i) white-rot fungi could chelate with Pb by the carboxyl, hydroxyl or other active functional groups on cell wall surface, (ii) white-rot fungi could improve the composting process. The humus formed during process could also bind Pb stably.

Liu et al. (2009) proved that the composting methods without inoculants and with inoculants of *Phanerochaete chrysosporium* could effectively transform Pb fractions, reduce active Pb and alleviate the potential harm of Pb-contaminated waste. The transformation behavior of Pb fractions might result from the fact that the Pb ions could be accumulated by fungal mycelium and chelated by the humus formed in the composting. The content of soluble-exchangeable Pb was positively correlated with pH value and microbial biomass, indicating that increasing pH and microbial biomass were important to the immobilization of Pb during composting. Furthermore the better immobilization effect of active Pb was found in the composting method with inoculants (*Phanerochaete chrysosporium)* compared without inoculants, which might be due to the more microbial biomass and higher pH value in composting of Pb-polluted waste with inoculants. It was also observed that the inoculants might be responsible for the increase of the content of residual Pb content during composting.

Using bacteria

The thermophilic bacteria in solid waste compost mainly belonged to the genus of *Bacillus* and there was a shift from several *Bacillus* species to one single *Bacillus* species, i.e., *B. stearothermophilus*, as the major dominant species when the temperature was higher than 65^{o}C whereas *Thermus* strains played an important role in organic matter degradation during the thermogenic phase (>70 ^{o}C) of the composting process (Fang and Wong, 2000). Siderophores are class of microbial chelating agents, which are low molecular weight ligands synthesized and excreted by bacteria for capturing and supplying Fe to support metabolic activity (Nair et al., 2008).

Bioleaching is process that reported to be an efficient and economical method for the removal of heavy metals from the sludge. The process has also been applied successfully for remediation of soils, sediments, industrial wastes and solid wastes contaminated with heavy metals (Pathak et al., 2009). The predominant metal-sulfide-dissolving microorganisms are extremely acidophilic bacteria (meaning organisms thriving at pH values below 3) that are able to oxidize either inorganic sulfur compounds and/or Fe (II) ions. The classical leaching bacteria now belong to the genus Acidithiobacillus (formerly Thiobacillus (Rohwerder et al., 2003). Bioleaching process uses the catalytic effect produced by the metabolic activities of iron-oxidizing and sulfur-oxidizing microorganisms resulting in an acceleration of the chemical degradation of the sulfides. It is a low cost and environmental friendly technique which is 80% cheaper in terms of chemical consumption compared to the traditional chemical methods employed for metals leaching from the sludge and recovery of metals from the leachate (Pathak et al., 2009).

Using earthworms

Vermicomposting is the usual method that managed by earthworms and in addition to decomposing of organic waste and reduction of metal bioavailability due to bioaccumulation of metals and formation of organo-metallic complex during the process (Ghyasvand et al., 2008). Li et al. (2010) showed that *E. fetida* can accumulate Cu, Zn, Pb and Cd. The adult earthworm was speculated to have such an ability to store high concentrations of heavy metals in the non-toxic forms. Introduction of earthworms for vermincomposting tended to increase the solubilisation of Fe, this behaviour was attributed to a greater rate of degradation in the presence of higher concentrations of different microorganisms within earthworm intestine (Bhattacharya and Chattopadhyay, 2006). It is reported that soluble and exchangeable metal concentrations were the best descriptors of bioaccumulation in *E. fetida*. Tubifex, one of the oldest described aquatic oligochaetes, was able to sequester superfluous Cd in the granules fraction and by proteins as metallothionein-like proteins in the heat stable fraction (Li et al., 2010). The soluble and exchangeable Pb content decreased as the pH increased. The pH is known to affect the ionic form and chemical mobility, so a high pH value can decrease the solubility of metals in the medium (Liu et al., 2009).

Jain et al. (2004) reported that the heavy metal concentration was reduced during vermicomposting. Metabolic conversion of highly toxic form Cr (VI) to nontoxic form Cr (III) through mitochondrial and cytoplasmic fractions has also been demonstrated in *E. fetida* (Jain et al., 2004). Suthar and Singh (2008) suggested that metal reduction during vermicomposting was related to the earthworm activity in the waste decomposition, furthermore earthworm could accumulate heavy metals in their tissues. Suthar (2009) reported that vermicomposting caused significant reduction in concentration of DTPA extractable metals like Cu, Fe, Mn and Zn during vermicomposting of sewage sludge spiked with sugarcane trash. The epithelial layer of gut absorbs bioavailable fractions of metals during the passage of wastes through it as the bioaccumulation of a high content of metals in earthworm tissues is well documented (Suthar and Singh, 2009; Suthar et al., 2008). The rate of bioaccumulation of water soluble fraction of metals could be increased when it passes through worm’s gut (Suthar, 2009). Vermicomposting of organic wastes accelerates organic matter stabilization and gives chelating and phyto-hormonal elements which have a high content of microbial matter and stabilized humic substances (Gupta and Garg, 2008; Suthar, 2009, Hait and Tare, 2012).

Hait and Tare (2012) studied vermicomposting of sewage sludge using *Eisenia fetida*; shows that earthworm reduced water solubility of some heavy metals such as Cu, Zn and Cr. When the organic matter was passing through the gut of earthworm some part of it’s digested, and pH and microbial activity of the gut increased. As a consequence, the possibilities of binding of metals to ions and carbonates (i.e. more soluble fractions) increased in ingested material. The decrease in water soluble Cr during vermicomposting probably caused by the reduction of Cr(VI) to Cr(III) by biological organisms, followed by the combination of Cr(III) with decomposed organic materials. The mobility and bioavailability of heavy metals were reduced through two major types of cellular adaptation to toxicity of metals: one involves binding of metals to nuclear proteins and the formation of inclusion nuclear bodies; the second type is a cytoplasmic process involving synthesis of a specific metal binding protein, metallothionein within the chloragogenous tissue. The interaction of the humic acid with metals that is one of the main factors affecting the partitioning of heavy metals as humic acid has been shown to have a stronger sorption effect on heavy metals, particularly Cu and Zn (Hait and Tare, 2012).

2.5 CONCLUDING REMARK

Water hyacinth is one of the most uncompromising weeds of the world. The composting followed by land application represents one of the most economical ways for treatment and final disposal of water hyacinth, due to it combines material recycling and biomass disposal. Heavy metals containing compost may change the physical, chemical and biological properties of soil. These metals uptake by plants from the soil, it reduces the crop productivity by inhibiting physiological metabolism. Heavy metals uptake by plants and successive accumulation in human tissues and biomagnifications through the food chain causes both human health and environment concerns.

Heavy metals containing agricultural runoff enter in aquatic environment, and harm to aquatic plants and animals. Therefore, if the compost has to be applied in agriculture it should be free from pathogens and heavy metals. Toxicity of heavy metals does not depend on its total concentration but depends on different bioavailable fractions in which metals are present. The water soluble fraction has highest potential of contamination to the food chain, surface water and ground water. Composting process can reduced toxicity of metals by reducing available fractions such as water soluble, DTPA extractable and leachable fractions.

The composting process can promote the complexation of heavy metals whose mobility and availability tend to decreases with reducing toxicity. In a risk assessment, pH is the most important factor that affects mobility and bioavailability of metals during the composting process. Analysis of heavy metals in the composts is very important for the routine monitoring and risk assessment and regulation of environment. Hence, the literature suggested that bioavailability and leachability of metals should be assessed before applying compost to soil which are derived from municipal solid waste, sewage sludge, industrial wastes and water hyacinth.

The sequential extraction enhanced over the use of single extraction is that the improved phase specificity and it provides useful information for risk assessment. The chemical speciation of heavy metals allowed the assessment of heavy metal bioavailability and is related to the different natures of the metals, their bonding strength and either in free ionic form or complexed by organic matter, or incorporated in the mineral fraction of the sample.

Specifically more effort should be in the following directions (i) interaction between organic matter and heavy metals (ii) focus on physico-chemical parameters which are responsible for transformation of heavy metals (iii) addition of materials which are not harmful for composting process and having cation exchange capacity, consequently reduce most bioavailable fractions of metals (iv) use of specific microorganisms in the compost which are able to convert easily available fractions (exchangeable and carbonate) into less mobile fractions (reducible and oxidizable) or inert fraction (residual).

During the composting process, the metals content can be reduced by addition some chemicals, microbial inoculants and earthworm. Addition of lime during composting process provides buffering against reduction in pH and calcium for metabolic activity of microorganisms consequently reduced bioavailability of heavy metals. Natural zeolites have potential for metal immobilization due to their high ion exchange capacities and highly porous structures. It has ability to exchange sodium and potassium with toxic metals. Care should be taken when using zeolites with high sodium content as they have been shown to increase soil salinity and exchangeable sodium concentrations, which could cause plant toxicity. Bamboo charcoal reduced bioavailability and leachability of heavy metals due to it has a large quantity of surface negative charge and a high charge density. Red mud affects the speciation of heavy metals through increasing the pH, solid-to-solution ratio, and available adsorption sites.

The inoculation of microorganisms could be very useful to improve the composting process by enhancing enzymatic activity and quality level of the compost is acceptable, with very low heavy metal content. During the vermicomposting earthworm can accumulate the high concentration of heavy metals in the non-toxic forms and capable be reducing possible toxic effects of unessential heavy metals by utilizing them for physiological metabolism. The principal advantage claimed for biological process over the use of chemicals during composting is the low capital cost.

Therefore, the aim of the thesis was to assess the total heavy metal concentration for the confirmation of pollutants in the water hyacinth compost. Assessment of bioavailability, leachability and speciation of heavy metals were carried out during agitated pile, rotary drum composting and vermicomposting of water hyacinth mixed with cattle manure and sawdust. In addition, effects of lime and natural zeolite on bioavailability and leachability of heavy metals were analyzed during the water hyacinth composting process.

Chapter 3

MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

In order to accomplish the objectives, the research was proposed to be carried out in different phases as summarized below. Fig. 3.1 shows experimental design of the research work and Fig. 3.2 shows detail experimental design of phase 4. In phase 1, 2 and 3, studies were conducted on bioavailability, leachability and speciation of heavy metals during agitated pile composting, rotary drum composting and vermicomposting respectively. Five different proportions of water hyacinth, cattle manure and sawdust were prepared for all three phases. In phase 4, best proportion was selected from each phase 1 and 2, and effects of lime (1, 2 and 3%) and natural zeolite (5, 10 and 15%) were studied on bioavailability, leachability and speciation of heavy metals.

3.2 FEEDSTOCK MATERIALS

Water hyacinth, cattle (cow) manure and sawdust were used for the preparation of different waste mixtures. Water hyacinth was collected from the Amingoan industrial area near Indian Institute of Technology Guwahati (IITG), Assam, India. Cattle manure was obtained from dairy farm near the IITG campus. Sawdust was purchased from nearby saw mill. Prior to composting, the maximum particle size in the mixed waste was restricted to <1 cm in order to provide better aeration and moisture control. Fig. 3.3 shows the pictorial view of water hyacinth, cattle manure and sawdust.

Exotic earthworm species *Eisenia fetida* was collected from Central Plantation Crops Research Institute (CPCRI), Indian Council of Agricultural Research, Regional Station, Kahikuchi, Guwahati, India. For developing the cultures, Perspex bin sizes 450×300×450 mm were fabricated in the laboratory. Fig. 3.4 shows pictorial view of Perspex bin and cultured earthworm. For aeration and drainage purpose 16 holes of 10 mm diameter were drilled along the longer sides and 16 at the bottom respectively. The earthworms, bedding was prepared using chopped hay (about 50mm), cow dung, banana pulp (chopped about 50 mm), tree leaves all were partially degraded. The bedding was watered to keep it moist to enable the worms to breathe. The earthworms were cultured in partially degraded cattle manure. Table 3.1a and b shows initial characterizations of water hyacinth, cattle manure and sawdust.

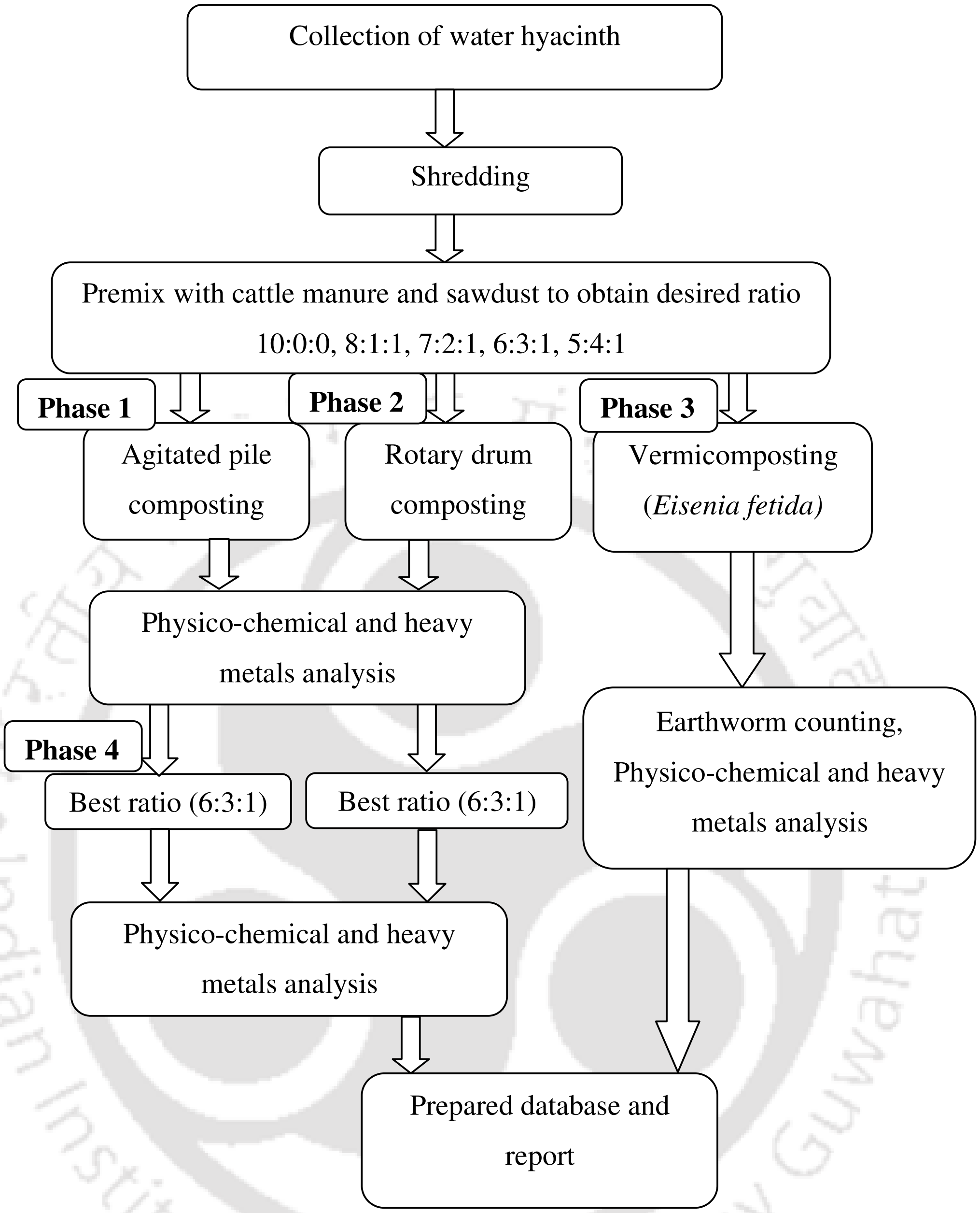


Fig. 3.1 Experimental design of the research work

Lime was collected from Assam Air Products Pvt. Ltd., Assam, India. Lime was dried at 105^{o}C in oven for 24 h. It was ground and passes from 0.22 mm sieve. The content of calcium oxide (CaO) in lime sludge was about 35-40%. Powder form of natural zeolite was purchased from G M Chemicals Pvt. Ltd., Gujarat India. Fig. 3.5 shows the pictorial view of lime and natural zeolite. Table 3.1a and b shows initial characterizations of lime and natural zeolite. The micro-porous structure and elemental composition of natural zeolite was analyzed by scanning electron microscope (Fig. 3.6), which may be beneficial for heavy metals uptake during composting process. Table 3.2 shows waste composition of different mixtures.

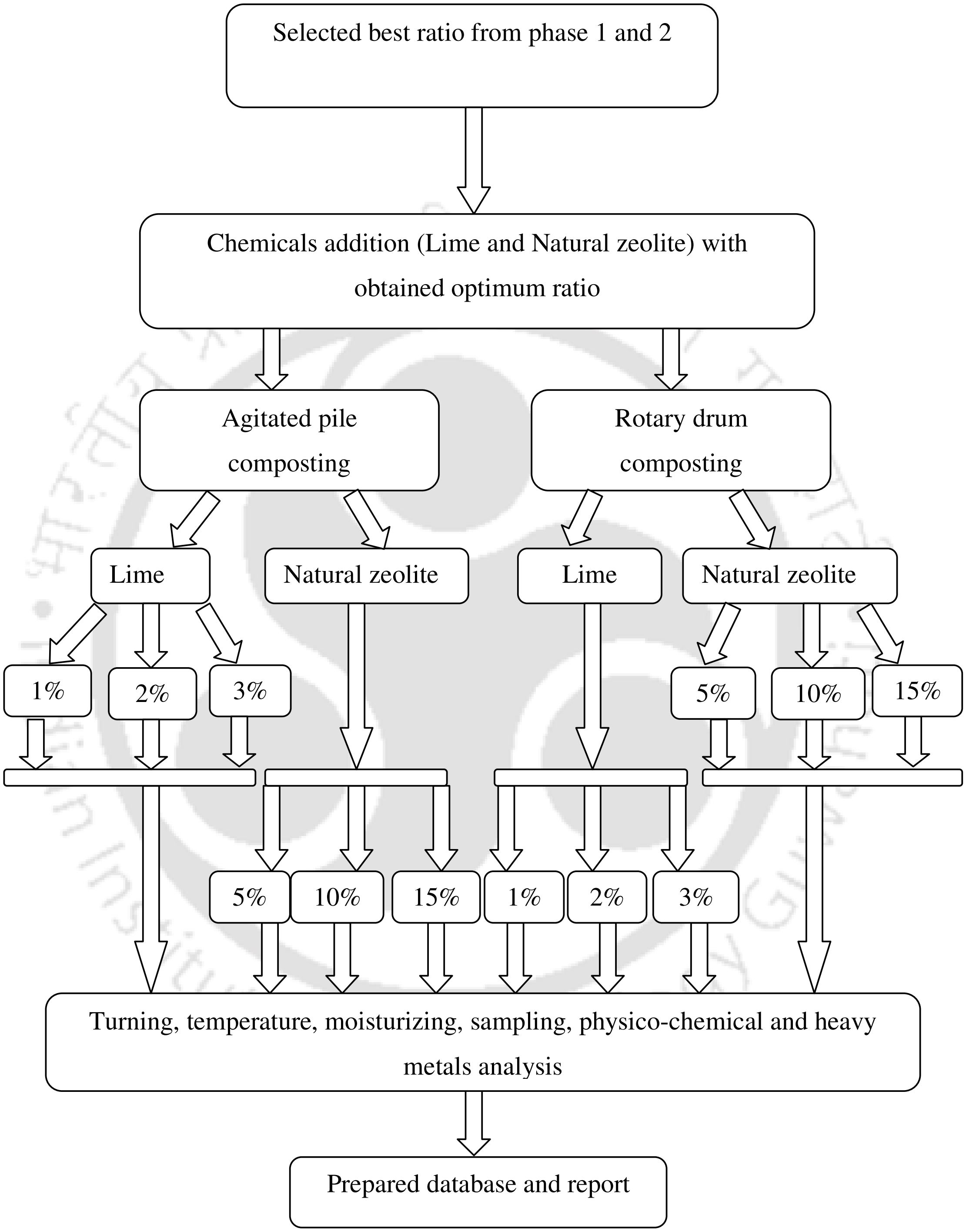


Fig. 3.2 Experimental design of phase 4 in the research work

Fig. 3.3 Pictorial view of water hyacinth, cattle manure and sawdust

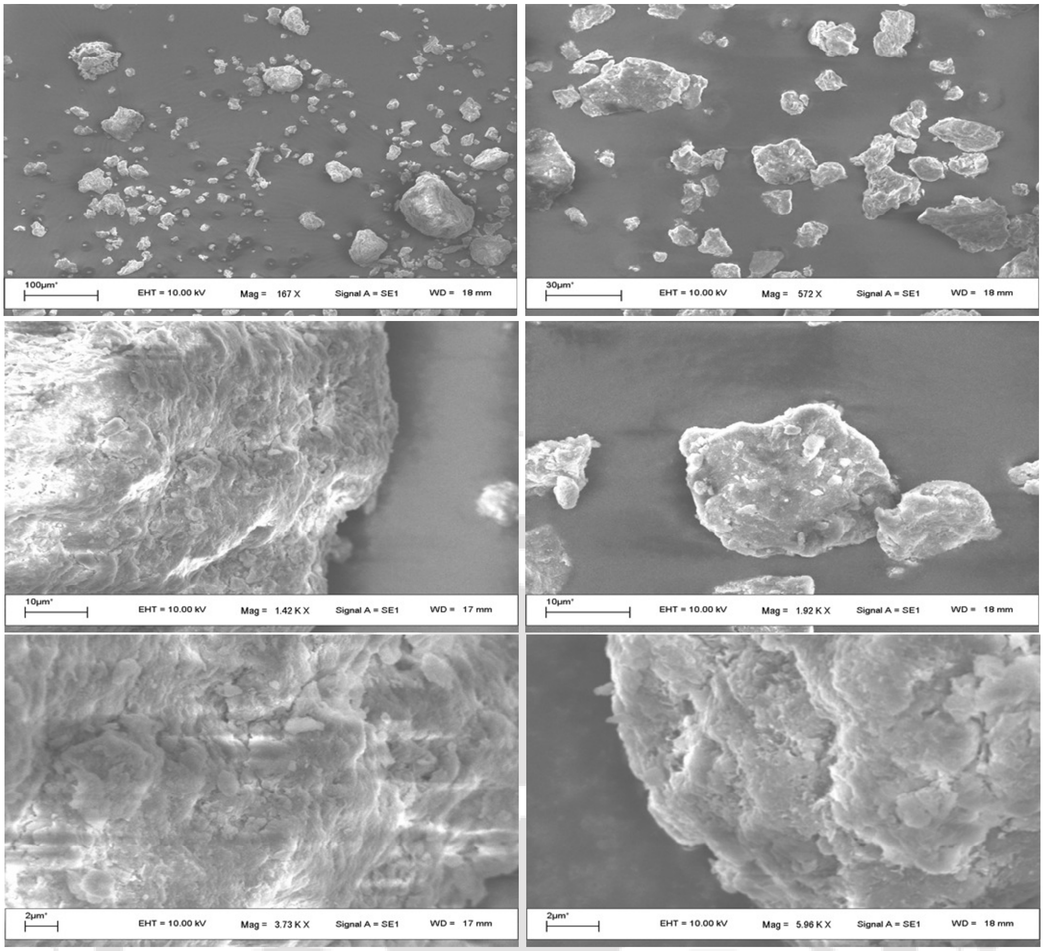
Fig. 3.4 Pictorial view of Perspex bin and cultured earthworms

Fig. 3.5 Pictorial view of lime and natural zeolite

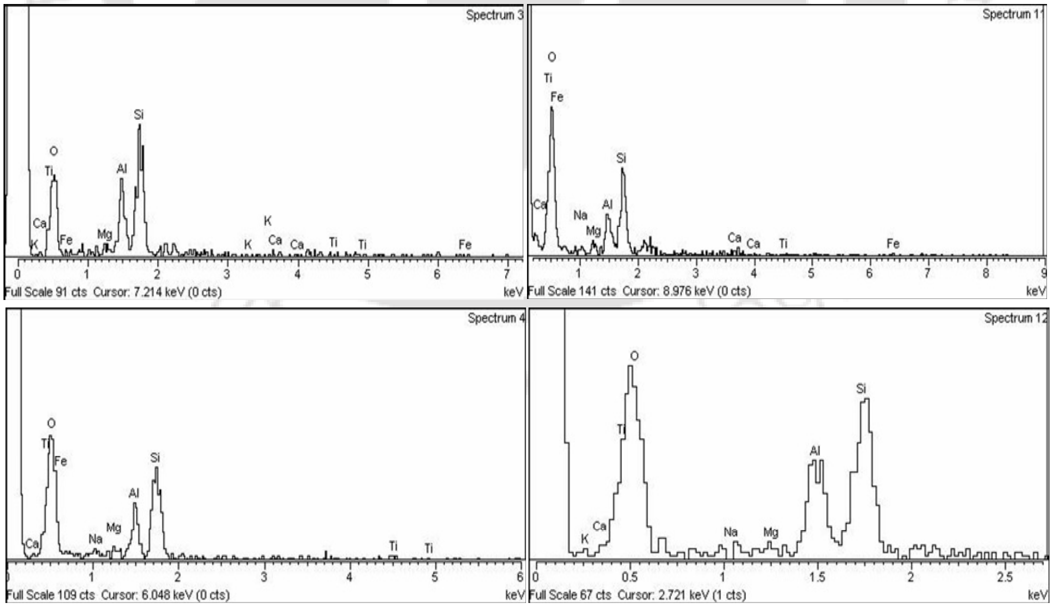
Table 3.1a Initial characterization of waste materials (mean ± SD, n=3)

Parameters	Waste materials (kg)				
	Water hyacinth	**Cattle manure**	**Sawdust**	**Lime**	**Natural zeolite**
pH	5.8±0.1	6.7±0.1	6.2±0.01	12.5±0.06	8.9±0.05
Electrical conductivity (EC) (dS/m)	4.9±0.01	3.3±0.03	0.4±0.002	8.5±0.13	1.6±0.03
Moisture content (%)	85.9±0.1	80.9±0.2	10.0±0.04	55±0.32	3.8±0.15
Volatile solids (%)	72.6±0.04	72.1±0.2	97.9±0.2	0.6±0.22	11.7±0.03
Nutrient (mg/kg dry matter)					
Na	8875±125	2400±5.0	1098±2.5	1.4±0.05	7100±100
K	18195±205	988±22.5	698±7.5	1.2±0.03	1850±100
Ca	6178±7.5	9893±17.2	2420±15	65650±350	6575±0.08
Mg	8304±233	75500±500	30206±28	4.7±1.27	1175±125
Total heavy metals (mg/kg dry matter)					
Zn	152±16	182.4±2.0	116±2.3	2.14±0.43	6.61±0.54
Cu	39.8±0.3	47.8±0.8	29.5±1.0	0.66±0.02	1.15±0.05
Mn	645±16.3	527.5±1.5	143±3.5	7.23±0.23	4.60±0.10
Fe	12925±10	1860.8±2.8	2749±13.3	3.00±0.06	8.10±0.26
Ni	180±10.3	235.9±2.6	279±1.5	0.20±0.01	ND
Cd	43.3±0.3	48±1.1	58±0.5	ND	0.72±0.07
Pb	1140±5	817.5±7.5	1000±5	ND	ND
Cr	301±0.2	124.5±0.5	89.23±0.2	ND	0.08±0.00

Note: SD- standard deviation, ND- Not detected



(a)



(b)

Fig. 3.6 Scanning Electron Microscopy (SEM) of natural zeolite (a) image analysis (b) elemental composition

Table 3.1b Initial characterization of waste materials (mean ± SD, n=3)

	Waste materials (kg)				
Parameters	**Water hyacinth**	**Cattle manure**	**Sawdust**	**Lime**	**Natural zeolite**
Water soluble metals (mg/kg dry matter)					
Zn	7.3±0.10	3.4±0.03	2.1±0.03	0.11±0.007	0.29±0.05
Cu	1.8±0.14	3.3±0.02	0.9±0.02	0.01±0.002	0.05±0.02
Mn	60.7±0.10	19.9±0.13	5.2±0.20	0.15±0.006	0.22±0.10
Fe	42.1±1.10	190±3.0	174±2.3	ND	ND
Ni	ND	ND	ND	ND	ND
Cd	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND
Cr	0.4±0.01	5.0±0.04	2.08±0.6	ND	ND
DTPA extractable metals (mg/kg dry matter)					
Zn	29.6±3.0	40.3±0.40	10.8±0.70	0.45±0.03	0.74±0.12
Cu	7.2±0.05	6.9±0.37	1.7±0.20	0.02±0.002	0.08±0.01
Mn	175±5	220.4±4.9	40.4±0.90	1.80±0.10	0.40±0.02
Fe	295±15	50.9±1.50	8.2±0.40	0.04±0.009	1.05±0.03
Ni	1.6±0.4	0.13±0.01	0.08±0.03	ND	ND
Cd	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND
Cr	1.4±0.15	0.7±0.05	0.6±0.1	ND	ND
Leachable metals (mg/kg dry matter)					
Zn	33.8±1.30	37.7±5.6	15.5±1.50	0.60±0.50	1.74±0.74
Cu	5.6±0.70	4.0±0.50	2.1±0.10	0.06±0.02	0.50±0.10
Mn	258.1±0.6	520±30	43.9±1.30	2.00±0.40	0.86±0.03
Fe	257.6±4.8	59±2.20	13.0±0.20	1.00±0.15	1.15±0.05
Ni	1.6±0.10	1.3±0.10	0.9±0.05	ND	ND
Cd	8.9±0.90	8.4±0.20	7.9±0.10	ND	0.02±0.00
Pb	1.0±0.03	1.2±0.04	0.9±0.04	ND	ND
Cr	4.1±0.20	4.2±0.20	4.0±0.15	ND	ND

Note: SD- standard deviation, ND- Not detected

Table 3.2 Waste composition of different mixtures

Composting Methods		Composition					
		Water hyacinth (kg)	Cattle manure (kg)	Sawdust (kg)	Ratios	Lime (%)	Natural Zeolite (%)
Phase 1: Agitated pile composing	Trial 1	150	-	-	10:0:0	-	-
	Trial 2	120	15	15	8:1:1	-	-
	Trial 3	105	30	15	7:2:1	-	-
	Trial 4	90	45	15	6:3:1	-	-
	Trial 5	75	60	15	5:4:1	-	-
Phase 2: Rotary drum composting	Trial 1	150	-	-	10:0:0	-	-
	Trial 2	120	15	15	8:1:1	-	-
	Trial 3	105	30	15	7:2:1	-	-
	Trial 4	90	45	15	6:3:1	-	-
	Trial 5	75	60	15	5:4:1	-	-
Phase 3: Vermi composting	Trial 1	1.5	-	-	10:0:0	-	-
	Trial 2	1.2	0.15	0.15	8:1:1	-	-
	Trial 3	1.05	0.30	0.15	7:2:1	-	-
	Trial 4	0.90	0.45	0.15	6:3:1	-	-
	Trial 5	0.75	0.60	0.15	5:4:1	-	-
Phase 4: Lime and natural zeolite addition	Lime 1	90	45	15	6:3:1	1	-
	Lime 2	90	45	15	6:3:1	2	-
	Lime 3	90	45	15	6:3:1	3	-
	Zeolite 1	90	45	15	6:3:1	-	5
	Zeolite 2	90	45	15	6:3:1	-	10
	Zeolite 3	90	45	15	6:3:1	-	15

3.3 EXPERIMENTAL SETUP

3.3.1 PHASE 1: AGITATED PILE COMPOSTING

Composting materials were formed into trapezoidal piles (length 2100 mm, base width 350 mm, top width 100 mm and height 550 mm) with length to base width (L/W) ratio 6. Fig. 3.7 shows schematic diagram and pictorial view of agitated pile. Agitated pile containing approximately 150 kg of composting materials with different waste combinations were manually turned on 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 days.

Composting period of total 30 days was decided for agitated pile composting (Das and Kalamdhad, 2011). After turning, about 100 g of each grab samples were collected on 0, 6, 12, 18, 24 and 30th day from six different locations, mostly at the mid span and end terminals of agitated pile and samples were mixed together and considered as homogenized sample.

Fig. 3.7 Schematic diagram and pictorial view of agitated pile composting

3.3.2 PHASE 2: ROTARY DRUM COMPOSTING

Fig. 3.8 shows the schematic diagram and pictorial view of a pilot-scale rotary drum composter of 550 L capacity; operated by batch-mode operation (Kalamdhad et al., 2009). The main unit of the composter i.e. the rotary drum is of 1.22 m in length and 0.76 m in diameter, fabricated by a 4 mm thick metal sheet.

The inner side of the drum is covered with anti-corrosive coating. The drum was mounted on four rubber rollers attached to a metal stand and rotated manually with its handle. In order to ensure appropriate mixing, agitation and aeration of the wastes during rotation, 40×40 mm angles are welded longitudinally inside the drum. In addition, two adjacent holes of 10 cm each are made on top of the drum to drain out the excess water.

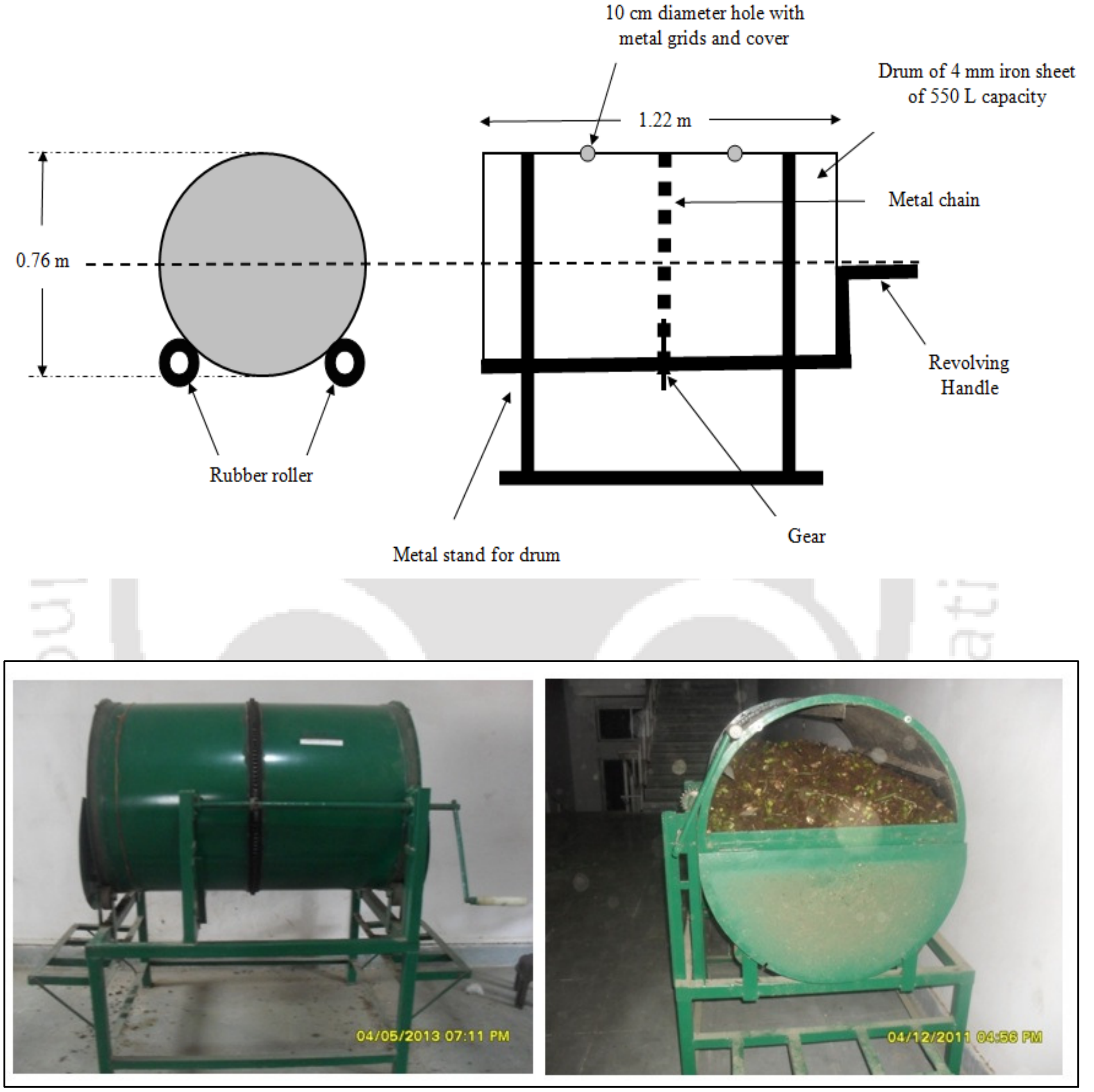


Fig. 3.8 Schematic diagram and pictorial view of a rotary drum composter for batch operation

The capacity of the pilot scale rotary drum composter was decided keeping in view of the capability of a single person who can handle around 150 kg of wastes by manual rotation. The composting period of 20 days was decided for both proper degradation and stabilization based on the performance of earlier studies regarding in-vessel composting reactors (Kalamdhad and Kazmi, 2009; Singh et al., 2009; Singh et al., 2012). Manual turning was done after every 24 hours through one complete rotation of the rotary drum to ensure that the material on the top portion moved to the central portion, where it was subjected to higher temperature. After that, aerobic condition was maintained by opening the top half side doors of the two circular faces. The samples were collected through grab sampling from different locations, mainly from the mid span and end terminals of the pilot-scale rotary drum composter with a compost sampler to minimize disturbance of the adjacent materials. All the grab samples were thoroughly mixed together to make a homogenized sample. Triplicates homogenized samples were collected on 0, 4, 8, 12, 16 and 20th day after turning by hand

3.3.3 PHASE 3: VERMICOMPOSTING

The experiments were conducted in duplicate in bamboo containers (reactors) of curved shaped (Radius; 120 mm, depth; 90 mm and volume; 904.70 cm^3). Temperature in the experimentation room was maintained at 25±1°C which is optimum temperature for *E. fetida.* These reactors were kept in room temperature. Fig. 3.9 shows the pictorial view of bamboo made vermicompost reactors and vermicompost. The earthworm weight was calculated according to the weight of the feedstock added and the number of days for experimentation, based on the literature suggested; earthworms can consume materials half of their body weight per day under favorable conditions (Haimi and Hutha, 1986; Khwairakpam and Bhargava, 2009). The moisture level was maintained about 60-70% throughout the study period by periodic sprinkling of adequate quantity of tap (potable) water. To prevent moisture loss, the reactors were covered with gunny bags.

The proper aeration was provided by perforated reactor design and periodic turning of waste mixture. The reactor was designed for a total weight of 1.5 kg for 45 days (based on worm mass added) composting period. Acclimatized 120 earthworms (adult and juvenile, average weight of 50 g) were randomly picked from the Perspex bin culture and used for the purpose of investigation. After turning and earthworm counting, about 80 g of each grab samples were collected on 0, 15, 30, and 45th day from three different locations of vermireactors and samples were mixed together and considered as homogenized sample.

Fig. 3.9 Pictorial view of bamboo reactors

3.3.4 PHASE 4: LIME AND ZEOLITE ADDITION

The best proportions were selected from phase 1 and 2 in which lime and natural zeolite were added. Three trials for each agitated pile and rotary drum were carried out with lime 1, 2 and 3% (Fang and Wong, 1999). Three trials for each agitated pile and rotary drum were conducted with natural zeolite 5, 10 and 15% (Zorpas et al., 2000). Turning and sampling was done similar as phase 1 and 2.

3.4 ANALYSIS OF COMPOST SAMPLES

Different experimental methods were required in the study to accomplish the stipulated objectives. Physico-chemical analysis of the compost samples carried out in Environmental Engineering laboratory (Departments of Civil Engineering, IIT Guwahati). Fig. 3.10 shows detail analysis of physico-chemical parameters during the composting process. Collected samples from agitated pile, rotary drum and vermireactors were kept in hot air oven at 105^{o}C for 24 h. Dried samples were ground and passes from 0.22 mm sieve, powder form of samples were used for physico-chemical analysis.

3.4.1 PHYSICO-CHEMICAL ANALYSIS

Temperature was monitored on the basis of 6 h time interval using a digital thermometer throughout the composting period within pile and rotary drum composter. The pH and electrical conductivity (EC) (1:10 w/v waste: water extract), about (10 ± 0.1g) of ground sample (screened through 0.22 mm sieve) was dissolved in 100 mL of distilled water and kept it in a horizontal shaker for 2 h, the sample was filtered through Whatman No. 42 filter paper.

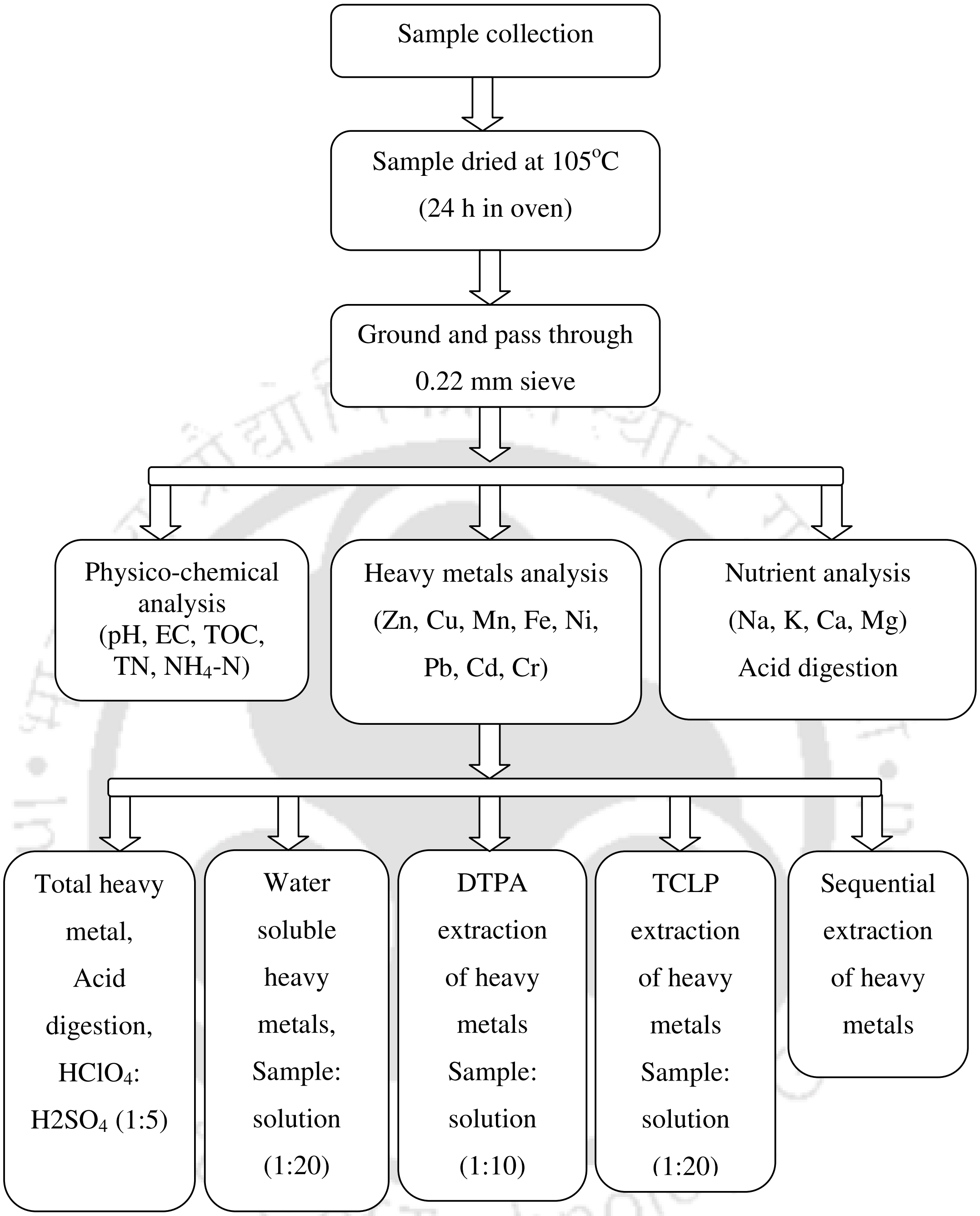


Fig. 3.10 Physico-chemical analysis of compost samples

The EC and pH was measured in filtered supernatant (BIS: 10158-1982). Volatile solid (VS) and ash content were also measured according to BIS, 10158-1982. Initial weight of the crucible was taken as W_1 g. Weigh (10 ± 0.1g) of ground sample (screened through 0.22 mm sieve) in crucible and kept it in a muffle furnace operating at a temperature of 550-600°C for 2 h. After 2 h crucible was taken out off the muffle furnace and kept in desiccator for ½ h for cooling and then final weight of crucible with sample was taken as W_2 g. Volatile solids content of the sample was calculated as:

$$VS\ (\%) = \frac{(5 - (W2 - W1)}{5} \times 100$$

$$\text{Ash content}\ (\%) = 100 - VS$$

Biodegradability coefficient (K_b) was calculated using the equation (Yadav and Garg 2009):

$$K_b = \frac{(OM_i - OM_f)100}{OM_i\ (100 - OM_f)}$$

where OM_f is the organic matter content at the end of process and OM_i is the organic matter content at the beginning of the process.

Total nitrogen (TN) was analyzed using the Kjeldahl method and NH_4-N using KCl extraction (Tiquia and Tam, 2000). For TN analysis 0.2 g of sample (passed through 0.22 mm sieve) was taken and catalyst mixture (potassium sulphate and cupric sulphate, 5:1) of 3 g was added, and digested with 10 mL conc. H_2SO_4 using digestion equipments at 400°C for 2 h (end color of digested sample was green). After digestion, make the digested sample 100 mL. 10 mL of diluted sample distillate using distillation unit (Pelican Equipments Chennai, India) with 40% NaOH and distilled water, distillate was collected in 25 mL boric acid with mixed indicator. Collected distillate (clear green color) and titrate with 0.02 N H_2SO_4 at end point purple colour.

The TN was calculated as follow:

$$TN\ (\%) = \frac{14x\ (S - B)x\ N}{wt.}$$

where, S = mL of standard sulfuric acid used for sample, B = mL of standard sulfuric acid used for blank, N = Normality of standard sulfuric acid, Wt. = Weight of compost sample in g.

For the analysis of NH_4-N, 5 g sample (passed through 0.22 mm sieve) was taken in a reagent bottle and shaken with add 50 mL of 2M KCl in a horizontal shaker for 2 h. After shaking sample was filtered and supernatant was taken for NH_4-N analysis using Phenate method of Standard methods (APHA, 2005).

The Flame photometer (Systronic 128) was used for analysis of Na, K and Ca concentration, and Mg concentration was measured by atomic absorption spectrometer (AAS) (Varian Spectra 55B) after the digestion of 0.2 g sample with 10 mL of H_2SO_4 and

$HClO_4$ (5:1) mixture in block digestion system (Pelican equipments, Chennai, India) for 2 h at 300°C.

3.4.2 HEAVY METAL ANALYSIS

The total concentration of Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr was measured using atomic absorption spectrometer (AAS) after the digestion of 0.2 g sample with 10 mL of H_2SO_4 and $HClO_4$ (5:1) mixture in block digestion system (Pelican equipments Chennai, India) for 2 h at 300°C.

The water-soluble heavy metals were determined after extraction of 2.5 g sample with 50 mL of distilled water (sample: solution ratio = 1:20) at room temperature for 2 h in a shaker at 100 rpm (Ciavatta et al., 1993).

The diethylene triamine penta-acetic acid (DTPA) extractable metals were obtained by mechanically shaking 4 g ground sample (screened through 0.22 mm sieve) with 40 mL of 0.005 M DTPA, 0.01M $CaCl_2$ and 0.1 M (triethanolamine) buffered to pH 7.3 at 100 rpm (Guan et al., 2011). Extraction efficiency of DTPA extractable heavy metals was calculated using following Eq. (Chen et al., 2010)

$$\text{Extraction Efficiency (\%)} = \frac{C_{\text{DTPA}}}{C_{\text{Total}}} \times 100$$

where, C_{DTPA}: concentration of DTPA extractable heavy metal; C_{Total}: concentration of total heavy metals.

The leachability of heavy metals was determined after extraction of 4 g of sample with 40 mL of 0.01 M $CaCl_2$ solution (sample: solution ratio = 1:10) at room temperature for 3 h in a shaker at 100 rpm (Qiao and Ho, 1997; Dean, 2007). The standard toxicity characteristic leaching procedure (TCLP) method according to EPA Method 1311 (1992) was applied to the solid samples in order to determine the potential leachability of heavy metals. According to this method, 5 g solid sample (size less than 9.5 mm) with 100 mL of acetic acid at pH 4.93 ± 0.05 (pH was adjusted by 1 N NaOH) (sample: solution ratio =1:20) was taken in 125 mL reagent bottle and kept at room temperature for 18 h in a shaker at 30±2 rpm. The suspensions of water, DTPA and TCLP extraction were centrifuged for five minutes at 10,000 rpm, and then were filtered through Whatman no. 42 filter paper and stored in a plastic reagent bottle at 4°C for analysis of selected heavy metals.

Sequential extraction of the heavy metals in the compost samples has been applied according to the method of Tessier et al. (1979). Five fractions were obtained: (i) exchangeable fraction (F1), (ii) carbonate fraction (F2), (iii) Reducible fraction (F3), (iv) organically bound fraction (F4) and (v) residual fraction (F5). A detail description of the sequential extraction procedure is presented in Table 3.3. The extraction was carried out with an initial mass of 1.0 g oven dried sample in polypropylene centrifuge tubes of 50 mL capacity (Fig 3.11). After each successive extraction, the supernatant liquid was removed with a pipette after centrifugation at 10,000 rpm for 5 min and made up to required volume for analysis of heavy metals. The residue was washed (except residual fraction) with 20 mL of Milli Q water by shaking for 15 minutes followed by centrifugation without loss of solids. The extracts were stored in plastic reagent bottles for heavy metal analysis. The concentration of heavy metals in each extract was determined by AAS. All extractions were performed in triplicate and the mean value was presented with standard deviation. Fig. 3.12 shows pictorial view of extracted samples. The bioavailability factor (BF) was calculated as follows (Liu et al., 2007)

$$\mathrm{BF} - \frac{\mathrm{F1} + \mathrm{F2} + \mathrm{F3} + \mathrm{F4}}{\mathrm{F1} + \mathrm{F2} + \mathrm{F3} + \mathrm{F4} + \mathrm{F5}}$$

Fig. 3.11 Pictorial view of polypropylene tubes with compost samples and solution

Fig. 3.12 Pictorial view of extracted samples from different steps of sequential extraction

3.4.3 STATISTICAL ANALYSIS

All the results reported are the means of three replicates. Repeated measures treated with analysis of variance (ANOVA) and Tukey's test were made using Statistica software. The objective of the statistical analysis was to determine any significant differences among the parameters analyzed for different trials. Microsoft excel was used for graphs plotting and determination of standard deviation & mean values.

3.5 INSTRUMENTS USED FOR THE STUDY

Instruments and accessories required for physico-chemical analysis of compost samples are given in table 3.4 and 3.5 respectively.

Table 3.3 The extractants, experimental conditions and properties of various extractable heavy metal forms with sequential extraction

S. N.	Speciation	Extractants	Environmental conditions	Characteristics
1	Exchangeable fraction (F1)	• 8 mL of 1.0 M $MgCl_2$ (pH = 7)	With agitation at 220 rpm for 1 h at 25°C	Readily influenced by changes of ionic composition in the liquid. Most available for plant uptake.
2	Carbonate fraction (F2)	• 8 mL 1.0 M NaOAc (pH = 5, adjusted with conc. HOAc).	With continuous agitation for 5 h at 25°C	Highly susceptible to pH variations. Available for plant uptake.
3	Reducible fraction (F3)	• 20 mL 0.04 M $NH_2OH.HCl$ in 25% HOAc (v/v).	Kept in water bath shaker for 6 h at 96°C with occasional agitation	Thermodynamically unstable under anoxic conditions. Available under extreme reducing conditions.
4	Organic matter bound fraction (F4)	• 3 mL 0.02 M HNO_3 and 5 mL 30% H_2O_2 (pH = 2, adjusted with conc. HNO_3). • After 2 h, 3 mL 30% H_2O_2 was added. • After cooling, 5 mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added.	Mixture was heated at 85°C for 2 h. Mixture was heated at 85°C for 3 h with occasional agitation. Agitated for 0.5 h at 25°C	It can be degraded (oxidizing) leading to a release of soluble metals under oxidizing conditions. Available under the extreme oxidizing conditions.
5	Residual fraction (F5)	• 10 mL of H_2SO_4 and $HClO_4$ (5:1) mixture	heated at 300°C 2 h	Fixed in crystal lattice and not entered in the food chain.

Table 3.4 Instruments used for physico-chemical analysis

S. N.	Parameters	Instruments/Equipments	Model/manufacture
1	Temperature	Digital thermometer	Mextech multi - therometer
2	Moisture content	Hot air oven	International Commercial Traders
3	pH	pH meter	Labtronics, microprocessor pH meter
4	EC	Conductivity meter	VSI, electronics Pvt. Ltd.
5	VS and ash content	Muffle furnace	International Commercial Traders
6	Total nitrogen	Kjeldahl distillation unit	Distyl-EM, Pelican equipments
7	NH_4-N	U.V. visible Spectrophotometer	Varian Cary 50 Bio
8	Na, K and Ca	Flame photometer	Systronic 128
9	Heavy metals	AAS	Varian Spectra 55B

Table 3.5 Other instruments and accessories used for physico-chemical analysis

S. N.	Instruments/Equipments	Purposes	Model/manufacture
1	Grinder	Grinding	Morphy richads
2	Sieve (0.22mm)	Sieving	SETHI standard test sieve
3	block digestion system	Solid sample acid digestion	Pelican Equipments Chennai-India
4	Hand gloved and apron	Hand and cloths precaution	Safety purpose
5	Centrifuge	Centrifuge	REMI instruments Limited, R-24
6	Water bath shaker	Temperature and shaking	IKON Instruments
7	Electronic balance	Weighing	WENSAR, weighing scale Ltd.
8	Shredder and grinder	Shredding and grinding	Shri Lakshmi industries

Chapter 4

BIOAVAILABILITY, LEACHABILITY AND SPECIATION OF HEAVY METALS

This chapter dealt with the bioavailability, leachability and speciation of heavy metals in agitated pile, rotary drum and vermicomposting of water hyacinth mixed with cattle manure and sawdust. The study was conducted in three phases.

1. Phase 1: Assessment of bioavailability, leachability and speciation during agitated pile composting
2. Phase 2: Assessment of bioavailability, leachability and speciation during rotary drum composting
3. Phase 3: Assessment of bioavailability, leachability and speciation during vermicomposting

4.1 PHASE 1: AGITATED PILE COMPOSTING

The bioavailability and leachability of heavy metals play an important role in the toxicity of heavy metals during composting followed by land application. Therefore, studies were carried out on bioavailability, leachability and speciation of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during 30 days agitated pile composting of water hyacinth. Five different proportions of water hyacinth, cattle manure and sawdust were prepared. The bioavailability of heavy metals was investigated in the form of water soluble and diethylene triamine penta-acetic acid (DTPA) extractable. Leachable fractions of heavy metals were extracted by 0.01 M $CaCl_2$ solution. The Tessier sequential extraction method was employed to investigate the changes in heavy metals speciation during the composting process. This study investigated the influence of parameters such as pH, temperature and organic matter content on distribution of heavy metals bioavailability, leachability and speciation during composting of water hyacinth.

4.1.1 PHYSICO-CHEMICAL ANALYSIS

As shown in Fig. 4.1, the composting pile temperature went through three typical phases (mesophilic, thermophilic and cooling phase) and ranged from 26°C to 56°C during the entire period of composting. The cattle manure affected the temperatures during the composting process in five different trials. Out of all five trials, trial 4 reached the highest temperature and longer due to higher addition of cattle manure indicating rapid development of microbial activity during the composting process (Chen et al., 2010a). In trial 1, a maximum temperature of 30^{o}C was observed, which contains only water hyacinth. These results indicate that cattle manure enhanced the composting process by providing easily available carbon for microorganisms, but in trial 5 which contained highest amount of cattle manure shows less temperature when compared to trial 4. Cattle manure in excess may increase the available carbon but inadequate nitrogen in trial 5 thereby reducing the temperature; furthermore compost microorganisms require optimum carbon and nitrogen for their growth.

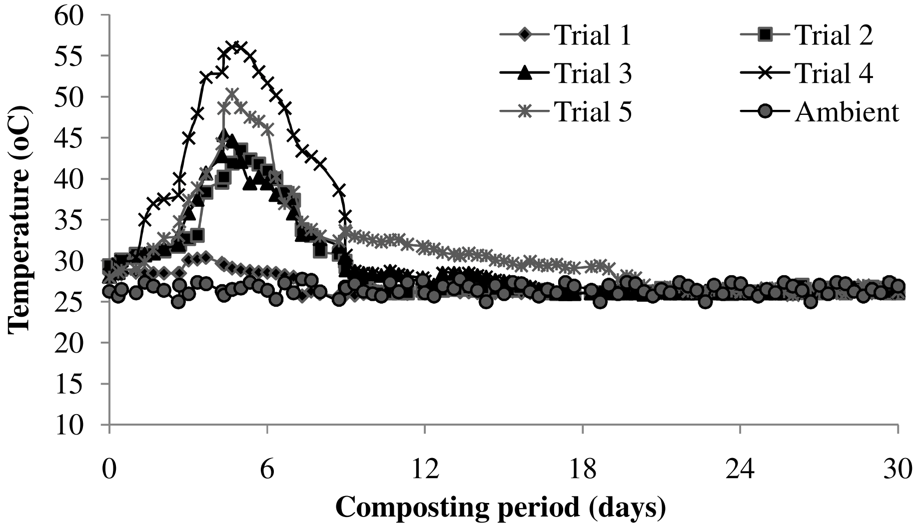


Fig. 4.1 Variation of temperature during pile composting process

Moisture loss during the composting process in the form of vapors by the heat generation can be viewed as an index of decomposition rate. The composting material requires optimum moisture content for survive of microorganisms (Kalamdhad et al., 2009). Initial moisture content was 86.0, 85.4, 83.5, 83.9 and 82.5% which reduced to 54.3, 42.9, 55.6, 37.5 and 47.9% within 30 days of composting period in trial 1, 2, 3, 4 and 5 respectively (Fig. 4.2). Highest moisture loss occurred in trial 4 (55%) and lowest moisture loss was observed in trail 1 (36.5%); it might be due to high heat generation in trial 4 and low heat generation in trial 1. On analyzing the results by ANOVA, the decrease in moisture content varied significantly between the days ($p < 0.05$). Leachate formation was not observed during the composting period.

The pH values were within the optimal range for the development of bacteria (6.0-7.5) and fungi (5.5-8.0) (Amir et al., 2005). Increase in the pH level during composting resulted in increase in volume of ammonia released due to protein degradation. Ammonia formation was very low after primary stabilization due to the low rate of organic matter degradation (Kalamdhad et al., 2009). Initially, pH was reduced in trial 2 and 3 within 6 days but it reached to almost neutral at end of composting process. Similar observations were found in all trials; the final compost pH was 7.3, 7.09, 7.3, 7.3 and 7.2 for trial 1, 2, 3, 4 and 5 respectively (Fig. 4.2). Significant difference in pH was observed in all the trials ($p < 0.05$). During the composting process sufficient aeration was provided by regular turning of pile consequences to decrease CO_2 level in the compost, which also leads to increase in pH and prevents the anaerobic conditions (Haug, 1993). The pH value of a solution strongly influences not only the site dissolution of biomass surface, but also the solution chemistry of heavy metals: hydrolysis, complexation by organic and or/inorganic legends, redox reaction, precipitation, the speciation and biosorption availability of the heavy metals (Wang et al., 2009).

Electrical conductivity (EC) is usually measured during composting because it reflects the salinity of the composting product and its suitability for plant growth (Fang and Wong 1999). High EC in the compost is undesirable because it will inhibit plant rooting and reduce the transportation of water and nutrients into the plants (Chiang et al., 2007). EC was increased from 3.9 and 4.1 dS/m to 7.9 and 5.7 dS/m in trial 1 and 2 respectively; it might be due to the net loss of weight and release of soluble salts through decomposition activity in the composting process. EC was decreased from 5.7, 6.4 and 4.7 dS/m to 5.4, 4.7 and 4.1 dS/m in trials 3, 4 and 5 respectively as shown in Fig. 4.2. The volatilization of ammonia and the precipitation of mineral salts could be the possible reasons for the decrease in EC at the later phase of composting (Kalamdhad et al., 2009). Furthermore, decrease in EC at the later phase of composting could be explained as, release of humic substances which had a capacity to interact with metal ions resulting in reduced water solubility (Amir et al., 2005). On analyzing the results by ANOVA, EC varied significantly between all trials ($p < 0.05$). During the composting process organic matter is decomposed and transformed into stable humic compounds. Humic substances had a competence to interact with metal ions and the ability to buffer pH and to act as a potential source of nutrients for plants (Amir et al., 2005; Singh et al., 2009). Fig. 4.2 shows the trend of organic matter degradation during 30 days of composting process in five different trials.

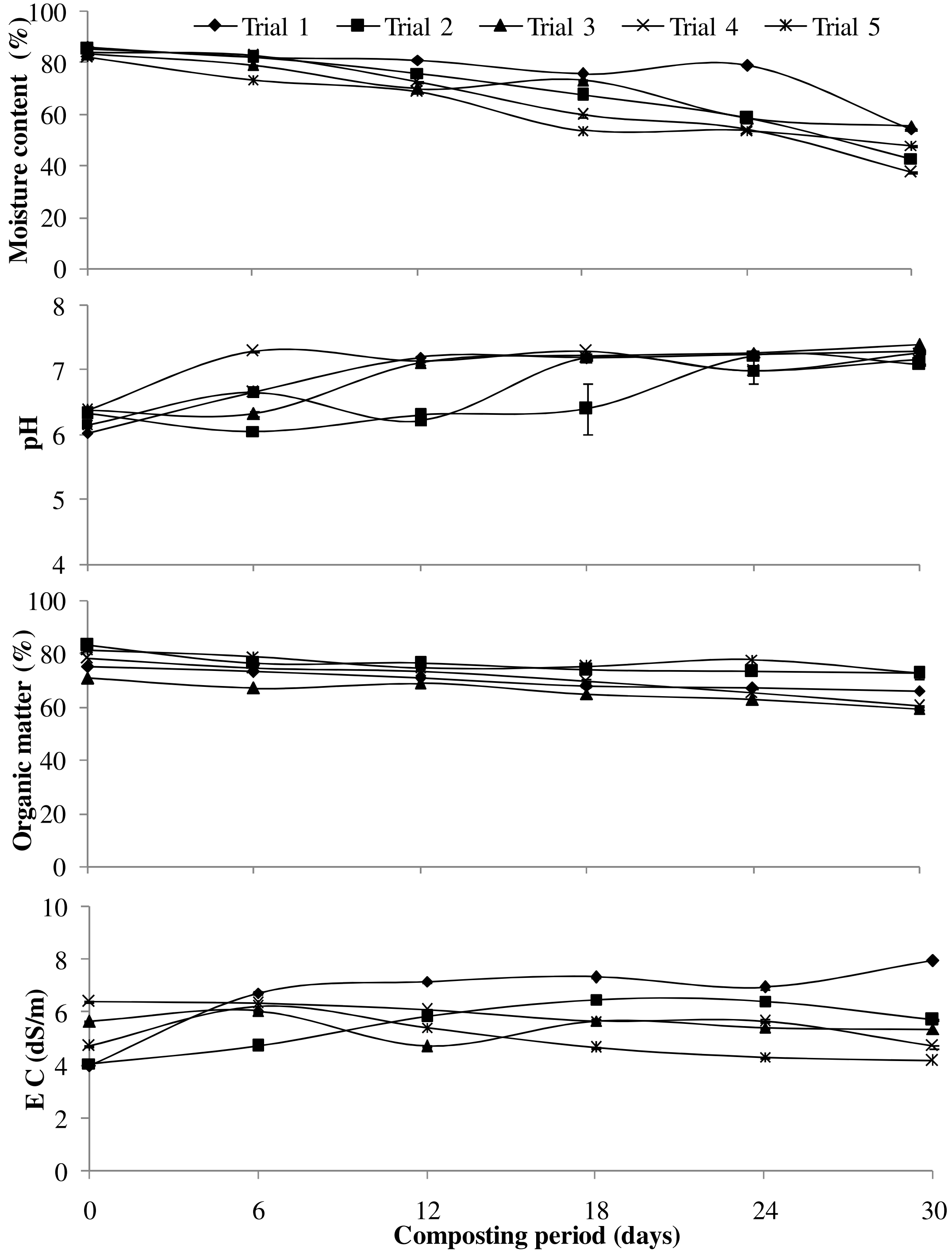


Fig. 4.2 Variation of moisture content, pH, electrical conductivity (EC) and organic matter during pile composting process

The content of organic matter was decreased as the decomposition progressed. Higher reduction of organic matter was observed in trial 4 (23%) as followed by trial 3 (16.1%), trial 2 (12.3%), trial 1 (11.7%), and trial 5 (11.0%).

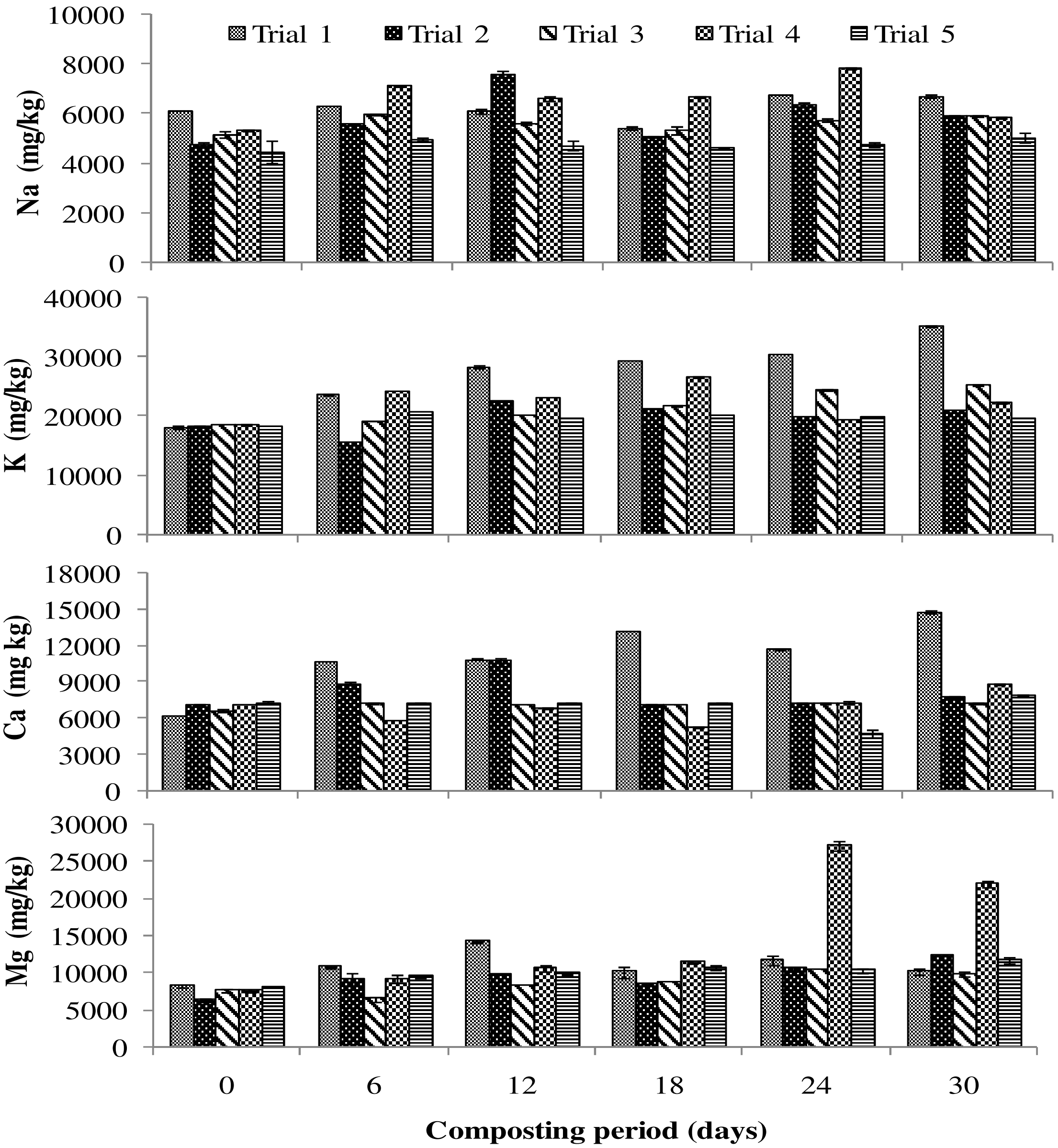


Fig. 4.3 Variation of nutrients (Na, K, Ca and Mg) during pile composting process

A significant variation in organic matter loss was observed in all the trials (P < 0.05). Fig. 4.3 illustrates the concentration of the nutrients such as Na, K, Ca and Mg was increased in all five trials throughout the composting process. These nutrients are required for plant growth. The concentration of Na, K, Ca and Mg was gradually increased till the end of the composting due to the net loss of dry mass. On analyzing the results by ANOVA, significant differences in nutrients were observed between all the trials ($p < 0.05$). An increase of metal concentrations in soil amended with compost in the long-term are generally reported to increase the concentrations of heavy metals in the tissues of plants growing in the soil (Li et al., 2010). Table 4.1a and b illustrates the total concentration of metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) in trials 1, 2, 3, 4 and 5 during 30 days of composting period. The order of total metal content in the composted water hyacinth was Fe > Pb > Mn > Ni > Cr > Zn > Cd > Cu. An increase in total metal concentration during composting process could be explained as reduction organic matter content (Zorpas et al., 2000). On analyzing the results by ANOVA, significant differences in total heavy metals (Zn, Cu, Mn, Fe, Pb, Cd, Ni and Cr) ($p < 0.05$) were observed between all trials. The potential toxic heavy metal concentrations of compost are high; the leachability of metal associated with compost is of concern (Amir et al., 2005). The total concentration of heavy metals indicates the extent of contamination, but they provide little information about the forms in which heavy metals are present, or about their potential for mobility and bioavailability in the environment (Cai et al., 2007).

4.1.3 BIOAVAILABILITY OF HEAVY METALS

- **Water soluble heavy metals**

Water soluble fractions of heavy metals are the most readily bioavailable fraction in compost applied to soils (Hsu and Lo, 2001). The water soluble fractions are positively more biologically dynamic and it has the highest prospective of contaminating food chain, surface water and ground water (Iwegbue et al., 2007). Metals in the water-soluble fraction may be readily leachable and bioavailable in the environment (Liu et al., 2008).

The water solubility of metals increase in thermophilic stage due to decomposition of organic matter and reduced in final compost due to changes in oxidizing and anionic conditions of the medium (Ahmed et al., 2007). Table 4.2 shows the changes in water soluble Zn, Cu, Mn, Fe and Cr concentration during the composting process. Water solubility of Ni, Pb and Cd were not detectable in all trials. The water solubility of Zn was reduced from 6.0, 1.5, 2.1 and 4.7% to 1.1, 1.0, 1.5 and 1.3% of total Zn in trials 2, 3, 4 and 5 respectively; however in trial 1 it was increased from 4.1 to 4.5% of total Zn at the end of the composting period. The concentration of Cu was reduced from 3.3, 4.8, 3.6 and 5.4% to 2.3, 1.6, 2.2 and 1.2% of total Cu in trials 2, 3, 4 and 5 respectively; however in trial 1 it was increased from 2.3 to 2.7% of total Cu.

Table 4.1a Total heavy metals concentration (Zn, Cu, Mn and Fe) during 30 days of composting period

Days	Total metals concentration									
	Zn (mg/kg dry matter)					**Cu (mg/kg dry matter)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	180.2±2.0a	115±0.7b	241.2±0.1c	161.1±1.6d	222.6±0.4e	77.5±1.5a	45.8±0.8b	46.8±0.8b	31±0.5c	49.3±0.8d
6	222.6±0.8a	125.8±0.8b	231.4±0.0c	200.8±0.2d	293.4±6.05e	78.8±1.3a	38.8±0.3b	38.8±0.3b	45.5±0.5c	58.8±1.3d
12	104.8±1.0ab	104.8±1.0a	239.8±1.8ab	282.8±7.2ab	282.8±7.3b	72.8±1.2a	31.3±0.3b	64.8±0.8c	51.8±0.8d	65.5±0.5c
18	195.8±3.0a	195.8±3.0a	261.7±0.9a	272.5±2.0a	272.5±2.0a	57.0±3a	44.5±5b	63.3±1.3c	46.8±0.8b	65.8±0.3c
24	179±1.6ab	179 ±1.6ab	242.9±0.4a	235.9±1b	235.9±1ab	59.3±0.8	89.75±7.8	61.3±0.8	49.0±0.5	67.3±1.8
30	197.3±3.8a	217.8±0.6b	291.0±2c	297.8±3c	269.7±5d	79.2±1.1a	61.5±3.5b	73.0±0.8a	103.3±0.8c	61.5±0.5a
Days	**Mn (mg/kg dry matter)**					**Fe (g/kg dry matter)**				
0	998±8a	356±0.5b	816±33c	573±10d	737.5±12e	17.5±0.03a	11±0.14b	6.2±0.02c	7.9±0.1d	9.6±0.05e
6	978±2.3a	405±1.8b	820±11c	626±6d	805±15c	23.9±0.16a	12.1±0.41b	7.7±0.01c	7.7±0.32c	9.7±0.1d
12	1416±6.3a	430±0.8b	813±2.8c	906±12d	852±17.5e	28.6±0.18a	12.9±0.01b	8.06±0.0c	11.0±0.02d	11.8±0.1e
18	1073±36.5a	727±6.3b	1226±1.8c	776.5±17b	970±10d	32.1±0.02a	11.9±0.07b	9.6±0.03c	12.4±0.54b	14±0.13d
24	1276 ±25a	760±5.5b	1125±75c	892±50d	801±1.5bd	30.6±0.01a	13.3±0.02b	10.1±0.77c	11.6±0.01d	13.6±0.1b
30	1182±9a	1069±30bd	1369±46c	1105±28ab	990±60d	20±0.04a	14.2±0.03b	11.7±0.2c	13.3±0.03d	14.3±0.2b

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$)

Table 4.1b Total heavy metals concentration (Cr, Ni, Cd and Pb) during 30 days of composting period

	Total heavy metals concentrations									
Days	**Cr (mg/kg dry matter)**					**Ni (mg/kg dry matter)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	149.5±1.0a	290.6±2.4b	180.5±3.5c	257.0±5d	247±5.3e	193±2.5a	158±7.3b	249±10.3c	187±1.8a	269±1.3d
6	175.8±1.8a	274.8±1.8b	184.0±8a	259.3±19b	215±2.5c	323±0.8a	187±7.0b	291±5.3c	223±3.3d	322±5.5a
12	174.8±8.8a	257.5±1.5b	219.5±6.5c	261±4.5c	220±1c	307±19a	161±6.8b	238±4.0c	293±2.0da	277±10.3d
18	190.5±1.5a	407.8±10b	221.3±2.3c	236±3.8d	226±2cd	274±0.8a	256±5.8b	259±2.0b	320±5.5c	291±1.0d
24	186.3±2.8a	287.25±0.8b	204.8±1.2ac	270.5±5.5c	309±15d	230±3.8a	267±3.0b	285±5.5c	285±9.0c	304±8.0d
30	183.3±8.8a	301±0.3b	227.5±2c	279.0±1.3c	281±2.5d	302±3a	317±8.0a	265±5.0b	236±1.8c	376±16d
Days	**Cd (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
0	60±0.02a	40±1b	57±2a	43.8±1.3c	63.5±0.5d	1140±5a	1030±25b	1185±5c	8723±12.5d	867±7.5d
6	70.8±0.3a	44.8±0.8b	72±2.5ac	71.8±0.3ac	75.5±2.0c	1200±5a	1090±4b	1275±1a	1032±27b	1200±25a
12	63.3±0.8a	45.8±1.3b	62.3±0.8a	69.5±0.5c	68.0±1.0c	1057±7.5a	1004±84b	1160±2c	1387±2.5d	1130±5e
18	68.8±0.8abc	64±1.5abc	62.5±0.5a	70±0.5c	62.5±5ab	880±25a	1055±5b	1353±8c	1372±58c	1245±5d
24	58±0.5a	67.3±1.3b	69±0.5b	60.3±0.8a	68.0±5.0b	1107 ±42a	1250±5b	1193±8b	1250±0.0b	1103±23a
30	65±2ad	75.3±.3b	64±0.5a	83.8±1.3c	67.0±0.5d	1187±17.5a	1582±2.5b	1568±6bc	1537±12.5c	1207±18a

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$)

The groups –OH and –COOH supplied by cattle manure increased the binding sites and combined with Cu to form insoluble and immobile complexes, thus the concentration of free Cu^{2+} decreased and the potential environmental risk was drastically reduced (Guan et al., 2011). Furthermore, the decrease of water soluble fraction of Cu and Zn was in agreement with increase of humic substances in the mature compost (Cai et al., 2007). Humic substances generally have higher affinity to complex with copper than with zinc. In water extracts, humic acid and fulvic acid mainly complex or bind with Cu when zinc coexists in manure. This is because water soluble humic substances always have high contents of carboxyl groups, which are ready to complex with Cu (Liu et al., 2008). Hydrolysis of Cu^{2+} reportedly occurs at pH of 5.9, whereas Zn^{2+} hydrolysis occurs at pH of 7.6 (Burton et al., 2003).

The concentration of Mn in trial 1, 2, 3, 4 and 5 were reduced from 1.6, 7.3, 3.6, 3.2 and 2.2% to 1.0, 1.3, 0.6, 1.2 and 0.7% of total Mn respectively; at the end of the composting period. The concentration of Cr in trial 1, 2, 3, 4 and 5 were reduced from 0.3, 0.6, 0.9, 0.8 and 0.4% to 0.2, 0.4, 0.7, 0.2 and 0.2% of total Cr respectively; at the end of the composting period. Water solubility of heavy metals reduced might be due to oxidation process and formation of organo-metallic complexes (Fang and Wong, 1999). The concentration of Fe in trial 1, 2, 3, 4 and 5 were increased from 0.2, 0.1, 0.1, 0.2 and 0.1% to 0.6, 0.3, 0.4, 0.6 and 0.2% of total Fe respectively at the end of the composting period. It had been observed that water solubility of Fe was increased in all five trials; it might be due to poor complexity of Fe with humic substances. The order of water soluble metal concentration in the composted water hyacinth was Fe > Mn > Zn > Cu > Cr. On analyzing the results by ANOVA, significant differences in water soluble heavy metals (Zn, Cu, Fe, Cr and Mn) ($P < 0.05$) were observed in all the trials.

During composting process, the heavy metals distribution is influenced by the release of heavy metals during the organic matter mineralization or metal solubilization by the decrease of pH, metal biosorption by the microbial biomass or metal complexation with the newly formed humic substances which makes the metals insoluble and therefore less easily extractable (Garcia et al., 1995; Castaldi et al., 2006; Cai et al., 2007). Water soluble organic matter in cattle manure decreased during the composting process (Liu et al., 2008). Water soluble humus was correlated with water soluble heavy metals, but the distribution of heavy metals in humic acid or fulvic acid depends on the type of heavy metals and not on the total content of the metal. The higher the affinity of cations with humic substances is, the more the cation will be complexed by water-soluble humic substances (Liu et al., 2008).

- **Plant availability of heavy metals (extraction with DTPA)**

Generally, metal fraction extracted with chelating agent DTPA can be considered as potentially available to plants (Garcia et al., 1995; Fang and Wong, 1999; Chiang et al., 2007). Fig. 4.4 shows the changes in extraction efficiency of DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr during 30 days of composting period. DTPA extractable Pb and Cd were not detected in all trials. The DTPA extraction of Zn was reduced 26.9, 39.87 and 29.5% of total Zn in trials 3, 4 and 5 respectively; however in trials 1 and 2 it was increased. The DTPA-extractability of Zn increased in the trials 1 and 2 might be due to dissolution of organic matter during the DTPA extraction procedure and its reduction in trials 3, 4 and 5 might due to the affinity for entering organic complexes like Cu (Walter et al., 2006).

Similar as Zn, DTPA extraction of Cu was reduced in trials 3, 4 and 5 about 21.3, 81.4 and 36.9% of total Cu respectively; however it was increased in trials 1 and 2 during the composting process. The total Cu concentration of all the five trials was very low, but it was extremely present in plant available form. The increased DTPA extractability of Cu in trial 1 might be due to absence of humic substances as results of incomplete degradation. The stability constant of metal-humic complexes depend on the nature of organics, metal, as well as other factors such as ionic strength and pH in the environment (Liu et al., 2008). The DTPA extraction of Mn was reduced about 40.0, 62.4, 81.7 and 21.2% of total Mn in trials 2, 3, 4 and 5 respectively; however it was of increased in trial 1. The DTPA extraction of Fe was increased in trials 1, 2, 3 and 4 however it was reduced in trial 5 about 60.7% of total Fe. Similar as Zn and Cu, DTPA extraction of Ni was reduced about 47.2, 64.2 and 17.1% of total Ni in trials 3, 4 and 5 respectively; however it was increased in trials 1 and 2. The DTPA extraction of Cr was reduced about 29.4, 31.1 and 27.1% of total Cr in trials 3, 4 and 5 respectively; however it was enhanced in trials 1 and 2 during the composting process. A DTPA extractable Cr concentration was very low in all trials except trial 1; it might be due to incomplete extraction or low mobility and availability of Cr in insoluble form (Walter et al., 2006). Heavy metal availability and mobility reduce due to formation of metal-humic complexes (Liu et al., 2008).

Table 4.2 Water soluble metals concentrations during 30 days of composting period

	Water soluble metals concentrations									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	7.3±0.1a	7±0.13b	3.6±0.06c	3.4±0.04c	10.4±0.04d	1.8±0.14a	1.5±0.06b	2.25±0.05c	1.1±0.04d	2.6±0.08e
6	8.7±0.03a	7.0±0.13b	1.9±0.03c	2.0±0.01c	3.61±0.1d	2.1±0.06a	1.3±0.02b	1.35±0.05b	1.8±0.02c	1.2±0.02d
12	2.1±0.08a	3.0±0.12b	4.3±0.07c	8.0±0.06d	8.7±0.03e	1.4±0.0a	1.7±0.1b	1.38±0.02a	1.8±0.04b	2.1±0.06c
18	4.5±0.13a	2.3±0.14b	3.3±0.13c	7.4±0.22d	7.0±0.54d	1.4±0.0a	1.75±0.05b	1.38±0.02a	2.1±0.06c	4.3±0.02d
24	3.6±0.09a	2.2±0.08b	7.7±0.21c	8.4±0.06d	4.5±0.13e	1.2±0.02a	2.48±0.04b	1.02±0.06c	1.8±0.08d	1.4±0.0e
30	8.9±0.02a	2.4±0.05b	2.7±0.02c	4.5±0.13d	3.6±0.09e	2.1±0.06a	1.4±0.04b	1.18±0.02c	2.3±0.01d	1.2±0.02c
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	15.8±0.35a	25.9±0.9b	29.5±0.5c	18.5±0.3d	16±0.24a	42.08±1.1a	13.8±0.04b	6.8±0.5c	16.14±1.1d	94.0±0.08bd
6	12.0±0.02a	22.1±0.02b	15.1±0.2c	5.1±0.02d	6.8±0.04e	126.4±2.02a	11.8±0.04b	8.0±0.4b	16.7±0.52b	85.0±9.24c
12	12.72±0.6a	23.8±0.3b	23.3±0.2b	7.5±0.1c	12±0.02d	57.8±0.92a	14.5±0.15b	28.2±0.28b	24.6±0.06b	108.5±15.8c
18	9.9±0.3ad	16.7±0.2b	12.7±0.1a	9.4±2.5d	4.89±0.3c	77.5±1.7a	14.3±0.25b	25.8±0.48c	77.5±1.7d	105.5±0.5e
24	6.8±0.04a	15.16±0.6b	9.7±1.0c	9.3±0.54c	9.9±0.26c	93.5±0.78a	61.8±1.04b	42.2±4.02c	93.5±0.78d	34.1±1.7e
30	12.0±0.0a	13.5±0.06a	8.2±1.4b	13.2±0.26a	6.8±0.04b	126.4±2a	36.4±0.2bd	42.2±4.02b	75.1±2.2c	35.3±0.3d
Days	**Cr (mg/kg dry matter)**					**Ni, Cd and Pb (mg/kg dry matter)**				
0	0.4±0.01a	1.6±0.06b	1.6±0.01b	2±0.07c	1.1±0.03d	ND	ND	ND	ND	ND
6	0.3±0.02a	2.1±0.15b	1.3±0.04c	1.7±0.07d	1.1±0.03c	ND	ND	ND	ND	ND
12	0.5±0.3a	1.8±0.06b	1.2±0.04c	1.8±0.02d	0.5±0.04a	ND	ND	ND	ND	ND
18	0.5±0.03a	1.4±0.03bc	1.5±0.02b	1±0.11c	0.7±0.28a	ND	ND	ND	ND	ND
24	0.4±0.1a	1.5±0.01b	1.3±0.02b	1.4±0.06b	0.7±0.2a	ND	ND	ND	ND	ND
30	0.4±0.05a	1.3±0.02b	1.7±0.04c	0.54±0.04d	0.5±0.0ad	ND	ND	ND	ND	ND

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$); ND- not detected

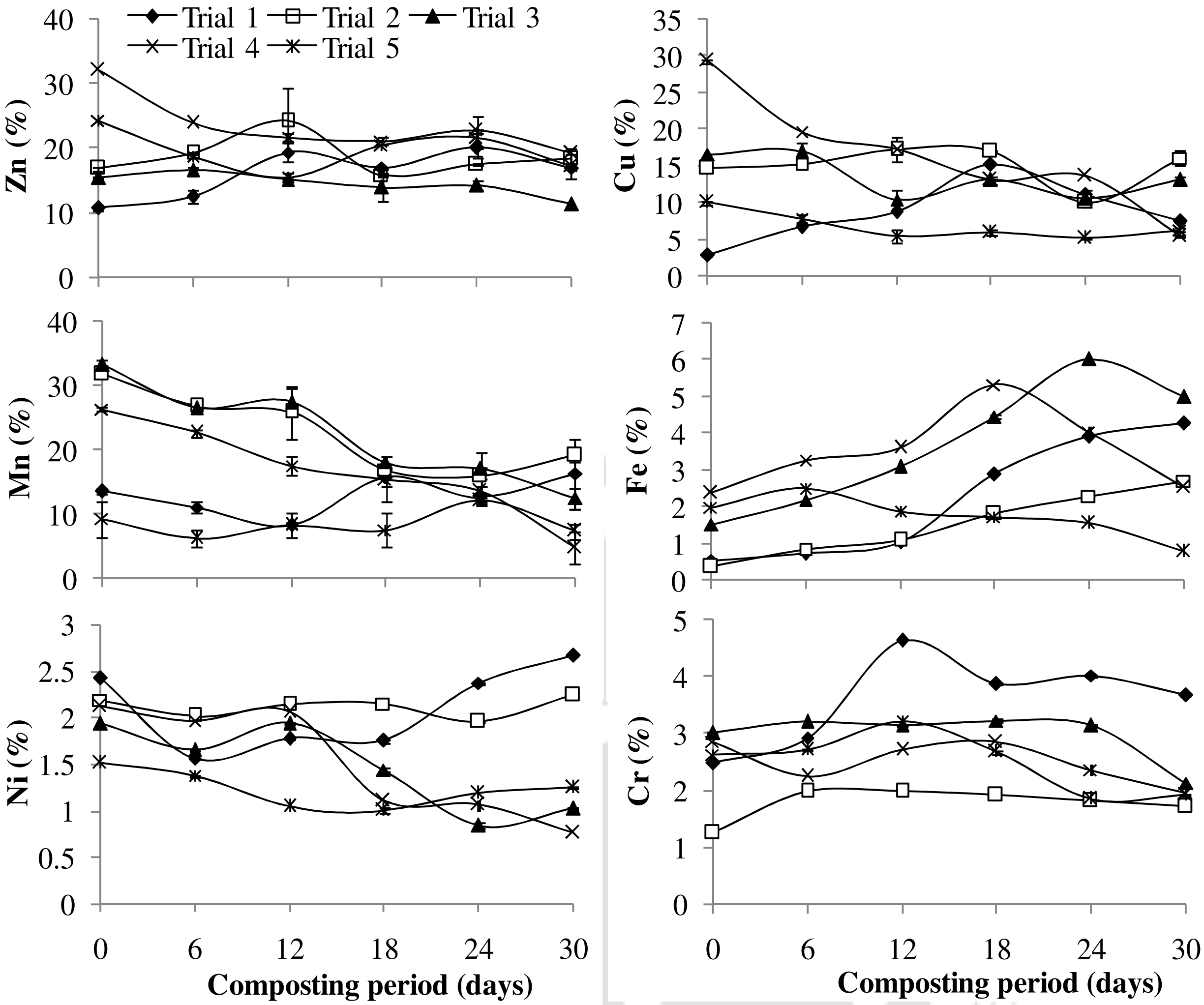


Fig. 4.4 Variation of DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr extraction efficiency during pile composting process

The order of plant available metals content in the final water hyacinth compos was: Fe > Mn > Zn > Ni > Cu > Cr. On analyzing the results by ANOVA, significant differences in DTPA extractable heavy metals (Zn, Cu, Mn, Fe, Ni and Cr) ($p < 0.05$) were observed between all the trials.

4.1.3 LEACHABILITY OF HEAVY METALS

The leachability of heavy metals was determined after extraction of 4 g of sample with 40 mL of 0.01 M $CaCl_2$ solution (Qiao and Ho, 1997). Table 4.3 illustrates the changes in leachable Zn, Cu, Mn, Fe and Cr concentration during 30 days of composting period. The leachable concentration of Zn was reduced from 2.9, 1.4, 2.0 and 2.0% to 1.5, 1.0, 1.6 and

1.0% of total Zn in trials 2, 3, 4 and 5 respectively; however its concentration was increased from 2.8 to 3.0% of total Zn in trial 1. The concentration of Cu was reduced from 2.0, 3.9, 5.2 and 1.5% to 1.1, 1.0, 0.7 and 1.4 % of total Cu in trials 2, 3, 4 and 5 respectively; however it was was increased from 1.6 to 1.6% of total Cu in trial 1. The leachability of Cu and Zn was reduced in all trials except trial 1; the reduction might be due to formation of Cu and Zn complex with organic matter or humic substances at maturity of the composting process. During the first phase, the neutral pH values caused a weak adsorption onto organic matter of Cu and Zn which probably leached out (Lazzari et al., 2000). The concentration of Mn was reduced from 7.8, 9.8, 5.5, 5.0 and 4.5% to 1.0, 1.2, 0.3, 1.2 and 1.0% of total Mn in trials 1, 2, 3, 4 and 5 respectively; at the end of the composting period. The concentration of Fe was reduced from 0.33 to 0.23% of total Fe in trial 5; however the concentration of Fe in trials 1, 2, 3 and 4 was increased in all trials at the end of the composting period. The leachability of Fe was increased in all the trials except trial 5 due its poor organometallic complex formation property. This might be due to small fraction of Fe attached with organic matter. In general the organometallic complexes follow the Irwing William series: Cu > Pb > Zn > Cd > Fe (Nomeda et al., 2008).

Leachability of Ni and Cd contents in all the trials was not detected because these metals were strongly complexed with organic matter and pH was neutral in all trials at end of composting. At higher pH (pH > 8) Ni and Cd may be leached due to break down of metal complex with organic matter (Lazzari et al., 2000). Pb contents were also not detectable in all trials because it has been considered that Pb forms strong complex with organic matter and it bioaccumulates in the humus rich surface layer compost. Pb is the least mobile heavy metal under reducing and non-acidic condition (Lazzari et al., 2000).

The leachable concentration of Cr was reduced from 3.7, 0.9, 1.5, 1.2 and 0.8% to 1.1, 0.7, 0.7, 0.8 and 0.2% of total Cr in trials 1, 2, 3, 4 and 5 respectively; at the end of the composting period. Cr^{3+} has an electron configuration closest to a noble gas with a high spherical symmetry and low polarization capacity. It has a valency of three and therefore it has a stronger electrostatic affinity for the sorption sites than divalent cations. Consequently it forms the most stable complex with humic substances (Qiao and Ho, 1997). The order of leachable heavy metals content in the composted water hyacinth was Mn > Zn > Fe = Cr > Cu. On analyzing the results by ANOVA, significant differences in leachable heavy metals (Mn, Fe, Zn, Cu and Cr) ($p < 0.05$) were observed between all trials.

Table 4.3 Leachable heavy metals concentration during 30 days of composting period

Days	Leachable heavy metals concentrations									
	Zn (mg/kg dry matter)					**Cu (mg/kg dry matter)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	5.0±0.29a	3.4±0.04b	3.3±0.15b	3.1±0.24b	4.4±0.03c	1.2±0.0a	0.9±0.0b	1.8±0.10c	1.6±0.03d	0.7±0.02e
6	3.9±0.2a	2.6±0.02b	1.0±0.04c	1.4±0.05d	2.05±0.1e	1.3±0.15a	1.8±0.05b	1.2±0.05a	1.9±0.05b	0.6±0.0c
12	2.9±0.1a	2.5±0.04b	2.6±0.0b	3.6± 0.04c	4.6±0.08d	1.6±0.12a	1±0.05b	1.6±0.07a	0.7±0.02c	1±0.05b
18	5.9±0.07a	2.7±0.07b	3.1±0.07c	2.9±0.04c	4.6±0.12d	1.4±0.0a	1.3±0.1a	0.5±0.03b	0.7±0.01c	0.9±0.0d
24	3.5±0.0a	2.06±0.16b	2.3±0.17b	3.4±0.05a	3.2±0.09a	1.1±0.0a	1.7±0.05b	0.6±0.01c	0.7±0.01c	1.2±0.13a
30	6±0.07a	3.2±0.04b	2.8±0.2b	4.8±0.12c	2.8±0.02b	1.3±0.02a	0.7±0.02b	0.7±0.02b	0.7±0.01b	0.8±0.1b
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	58±3a	34.9±0.5b	44.8±1.3c	28.5±0.5d	33.4±0.42b	21.4±0.5a	10.6±0.5b	16.0±3ab	15.6±4.2ab	28.5±5ac
6	49.3±4.5a	47.2±0.2a	27.2±0.2b	9.6±0.5c	14.14±0.5a	70.3±0.12a	10.8±0.4b	18.0±1c	13.4±0.4d	40.1±0.7e
12	19.5±0.4a	33.05±0.4b	14±0.4c	24±0.72d	24.5±0.3d	93.6±2.08a	10.8±0.5bc	32.5±4.5cd	17.5±0.6c	57.1±8.7a
18	11.5±0.04a	29.3±0.2b	5±0.08c	11.3±1.6a	28.5±0.9b	92.1±5.9a	10.9±0.1b	20.0±2c	16.4±1.6c	32.6±2.4d
24	9.3±0.0a	16.1±0.12b	4±0.02c	10.3±0.6d	9.6±0.5ad	73.9±2.7a	12.3±0.1bc	21.4±0.7cd	18.1±0.9c	33.8±1.4a
30	11.3±0.28a	12.6±0.17b	4.2±0.04c	13.7±0.6d	9.5±0.3e	51.0±14.8a	23.5±0.1b	25.4±1.6c	26.5±0.3d	39.3±0.8a
Days	**Cr (mg/kg dry matter)**					**Ni, Cd and Pb (mg/kg dry matter)**				
0	2.6±0.2ab	2.7±0.5abd	2.7±0.13ab	3.2±0.07bd	1.9±0.04c	ND	ND	ND	ND	ND
6	2.2±0.2a	2.4±0.01a	2.0±0.11ac	2.5±0.2ad	1.6±0.01b	ND	ND	ND	ND	ND
12	2.0±0.6a	2.3±0.11a	1.6±0.09a	2.2±0.3a	1.6±0.09a	ND	ND	ND	ND	ND
18	2.0±0.5a	2.0±0.18a	2.2±0.15a	1.2±0.05a	1.7±0.00a	ND	ND	ND	ND	ND
24	2.1±0.4a	2.2±0.05a	2.1±0.09a	1.6±0.12a	1.7±0.21a	ND	ND	ND	ND	ND
30	2.4±0.24ab	2.1±0.10ab	1.8±0.12bd	1.1±0.05c	1.8±0.03abd	ND	ND	ND	ND	ND

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$); ND- Not detected

4.2.4 SPECIATION OF HEAVY METALS

- **Speciation of Zn, Cu, Mn, Fe and Ni**

The Zn, Cu, Mn, Fe and Ni are essential elements required for normal plant growth and their metabolism, but higher concentration of these elements may be toxic for living organisms (Garrido et al., 2002), however toxicity of these metals depend on easily available fractions.

In water hyacinth, sawdust and cattle manure; the order of different fractions of Zn is: F5 (63.6%) > F4 (12.4%) > F1 (10.4%) > F3 (8.1%) > F2 (5.6%), F5 (76.7%) > F1 (9.9%) > F2 (7.2%) > F3 (4.5%) > F4 (1.6%) and F5 (53.3%) > F2 (23.5%) > F3 (9.5%) > F4 (8%) > F1 (5.6%) respectively (Fig. 4.5). For Zn it could be found that the residual fractions were dominant in all three raw materials.

Speciation of Zn in trial 1, 2 and 4 is given in Table 4.4a, 4.5a and 4.6a respectively, and speciation in trial 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. In trials 1 and 2, F1 and F5 fractions of Zn were decreased; however the F2, F3 and F4 fractions were increased of the total fraction of Zn during the composting process. The F4 and F5 fractions of Zn were the predominant speciation for Zn in trial. In trials 2 and 5, F1, F2 and F4 fractions of Zn were decreased; however the F3 and F5 fractions were increased of the total fraction of Zn during the composting process. In trial 4, all movable fractions (from F1 to F4) were reduced (percentage of total fraction) in comparison to other trials; however F5 fraction was rapidly decreased in initial phase of composting but increased at the end of composting. The reduction of all movable forms in trial 4 may be due to the formation of Zn complex with humic substances formed at the maturity stage of compost. Humic substances contain various organic functional groups that can sorbs metal ions through ionic force (Cai et al., 2007). Zn can precipitate with hydroxides, carbonates, phosphates, sulfides and several other anions as well as form complexes with organic legends. Cation exchange and complexation by organic legends were suggested to be the main Zn mobility controlling mechanisms (Kumpiene et al., 2008).

The fraction of F5 was the predominant speciation for Zn in all trials. In this study, pH of the final composts ranged from 6.0 to 7.4 (Fig. 4.2). The pH and transformation of organic matter into humic substances during the composting process might affect the transformation of Zn and consequently the speciation distribution. The Bioavailability factors (BF) of Zn in trial 1, 2 and 5 was increased from 0.36, 0.42 and 0.52 (initial) to 0.4, 0.44 and 0.53 (final); however, in trial 3 and 4 it was decreased from 0.53 and 0.75 (initial) to 0.4 and 0.48 (final) respectively during the composting period. Phytotoxicity could be caused by high Zn bioavailability factor (Fuentes et al., 2004). The Zn availability is influenced by the total Zn content, pH, organic matter content and the availability of adsorption sites (Achiba et al., 2009). Significant difference in F1, F2, F3, F4 and F5 fractions of Zn was observed in all the trials ($p < 0.05$).

In water hyacinth, sawdust and cattle manure; the order of different fractions of Cu is: F5 (55.6%) > F4 (34%) > F1 (4.7%) > F2 (3.2%) > F3 (2.4%), F5 (59.1%) > F4 (29.9%) > F1 (5.8%) > F3 (3.2%) > F2 (2.1%) and F5 (48.9%) > F4 (38.2%) > F1 (7.0%) > F2 (3.9%) > F3 (2.0%) respectively (Fig. 4.5). For Cu it could be found that the F4 and F5 fractions were predominant in all three raw materials. Speciation of Cu in trial 1, 2 and 4 is given in Table 4.4a, 4.5a and 4.6a respectively, and speciation in trials 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. In trial 1, F4 fraction of Cu was decreased though the F2, F3 and F5 fractions were increased of the total fraction of Cu during the composting process. The percentage change of fraction F1 was remaining same. The F1 and F5 fractions of Cu were decreased, however the F2, F3 and F4 fractions were increased of the total fraction of Cu in trial 3during the composting process.

The F1, F2, F3 and F4 fractions of Cu were decreased, however the F5 fraction was increased (percentage of total fraction) in trials 2, 4 and 5. In trial 2, initially Cu was bound dominantly in F4 fraction but after composting F4 fraction was converted into F5 fraction. In trials 4 and 5 movable form of Cu (F1, F2, F3 and F4 fraction) has been converted into F5 fraction after composting. A maximum reduction of F1 and F2 fractions of Cu were observed from 7 and 10% to 1.6 and 2.5% respectively in trial 4, which may be due to the formation of Cu ion complex with two or more organic functional groups mainly carboxylic, carbonyl and phenolic, so that the ion was immobilized in a rigid inner-sphere complex (Qiao and Ho, 1997).

BF of Cu in trial 1, 2, 4 and 5 were decreased from 0.44, 0.69, 0.63 and 0.59 (initial) to 0.4, 0.35, 0.28 and 0.35 (final); however in trial 3, it was increased from 0.42 (initial) to 0.75 during the composting period. The maximum reduction of movable fractions (from F1 to F4) and BF of Cu was observed in trial 4; it was due to maximum organic matter loss, high heat generation and organic mature converted into humic substances. Humic substances generally have higher affinity to complex with copper than zinc. This is because water soluble humic substances always have high contents of carboxyl groups, which are ready to complex with Cu (Liu et al., 2008). The stability constants of metal-humic complexes depend on the nature of organics, metal, as well as other factors such as ion strength and pH in environment (Liu et al., 2008). Significant differences in F1, F2, F3, F4 and F5 fractions of Cu were observed in all the trials ($p < 0.05$).

Table 4.4a Speciation of heavy metals (Zn, Cu, Mn and Fe) in trial 1 during 30 days of composting period

	Speciation of heavy metals									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F 1**	**F 2**	**F 3**	**F4**	**F 5**
0	17.9±0.1	9.7±0.08	13.9±0.16	21.4±0.02	109.9±4.8	3.3±0.03	2.3±0.03	1.7±0.1	24±0.1	39.2±0.8
6	5.7±0.03	19.2±0.01	19.2±5.5	41.6±0.2	131.5±0.6	2.0±0.03	2.6±0.03	1.0±0.3	25.7±0.3	39.8±0.8
12	4.7±0.03	19.8±0.02	26.3±0.13	32.7±0.7	124.8±0.2	2.9±0.03	2.7±0.03	1.3±0.2	13.8±0.05	48.3±0.3
18	3.8±0.01	16.0±0.01	22.1±4.1	32.6±0.8	127.9±1.1	2.7±0.03	2.5±0.03	1.1±0.3	15.2±0.05	30.5±0.5
24	4.3±0.02	16.1±0.13	22.0±2.5	29.9±0.08	136.6±1.7	2.8±0.03	2.5±0.03	1.2±0.4	12.2±0.05	36.3±1.2
30	4.9±0.33	13.7±0.7	19.7±2.4	35.4±0.4	107.7±0.9	2.4±0.06	2.4±0.03	1.5±0.2	15.3±0.6	29.3±0.3
Days	**Mn (mg/kg dry matter)**					**Fe (g/kg dry matter)**				
0	273.6±0.9	81.5±0.7	111.8±0.2	124.7±1.7	399.6±0.4	0.13±0.0	0.05±0.0	5.06±0.1	9.2±0.08	2.3±0.0
6	199.1±1.9	232.8±4.6	146.8±7.6	162.3±3.1	217.8±0.3	0.1±0.0	0.08±0.0	4.12±0.2	9.6±0.03	7.7±0.1
12	143.7±0.4	246.6±0.6	267±23.3	264±7.5	441.7±1.7	0.06±0.0	0.06±0.0	6.6±0.09	14.6±0.01	5.1±0.02
18	131.6±0.3	217.2±6.6	288.8±34	263.1±0.3	173±17	0.1±0.0	0.11±0.0	9.9±0.06	14.9±0.03	5.2±0.01
24	120.3±0.7	229.8±1.2	339.8±47	308.3±1.1	246.7±3	0.11±0.0	0.05±0.0	8.5±0.08	15.1±0.04	4.1±0.05
30	145.7±24	226.4±1.1	278.2±16	281.6±1.1	182.0±1	0.08±0.0	0.05±0.0	4.3±0.0	12.5±0.2	2.4±0.01

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$)

Table 4.4b Speciation of heavy metals (Ni, Pb, Cd and Cr) in trial 1 during 30 days of composting period

	Speciation of heavy metals									
Days	**Cr (mg/kg dry matter)**					**Ni (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F 1**	**F 2**	**F 3**	**F4**	**F 5**
0	21.6±0.05	10.4±0.1	2.5±0.8	13.1±1	94.0±5.0	12.2±0.1	1.6±0.2	ND	ND	177±1.5
6	24±0.3	11.3±0.2	5.7±0.05	14±1.0	112.3±1.8	9.0±0.5	2.8±0.09	ND	ND	282±2.5
12	30.5±0.3	10.4±0.8	5.7±0.2	14.7±0.1	106.3±5.8	10.8±0.4	3.2±0.06	ND	ND	276±4.5
18	31.5±0.5	9.4±0.4	5.1±0.1	14.4±0.1	115.8±4.3	10.7±0.2	3.15±0.09	ND	ND	242±1.0
24	29.0±0.4	9.9±0.6	4.6±0.3	14.3±1.4	112.8±0.3	10.8±0.3	2.73±0.03	ND	ND	200±1.0
30	29.6±0.5	12.7±0.2	3.5±0.3	12.6±0.8	115.3±1.3	9.5±0.7	3.4±0.03	ND	ND	260±0.3
Days	**Cd (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
0	1.7±0.06	0.09±0.03	ND	ND	54.5±1.5	27.0±1.2	9.0±0.6	ND	ND	1092±33
6	1.4±0.03	1.05±0.03	ND	ND	63.0±1.0	32.7±0.3	6.9±1.5	ND	ND	1065±10
12	2.2±0.03	0.7±0.09	ND	ND	59.8±0.3	19.5±0.3	4.5±0.3	ND	ND	967±23
18	2.3±0.09	0.7±0.03	ND	ND	61.0±0.5	28.5±2.1	5.1±0.3	ND	ND	780±25
24	2.3±0.03	0.8±0.03	ND	ND	52.5±1.5	30.6±0.0	5.4±0.6	ND	ND	1065±30
30	2.3±0.12	0.8±0.03	ND	ND	59.0±3.0	29.7±0.3	6.9±0.9	ND	ND	1077±13

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$); ND- Not detected

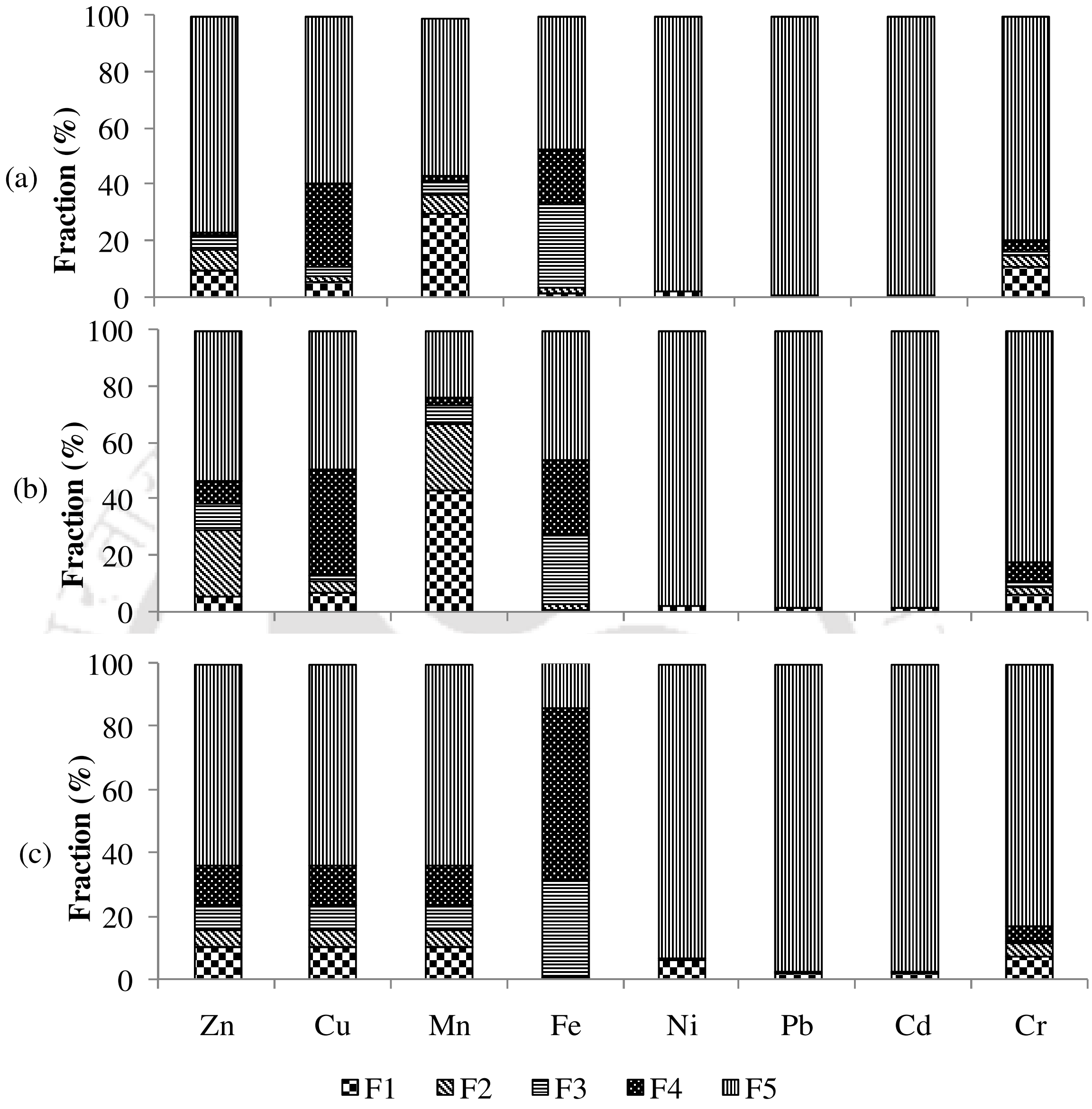


Fig. 4.5 Distribution of metal species in (a) sawdust (b) cattle manure (c) water hyacinth

In water hyacinth, sawdust and cattle manure, the order of different fractions of Mn is: F5 (40.3%) > F1 (27.6%) > F4 (12.6%) > F3 (11.3%) > F2 (8.2%), F5 (56.5%) > F1 (30.3%) > F2 (6.4%) > F3 (5.1%) > F4 (1.7%) and F1 (43.5%) > F5 (23.5%) > F2 (23.2%) > F3 (7%) > F4 (2.8%) respectively (Fig. 4.5). In water hyacinth and sawdust F1 and F5 fractions were predominant but in cattle manure F1 fraction of Mn was predominant. Speciation of Mn in trial 1, 2 and 4 is given in Table 4.4a, 4.5a and 4.6a respectively, and speciation in trial 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. In trial 1, F1 and F5 fractions of Mn was decreased; however the F2, F3 and F4 fractions were increased of the total fraction of Mn during the composting process. The F1, F3 and F4 fractions of Mn were decreased however the F2 and F5 fraction were increased of the total fraction of Mn during the composting process. In trial 3; F1, F2 and F4 fractions of Mn was decreased however the F3 and F5 fractions were increased of the total fraction of Mn during the composting process. In trial 4; F1 and F3 fractions of Mn was decreased however the F2, F4 and F5 fractions were increased of the total fraction of Mn during the composting process. In trial 5, F1 and F5 fractions of Mn was decreased however the F2, F3 and F4 fractions were increased of the total fraction of Mn during the composting process. The reduction in both mobile fractions (F1 and F2) of Mn was observed in trial 3 which is due to the increase in pH from 6.4 to 7.4 during the composting process. These fractions are easily available for plants. BF of Mn in trial 1 and 5 were increased from 0.6 and 0.54 (initial) to 0.8 and 0.72 (final) respectively; however, in trial 2, 3 and 4 BF was decreased from 0.75, 0.73 and 0.65 (initial) to 0.5, 0.6 and 0.5 during the composting period. Significant differences in F1, F2, F3, F4 and F5 fractions of Mn were observed in all the trials ($p < 0.05$).

Fig. 4.5 explains the speciation of Fe in raw materials and the order of different fractions of Fe in water hyacinth, sawdust and cattle manure: F4 (54.8%) > F3 (30.2%) > F5 (13.9%) > F1 (0.8%) > F2 (0.3%), F5 (47.3%) > F3 (29.5%) > F4(19.3%) > F2 (2.4%) > F1 (1.6%) and F5 (45.8%) > F4 (26%) > F3 (25.2%) > F2 (1.9%) > F1 (1.1%) respectively. In the water hyacinth, F3 and F4 fractions of Fe were predominant. Speciation of Fe in trial 1, 2 and 4 is given in Table 4.4a, 4.5a and 4.6a respectively, and speciation in trial 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. In trial 1; F1, F2, F3 and F5 fractions of Fe was decreased, but F4 fraction was increased. In trial 2; F1, F2, F4 and F5 fractions of Fe was decreased; though the F3 fraction was increased. In trial 3; F1, F4 and F5 fractions of Fe was decreased; however the F2 and F3 fractions were increased of the total fraction of Fe during the composting process. In trial 4; F1, F2, F3 and F4 fractions of Fe was decreased from 0.37, 0.9, 21.6 and 59.3% to 0.36, 0.2, 16 and 50% respectively; however the F5 fraction was increased from 18 to 33.4% of the total fraction of Fe during the composting process.

The concentration of Fe was mainly present in F4 fraction during the composting process. In trial 5, F1 fraction of Fe was decreased, however F2, F3, F4 and F5 fractions were increased of the total fraction of Fe during the composting process.

Table 4.5a Speciation of heavy metals (Zn, Cu, Mn and Fe) in trial 2 during 30 days of composting period

Days	Speciation of heavy metals									
	Zn (mg/kg dry matter)					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F 1**	**F 2**	**F 3**	**F4**	**F 5**
0	9.5±0.8	11.5±0.5	15.3±0.5	21.6±0.0	50.8±0.7	2.4±0.1	1.9±0.08	1.05±0.3	23.4±0.6	12.9±0.4
6	8.2±0.6	9.7±0.5	14.1±0.6	26.8±0.7	58.7±0.9	1.8±0.03	1.6±0.2	1.2±0.05	19.2±0.8	12.2±0.1
12	7.2±0.8	11.7±0.4	16.2±0.2	31.7±1.0	29.4±0.4	2.1±0.02	2.0±0.3	1.2±0.1	19.5±0.2	5.5±0.4
18	6.3±0.02	21.5±3.3	25.6±0.6	25.7±0.1	103.4±2.7	2.9±0.13	1.4±0.05	1.6±0.05	15.2±0.05	21.8±1.6
24	5.7±0.3	16.6±0.6	25.4±0.7	23.2±0.6	103.4±2.6	3.0±0.2	2.4±0.3	2.9±0.05	36.5±0.04	14.5±0.5
30	4.7±0.1	20.8±0.9	28.2±2.7	24.8±0.08	120.4±0.8	2.5±0.05	1.6±0.08	1.2±0.05	14.5±0.4	36.1±1.9
Days	**Mn (mg/kg dry matter)**					**Fe (g/kg dry matter)**				
0	97.2±2.6	36.3±2.4	63.8±2.6	63.3±2.8	83.5±5	0.034±0.0	0.026±0.0	1.4±0.02	4.8±0.08	4.4±0.0
6	69.8±0.5	40.1±1.6	68.8±6.7	99.8±0.2	88.6±0.9	0.03±0.0	0.023±0.0	1.8±0.03	7.4±0.01	2.4±0.01
12	81.3±0.3	72.5±2.2	65.5±2.8	87.8±2.3	82.3±3.4	0.055±0.0	0.03±0.0	2.9±0.04	6.4±0.04	2.4±0.01
18	116.9±9.4	146.8±20	146.2±6	61.7±0.7	189.3±1.8	0.023±0.0	0.023±0.0	2.8±0.07	7.8±0.06	1.0±0.01
24	126.1±7.5	160.9±9.9	174.6±20	43.2±5.9	243±9.5	0.023±0.0	0.03±0.0	2.2±0.03	8±0.02	3.0±0.0
30	119.7±1.3	169.5±10	159.9±9.6	52±2.1	505.8±45	0.021±0.0	0.03±0.0	2.7±0.0	6±0.03	5.1±0.0

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$)

Table 4.5 b Speciation of heavy metals (Cr, Ni Cd and Pb) in trial 2 during 30 days of composting period

	Speciation of heavy metals									
Days	**Cr (mg/kg dry matter)**					**Ni (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F 1**	**F 2**	**F 3**	**F4**	**F 5**
0	30.1±0.5	8.3±0.4	3.4±0.3	12.2±0.3	225.3±2.8	13.4±0.1	7.5±0.2	ND	ND	111.5±0.5
6	29.9±0.5	7.7±0.2	3.3±0.4	14.9±1.8	196.3±8.3	11.8±0.2	5.6±0.2	ND	ND	136.3±1.6
12	30.0±1.4	8.1±0.1	4.3±0.3	12.5±0.05	189±4.0	13.2±0.02	3.4±0.1	ND	ND	124.5±1.1
18	32.0±1.3	8.6±0.4	4.6±0.3	13.7±0.9	232±4.0	21.9±0.7	8.5±0.3	ND	ND	202.0±1.0
24	29.6±1.1	6.2±0.2	5.0±0.5	13.6±0.9	212.5±1.5	23.3±2.0	9.7±0.3	ND	ND	206.8±0.8
30	31.0±0.4	6.9±0.3	5.6±0.5	13.5±0.6	233.8±11	12.0±1.0	9.0±0.4	ND	ND	284.8±0.8
Days	**Cd (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
0	ND	0.5±0.0	ND	ND	38.5±1.0	28.3±0.8	ND	ND	ND	1030±25
6	ND	0.33±02	ND	ND	43.0±0.5	19.0±2.0	4.3±2.3	ND	ND	1090±40
12	1.0±0.3	0.13±0.1	ND	ND	41.8±0.3	19.8±2.3	10.3±1.3	ND	ND	1004±3.9
18	2.9±0.1	1.2±0.03	ND	ND	58.3±2.3	14.8±4.3	26.8±3.3	ND	ND	1055±5.0
24	3±0.2	1.8±0.1	ND	ND	59.8±2.8	13.5±1.0	27.5±1.5	ND	ND	1082±7.5
30	2.5±0.1	1.05±0.1	ND	ND	66.5±0.5	19.0±2.0	25.8±1.8	ND	ND	1340±5.0

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$); ND - Not detected

BF of Fe in trial 1, 2, 3 and 5 were increased from 0.86, 0.58, 0.76 and 0.71(initial) to 0.87, 0.63, 0.82 and 0.73 (final) respectively; however in trial 4, BF was decreased from 0.82 (initial) to 0.67 during the composting period. It has been observed that in trial 4 all mobile fractions (F1, F2, F3 and F4) and BF were decreased, it could be explained as Fe formed a complex compound with humic substances. Fe was mainly present in F4 fraction in all trials. Significant differences in F1, F2, F3, F4 and F5 fractions of Fe were observed in all the trials ($P < 0.05$).

Fig. 4.5 reveals the speciation of Ni in raw materials and the order of different fractions of Ni in water hyacinth, sawdust and cattle manure: F5 (92.8%) > F1 (6.4%) > F2 (0.8%), F5 (98%) > F1 (2.0%) and F5 (97.6%) > F1 (2.4%) respectively. Ni occurred predominantly in the F5 fraction, which agreed with the report of Wang et al. (2008). After composting, the residual fraction was still the dominant fraction. In water hyacinth composting F3 and F4 fraction were not detectable; in sawdust and cattle manure F2, F3 and F4 fractions were not detectable.

Speciation of Ni in trials 1, 2 and 4 is given in Table 4.4b, 4.5b and 4.6b respectively, and speciation in trial 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. In trial 1, F1 fraction of Ni was decreased; however the F2 and F5 fractions were increased of the total fraction of Ni during the composting process. However in trial 2, 3, 4, and 5; F1 and F2 fractions of Ni were decreased, but the F5 fraction was increased of the total fraction of Ni during the composting process. The F3 and F4 fractions of Ni were not detected in all trials during the composting period. The maximum reduction of F1 and F2 fractions of Ni were observed in trial 4. During composting process, the proportion of F1 and F2 fractions of Ni were decreased while the proportion of F5 fraction of Ni was increased; it was due to F1 and F2 fractions of Ni were transformed to the F5 fraction during the composting process (Zheng et al., 2007; Wang et al., 2008). Su and Wong (2003) reported that the F5 fraction contributes 52% of the total Ni content; followed by the reducible form in sewage sludge; whereas, in present study F5 fraction contributed about 95%. Fuentes et al., (2004) also reported that maximum amount of Ni was bound with F5 fraction. A significant increase in the level of Ni with F5 fraction may be due to alkaline stabilization process.

The Ni cation has less stability to form complex with organic ligands than Cu in the transition metal cations (Qiao and Ho, 1997). Both F1 and F2 fractions contributed < 10% of the total fraction of Ni in all trials. About 15-30% in F1 and F2 fractions can cause environmental toxicity during mobility (Venkateswaran et al., 2007).

BF of Ni was decreased from 0.07, 0.15, 0.1, 0.12 and 0.08 (initial) to 0.05, 0.09, 0.08, 0.06 and 0.04 (final) in all trials 1, 2, 3, 4 and 5 respectively during the composting process. The BF of Ni was decreased in all trials, but maximum reduction of BF was observed in trial 4. A significant decrease of BF in trial 4 confirmed that the addition of cattle manure in an appropriate proportion could effectively prevent the availability of Ni for plant uptake. Significant differences in F1, F2 and F5 fractions of Ni were observed in all the trials ($p < 0.05$).

- **Speciation of Pb, Cd and Cr**

Pb, Cd and Cr are not essential for plants growth, since they do not perform any known physiological function of the plants. Even very small quantity of these heavy metals may be harmful to plants and soil microbes (Garrido et al., 2002), but toxicity of these metals depends on its different forms. In water hyacinth, sawdust and cattle manure, the order of different fractions of Pb are: F5 (96.8%) > F1 (2.4%) > F2 (0.8%), F5 (98.7%) > F1 (1.3%) > F2 (0.06%) and F5 (98.4%) > F1 (1.6%) respectively (Fig. 4.5). Pb occurred predominantly in the F5 fraction in all the raw materials. In water hyacinth and sawdust, F3 and F4 fractions were not detected and in cattle manure F2, F3 and F4 fractions were not detected. Similar as Ni, F3 and F4 fractions of Pb were almost absent in all trials till at the end of composting. Speciation of Pb in trial 1, 2 and 4 is given in Table 4.4b, 4.5b and 4.6b respectively, and speciation in trial 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. There was very little change in speciation of Pb observed in trial 1 due to poor degradation. The proportion of cattle manure and sawdust was nil in trial 1. The F1 and F5 fractions of Pb were decreased in trials 2 and 3. Initially F2 fraction of Pb was not detected but after composting the percentage was increased from 0 to 1.9%, it might be due to F5 fraction was converted into F2 fraction during the course of composting. In trial 3, F1 and F5 fractions of Pb was increased whereas F2 fraction was decreased of the total fraction of Pb during the composting process. In trials 4 and 5, F1 and F2 fractions of Pb were reduced whereas F5 fraction was increased of the total fraction of Pb during the composting process. The maximum reduction of F1 and F2 fractions of Pb were observed in trial 4.

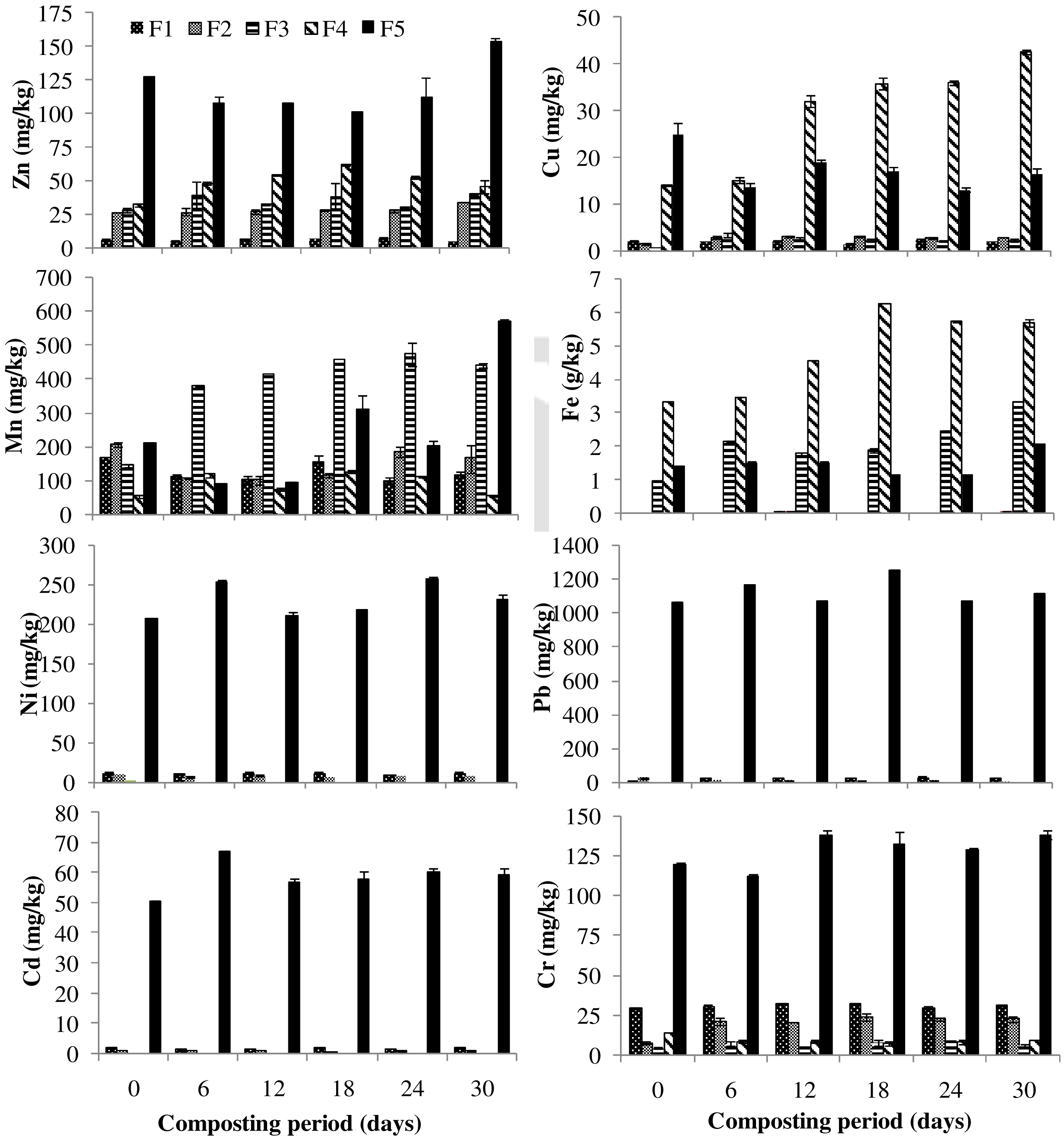


Fig. 4.6 Changes in speciation of heavy metals during pile composting in trial 3

During the course of composting, the thermophilic phase showed intense microbial decomposition of organic matter with release of Pb from organic site. A similar observation was also reported during the composting of sewage sludge by Amir et al. (2005). Both F1 and

F2 fractions contributed < 5% of the total fraction of Pb in all the trials. After composting, the residual fraction of Pb was still the dominating fraction in all trials.

In this study, the mobility of Pb was decreased during the composting process, a similar result was also observed by Wong and Selvam (2006). Slightly alkaline medium can reduce mobility of Pb by forming Pb-organic matter complex, but higher alkaline conditions can have a reverse effect on Pb stability due to the amphoteric nature of Pb. However at pH > 11-12, it might form soluble hydroxide complexes that can increase Pb mobility (Kumpiene et al., 2008). Pb occurs mainly in the moderately mobile form of carbonates and less mobile, but for the plants potentially available, organically bound form (Ciba et al., 1999). The BF of Pb was increased from 0.027 (initial) to 0.032 (final) in trial 2 respectively, however it was reduced from 0.033, 0.06 and 0.061 (initial) to 0.027, 0.03 and 0.04 (final) in trial 3, 4 and 5respectively, during the composting process. The BF of Pb was slightly increased in trial 1, which was due to poor formation of humic substances. Significant differences in F1, F2 and F5 fractions of Pb were observed in all the trials ($p < 0.05$).

In general, Cd is considered as a hard, high melting point and un-reactive substance that exhibits a wide range of oxidation states and tends to form strong covalent bonds (Haroun et al., 2007). Fig. 4.5 illustrates the speciation of Cd in water hyacinth, sawdust and cattle manure. The order of different fractions of Cd in the water hyacinth, sawdust and cattle manure: F5 (96.9%) > F1 (3.0%) > F2 (0.2%), F5 (98.7%) > F1 (1.3%) and F5 (92.1%) > F1 (7.9%) respectively. The F5 fraction of Cd was dominant in all raw materials. The F3 and F4 fractions were absent in raw materials from initial to final compost. The concentration of Cd was very less in cattle manure but the percentage of F1 fraction was higher than other two materials. Speciation of Cd in trials 1, 2 and 4 is given in Table 4.4b, 4.5b and 4.6b respectively, and speciation in trial 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. The F1 and F2 fractions of Cd were increased but F5 fraction was reduced in trials 1 and 2. In trial 2, F1 fraction of Cd was not detected initially, but after composting the percentage of total fraction was increased from 0 to 3.6%, it might be due to decomposition of organic matter during the course of composting. In trials 3, 4 and 5; F5 fraction of Cd was increased but F1 and F2 fractions were declined during the composting process. One possible explanation for reduction of F1 and F2 fractions and raise in F5 fraction may be due to the ability of Cd to chemically bond strongly with organic materials (Haroun et al., 2007). The decrease in F1 and F2 fractions of Cd could be due to increase of pH during the composting process (from 6.0 to 7.4). A similar observation was also reported by Hanc et al. (2009) during composting of sewage sludge. Furthermore Cd ion is directly bound to two or more organic functional groups mainly carboxylic, carbonyl and phenolic, so that the ion was immobilized in a rigid inner-sphere complex. The F1 fraction indicates that Cd can be available for plant uptake. The BF of Cd in trial 1 and 2 was increased from 0.031 and 0.012 (initial) to 0.052 and 0.05 (final); however, in trial 3, 4 and 5 BF was declined from 0.063, 0.063 and 0.07 (initial) to 0.048, 0.053 and 0.046 (final) during the composting process. Significant differences in F1, F2 and F5 fractions of Cd were observed in all the trials ($p < 0.05$).

Fig. 4.5 demonstrates the speciation of Cr in water hyacinth, sawdust and cattle manure. The order of different fractions of Cr in the water hyacinth, sawdust and cattle manure: F5 (82.7%) > F1 (7.9%) > F4 (4.8%) > F2 (3.8%) > F3 (0.9%), F5 (59.2%) > F1 (31.6%) > F4 (4.5%) > F2 (3.4%) > F3 (1.3%) and F1 (34%) > F5 (31.4%) > F4 (28.3%) > F2 (4.2%) > F3 (2.1%) respectively. The F5 fraction of Cr was predominant in water hyacinth and sawdust; however in cattle manure F1 fraction was predominant. The concentration of Cr was very low in cattle manure but the percentage of F1 fraction was higher than other two materials.

Speciation of Cr in trial 1, 2 and 4 is given in Table 4.4b, 4.5b and 4.6b respectively, and speciation in trial 3 and 5 is given in Fig. 4.6 and 4.7 respectively during the composting process. In trial 1, F1 and F3 fractions of Cr was increased but F4 fraction was reduced, a very little change in F2 and F5 fractions of Cr has been observed from initial to final compost. The F3 and F4 fractions of Cr were increased but F1, F2 and F5 fractions were reduced in trial 2 at the end of composting. The F2 and F3 fractions of Cr were increased whereas F1, F4 and F5 fractions were reduced in trial 3. In trial 4, F5 fraction of Cr was increased whereas F1, F2, F3 and F4 fractions were reduced from 11.5, 9.1, 3.6 and 4.6% to 9.1, 3.7, 2.4 and 2.8% of total fraction of Cr during the composting process. The significant reduction in fractions (F1- F4) was observed in trial 4 in comparison to other trials. It could be explained that, the F1 and F2 forms may bound with various organic functional groups present in humic substances, while the F3 and F4 fractions might be converted into F5 fraction during the stabilization of compost. The F1 and F4 fractions of Cr were reduced but F5 fraction was raised of the total fraction in trial 5 (Table 4.8). Cr was present predominantly in residual stable form in all trials from initial to final compost and consequently had extremely low bioavailability, which agrees with the report made by Smith (2009).

Table 4.6a Speciation of heavy metals (Zn, Cu, Mn and Fe) in trial 4 during 30 days of composting period

	Speciation of heavy metals									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F 1**	**F 2**	**F 3**	**F4**	**F 5**
0	11.0±0.2	25.4±0.2	41.7±0.7	41.0±0.01	39±0.8	2.0±0.03	2.8±0.2	1.4±0.1	11.7±0.2	10.5±0.5
6	5.9±0.02	21.8±0.9	25.2±1.5	46.4±4.5	97±18	2.4±0.3	2.9±0.05	1.7±0.2	17.7±0.4	19.0±0.5
12	5.4±0.4	19.8±0.3	33.8±0.8	85.7±12	117±2.5	1.8±0.1	2.6±0.1	1.4±0.2	16.9±0.7	27.5±0.5
18	17.0±9.0	22.1±0.5	37.8±4.2	54.9±2.9	120±0.4	1.7±0.2	2.5±0.1	1.1±0.2	15.2±0.1	23.5±2.5
24	6.9±0.03	24.3±3.3	37.8±2.1	54.9±6.3	120±2.5	1.7±0.05	3.2±0.5	1.3±0.2	14.8±0.2	25.0±1.0
30	5.7±0.07	23.2±0.8	46.1±0.9	56.8±0.3	143±25	1.6±0.05	2.6±0.2	1.2±0.1	22.8±1.3	72.3±1.3
Days	**Mn (mg/kg dry matter)**					**Fe (g/kg dry matter)**				
0	95±5.4	37.8±0.5	176.0±2	27.1±4.8	179.5±4	0.03±0.0	0.07±0.0	1.6±0.02	4.5±0.01	1.4±0.03
6	84±7.6	96.2±5.1	83.7±4.7	53.2±1.7	276±6.5	0.043±0.0	0.027±0.0	1.5±0.02	4.3±0.04	1.2±0.08
12	91±16.8	104.5±2.3	176±7.9	92.9±5.1	368.8±8.3	0.05±0.0	0.028±0.0	1.9±0.04	6.8±0.07	2.2±0.01
18	90±0.1	113.7±7.2	186.1±16	93±14.7	273.5±19.5	0.044±0.0	0.03±0.0	2.0±0.03	6.1±0.08	3.2±0.01
24	71.3±0.8	145.6±31	167.9±28	87.8±0.3	361.8±1.3	0.045±0.0	0.029±0.0	2.03±0.04	6.3±0.2	5.2±0.13
30	69.5±1.4	120.7±10	220.5±6.3	84.2±0.8	548.8±56	0.045±0.0	0.028±0.0	2.0±0.0	6.2±0.05	4.2±0.36

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$)

Table 4.6b Speciation of heavy metals (Cr, Ni, Cd and Pb) in trial 4 during 30 days of composting period

	Speciation of heavy metals									
Days	**Cr (mg/kg dry matter)**					**Ni (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F 1**	**F 2**	**F 3**	**F4**	**F 5**
0	29.5±1.9	23.1±0.3	9.1±1.3	11.7±0.5	180±3.8	16.0±1.0	6.6±0.2	ND	ND	163.8±1.8
6	29.7±0.5	24.0±0.0	10.0±0.4	10.0±2.0	162±1.5	13.7±0.3	6.2±0.2	ND	ND	185.5±6.5
12	27.4±0.7	23.2±0.3	10.4±0.9	13.8±1.7	180±2.0	12.9±0.2	6.1±0.3	ND	ND	269.8±2.3
18	26.8±0.8	9.5±1.3	7.2±0.5	8.2±0.3	165±1.3	13.4±0.4	5.2±0.1	ND	ND	270.3±2.5
24	24.5±0.7	9.1±0.4	3.7±0.1	10.8±0.7	199±5.8	13.8±0.4	6.5±0.8	ND	ND	240.3±16
30	22.9±0.6	9.8±0.7	6.4±0.2	7.4±0.9	205±5.3	11.6±1.2	3.4±0.3	ND	ND	227.0±4.0
Days	**Cd (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
0	3.6±0.0	2.6±0.1	ND	ND	38.3±0.8	33.7±1.4	13.7±0.9	ND	ND	800±20
6	3.0±0.0	1.5±0.2	ND	ND	66.0±1.0	41.3±0.3	11.8±0.3	ND	ND	1060±115
12	2.8±0.02	1.7±0.0	ND	ND	59.8±1.8	41.5±0.0	13.3±1.3	ND	ND	1207±17
18	2.7±0.1	1.6±0.03	ND	ND	60.8±8.3	36.2±0.8	12.5±0.5	ND	ND	1240±25
24	2.9±0.03	1.4±0.03	ND	ND	56.0±12	29.9±0.4	13.3±0.8	ND	ND	1172±2.5
30	2.6±0.02	1.5±0.2	ND	ND	72.3±0.8	25.1±0.9	10.0±0.4	ND	ND	1375±15

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$); ND - Not detected

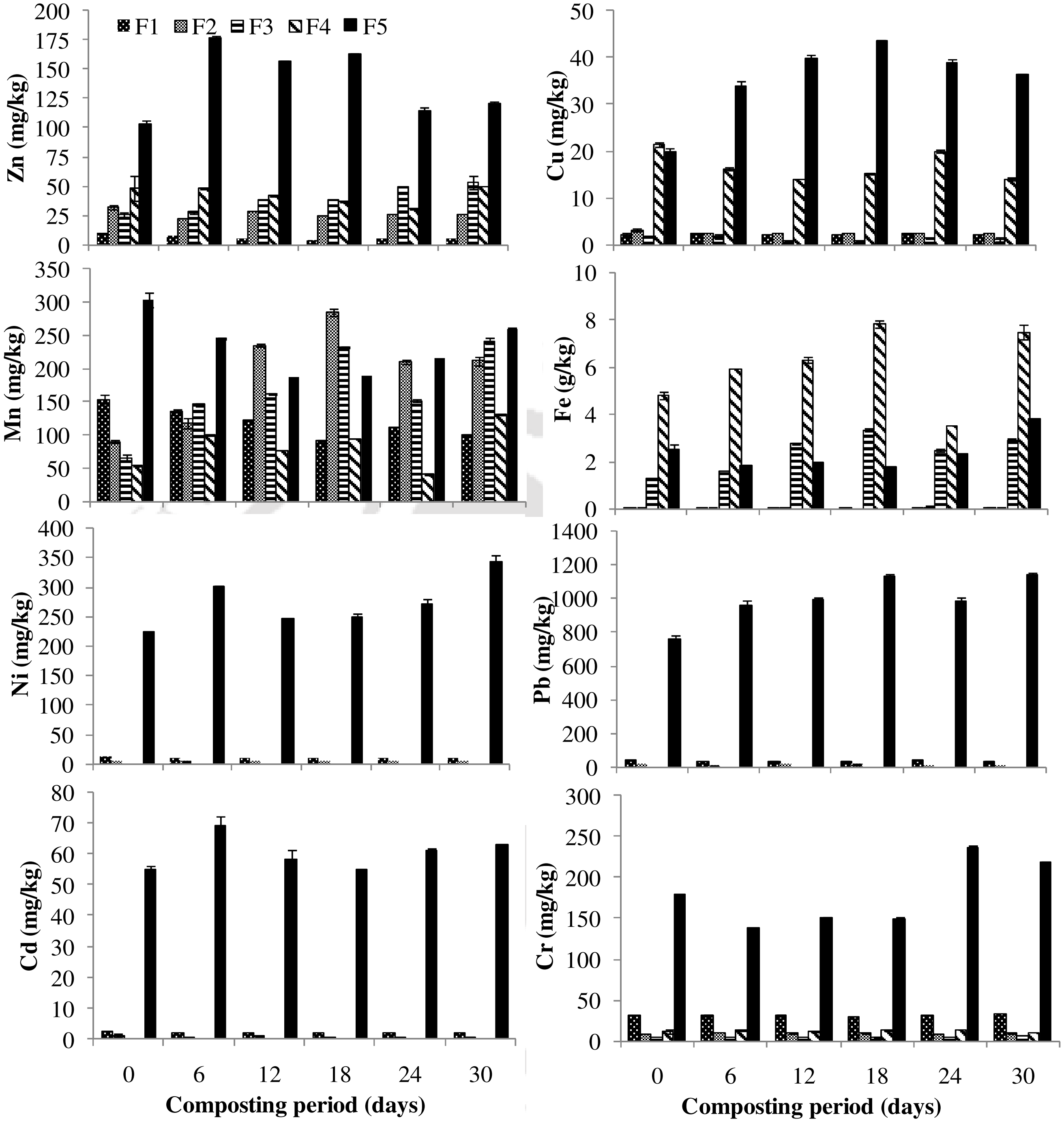


Fig. 4.7 Changes in speciation of heavy metals during pile composting in trial 5

BF of Cr in trial 4 and 5 was reduced from 0.29 and 0.24 (initial) to 0.22 and 0.21(final) respectively; however in trial 3, BF was increased from 0.32 (initial) to 0.33 (final) during the composting process. A very little change in BF of trial 1 and 2 has been observed from initial to final compost. The reduction of BF in trial 4 and 5 could be explained as addition of cattle manure can reduce the Cr availability by increasing humic substances. Significant differences in F1, F2, F3, F4 and F5 fractions of Cr were observed in all the trials ($p < 0.05$).

4.1.5 CONCLUSION OF PHASE 1

Assessment of heavy metals during composting process shows that bioavailability, leachability and speciation of heavy metals were depends on other physicochemical properties of the medium besides total metal contents such as decomposition of organic matter and pH. Water soluble and leachable Ni, Pb and Cd contents and DTPA extractable Pb and Cd were not detected in throughout composting period. The total concentration of Cu and Zn were low but their percentage of water soluble, plant available (DTPA extractable) and leachable fractions were similar as Fe, Cr and Mn, which was observed in high concentration. Total Cu content was less than total Cr content but water solubility of Cu was higher than Cr, which indicates toxicity risk of Cu higher than Cr.

The concentrations of Cu and Cd were very low as compared to the other metals, but the percentage of F1 and F2 fractions were similar as other metals. Ni, Pb and Cd were not bound with F3 and F4 fractions during the water hyacinth composting. The maximum reduction in F1 and F2 fractions of all metals except Mn and Cd were found in trial 4 in comparison to other trials, which are more toxic and easily bioavailable fractions. The optimum proportion of cattle manure addition (trial 4) can enhance organic matter degradation and humification process; consequently it reduced the bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate) of heavy metals during the composting process.

4.3 PHASE 2: ROTARY DRUM COMPOSTING

In this phase study was carried out on bioavailability, leachability and speciation of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during rotary drum composting of water hyacinth for 20 days. Similar as agitated pile, five different proportions of water hyacinth, cattle manure and sawdust were prepared for composting process. The bioavailability of heavy metals was investigated in the form of water soluble and diethylene triamine penta-acetic acid (DTPA) extractable. Leachable fractions of heavy metals were extracted by TCLP test. The Tessier sequential extraction method was employed to investigate the changes in heavy metals speciation during the composting process. The influence of parameters such as pH, temperature and organic matter content on bioavailability, leachability and speciation of heavy metals were studied during rotary drum composting of water hyacinth.

4.2.4 PHYSICO-CHEMICAL ANALYSIS

The evolution of temperature during high rate composting in the rotary drum composter is presented in Fig. 4.8. Microbial activity started during initial hours of operation caused an increase in temperature. Temperature from 52 to 60^{o}C is considered to maintain the greatest thermophilic activity in composting systems (Kalamdhad et al., 2009). As shown in Fig. 4.8, the composting trials temperature went through three typical phases (mesophilic, thermophilic and cooling phase) and ranged from 22^{o}C to 60^{o}C during the entire period of composting. The similar trend of temperature was also observed by Singh et al. (2012) during rotary drum composting of water hyacinth. Nomeda et al. (2008) reported temperature in the range of 30-74^{o}C during pile composting of sewage sludge. The cattle manure affected the temperature during the composting process in five different trials. Out of all five trials, trial 4 reached the highest temperature due to higher addition of cattle manure indicating quick establishment of microbial activity during the composting process (Chen et al., 2010b). These results indicate that cattle manure enhanced the composting process by providing easily available carbon for microorganisms, but in trial 5 which contained highest amount of cattle manure shows less temperature when compared to trial 4. Cattle manure in excess may increase the available carbon but inadequate nitrogen in trial 5 thereby reducing the temperature; furthermore the compost microorganisms require optimum carbon and nitrogen for their growth.

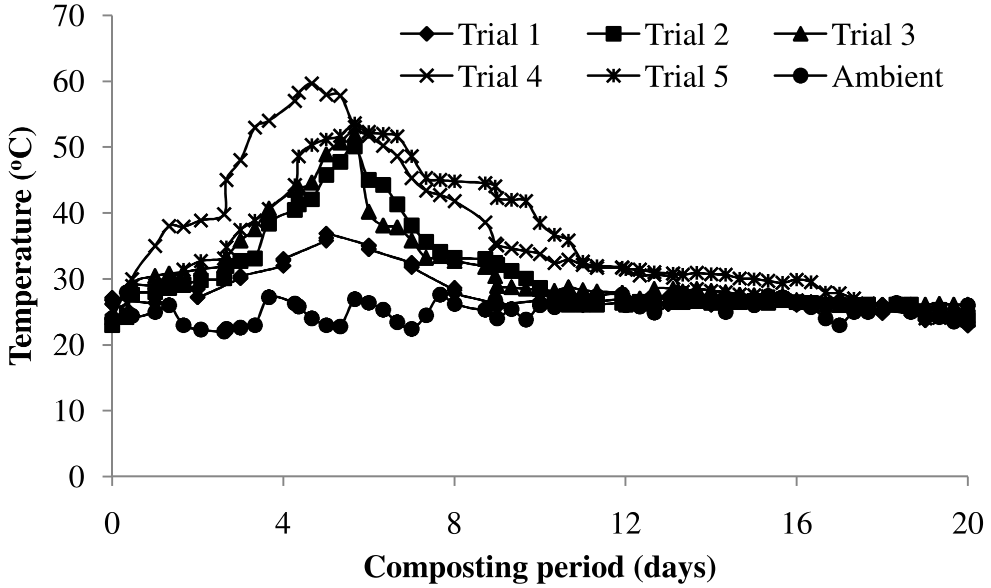


Fig. 4.8 Variation of temperature during drum composting process

Initial moisture content was 88.8, 86.9, 81.9, 89.9 and 79.5% which reduced to 86.2, 80.7, 76.2, 65.3 and 78.7% during the composting process in trials 1, 2, 3, 4 and 5 respectively (Fig. 4.9). Highest moisture loss occurred in trial 4 (27.4%) and lowest moisture loss was observed in trial 1 (8.9%), due to high heat generation in trial 4 and low heat generation in trial 1. On analyzing the results by ANOVA, the decrease in moisture content varied significantly between the days ($p < 0.05$). Leachate formation was not observed during the composting period.

The pH is a parameter which greatly affects the composting process. Fig. 4.9 shows the variation in pH in five different trials during the composting process. The pH values were increased in all trials and the maximum pH 7.4 was observed in trial 4 at the end of composting process. Significant difference in pH was observed in all the trials ($p < 0.05$). Electrical conductivity (EC) of all trials were increased except trial 4, in which it decreased from 3.4 to 2.1 dS/m during the composting process (Fig. 4.9). The increases in EC might be due to the net loss of weight and release of soluble salts through decomposition activity in the composting process. The volatilization of ammonia and the precipitation of mineral salts could be the possible reason for the decrease in EC at the later phase of composting (Fang and Wong, 1999). On analyzing the results by ANOVA, EC varied significantly between all the trials ($p < 0.05$). The similar trend of pH and EC was also reported by Singh et al. (2012).

Fig. 4.9 shows the decreasing trend of organic matter degradation during 20 days of composting process in five different trials. The maximum organic matter loss was observed in trial 4 (32.6%) followed by trial 3 (28%), trial 5 (24.5%), trial 2 (10.6%) and trial 1 (8.0%). Singh et al. (2012) observed maximum reduction of organic matter about 25.7% in rotary drum composting of water hyacinth but in present study about 33% organic matter loss was observed. Higher organic matter loss occurred during composting process as a result of higher temperature during themophilic phase of trial 4. A significant variation in organic matter loss was observed in all the trials ($p < 0.05$). Fig. 4.10 illustrates the concentration of the nutrients such as Na, K, Ca and Mg in all five trials throughout the composting process. These nutrients are required for plant growth.

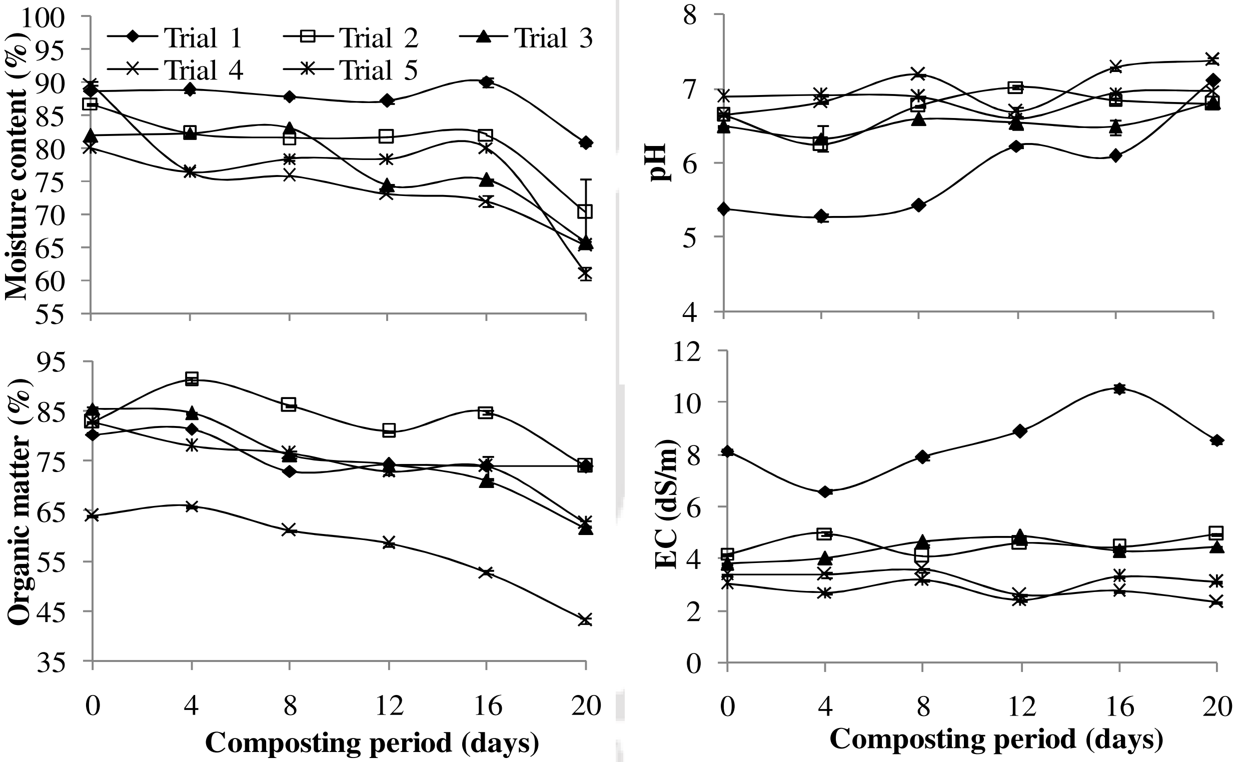


Fig. 4.9 Variation of moisture content, pH, electrical conductivity (EC) and organic matter during drum composting process

The concentration of Na, K, Ca and Mg was gradually increased till the end of the composting due to the net loss of dry mass. The increases in these nutrients were also observed by Amir et al. (2005). On analyzing the results by ANOVA, significant differences in nutrients were observed between the all trials ($p < 0.05$). Increase of metal concentrations in soil amended with compost in the long-term are generally reported to increase the concentrations of heavy metals in the tissues of plants growing in the soil (Li et al., 2010). Table 4.7a and b illustrates the total concentration of metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) in trials 1, 2, 3, 4 and 5 during 20 days of composting period. The order of total metal content in the composted water hyacinth was Fe > Mn > Pb > Ni > Zn > Cu > Cr > Cd. These heavy metals were concentrated during the composting process, due to weight loss in the course of composting following organic matter decomposition, release of CO_2 & water and mineralization process (Zorpas et al., 2000).

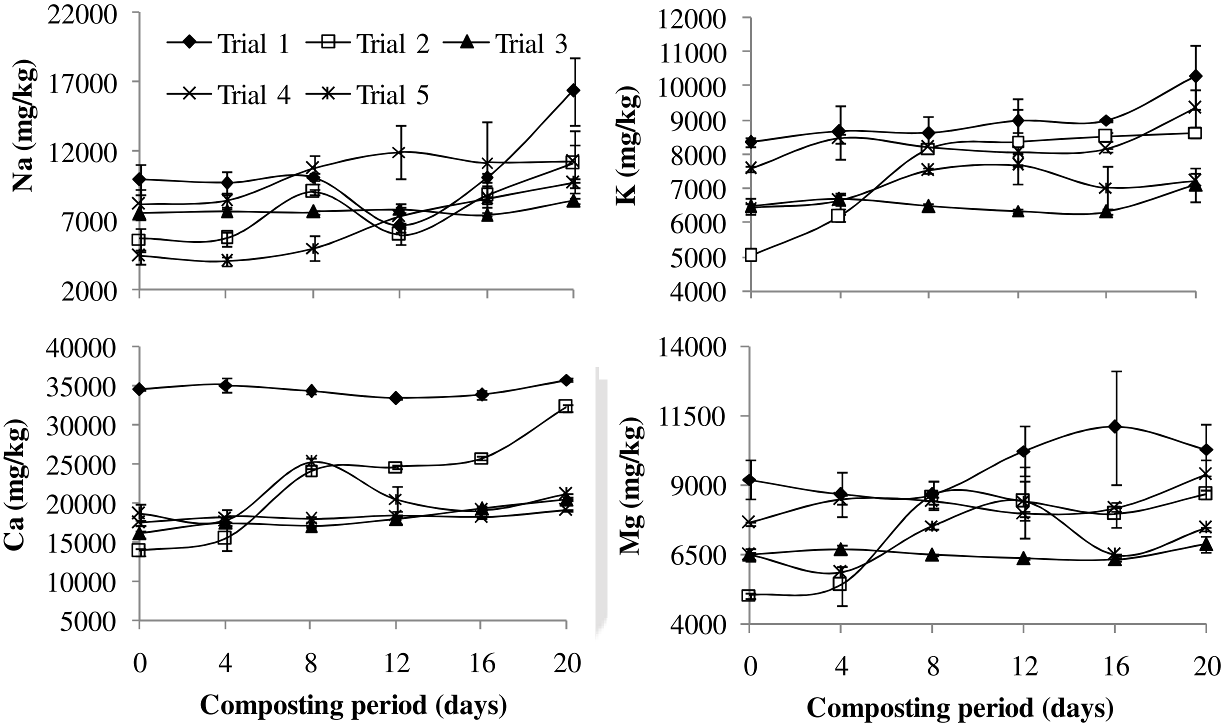


Fig. 4.10 Variation of nutrients (Na, K, Ca and Mg) during drum composting process

On analyzing the results by ANOVA, significant differences in total heavy metals (Zn, Cu, Mn, Fe, Pb, Cd, Ni and Cr) ($p < 0.05$) were observed between the trials. The total concentration of heavy metals indicates the extent of contamination, but they provide little information about the forms in which heavy metals are present, or about their potential for mobility and bioavailability in the environment (Cai et al., 2007). The total concentration of heavy metals is usually assessed by their potential toxicity for land application of water hyacinth compost.

Table 4.7a Total heavy metals concentration (Zn, Cu, Mn and Fe) during 20 days of composting period (mean ± SD, n=3)

	Total metals concentrations									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	137.7±10a	103.8±9.9a	149.6±5.1b	147.3±2.0b	137.0±1.0c	51.5±2.5a	70.4±5b	43.2±0.4a	70.5±1b	44±5.5a
4	135.5±6.7a	106.8±4.2a	154.2±3.4b	155.4±1.2b	153.0±18.5b	47.0±2.5a	63.5±1.5b	91.0±1.5c	71.7±1.2b	40.5±9a
8	156.0±4.5a	142.2±24a	150.1±0.8b	177.6±2.3c	147.7±7.1b	55.0±2.5a	87.5±11.5b	44.5±4.5a	71.3±0.5ab	47.0±7a
12	225.2±24a	140.3±2.0a	130.3±8.0b	153.9±3.1bc	140.9±8.5b	76.5±5.5a	76.1±2.1a	47.0±3b	73.0±0.4a	70.3±1.3a
16	186.9±7.7a	167.2±17.7a	146.4±2.4b	141.0±12.8bc	128.0±4.3c	56.7±1.4a	72.0±2.0b	33.8±5.8c	76.9±1.6b	38.8±5.3c
20	177.1±8.2a	149.1±6.5a	187.7±7.5b	155.0±3.0c	144.0±2.0c	54.1±1a	75.5±2.5b	48.0±4b	91.6±2b	50.3±1.8c
Days	**Mn (mg/kg dry matter)**					**Fe (g/kg dry matter)**				
0	1775±25a	632.5±67b	760±20b	538±18b	1063±13c	36.4±0.2a	13.6±0.1b	15.0±0.3c	11.0±0.3d	10.3±0.3e
4	2220±40a	555±19b	980±70c	700±95b	1008±13c	38.0±0.3a	12.1±1b	18.4±1.8c	13.4±0.8bc	10.6±4.3b
8	1908±17.5a	938±48b	855±90b	705±40b	1247±113c	46.7±2.0a	15.7±0.4b	13.7±1c	12.8±0.2bc	12.2±0.4b
12	1800±35.5a	855±70b	783±43b	550±85c	852.5±93b	39.0±1.0a	17.5±0.7b	14.3±2.7b	13.2±0.2cb	12.2±0.6cb
16	2045±25a	835±50b	961±15b	670±19bd	1205±30c	40.0±1.6a	17.3±1.8b	15.6±0.8b	13.3±0.4cb	12.9±0.6cb
20	1928±7.5a	1031±44b	774±10c	658±13d	1288±43e	43.0±0.8a	23.4±0.3b	15.9±0.3c	14.1±0.4d	15.9±0.4c

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$)

Table 4.7b Total heavy metals concentration (Cr, Ni, Cd and Pb) during 20 days of composting period (mean ± SD, n=3)

	Total metals concentration									
	Cr (mg/kg dry matter)					**Ni (mg/kg dry matter)**				
Days	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	52.3±2.3a	36±1b	40±4.5b	59±3.3a	39±1.2b	230±1.5a	144±6b	221±11a	240±11a	220±4.5a
4	46±1.5a	41±2.3a	48±3ad	66±2.3b	38±1ac	239±2.5a	178±26b	190±9a	233±3.3a	186±4.5b
8	53±1.5a	85±2b	47±3a	78±4.1b	40±0.4c	228±7a	220±10a	212±6ab	240±3.5ab	222±18a
12	51±5a	46±4a	42±2.9a	66±2.5b	48±1.5a	270±19a	200±1b	202±2.3b	224±9b	204±2.3b
16	46.8±2a	66±3b	45±4a	77±2.1c	47±4.3a	208±13ab	235±9a	218±6b	234±4.5b	216.5±4ab
20	54.5±1.5a	56±0.8a	50±1ac	76.3±2.4b	58±2.5a	246±3.5a	196±3b	225±2a	245±2.5a	237±13ac
Days	**Cd (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
0	54.3±3.3a	39±1.5b	45.1±0.7c	51.5±1a	52.7±0.4a	911±1a	660±35b	940±50ad	958±7.5ad	853±7.5ac
4	57.5±3.5a	47.8±2.3a	46.1±2.6ac	54.3±1.3a	52.4±1.4ab	908±73a	883±27.5a	945±33ac	930±35a	828±8.4ab
8	55±1.5a	57.3±3.3ab	56.3±2.8a	56.6±2.1a	63.2±0.8b	850±50a	918±57.5ab	1008±18b	950±20b	957±12b
12	61.8±5.8a	56.1±0.3a	54.7±0.9a	56.9±2.9a	64.9±1.4ab	988±2a	815±25b	973±14a	919.9±5c	866±6.0d
16	57.3±1.7a	53.6±0.9ab	57±2.5ab	57.3±3.8a	62.7±3.8abc	836±38a	885±50a	1003±18b	980±35b	878±23ab
20	57.5±3.5a	56.2±2.3a	58.3±1a	60.7±1.8abc	65.9±3.6b	1038±23a	843±22.5b	1055±65a	1111±53a	956±6.1ac

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$)

However, the present study shows that the level of heavy metal toxicity to the environment is not only related to its total concentration in water hyacinth compost, but also to its bioavailability and leachability.

4.2.5 BIOAVAILABILITY OF HEAVY METALS

- **Water solubility of heavy metals**

Table 4.8 shows the changes in water soluble Zn, Cu, Mn, Fe and Cr contents during 20 days composting period. Water soluble concentrations of Zn in trials 1, 2, 4 and 5 was reduced from 3.8, 2.9, 1.5 and 2.2% to 1.7, 1.0, 0.8 and 1.3% of total Zn respectively; however the concentration of Zn in trial 3 was increased slightly from 2.4 to 2.6% of total Zn, at the end of the composting period. The reduction in water solubility of Zn also reported by Castaldi et al. (2005) during composting of municipal solid waste.

The concentration of Cu in all trials (from 1 to 5) were reduced from 10.4, 7.4, 5.9, 2.8 and 3.2% to 4.9, 5.3, 5.4, 1.2 and 1.7% of total Cu respectively at the end of the composting period but Hsu and Lo (2001) reported water soluble Cu concentration was increased about 3% to 16% at day 18 and gradually decreased to 3% in the mature compost of swine manure. Guan et al., (2011) reported that addition of cattle manure reduced potential environmental risk due to binding of metal ions with hydroxide and carboxylic groups present in cattle manure, and redued solubility of metals. Furthermore, the decrease of water soluble fraction of Cu and Zn was in agreement with increase of humic substances in the water hyacinth compost (Cai et al., 2007). This is because water soluble humic substances always have high contents of carboxyl groups, which are ready to complex with Cu (Liu et al., 2008).

The concentration of Mn in all trials (from 1 to 5) was reduced but maximum reduction was observed in trial 4 (from 1.9 to 0.5% of total Mn) during the composting process. Hsu and Lo (2001) also reported that the water soluble Mn concentration was reduced from 1% to 0.5% of total Mn during swine manure composting. The concentration of Cr in trials 3, 4 and 5 were reduced, but the maximum reduction was observed in trial 4 (from 2.9 to 0.5% of total Cr); however the concentration of Cr in trials 1 and 2 was increased (percentage of total Cr) at the end of the composting period. Sims and Kline (1991) reported water extractable Cr about 1.4% of total Cr during commercial waste composting. Fang and Wong (1999) suggested that the solubility of metals reduced may be due to oxidation process and the formation of organo-metallic complexes taking place during composting process.

Table 4.8 Water soluble metals concentration during 20 days of composting period (mean ± SD, n=3)

Days	Water soluble metals concentration									
	Zn (mg/kg dry matter)					**Cu (mg/kg dry matter)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	5.3±0.1a	3.1±0.02b	3.6±0.0c	2.3±0.01d	3.0±0.03b	5.3±0.1a	5.2±0.4a	2.6±0.08b	2.3±0.2b	1.4±0.07c
4	3.9±0.0a	3.5±0.02b	2.8±0.04c	2.4±0.01d	2.5±0.02e	3.6±0.04a	4.3±0.4b	4.7±0.08c	1.7±0.03d	1.3±0.01e
8	3.6±0.1a	2.4±0.0b	4.5±0.01c	3.0±0.03d	3.1±0.04d	2.0±0.07a	6.4±0.1b	3.3±0.01c	1.7±0.01d	1.5±0.01e
12	3.0±0.1a	2.4±0.0b	4.5±0.15c	1.8±0.04d	7.0±0.15e	1.5±0.47a	4.3±0.01b	3.7±0.05b	1.3±0.01a	1.4±0.03a
16	4.7±0.1a	1.9±0.03b	3.7±0.06c	1.4±0.02b	1.7±0.46b	1.4±0.35a	3.4±0.1b	3.5±0.31b	1.1±0.04a	1.6±0.01a
20	3.0±0.0a	1.5±0.02b	5.0±0.01c	1.2±0.02d	1.9±0.02e	2.7±0.15a	4±0.1b	2.6±0.06a	1.1±0.01c	0.8±0.04d
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	57.8±1a	40.3±0.04b	59.7±0.3c	10±0.01d	32.4±0.6e	124.7±0.8a	39.3±0.6b	66.8±0.02c	19.3±0.3d	25.7±0.3e
4	56.0±2.8a	28.5±0.4b	39.3±.2c	11.6±0.2d	26.1±0.1b	126.8±0.8a	31.4±0.2b	63.8±0.03c	23.4±0.3d	21.3±0.2e
8	59.7±3.9a	22.6±0.2b	43.8±0.1c	6.9±0.1d	31.6±0.3e	65.7±12.6a	55.2±0.03b	61.6±0.6a	21.6±0.1b	33.7±1b
12	26.0±0.9a	20.7±0.1b	44.7±0.4c	5.4±0.03d	20.3±0.2b	106.3±6.9a	51.0±0.3b	70.6±1c	22.2±0.1d	11.3±0.03e
16	26.6±1.2a	15.4±0.1b	31.3±0.6c	4±0.04d	22.3±0.4e	116.8±3a	43.0±0.3b	51.3±0.2c	14.3±0.2d	22.6±0.4e
20	34.6±0.6a	15.1±0.1b	27.3±0.4c	3.4±0.04d	25.6±0.1e	111.2±8.9a	58.8±0.2b	57.9±1.5b	20.3±0.2c	30.2±0.2c
Days	**Cr (mg/kg dry matter)**					**Ni, Cd and Pb (mg/kg dry matter)**				
0	1.8±0.03a	0.3±0.2b	2±0.02ac	1.7±0.2a	2.0±0.1a	ND	ND	ND	ND	ND
4	1.5±0.01a	0.6±0.2b	1.3±0.1ac	1.6±0.1a	1.6±0.1a	ND	ND	ND	ND	ND
8	1.8±0.03a	0.9±0.1b	1.5±0.1c	1.4±0.2cd	1.6±0.04ac	ND	ND	ND	ND	ND
12	2.2±0.2a	0.7±0.1b	1.2±0.2c	1.3±0.1c	1.9±0.1a	ND	ND	ND	ND	ND
16	1.9±0.04a	1.0±0.2b	1.7±0.7c	0.6±0.03c	1.7±0.1a	ND	ND	ND	ND	ND
20	2.0±0.08a	1.2±0.01b	1.3±0.1b	0.4±0.1c	1.4±0.01b	ND	ND	ND	ND	ND

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$); ND- Not detected

The water soluble concentration of Fe was decreased in all trials 1, 2, 3, 4 and 5, but the maximum reduction was observed in trial 5 (from 0.3 to 0.2% of total Fe) during the composting process.

It has been observed that water solubility of Fe decreased in all five trials, which might be due to formation of insoluble organo-metallic complexes, during composting process, as a result reduced metal availability (Wong and Fang, 2000). In water, COOH groups of humic and fulvic acids dissociate to form COO^- and H^+, and the organic molecule then stretches out due to repulsion of the negative charges. When metal ions are then introduced into the system, humic and fulvic salts form and the negative charges become satisfied. The organic molecule then collapses on itself and may become insoluble (Talbot, 2006). Furthermore the reduction a fraction of the water-soluble metals might be due to transformation of water soluble metals into a more stable form during composting process (Castaldi et al., 2005). Water soluble concentrations of Ni, Pb and Cd contents in all trials were not detected in mature compost of water hyacinth. Castaldi et al. (2005) reported water-soluble Pb and Cd about 0.89 and 0.005 mg/kg (dry maater) respectively in the mature compost of municipal solid waste. Sims and Kline (1991) reported water extractable Ni about 7.2% of total Ni during sewage sludge composting.

The order of water soluble metal concentration in the composted water hyacinth was: Fe > Mn > Cu > Zn > Cr. On analyzing the results by ANOVA, significant differences in water soluble heavy metals (Zn, Cu, Fe, Cr and Mn) ($p < 0.05$) were observed in all the trials. Heavy metals distribution was affected by the release of heavy metals through the organic matter mineralization or metal solubilization by the decrease of pH during the composting process. Furthermore, reduction of metal solubility could be explained as metal biosorption by the microbial biomass or metal complexation with the newly formed humic substances formed in mature compost (Garcia et al., 1995; Castaldi et al., 2006; Cai et al., 2007).

- **DTPA extraxtable heavy metals**

DTPA solution is assumed to extract both carbonate-bound and organically bound metal fractions in calcareous soils (Walter et al., 2006). DTPA extractable metals (Zn, Cu, Mn, Fe, Ni and Cr) extraction efficiency during composting (Fig. 4.11). DTPA extractable Pb and Cd content in all trials were not detectable. The concentration of Zn in all trials (from 1 to 5) was increased (percentage of total Zn) during the composting process. The DTPA extractable Zn was increased in all trials, which might be due to dissolution of organic matter during the DTPA extraction procedure.

The concentration of Cu in trials 1, 4 and 5 was reduced but maximum reduction was observed in trial 4 (from 22.5 to 7.5% of total Cu), however in trials 2 and 3 it was increased during the composting process.

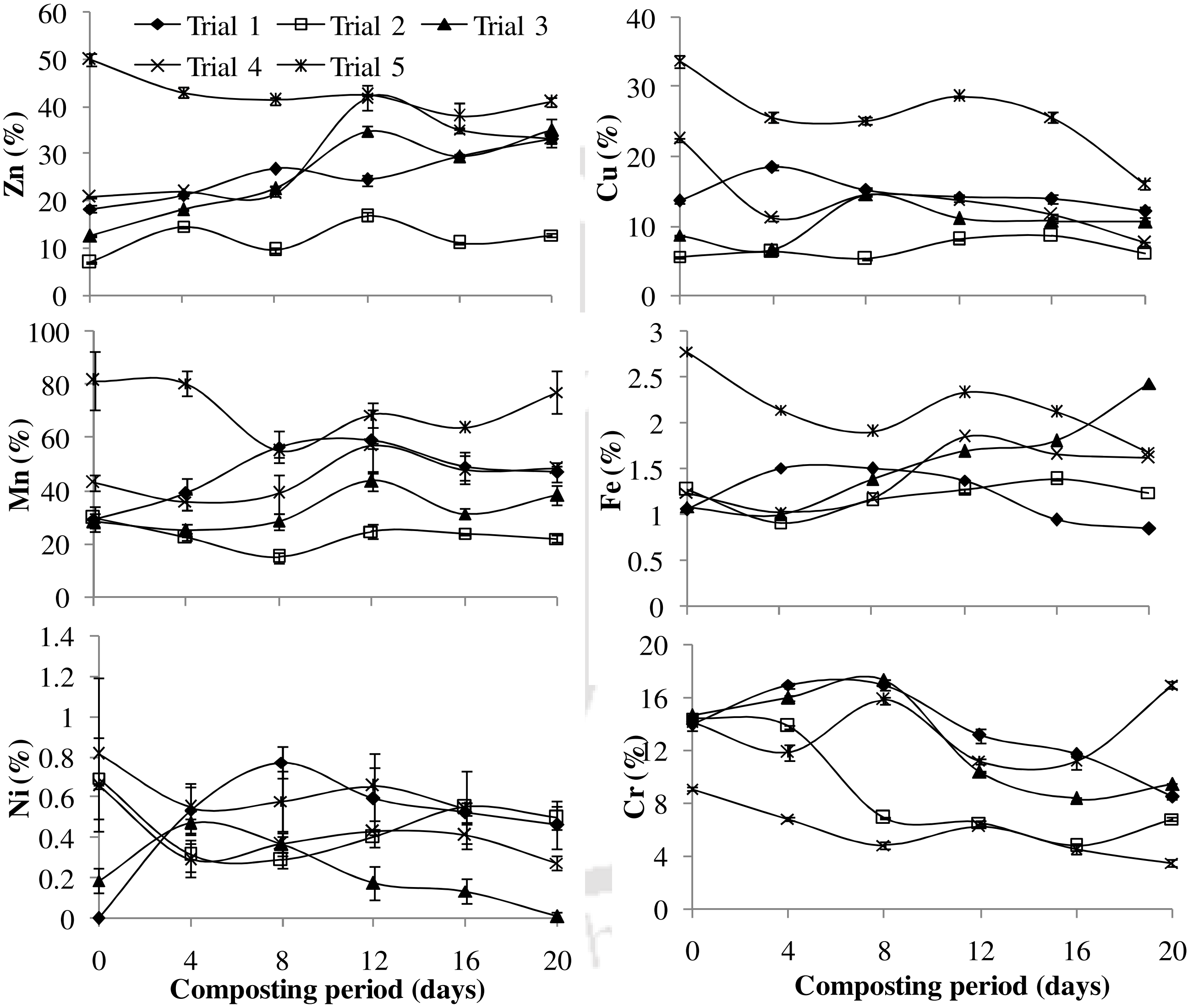


Fig. 4.11 DTPA extraction efficiency of Zn, Cu, Mn, Fe, Ni and Cr during drum composting process

Electrolyte composition is also significant for Cu uptake by humic substances with increases in ionic strength through promoting ionization of the functional groups. The complexation capacity of a humic acid is drastically reduce with increases in salinity (Talbot, 2006). The total Cu concentration of all the five trials was very low, but it was present extremely in plant available form. The maximum plant available Cu (percentage of total Cu) was observed in trial 1 due to poor formation of humic substances. The stability constant of metal-humic complexes depend on the nature of organics, metal, as well as other factors such as ion strength and pH in the environment (Liu et al., 2008). The concentration of Mn in trials 1, 3 and 4 was enhanced, however the concentration of Mn in trials 2 and 5 was decreased (percentage of total Mn) at the end of the composting period. In other finding DTPA extractable Mn was decreased from 22.6 to 9.8% of total Mn end of composting (Iwegbue et al., 2007). The concentration of Fe in trials 1, 2 and 5 was reduced; however the concentration of Fe in trials 3 and 4 was increased from 1.1 and 1.2% to 2.4 and 1.9% of total Fe respectively at the end of the composting period. In the present study DTPA extractable Fe was <5% of total Fe in all trials but Walter et al. (2006) reported about 3-9% of total Fe in sewage sludge compost. On analyzing the results by ANOVA, significant differences in DTPA extractable heavy metals (Zn, Cu, Mn, Fe, Ni and Cr) ($p < 0.05$) were observed between all the trials.

4.2.6 LEACHABILITY OF HEAVY METALS

Table 4.9a illustrates the changes in leachable Zn, Cu, Mn and Fe. Table 4.9b illustrates changes in Ni, Cd, Pb and Cr concentration during 20 days of composting period. The TCLP is designed to determine the mobility of both organic and inorganic analytes present in liquid, solid, and multiphasic wastes. If an analysis of any one of the liquid fractions of the TCLP extract indicates that standardized heavy metals are present in high concentrations that, even after accounting for dilution from the other fractions of the extract, the concentration would be above the regulatory level for those metals, then the waste is hazardous. The threshold limit for heavy metals contamination in mg/kg is as follow: Cd-20, Cr-100 and Pb-100 (US EPA method 1311, 1992). The concentrations of Zn and Mn in trials 2, 3, 4 and 5 were reduced, but the concentration of these metals in trial 1 was increased slightly from 20.9 and 31.2 to 40.3 and 21% of total Zn and Mn, respectively, at the end of the composting period. The concentration of Cu in all trials (from 1 to 5) was reduced but the maximum reduction was observed in trial 4 (from 12.2 to 5.3% of the total Cu) during the composting process.

The leaching concentration of Cu, Mn and Zn reduced in all trials except trial 1, the reduction may be due to Cu, Mn and Zn complexed with organic matter or humic substances at maturity of compost. During the first phase, the neutral pH values caused a weak adsorption onto organic matter of Cu and Zn which probably leached out (Lazzari et al., 2000). The reduction of leachable fraction of Zn and Cu during composting process was consistent with other finding (Chiang et al., 2007). Leachable fraction of Fe in trials 1, 2, 4 and 5 was reduced, but the maximum reduction was observed in trials 4, although the concentration of Fe in trial 3 was increased (percentage of total Fe) at the end of the composting period. The leachability of Fe was decreased in trials 1, 2, 4 and 5; this might be due to small fraction of Fe attached with organic matter during composting process. The enhancement of Fe concentration in trial 3 might be due to high EC in this trial. The complexation capacity of a humic acid has been found to drastically reduce with increases in EC (Talbot, 2006).

The leachable concentration of Cr in trials 1, 2, 3 and 4 was reduced but the maximum reduction was observed in trial 4 (from 10.3 to 4.8% of the total Cr); however it was increased in trial 5 was during the composting process. Cr has an electron configuration closest to a noble gas with a high spherical symmetry and low polarization capacity. It has a valance of three and therefore it has a stronger electrostatic affinity for the sorption sites than divalent cations. The leachable concentration of Cd in all trials (from 1 to 5) was reduced during the composting process, it might be due to pH was raised in all trials at the end of composting period. The influence of pH on trace element solubility is well known. Either through solubility equilibria, or due to complexation by soluble and surface legends, increasing pH, within an ordinary range, decreases solubility and bioavailability of divalent trace metals (Cambier and Charlatchka, 1999). The concentrations of Ni and Pb in trials 1, 2 and 4 were reduced but the maximum reduction of concentration of these metals were observed in trial 4, however the concentrations of Ni and Pb in trials 3 and 5 were increased during the composting process. Chiang et al. (2007) also reported reduction of Ni with increase in composting time. The concentrations of Ni and Pb raised in trials 3 and 5 may be due lower pH (below 7) in comparison to other trials. There was no Pb, Cd and Cr detected in the compost of sewage sludge with fly ash, lime and zeiolite (Chiang et al., 2007), however in the present study these metals were found in the range of 1.0-6.8% of total Pb, 1.4-23.6% of total Cd and 7.5-23.7% of total Cr respectively.

Table 4.9a TCLP concentrations of heavy metals (Zn, Cu, Mn and Fe) during 20 days of composting period (mean ± SD, n=3)

	Leachable metals concentration									
	Zn (mg/kg dry matter)					**Cu (mg/kg dry matter)**				
Days	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	28.8±1.2a	23.5±2b	54.8±0.3c	22.2±0.3b	28.8±1a	8.2±1a	10.5±0.7ac	6.6±2.4a	6.6±0.2a	5.3±0.1ab
4	21.7±0.1a	20.6±0.1a	39.5±1.1b	21.1±0.2a	28.7±1.2c	7.8±0.6a	6.5±1.1a	8.6±2.6a	6.7±0.3a	6.9±1.1a
8	35.7±1.1a	21.8±0.2b	42.9±0.1c	24.5±1.3d	30.5±0.6e	7.1±0.5a	9.8±1b	6.1±1.3a	7.9±0.36ab	6.5±0.5a
12	31.5±3.1a	23.4±0.9b	27.4±0.9ab	39.8±0.4c	28.7±2.2a	6.7±0.5a	6.5±0.3a	7.3±2.5a	9.4±0.4a	6.2±0.4a
16	30.7±4.3a	22.8±0.4b	34.2±1c	26.6±1.0ac	28.5±2abc	5.3±0.5a	6.6±1a	11.5±1.1a	7.6±0.6a	4.9±0.5a
20	37.2±0.9a	28.3±0.8b	35.4±0.8a	21.3±0.1c	24.3±0.7d	5.4±0.2a	4.9±0.1a	5.3±0.5a	4.7±0.2a	4.5±0.1a
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	553±28a	217.9±7.3b	292±0.4c	205.1±9.7b	544±3.6a	467.5±55.5a	242.5±4.3b	163.2±30.6c	121.7±8.1c	88.1±6.3c
4	829.9±33a	140.9±11.5b	247.8±6.4c	181.8±11b	455±17d	724.4±19.6a	189.8±3.4b	141.2±3.8c	159.4±11c	104.7±2.3d
8	998.2±3.8a	182.4±2.6b	276.2±11.2b	191±1.6b	495±99c	647.0±10a	182.5±0.9b	175.2±2.2b	145.5±6.1c	111.6±6.2d
12	820.0±4.0a	227.3±13.3b	259.3±3.7c	177.0±3.0d	449±13.2e	383.6±10.8a	166.2±8.4b	212.3±2.5c	107.4±7.8d	82.5±5.7e
16	968.2±10.2a	188.4±5.2b	321.7±8.9c	176.7±2.0b	486±77.9d	330.2±10.2a	179.1±2.3b	280.7±11.7c	98.9±1.5d	108.5±0.9d
20	777.6±12.2a	276.5±11.5b	288.8±9.2b	175.5±8.5c	405±20d	305.9±6.3a	184.4±0.8b	238.3±10.9c	63.3±2.3d	85.8±11e

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$)

Table 4.9b TCLP concentrations of heavy metals (Cr, Ni, Cd and Pb) during 20 days of composting period (mean ± SD, n=3)

	Leachable metals concentration									
Days	**Cr (mg/kg dry matter)**					**Ni (mg/kg dry matter)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	9.1±1.5a	6.5±0.9b	6.4±0.0b	6.1±0.7b	6.8±0.4ab	10.5±0.5a	6.5±0.5b	5.5±0.5b	8.6±0.6c	1.8±0.4d
4	8.7±0.9a	5.8±0.6b	8±1ab	7.1±0.5ab	8.8±1ab	9.1±0.7a	7.5±0.5b	4.7±0.3bc	6.1±0.5bc	13.2±0.8d
8	6.8±0.8a	7±0.8a	6.8±1.2a	6.4±0.4a	7.8±0.2a	14.3±0.3a	8.8±0.6b	7.3±0.9b	5.4±3.6bc	5.1±0.7d
12	9.3±0.3a	5.4±0.2b	6.4±1.2bc	7.1±0.3bc	8.8±0.4a	7.3±0.3a	8.9±0.7abd	6.4±0.4a	4.6±0.2c	8.5±0.5ad
16	11.1±0.7a	5.9±0.1b	6.7±0.3b	6.9±1.1bc	6.5±0.5bc	10±0.2a	11.6±0.4b	6.1±0.1c	5.5±0.3c	8.9±0.5d
20	8±0.2a	5±0.8b	4±0.2b	3.65±0.2b	10.8±1.6d	8.6±0.2a	4±1b	9.9±0.5a	3.6±0.2cb	9.2±0.4a
Days	**Cd (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
0	0.8±0.03a	2.3±0.1b	2.4±0.02	1.9±0.02c	1.4±0.02d	43±1a	24±2b	30±12abc	53±16.9a	24±2bc
4	0.9±0.04a	2.1±0.1b	2.3±0.1b	2.1±0.2b	1.4±0.09c	37±1a	33±1bc	43±3ad	40±15.5a	14±4b
8	0.9±0.07a	2.3±0.05b	2.4±0.1b	1.9±0.1c	1.3±0.03d	46±4a	20±4b	24±2b	55±25.6c	11±3d
12	0.8±0.05a	2.4±0.1b	2.4±0.2b	1.8±0.02c	1.2±0.03d	30±6a	30±2a	15±3b	23±4.8abc	15±1bc
16	0.8±0.05a	2.4±0.04b	2.4±0.1b	1.6±0.04b	1.0±0.03d	32±2a	60±10b	45±1c	35±7ac	23±1a
20	0.8±0.0a	2.5±0.1b	2.0±0.1c	1.0±0.04c	1.0±0.02d	36±2a	26±2b	50±4c	17±5.9d	27±1b

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.05$)

The order of leachable heavy metal content in the composted water hyacinth was: Mn > Fe > Pb >Zn > Ni > Cr > Cu > Cd. On analyzing the results by ANOVA, significant differences in leachable heavy metals (Mn, Fe, Zn, Cu, Cd, Pb, Ni and Cr) ($p < 0.05$) were observed between all the trials.

4.2.7 SPECIATION OF HEAVY METALS

- **Speciation of Zn, Cu, Mn, Fe and Ni**

Speciation of Zn in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Zn in trial 5 during the process. In trial 1; F1, F2, and F5 fractions of Zn were decreased however the F3 and F4 fractions were increased (percentage of the total fraction of Zn). In trial 2; F1, F2, F3 and F4 fractions of Zn were decreased, however the F5 fraction was increased (percentage of the total fraction). In trial 3; F1, F2, F3 and F4 fractions of Zn were decreased, however the F5 fraction was increased (percentage of the total fraction). In trial 3, F2, F3, F4 and F5 fractions of Zn were increased, alternatively F1 fraction was increased of the total fraction at the end of composting process. The stabilization of raw organic matter during composting process transformed Zn from F1 fraction to more stable F4 fraction (Smith, 2009). In trial 4; F1, F4 and F5 fractions were decreased while F2 and F3 fractions were increased of total fraction during the composting process. In trial 5, F1 and F4 fractions were decreased while F2, F3 and F5 fractions were increased (parentage of total fraction) during the composting process. The reduction of movable forms of Zn might be due to the formation of Zn complex with humic substances formed at the maturity stage of compost (Cai et al., 2007). F5 fraction was the dominant fraction of Zn in all trials. In this study, pH of the final composts ranged from 5.3 to 7.4 (Fig. 4.9). BF of Zn was reduced from 0.55, 0.36, 0.37 and 0.49 to 0.35, 0.23, 0.23 and 0.41 in trials 2, 3, 4 and 5 respectively however it was increased in trial 1 during the composting period (Fig. 4.13). The higher reduction in BF of Zn was observed in trial 4 (37.5%) followed by trial 3 (36.5%), trial 2 (35.8%) and trial 5 (14.9%) during the process. Reduction in BF of Zn could be attributed as, bioavailable fractions (F1, F2, F3 and F4) precipitated with hydroxides, carbonates, phosphates, sulfides and some other anions form complexes with organic legends. Cation exchange and complexation by organic ligands were suggested to be the main Zn mobility controlling mechanism (Kumpiene et al., 2008).

Table 4.10 Speciation of heavy metals during 20 days of composting period in trials 1 and 2 (Mean ± SD, n = 3)

Days	Trial 1					Trial 2				
					Zn (mg/kg dry matter)					
	F1	F2	F3	F 4	F5	F1	F2	F3	F 4	F5
0	11.0±0.13	13.0±0.05	11.6±0.52	5.9±0.42	86.5±1.5	5.8±0.5	16.9±1.3	20.84±0.52	13.56±2.65	41.8±0.3
4	7.7±0.21	13.5±0.18	10.0±0.78	12.2±0.25	81.5±5.5	7.8±0.2	11.3±1.3	17.02±3.24	12.90±2.35	62.5±2.5
8	6.6±0.02	14.4±0.15	17.8±3.0	13.8±0.34	91±9.0	5.6±0.07	14.2±1.0	16.98±3.4	14.79±0.69	87.4±2.6
12	5.5±0.32	13.5±0.29	21.9±1.17	14.5±0.24	62.5±2.5	4.8±0.19	11.5±0.4	20.17±0.23	17.08±0.26	97.0±5.0
16	6.4±0.20	16.4±0.11	23.0±0.14	14.6±0.63	112.5±7.5	4.9±0.04	11.0±0.3	17.5±1.22	19.15±0.37	122.0±2.0
20	7.6±2.26	16.3±0.25	36.4±0.43	18.5±0.48	90.5±1.5	4.0±0.0	13.2±0.2	17.53±1.87	19.36±0.12	105.7±3.3
Days					**Cu (mg/kg dry matter)**					
0	3.8±0.13	2.2±0.05	1.4±0.30	0.0±0.0	41.7±1.15	5.9±0.2	8.0±0.1	9.9±0.7	11.6±1.1	32.5±0.5
4	2.5±0.03	2.2±0.10	1.7±0.25	2.6±0.24	34.2±0.4	3.1±0.3	1.5±0.4	5.2±0.6	12.2±1.4	36.5±1.5
8	2.5±0.25	1.9±0.13	1.5±0.20	3.6±0.40	43.0±2.3	7.0±0.5	6.3±0.3	7.3±0.3	13.1±0.3	50.1±1.1
12	0.8±0.15	1.2±0.03	1.6±0.20	1.4±0.08	65.0±1.1	3.1±0.4	1.2±0.2	1.6±0.2	12.5±0.6	53.1±2.1
16	1.3±0.05	1.4±0.13	4.2±0.35	1.0±0.02	44.4±1.5	2.5±0.4	1.3±0.1	0.9±0.7	12.0±0.8	49.6±0.6
20	1.3±0.1	1.5±0.15	3.8±0.25	1.1±0.32	44.0±3.5	1.9±0.1	0.9±0.03	1.2±0.6	10.2±0.8	56.5±1.5
Days					**Mn (mg/kg dry matter)**					
0	450.0±6.5	332.0±2.0	146.1±2.7	116.8±8.0	563.2±7.1	163.4±21.2	103.3±2.8	58.1±0.7	74.6±0.6	223.0±2.9
4	557.5±3.0	609.0±10.5	249.4±7.0	272.8±2.4	338±3.0	123.0±2.5	41.1±2.8	36.5±1.1	22.1±2.4	325.0±15.0
8	533.8±19.8	620.0±1.0	303.2±13.6	199.2±5.6	221.4±23.1	150.4±2.4	105.7±22	61.3±9.3	56.7±1.0	477.8±5.4
12	319.6±1.1	688.7±1.3	441.6±11.5	214.4±16.0	122.9±1.0	138.6±4.9	93.6±5.3	55.6±1.4	62.9±1.0	431.1±5.1
16	241.8±25.3	764.8±15.3	546.0±0.0	203.2±3.2	239.3±29.3	93.8±2.6	68.6±2.6	41.8±0.2	26.5±0.1	526.8±14.4
20	246.8±2.3	670.3±28.3	621.0±1.0	211.2±6.4	162.8±6.0	104.7±6.3	99.4±4.4	54.0±1.0	47.4±7.5	646.9±11.9
Days	**Fe (mg/kg dry matter)**									
0	152.6±4.4	142.8±30.3	5135±210	6125±49.6	24130±720	26.6±2.0	37.8±0.6	2069.7±24.7	2800.5±444	8561.5±38
4	145.8±2.4	108.6±10.2	5448±46	9237±184	30957±703	23.4±0.4	39.4±0.1	1856.5±6.5	1680.7±14	8238.5±206
8	150.2±0.9	65.7±4.3	7627±99	11527±362	24136±395	35.8±1.5	38.9±1.0	2675.2±80.2	4580.8±8	8010.5±45
12	79.5±1.8	68.3±0.8	8560±66	12565±437	16569±211	25.5±0.6	32.7±1.2	2242.2±46.2	3973.9±68	9901.5±334
16	85.4±2.8	75.1±0.6	7275±350	10470±362	35892±357	30.3±0.3	31.0±0.03	1661±97.0	1973.3±137	12894.5±894
20	78.9±0.7	58.8±0.2	11173±438	13481±215	12846±482	27.7±0.4	34.0±0.1	2012.8±28.6	4314.8±67	14933.0±668

Days					**Ni (mg/kg dry matter)**					
0	5.0±0.43	4.7±0.30	ND	ND	211.0±2.1	4.6±0.22	3.7±0.08	ND	ND	127.0±3.0
4	4.3±0.15	5.0±0.10	ND	ND	225.0±6.0	4.0±0.05	2.5±0.08	ND	ND	166.6±11.6
8	5.0±0.08	4.0±0.35	ND	ND	216.4±0.8	4.5±0.08	4.1±0.33	ND	ND	199.9±1.6
12	6.4±0.38	4.2±0.20	ND	ND	241.9±12.9	4.3±0.08	4.2±0.15	ND	ND	184.9±1.2
16	4.5±0.45	5.0±0.05	ND	ND	187.9±1.4	3.7±0.05	3.4±0.05	ND	ND	214.5±10.5
20	4.4±0.48	4.3±0.13	ND	ND	230.7±9.4	4.3±0.03	3.9±0.05	ND	ND	184.8±1.2
Days					**Pb (mg/kg dry matter)**					
0	16.8±0.3	15.8±0.3	ND	ND	847.3±17.9	13.8±0.8	14.5±0.5	ND	ND	599.6±53.1
4	18.0±1.5	15.5±1.0	ND	ND	863.2±5.0	13.5±0.5	13.8±0.8	ND	ND	784.6±27.8
8	8.5±1.0	21.3±1.3	ND	ND	817.6±28	14.0±0.5	17.5±0.5	ND	ND	838.8±17.4
12	14.8±0.8	20.5±0.5	ND	ND	941.3±15.0	14.8±0.8	18.3±0.8	ND	ND	775.5±11.5
16	26.8±1.3	25.8±0.3	ND	ND	778.2±1.3	13.3±0.8	12.8±0.8	ND	ND	813.8±51.3
20	17.8±0.8	22.0±1.0	ND	ND	952.0±16.2	15.3±0.3	14.0±0.5	ND	ND	804.1±5.6
Days					**Cd (mg/kg dry matter)**					
0	0.75±0.05	0.95±0.50	ND	ND	51.4±1.3	0.15±0.05	0.28±0.02	ND	ND	36.9±1.3
4	0.80±0.10	1.33±0.03	ND	ND	52.4±0.7	0.38±0.03	0.28±0.03	ND	ND	46.0±0.3
8	0.90±0.05	1.10±0.10	ND	ND	51.0±0.9	0.08±0.03	0.19±0.01	ND	ND	55.1±0.2
12	0.85±0.05	1.30±0.05	ND	ND	54.3±0.9	0.13±0.03	0.08±0.03	ND	ND	53.1±0.7
16	0.93±0.08	1.30±0.10	ND	ND	53.7±1.7	0.18±0.03	0.30±0.02	ND	ND	53.0±0.9
20	0.78±0.03	1.35±0.10	ND	ND	54.5±0.4	0.23±0.02	0.36±0.03	ND	ND	54.5±0.8
Days					**Cr (mg/kg dry matter)**					
0	3.9±0.1	2.5±0.2	0.6±0.1	4.4±0.24	36.3±2.7	7.0±0.13	2.6±0.10	3.8±0.2	8.4±0.3	12.8±1.0
4	3.3±0.1	1.8±0.2	0.9±0.2	4.3±0.16	32.2±0.4	7.1±0.08	2.3±0.13	3.0±0.6	7.6±0.4	19.7±0.8
8	4.4±0.1	1.3±0.1	0.5±0.4	5.5±0.24	36.8±0.0	5.5±0.08	2.7±0.18	4.2±0.2	9.5±0.5	59.0±3.9
12	4.5±0.2	2.3±0.1	1.8±0.2	5.8±0.16	34.4±1.3	7.0±0.35	2.0±0.05	3.4±0.4	9.2±0.3	22.9±0.9
16	2.9±0.1	1.0±0.1	1.4±0.3	2.9±0.16	35.2±1.4	7.5±0.25	2.3±0.05	3.6±0.2	7.4±0.4	41.2±1.7
20	3.5±0.1	1.8±0.1	2.0±0.3	5.2±0.40	37.5±3.0	6.5±0.08	1.7±0.10	2.9±0.7	7.5±0.2	32.8±1.1

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$); ND - Not detected)

Speciation of Cu in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Cu in trial 5 during the process. In trials 2; F1, F2, F3 and F4 fractions of Cu were decreased, however the F5 fraction was increased of the total fraction of Cu during the composting process. In trial 3, F1 and F2 fractions of Cu were decreased, however the F3, F4 and F5 fractions were increased of the total fraction of Cu during the composting process. In trials 4 F1, F2, F3 and F4 fractions of Cu were decreased from 5.2, 2.9, 3.1 and 40.8% to 1.1, 2.6, 1.9 and 18.6% of the total fraction of Cu whereas the F5 fraction was increased from 48.9 to 76% of total fraction during the composting process. In this trial all movable fractions of Cu (F1, F2, F3 and F4 fraction) has been converted into F5 fraction at the end of composting process, which could be explained as Cu formed complex with organic functional groups mainly carboxylic, carbonyl and phenolic, resulting reduction in most bioavailable fractios (Qiao and Ho, 1997). In trial 5, F2 and F4 fractions of Cu were decreased whereas the F1, F3 and F5 fractions were increased (percentage of total fraction) during the composting process. In this trial F2 and F4 fractions were might be converted in to F5 fraction during the process. The reduction of all movable fractions (from F1 to F4) of Cu was observed in trial 4 in comparison to other trials; it was due to maximum organic matter loss, high heat generation and organic matter converted into humic substances.

The BF of Cu was reduced from 0.16, 0.52, 0.40, 0.52 and 0.28 to 0.15, 0.24, 0.35, 0.22 and 0.17 in trials 1, 2, 3, 4 and 5 respectively during the composting period (Fig. 4.13). The higher reduction in BF of Cu was observed in trial 4 (58.7%) followed by trial 2 (53.7%), trial 5 (40.0%), trial 3 (11.8%) and trial 1 (6.0%) during the process. The maximum reduction of BF of Cu was observed in trial 4 due to presence of hydroxyl and carboxylic groups supplied by cattle manure increased the binding sites and combined with Cu to form insoluble and immobile complexes (Guan et al., 2011). Furthermore addition of appropriate proportion of cattle manure enhanced composting process and consequently improved humic substances formation during the process. Significant differences in F1, F2, F3, F4 and F5 fractions of Cu were observed in all the trials ($p < 0.05$).

Speciation of Mn in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Mn in trial 5 during the process. In trial 1, F1 and F5 fractions of Mn was decreased, however the F2, F3 and F4 fractions were increased of the total fraction of Mn during the composting process. In trial 2; F1, F2, F3 and F4 fractions of Mn were decreased however the F5 fraction was increased of the total fraction of

Mn during the composting process. In trial 3; F3 and F4 fractions of Mn were decreased however the F1, F2 and F5 fractions were increased of the total fraction of Mn during the composting process. In trials 4 and 5; F1 and F4 fractions of Mn was decreased however the F2, F3 and F5 fractions were increased of the total fraction of Mn during the composting process. These fractions are easily available for plants. The rise in pH can decrease Mn mobility by precipitate formation, thus increasing the number of adsorption sites and decreasing the competition of H^+ for adsorption, followed by increase in metal stability with humic substances (Achiba et al., 2009). BF of Mn in trials 1 and 3 were increased from 0.7 and 0.3 (initial) to 0.9 and 0.4 (final) respectively; however, in trial 2, 4 and 5, BF was decreased from 0.6, 0.7 and 0.94 (initial) to 0.3, 0.6 and 0.93 during the composting period (Fig. 4.13). The higher reduction in BF of Mn was observed in trial 2 (50.0%) followed by trial 4 (29.6%) and trial 5 (0.94%) during the process. Significant differences in F1, F2, F3, F4 and F5 fractions of Mn were observed in all the trials ($p < 0.05$).

Speciation of Fe in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Fe in trial 5 during the process. In trial 1; F1, F2 and F5 fractions of Fe was decreased, but F3 and F4 fractions were increased. In trial 2; F1, F2, F3 and F4 fractions of Fe was decreased; though the F5 fraction was increased. In trial 3; F1, F2, F3 and F5 fractions of Fe was decreased; however the F4 fractions were increased of the total fraction of Fe during the composting process. In trial 4; F1, F2, F3 and F4 fractions of Fe was decreased however the F5 fraction was increased percentage of the total fraction of Fe during the composting process. In trial 5; F1, F2 and F4 fractions of Fe was decreased; however F3 and F5 fractions were increased of the total fraction of Fe during the composting process. In trials 1, 2, and 3; F5 fraction was dominant from initial to final stage of composting process due addition of less quantity of cattle manure in trials 2 and 3, and nil in trial 1. In trials 4 and 5; Fe was mainly present in F4 fraction. Due to proper degradation in trials 4 and 5, F5 fraction of Fe was converted in to F4 fraction. The F1 and F2 fractions of Fe were < 2% of total fractions, despite the fact that total concentration was very high. BF of Fe in trials 1 and 3 were increased from 0.32 and 0.38 (initial) to 0.66 and 0.42 (final) respectively; however in trials 2, 4 and 5; BF of Fe was decreased from 0.37, 0.82 and 0.84 (initial) to 0.30, 0.56 and 0.63 respectively during the composting period (Fig. 4.13). The higher reduction in BF of Fe was observed in trial 4 (32.15%) followed by trial 5 (25.1%) and trial 2 (18.1) during the process. The reduction in BF of Fe could be explained as humic substances formed a complex compound with Fe (Cai et al, 2007). Fe was mainly present in F4 fraction in all trials. Significant differences in F1, F2, F3, F4 and F5 fractions of Fe were observed in all the trials ($p < 0.05$).

Speciation of Ni in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Ni in trial 5 during the process. In trial 1, F1 and F2 fractions of Ni was decreased, however the F5 fraction was increased of the total fraction of Ni during the composting process.

In trial 1, F1 and F2 fractions were decreased though the F3, F4 and F5 fractions were increased of the total fraction of Cu during the composting process. Initially F4 fraction of Cu was not present but at the end of composting this fraction was increased to 2.2% of total fraction. However in trial 2 and 4; F1 and F2 fractions of Ni were decreased, but the F5 fraction was increased of the total fraction of Ni during the composting process. The F3 and F4 fractions of Ni were not detected in all trials during the composting period. The maximum reduction of F1 and F2 fractions of Ni were observed in trial 4. In trials 3 and 5, F1 fraction was reduced but the F2 and F5 fraction were increased. During composting process, the proportion of F1 and F2 fractions of Ni were decreased while the proportion of F5 fraction of Ni was increased; it was due to F1 and F2 fractions of Ni were transformed into the F5 fraction (Zheng et al., 2007; Wang et al., 2008). Both F1 and F2 fractions contributed < 10% of the total fraction of Ni in all trials. Venkateswaran et al. (2007) reported that about 15-30% in F1 and F2 fractions can cause the environmental toxicity during mobility. A significant increase in the level of Ni with F5 fraction could be due to alkaline stabilization process. The stable complex formation ability of Ni is less than Cu^{2+} in the transition metal cations (Qiao and Ho, 1997).

The BF of Ni was reduced from 0.044, 0.061, 0.034, 0.041 and 0.043 (initial) to 0.036, 0.042, 0.033, 0.014 and 0.025 (final) in trials 1, 2, 3, 4 and 5 respectively (Fig. 4.13). The higher reduction in BF of Ni was observed in trial 4 (65.0%) followed by trial 5 (42.6%), trial 2 (31.3%), trial 1 (17.1%) and trial 3 (6.2%) during the process. A significant decrease of BF in trial 4 confirmed that the addition of cattle manure in an appropriate proportion could effectively prevent the availability of Ni for plant uptake.

Table 4.11 Speciation of heavy metals during 20 days of composting period in trials 3 and 4 (Mean ± SD, n = 3)

Days	Trial 3					Trial 4				
	Zn (mg/kg dry matter)									
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	8.4±0.02	12.87±0.62	10.09±0.18	20.58±5.16	93.5±3.5	4.3±0.6	12.6±0.47	14.3±0.53	21.3±2.3	89.7±4.7
4	8.4±0.20	11.95±0.08	10.70±0.28	19.42±0.49	102.1±6.0	4.6±0.4	13.3±0.24	14.2±0.63	23.7±0.0	95.7±1.4
8	8.5±0.07	10.94±0.14	9.85±0.06	17.22±0.37	101.0±1.0	3.6±0.2	14.8±0.28	15.8±0.45	26.9±1.1	105.0±2.1
12	6.6±0.33	10.77±0.72	11.95±0.58	16.27±0.71	83.0±1.0	4.8±0.1	25.6±0.73	39.0±0.50	20.9±1.1	77.0±1.0
16	5.5±0.10	8.19±0.97	10.90±0.51	16.19±0.53	101.5±3.5	4.2±0.03	18.4±0.77	22.8±0.10	22.9±0.3	70.5±2.5
20	6.4±0.22	8.97±1.78	10.13±0.01	14.97±0.08	138.5±2.5	4.0±0.03	13.8±0.25	26.7±1.1	21.2±0.9	83.0±3.0
Days	**Cu (mg/kg dry matter)**									
0	7.1±0.50	1.7±0.73	1.5±0.42	6.3±4.10	24.6±4.1	3.4±0.13	1.9±0.28	2.0±0.20	26.5±0.49	31.3±3.30
4	5.9±0.13	3.0±0.08	3.5±0.56	23.2±0.75	50.1±0.8	2.0±0.13	1.0±0.08	3.0±0.25	25.3±1.75	36.4±1.85
8	1.9±0.03	1.6±0.28	2.5±0.56	9.5±0.60	28.0±0.6	2.2±0.10	1.5±0.05	2.7±0.15	19.0±2.10	44.6±1.75
12	1.9±0.23	1.9±0.20	3.6±0.21	12.1±0.55	25.2±0.6	1.3±0.08	2.2±0.10	2.9±0.25	31.8±2.96	33.5±2.50
16	1.0±0.10	0.8±0.10	2.3±0.21	6.4±0.50	23.1±0.5	0.9±0.05	2.3±0.03	1.8±0.10	14.0±0.40	51.4±0.75
20	1.4±0.18	0.8±0.05	3.6±0.21	10.4±1.50	29.5±1.5	0.9±0.08	2.1±0.10	1.7±0.03	15.9±0.24	65.1±3.85
Days	**Mn (mg/kg dry matter)**									
0	119.8±21.6	40.0±4.9	36.5±0.6	29.1±0.4	475.4±34.6	145.8±3.3	89.6±3.6	61.4±2.0	80.6±2.2	142.3±7.3
4	149.4±5.9	83.5±2.3	63.0±0.9	70.5±5.4	524.2±3.1	127.2±2.4	89.6±4.2	61.1±2.5	85.3±0.1	267.9±6.6
8	105.6±0.5	56.2±0.4	41.2±1.7	34.1±0.6	586.3±23.9	94.8±3.8	135.5±3.5	85.7±0.4	110.5±1.3	210.7±2.1
12	96.2±2.5	81.4±2.9	46.6±2.6	31.8±2.5	505.2±36.1	81.3±2.0	130.5±4.0	90.2±2.7	30.4±1.6	189.8±21.0
16	101.1±3.0	58.3±2.3	45.1±1.1	27.0±0.6	636.2±4.6	83.4±1.9	143.5±1.4	105.1±5.1	54.4±3.2	242.3±20.9
20	150.1±2.1	64.1±1.1	43.9±1.4	42.5±3.6	455.0±3.0	82.3±0.3	144.8±1.4	107.2±1.0	59.2±1.6	237.9±7.1
Days	**Fe (mg/kg dry matter)**									
0	70.0±0.2	29.5±0.5	2721.0±56	2541±96	8840.5±96	44.7±0.9	28.2±0.2	2484.5±4.5	6386.0±489	1953.0±86
4	13.2±0.9	35.4±0.1	3108.5±3.5	4704±56	9809.5±56	15.1±0.4	29.1±0.8	2558.5±33.5	7788.9±2.1	2467.5±429
8	11.0±0.3	32.5±1.1	2482.0±78	2598±10	8040.0±10	15.5±0.2	30.5±1.3	2359.5±7.5	6789.0±109	2731.5±164
12	7.5±0.1	33.5±0.5	2797.0±51	2548±107	8462.5±107	40.5±0.2	27.0±1.0	3305.0±20.0	5465.4±97	4176.5±53.5
16	9.4±0.5	23.9±0.1	2473.0±52	2020±261	9586.0±261	9.8±0.3	26.5±0.1	2141.0±30.0	5360.7±10.5	4582.5±147
20	8.9±0.2	25.1±0.2	2464.5±18	3566±475	8465.0±475.0	40.6±0.7	33.6±0.1	2901.5±48.5	4639.2±66	6058.4±595

Days					**Ni (mg/kg dry matter)**					
0	4.9±0.20	2.5±0.05	ND	ND	205.0±6.0	5.3±0.08	3.9±0.05	ND	ND	214.6±14.1
4	4.8±0.10	3.4±0.20	ND	ND	177.1±1.8	5.3±0.18	3.7±0.13	ND	ND	219.3±14.8
8	4.8±0.20	4.2±0.40	ND	ND	183.5±6.6	5.2±0.20	3.7±0.05	ND	ND	224.2±0.80
12	4.1±0.20	3.1±0.13	ND	ND	188.9±6.6	4.6±0.10	3.7±0.08	ND	ND	200.0±7.5
16	3.7±0.03	2.7±0.20	ND	ND	192.3±4.5	4.2±0.10	4.0±0.08	ND	ND	220.5±10.6
20	4.5±0.03	2.7±0.23	ND	ND	213.0±4.5	4.0±0.08	3.0±0.05	ND	ND	227.0±6.0
Days					**Pb (mg/kg dry matter)**					
0	26.0±1.5	14.8±0.3	ND	ND	855.0±15	25.0±1.2	15.6±0.8	ND	ND	895.4±40
4	13.0±0.5	6.3±0.8	ND	ND	890.0±30	20.0±1.0	8.8±0.8	ND	ND	892.9±3.2
8	11.8±0.8	6.5±0.5	ND	ND	928.2±28	20.8±1.3	7.5±2.0	ND	ND	916.0±16
12	12.0±0.5	8.3±0.3	ND	ND	907.2±4.9	18.5±0.5	8.5±0.5	ND	ND	879.8±3.4
16	12.5±1.0	9.5±0.5	ND	ND	975.6±3.1	21.8±0.8	10.8±0.8	ND	ND	925.0±10
20	11.3±1.3	7.0±0.5	ND	ND	1027.5±23	16.5±0.6	8.9±0.3	ND	ND	992.5±23
Days					**Cd (mg/kg dry matter)**					
0	0.10±0.05	0.10±0.01	ND	ND	42.7±0.4	1.1±0.16	1.1±0.12	ND	ND	47.5±0.6
4	0.08±0.03	0.18±0.04	ND	ND	44.6±1.1	0.3±0.05	0.1±0.01	ND	ND	52.8±1.6
8	0.17±0.03	0.16±0.03	ND	ND	55.3±0.8	0.1±0.03	0.1±0.05	ND	ND	54.8±2.1
12	0.13±0.08	0.15±0.04	ND	ND	54.1±1.3	0.6±0.08	1.2±0.08	ND	ND	53.9±0.3
16	0.14±0.03	0.17±0.05	ND	ND	51.6±1.5	0.7±0.18	0.9±0.10	ND	ND	54.3±1.6
20	0.13±0.03	0.28±0.03	ND	ND	53.8±0.2	0.4±0.05	0.7±0.03	ND	ND	54.1±0.3
Days					**Cr (mg/kg dry matter)**					
0	6.7±0.1	2.0±0.03	2.2±0.05	9.3±0.77	17.8±2.3	5.8±0.28	1.8±0.25	3.4±0.20	15.4±0.28	28.5±3.5
4	6.0±0.5	2.3±0.02	2.5±0.30	7.9±0.21	25.3±1.0	6.5±0.05	2.8±0.55	3.6±0.20	15.6±0.07	35.7±2.5
8	8.1±0.1	2.0±0.08	1.7±0.25	9.2±0.56	24.3±1.2	5.8±0.08	2.8±0.18	3.5±0.30	17.0±1.47	43.8±2.2
12	7.0±0.1	2.5±0.05	2.0±0.20	9.0±0.49	21.5±1.1	3.0±0.22	2.7±0.10	2.3±0.10	7.84±0.16	47.3±1.5
16	6.3±0.3	1.8±0.07	2.8±0.10	10.1±0.42	21.9±0.6	4.0±0.25	2.9±0.13	0.3±0.05	7.76±0.24	58.9±3.6
20	6.2±0.2	1.3±0.13	2.7±0.15	9.6±0.49	26.0±0.9	3.9±±0.1	1.4±0.03	0.8±0.10	4.32±0.32	62.8±2.6

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$); ND - Not detected

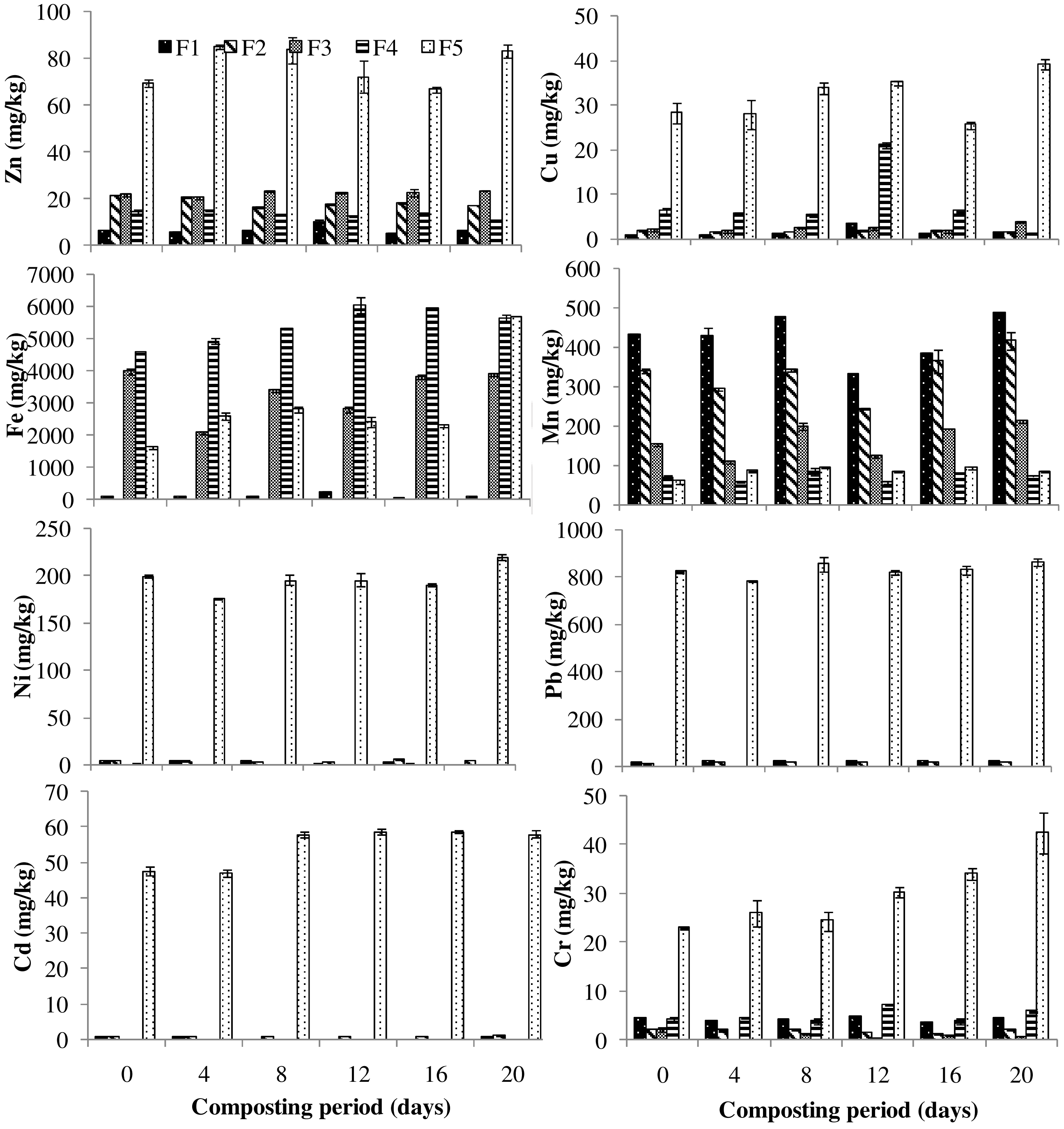


Fig. 4.12 Speciation of heavy metals in trial 5 during drum composting process

Furthermore reduction in BF of Ni might be due to formation of organometallic complex at the maturity of composting process (Qiao and Ho, 1997). A significant decrease of BF in trial 4 confirmed that the addition of cattle manure in an appropriate proportion could effectively prevent the availability of Ni for plant uptake. Significant differences in F1, F2 and F5 fractions of Ni were observed in all the trials ($p < 0.05$).

- **Speciation of Pb, Cd and Cr**

Speciation of Pb in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Pb in trial 5 during the process. In trials 1 and 5; F1, F2 and F5 fraction of Pb was increased (percentage of total fraction) during the composting process.

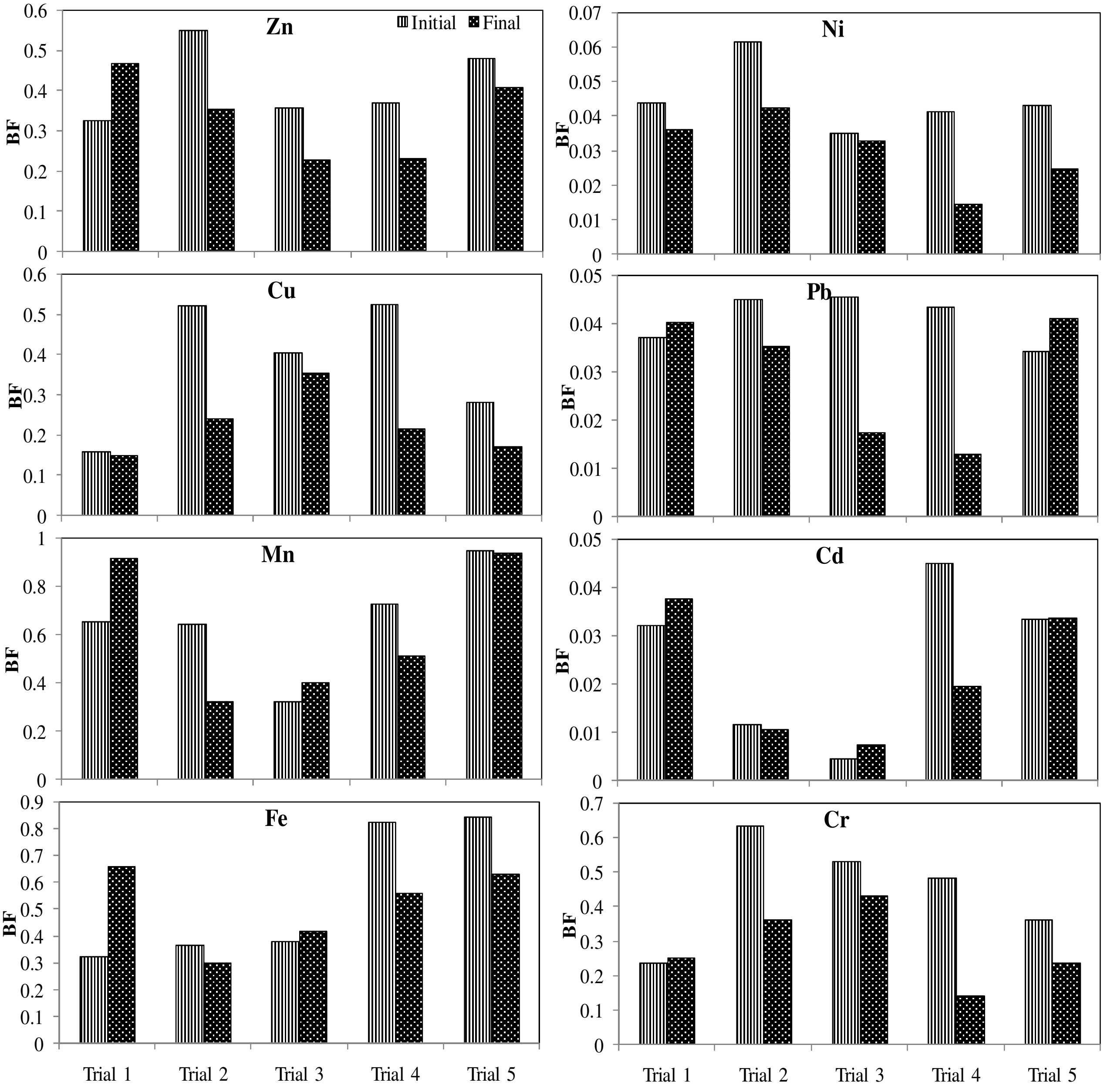


Fig. 4.13 Variation in bioavailability factor (BF) of heavy metals during rotary drum composting

It might be due to mass loss and poor formation of humic substances during the process. In trials 2, 3 and 4, F1 and F2 fractions of Pb was decreased but the F5 fraction was increased (percentage of total fraction) during the composting process. During the course of composting, the thermophilic phase showed intense microbial decomposition of organic matter with release of Pb from organic site. A similar results also reported by by Amir et al. (2005) during the sewage sludge composting.

Both F1 and F2 fractions contributed < 5% of the total fraction of Pb in all the trials. After composting, the F5 fraction of Pb was still the dominating fraction in all trials. In this study, the mobility of Pb was decreased during the composting process; a similar result was also reported by Wong and Selvam (2006). The BF of Pb in trials 1 and 5 was increased from 0.037 and 0.034 (initial) to 0.040 and 0.041 (final) respectively, while in trial 2, 3, and 4; BF was decreased from 0.045, 0.045 and 0.043 (initial) to 0.035, 0.017 and 0.025 (final) respectively, during the composting process (Fig. 4.13). The higher reduction in BF of Pb was observed in trial 4 (70.2%) followed by trial 3 (61.6%) and trial 2 (22.0%) during the process. Reduction in BF of Pb could be explained as, slightly alkaline medium can decreases the F1, F2, F3 and F4 fractions of Pb by forming Pb-organic matter complex, but higher alkaline conditions can have a reverse effect on Pb stability due to the amphoteric nature of Pb (Kumpiene et al., 2008). Significant differences in F1, F2 and F5 fractions of Pb were observed in all the trials ($p < 0.05$).

Speciation of Cd in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Cd in trial 5 during the process. The F1, F2 and F5 fractions of Cd in trials 1, 2, 3 and 5 were increased of the total fraction of Cd during the composting process. In trials 4; F5 fraction of Cd was increased but F1 and F2 fractions were declined with composting age. One possible explanation for reduction of F1 and F2 fractions and raise in F5 fraction in trial 4 may be due to the ability of Cd to chemically bond strongly with organic materials (Haroun et al., 2007). The decrease in F1 and F2 fractions of Cd could be due to increase in pH during the composting process (from 5.3 to 7.4). A similar results also reported by Hanc et al. (2009) during composting of sewage sludge. Additionally Cd was directly bound to two or more organic functional groups mainly carboxylic, carbonyl and phenolic, so that the ion was immobilized in a rigid inner-sphere complex. The F1 and F2 fraction indicates that Cd can be available for plant uptake. The BF of Cd in trials 1, 3 and 5 was increased from 0.032, 0.005 and 0.033 (initial) to 0.038, 0.007 and 0.034 (final); however in trials 2 and 4, BF was declined from 0.012 and 0.045 (initial) to 0.010 and 0.019 (final) during the composting process (Fig. 4.13). The higher reduction in BF of Cd was observed in trial 4 (56.4%) followed by trial 2 (8.62%) during the process. Reduction in BF of could be attributed as; most bioavailable fractions (F1 and F2) were bound to two or more organic functional groups mainly carboxylic, carbonyl and phenolic, so that the ion was immobilized in a rigid inner-sphere complex (Qiao and Ho, 1997). Significant differences in F1, F2 and F5 fractions of Cd were observed in all the trials ($p < 0.05$).

Speciation of Cr in trials 1 and 2 is given table 4.10, and speciation in trials 3 and 4 are given in table 4.11. Fig. 4.12 shows speciation of Cr in trial 5 during the process. The F1 and F2 fractions of Cr were decreased but F3, F4 and F5 fractions were increased (percentage of total fraction) in trial 1 during the composting process. In trials 2, 4 and 5, all movable fractions (F1 - F4) of Cr were decreased but F5 fraction was enhanced at the end of composting. The significant reduction in fractions F1, F2, F3 and F4 from 10.6, 3.2, 6.2 and 28.1% to 5.3, 1.9, 1.1 and 5.9% of total fraction was observed in trial 4 in comparison to other trials. It could be explained that, the F1 and F2 forms might bound with various organic functional groups present in humic substances, while the F3 and F4 fractions might be converted into F5 fraction during the composting process.

The BF of Cr in trials 2, 3, 4 and 5 was reduced from 0.63, 0.53, 0.48 and 0.36 (initial) to 0.36, 0.43, 0.14 and 0.24 (final) respectively; however in trial 1, it was increased slightly from 0.24 (initial) to 0.25 (final) during the composting process (Fig. 4.13). The higher reduction in BF of Cr was observed in trial 4 (70.5%) followed by trial 2 (42.6%), trial 5 (34.4%) and trial 3 (18.5%) during the process. Reduction in BF of Cr could be explained as; F1 and F2 forms may bound with various organic functional groups present in the humic substances, while F3 and F4 fractions might be converted into F5 fraction during the process. Furthermore the maximum reduction of BF was observed in trial 4 could be explained as addition of appropriate proportion of cattle manure can reduce the Cr availability by increasing humic substances. Significant differences in F1, F2, F3, F4 and F5 fractions of Cr were observed in all the trials ($p < 0.05$).

4.2.5 CONCLUSION OF PHASE 2

The better trends of physicochemical parameters were found in rotary drum composting in comparison to agitated pile composting, it might be due to appropriate agitation, mixing and aeration of composting materials. The total Cu concentration was less than total Zn concentration but water solubility of Cu was similar as Zn, which indicates equal toxicity of both metals. The lowest water solubility of Cr was observed in all trials. The finding of TCLP test pointed out that the heavy metals concentrations in all trials did not go beyond the threshold limits for compost use for agriculture purposes. Similar as agitated pile composting, maximum reduction of water soluble, plant available and leachable metals were observed in trial 4.

The total concentration of Cu, Cr and Cd was very low comparatively to the other metals (Zn, Mn, Fe, Ni and Pb), but the percentage of exchangeable and carbonate fractions of these metals were similar as other metals. These results confirmed that the bioavailability of metals does not depend on total concentration of metals. The total concentration of Cu, Cr and Cd was approximately similar but F1 and F2 fractions of Cr was twofold than Cu and fifty fold than Cd, which indicates Cr is more available for plants and it may be toxic to environment. Similar as agitated pile composting, the F3 and F4 fractions of Ni, Pb and Cd were not found in all trials. However, the F1 and F2 fractions were found in all trials which are more toxic and easily bioavailable fractions. The appropriate proportion of cattle manure addition (trial 4) significantly reduced the bioavailability, leachability and easily available fractions (F1 and F2) of heavy metals.

4.3 PHASE 3: VERMICOMPOSTING

Vermicomposting of water hyacinth is a good alternative for the treatment of water hyacinth and subsequentially beneficial for agriculture purposes. The bioavailability, leachability and speciation of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) were evaluated during vermicomposting of water hyacinth employing *Eisenia fetida* earthworm. Five different proportions (trials 1, 2, 3, 4 and 5) of cattle manure, water hyacinth and sawdust were prepared and composted for 45 days in the laboratory. Similar as agitated pile and rotary drum composting, the bioavailability of heavy metals was investigated in the form of water soluble and diethylene triamine penta-acetic acid (DTPA) extractable. TCLP test was performed for determination of leachable fraction of heavy metals. The Tessier sequential extraction method was employed to investigate the changes in heavy metals speciation during the vermicomposting process.

4.3.1 EARTHWORM POPULATION AND BIOMASS IN DIFFERENT TREATMENT

Earthworm production is an incorporated and important feature of any vermicomposting process (Deka et al., 2011). The survival of earthworms was very less found in trial 1 which contains 100% water hyacinth in comparison to other trials. The feed with higher proportion of water hyacinth might not have adequate amount of easily metabolizable organic matter and non-assimilated carbohydrates which could be essential for the growth and reproduction of the earthworms (Gupta et al., 2007). The number of earthworms showed statistical difference ($F = 16.4$, $p < 0.001$) in different feed mixtures. The number of earthworms increased on 30th day of experiment in all trials but maximum biomass growth was observed in trial 5 followed by trials 4, 3, 2 and 1 (Fig. 4.14). The earthworms were started to decrease after 30th day due to the exhaustion of food at end of composting (Yadav and Garg, 2009). Furthermore, the higher population of earthworms could increase mortality, reduced cocoon production per earthworm and reduced growth rate due to conversion of fresh organic matter into earthworm casts (Yadav and Garg, 2011). Similar observations have been reported by Vig et al. (2011) during vermicomposting of tannery sludge with cow dung. Initially cocoons production rate was lower and with time it was enhanced but new hatchlings were declined after 30th days. The maximum number of cocoons and hatchlings were produced in trial 5 and minimum in trial 1 (Fig. 4.14). The total earthworm biomass (in number) was increased from 120 to 205, 278, 287 and 350 in trials 1, 2, 3, 4 and 5 respectively at 30th day of vermicomposting process.

The total biomass was reduced and become 142, 229, 235, 229 and 232 in trials 1, 2, 3, 4 and 5 respectively at 45th day of vermicomposting. The highest biomass growth was observed in trial 5 might be due to higher addition of cattle manure. Higher percentage of water hyacinth in the feed mixture was not favourable for earthworm's growth and causing affected the biomass production during the vermicomposting process.

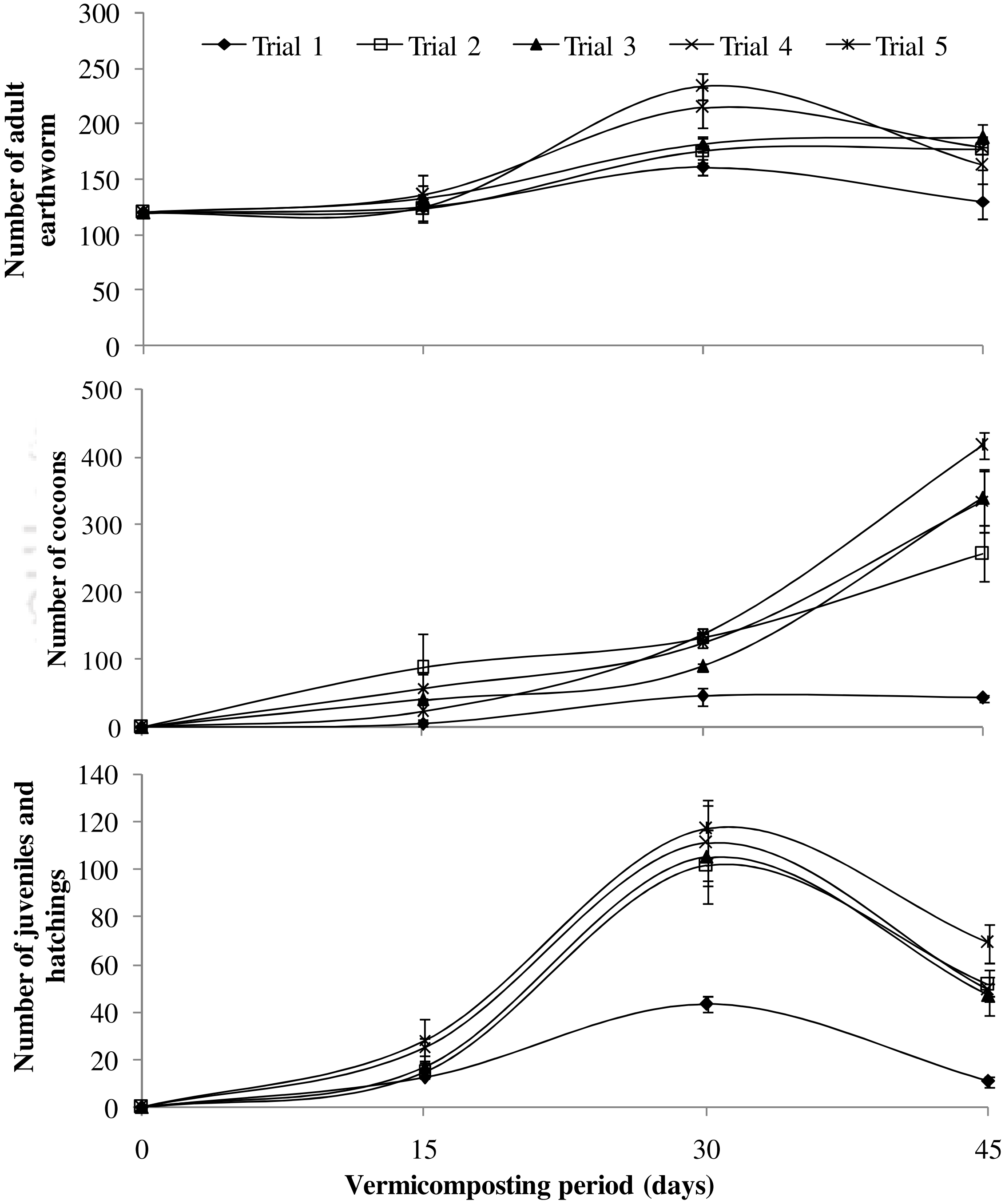


Fig 4.14 Variation in number of adult earthworms, cocoons and hatchlings during the vermicomposting in different trials

This might be due to the fact that higher proportion of water hyacinth in the feed mixture made it harder and more tensile, which was not easily used by the earthworms (Gupta et al., 2007). The higher increase in the biomass production resulted from the higher percentage of cattle manure addition. The continued existence, biomass production and reproduction of earthworms are the best indicators to assess the vermicomposting process. The number of earthworms biomass ($F = 50.7$, $p < 0.001$) number of cocoons ($F = 31$, $p < 0.001$) and number of hatchlings ($F = 104.9$, $p < 0.001$) shows statistical differences in different feed mixtures.

4.3.2 PHYSICO-CHEMICAL ANALYSIS

The water hyacinth and cattle manure containing feed mixtures had higher moisture and organic contents initially. Sufficient moisture is one of the most important requirements of earthworms during vermicomposting. They require moisture in the range 60-70%. Excess moisture content may create anaerobic conditions which may be fatal to earthworms (Garg and Gupta, 2011). The feed mixtures used in the present study were having moisture within the recommended range. The pH is an important parameter which greatly affects the vermicomposting process. The acceptable pH range, suitable for earthworms and microorganisms activity, is 5.5-8.5 (Yadav and Garg, 2011). The pH was increased significantly ($F = 90.1$, $p < 0.001$) from 6.07, 6.08, 6.50, 6.75 and 6.80 to 7.53, 7.57, 7.64, 7.68 and 7.70 in trials 1, 2, 3, 4 and 5 respectively during the vermicomposting process (Table 4.12). An increase in the pH of final vermicompost may be due to excess of organic nitrogen not required by microbes is released as ammonia, which gets dissolved in water and increases the pH of the vermicompost (Vig et al., 2011). Composting and vermicomposting process itself having buffering capacity, it always stays near neutrality either initial waste material with alkaline or acidic pH. The neutralization reaction occurring due presence of carboxylic and phenolic groups in humic acid, and ammonium ions formed at the end of process (Garg and Gupta, 2011). Electrical conductivity (EC) reflects the salinity of the composting product and its suitability for plant growth. High EC in the final product is undesirable because it will inhibit plant rooting and also reduce the transportation of water and nutrients to the plants (Fang and Wong, 1999; Chiang et al., 2007). The EC was decreased significantly ($F = 9.8$, $p < 0.001$) in the range of 5.7-27.3% in all trials during the process (Table 4.12). The decreasing trend of EC during vermicomposting agrees with the finding of other researcher (Vig et al., 2011).

Organic matter of the final vermicompost was significantly ($F = 131$, $p < 0.001$) lower as compared to the initial composted materials. The maximum reduction of organic matter was observed in trial 5 (54.5%) followed by trial 4 (51.6%), trial 3 (38.6%), trial 2 (16.4%) and trial 1 (5.0%) during the process. The reduction of organic matter during vermicomposting consistent with others finding (Gupta et al., 2007; Khwairakpam and Bhargava, 2009; Garg and Gupta, 2011). The reduction of organic matter might be due to the loss of CO_2 as well as due to the consumption of the available carbon as a source of energy by the earthworms and the microorganisms in all the trials (Khwairakpam and Bhargava, 2009). The highest values of biodegradability constant (K_b) was observed in trial 5 (0.79) followed by trial 4 (0.78), trial 3 (0.68), trial 2 (0.40), and trial 1 (0.11). The ash content is an important investigative parameter for decomposition and mineralization of the substrates. The ash content was increased in all trials significantly ($F = 83.6$, $p < 0.001$) in the range of 6.2-54.5% during the vermicomposting process. Significantly increase in ash content indicated the higher rate of volatilization and mineralization of the organic matter (Gupta et al., 2007; Khwairakpam and Bhargava, 2009). The macro-nutrients (Na, K, Ca and Mg) are required in very less quantity for adaptation of earthworms during the vermicomposting process and these nutrients are also required for plant growth. Table 4.12 illustrates the concentration of the macro-nutrients Na ($F = 51$, $p < 0.001$), K ($F = 137$, $p < 0.001$), Ca ($F = 33.5$, $p < 0.001$) and Mg ($F = 43.3$, $p < 0.001$) were increased significantly in all trials throughout the vermicomposting process. Augmented number of micro flora present in the gut of earthworms in the case of vermicomposting might have played an important role in this process and increased nutrients concentration in the vermicompost (Khwairakpam and Bhargava, 2009; Hait and Tare, 2012). Furthermore, nutrients were increased during process might be due to net loss of dry mass (Amir et al., 2005).

Heavy metals are important trace elements for well being of plants, animals and humans, but their excess is known to have toxic effects. Heavy metals were increased from initial feed mixtures but their enrichment was within permissible limit. The increased in the content of total metals in vermicompost may be due to a reduction in the weight and volume of the final product (Vig et al., 2011). An increase in heavy metals concentration in final vermicompost of different wastes was reported by other researcher (Kaushik and Garg, 2003).

Table 4.12 Physico-chemical parameters during vermicomposting (mean ± SD, n=3)

	Vermireactors									
Days	**pH**					**EC (dS/m)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
0	6.07±0.13a	6.08±0.12a	6.48±0.04b	6.75±0.10cd	6.80±0.06cd	4.80±0.72a	3.13±0.01bc	3.00±0.03bc	3.10±0.08bc	3.03±0.05bc
15	7.08±0.16a	6.80±0.12b	6.98±0.07ab	7.16±0.05a	7.30±0.01ac	5.32±0.61a	3.15±0.12bc	3.14±0.34bc	2.96±0.13bc	2.57±0.02bc
30	7.17±0.01a	5.91±0.86b	7.47±0.0a	6.85±0.18ab	7.20±0.25a	3.68±0.04a	3.44±0.63ab	3.17±0.27bc	3.09±0.03ab	2.71±0.08ab
45	7.53±0.06a	7.57±0.08a	7.64±0.0abc	7.68±0.07ab	7.65±0.01a	3.49±0.13a	2.66±0.09bc	2.83±0.06ab	2.63±0.13bc	2.57±0.18bc
Days	**Organic matter (%)**					**Ash content (%)**				
0	55.32±0.82a	70.26±2.5b	70.86±0.7b	69.57±0.6b	68.71±0.8b	44.78±0.7a	29.74±2.5b	29.14±0.7b	30.43±0.6b	33.47±0.8b
15	52.30±2.76a	63.89±2.9b	63.72±0.7b	63.34±1.2b	51.8±1.3a	47.70±0.9a	36.11±2.9b	36.28±0.7b	36.66±1.2b	48.2±1.3a
30	51.73±1.79a	61.30±2.5b	60.56±0.4b	63.09±0.3b	44.45±3.8c	48.27±2.4a	38.70±2.5b	39.44±0.4b	36.91±0.3b	55.55±3.8c
45	52.53±4.9a	58.73±5.8ac	43.89±5.0ab	33.62±6.0ad	31.27±3.03b	47.70±2.0a	41.27±5.8ac	56.11±5.0ab	66.38±14ad	68.73±3.03b
Days	**Na (g/kg)**					**K (g/kg)**				
0	7.68±0.12a	5.90±0.3b	5.67±0.07b	5.73±0.23b	7.00±0.14c	20.78±5.6a	15.15±0.3a	15.10±0.2b	15.98±0.9c	14.05±0.3d
15	8.45±0.25ac	6.55±0.15b	6.10±0.2b	7.10±0.8b	7.70±1.8abc	26.18±0.9a	18.10±1.0b	19.40±1.0b	17.75±0.4b	12.60±1.1c
30	8.85±0.25a	7.20±0.4b	8.05±0.15d	8.55±0.4acd	8.43±0.1acd	25.13±1.6a	17.70±0.4b	20.78±0.1c	17.20±0.1b	17.95±0.7b
45	9.20±0.3ac	9.05±0.65a	9.72±0.09ab	9.20±0.4a	10.59±0.5b	22.03±0.3a	17.28±0.9b	18.48±0.6bd	18.45±0.3b	16.73±0.6bc
Days	**Ca (g/kg)**					**Mg (g/kg)**				
0	13.05±0.3a	12.40±0.3b	10.83±0.22c	11.1±0.03c	10.93±0.13c	8.32±0.0a	7.89±0.3b	6.79±0.2b	7.12±0.1c	6.69±0.14d
15	13.30±0.9a	13.38±0.33a	12.13±0.33ab	12.9±0.55ab	11.35±1.1b	11.11±0.8a	8.65±0.3ab	8.64±0.4ab	8.44±0.3b	7.29±1.8b
30	14.25±0.5a	14.03±0.53a	14.13±0.38a	13.8±0.73a	15.13±0.93a	10.70±0.1a	10.88±0.6a	8.43±0.1b	8.67±0.3b	8.87±0.06b
45	14.20±0.4a	15.85±0.35b	15.06±0.24ab	16.5±0.3bc	16.68±0.47ac	9.42±0.05a	10.89±0.3b	9.39±0.5a	8.69±0.3ac	8.16±0.5c

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.001$)

The total concentration of Cu, Fe and Ni was reduced in trial 4 about 9.6, 17.2 and 8.0% respectively from the initial concentration. Very little amount of Fe was also reduced in trials 3 and 5 about 11.6 and 5.6% respectively. The total concentration of Mn and Cd was reduced about 12.1 and 24.6% respectively in trial 3 from the initial concentration (Table 4.17).

The reduction in metal content was in trials 3, 4 and 5, which showed the better decomposition as well as earthworm growth activities. Therefore, it is suggested that metal loss was related to the earthworm activity in the waste decomposition, furthermore earthworm can accumulate heavy metals in their tissues if reared in contaminated soils for long durations (Suthar and Singh, 2008). The reduction of heavy metals was also reported by other researchers (Jain et al., 2004). The variation in Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in different trials were significant ($F = 242$, $p < 0.001$ for Zn, $F = 9$, $p < 0.001$ for Cu, $F = 49$, $p < 0.001$ for Mn, $F = 32.6$, $p < 0.001$ for Fe, $F = 92.6$, $p < 0.001$ for Ni, $F = 34.8$, $p < 0.001$ for Pb, $F = 471$, $p < 0.001$ for Cd, $F = 6.2$, $p < 0.001$ for Cr).

4.3.3 BIOAVAILABILITY OF HEAVY METALS

- **Water solubility of heavy metals**

It is extremely necessary to determine the water soluble heavy metals in vermicompost before agronomic application because these metal fraction are positively more biologically dynamic and it has the highest prospective of contaminating to food chain (Hsu and Lo, 2001; Iwegbue et al., 2007; Hait and Tare, 2012). Fig. 4.15 illustrates the changes in water soluble Zn, Cu, Mn, Fe and Cr contents during 45 days of vermicomposting period. Water soluble Ni, Pb and Cd contents were not detectable in initial materials and during the vermicomposting process. The water soluble concentration of Zn was reduced in all trials in the range of 35.8-65.5% of the total Zn during the process. The water solubility of Cu reduced in the range of 19.3-64% of the total Cu in all trials except trial 1 during the process. The water solubility of Cu increased slightly in trial 1 might be due to poor growth of earthworm biomass, resulting incomplete degradation of organic matter (Gupta et al., 2007). The decrease in water solubility of Cu and Zn might be due to formation of humic substances during vermicomposting process, humic substances contain carboxyl groups, which can form complex with metals (Liu et al., 2008).

The water soluble concentration of Mn was reduced in the range of 46.2-84.6% of the total Mn in all trials during the process. The water soluble concentration of Fe was reduced in the range of 6.7-58.8% of the total Fe in all trials except trial 4. The water solubility of Fe increased in trial 4 can be attributed to a greater rate of degradation in the presence of higher concentrations of different microorganisms within earthworm intestine (Hait and Tare, 2012). The water solubility of Cr was reduced in all trials in the range of 35.5-76.7% of the total Cr during the process.

Hait and Tare (2012) have demonstrated that vermicomposting caused considerable reduction in concentration of water soluble metals like Cu, Zn and Cr during vermicomposting of sewage sludge. When the organic matter was passing through the gut of earthworm; some part of its digested, and pH and microbial activity of the gut were increased. Therefore, the rate of bioaccumulation of water soluble fraction of metals could be increased when it passes through worm's gut (Suthar, 2009). The order of water soluble metal concentration in the vermicomposted water hyacinth was Mn > Cu > Zn > Cr = Fe. The bioavailability of heavy metals decreased during vermicomposting process, it might be due to bioaccumulation of metals by *E. fetida* and organometallic complex formation (Li et al., 2010). The mobility and bioavailability of heavy metals decreased during vermicomposting in the course of two major types of cellular adaptation to toxicity of metals: one involves binding of metals to nuclear proteins and the formation of inclusion nuclear bodies; the second type is a cytoplasmic process involving synthesis of a specific metal binding protein, metallothionein within the chloragogenous tissue (Hait and Tare, 2012). The variation in water soluble Zn, Cu, Mn, Fe and Cr concentrations in different trials were significant ($F = 15.1$, $p < 0.001$ for Zn, $F = 55$, $p < 0.001$ for Cu, $F = 247.9$, $p < 0.001$ for Mn, $F = 1210$, $p < 0.001$ for Fe, $F = 17.9$, $p < 0.001$ for Cr). Water soluble fraction of heavy metals (Zn, Cu, Mn, Fe and Cr) was significantly correlated with pH, organic matter, electrical conductivity, ash content and total metal concentration (Table 4.13).

- **Plant availability of heavy metals (extraction with DTPA)**

The metal toxicity is not caused by the presence of heavy metals, but it depends on metal concentration, toxicity, mobility in free form, the route of uptake mechanism and bioavailability if it is accumulated in plants (Vig et al., 2011). The immobilization of metals during vermicmposting process appears to be a valuable and easy substitute. The DTPA extractable Pb and Cd were not detectable during the vermicomposting process.

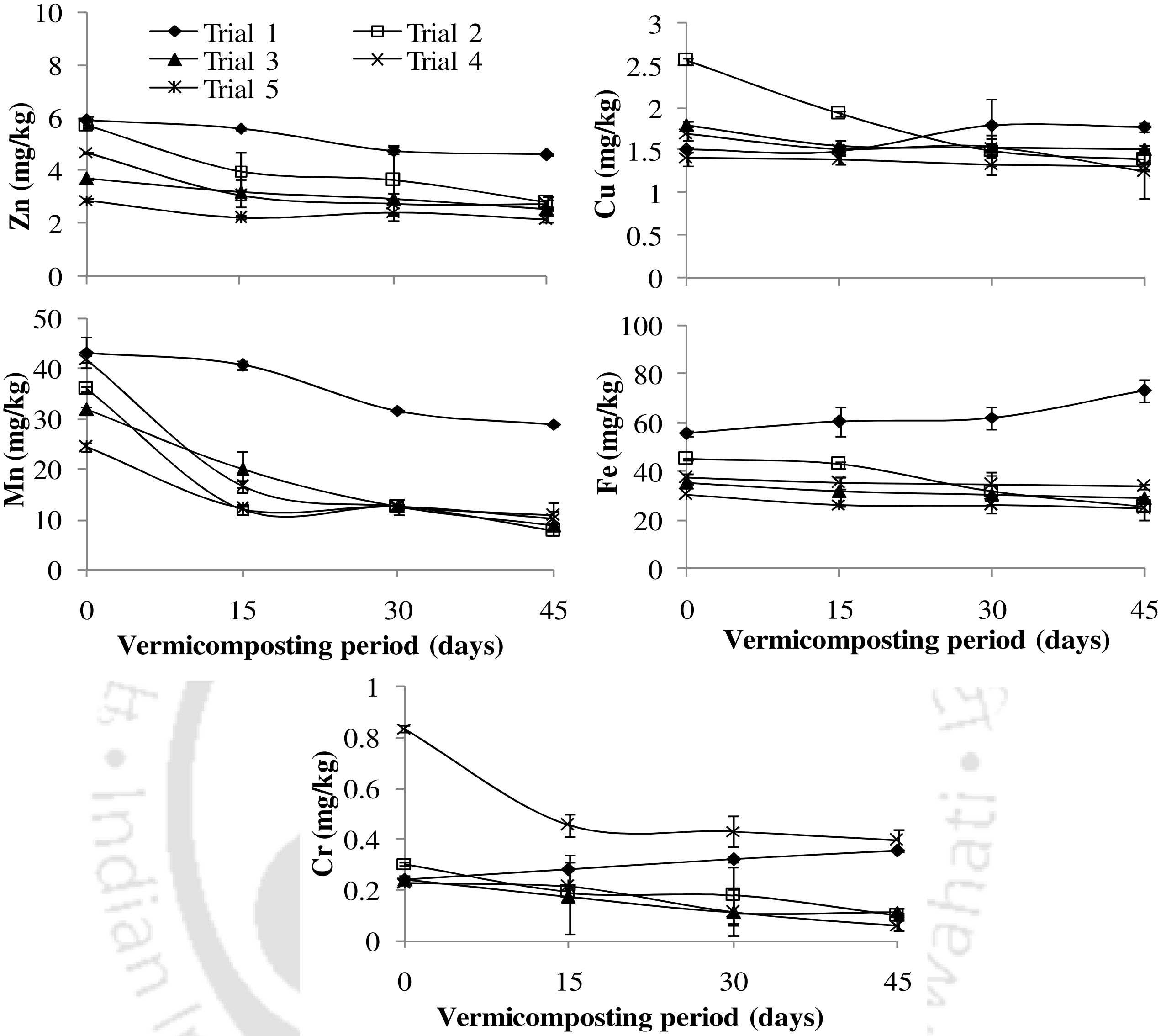


Fig 4.15 Variation of water soluble Zn, Cu, Mn, Fe and Cr concentration in different trials during the vermicomposting

The DTPA extraction efficiency was reduced in the ranges: 4.0-33.6% for Zn, 9.2-57.7% for Cu, 36.5-54.6% for Fe, 63.7-73.7% for Ni except trial 1 and 15.2-51.3% for Cr (Fig. 4.16) during the vermicomposting process. The percentage reduction of Zn and Cu might be due to immobilization of Cu and Zn atoms in microorganism cells decomposing the organic materials and to the formation of organo-metallic complex by earthworms (Bhattacharya and Chattopadhyay, 2006). The DTPA concentration of Mn was increased in all trials except trial 2 in the range of 3.7-53% of the total Mn during the process. In trial 2, concentration of Mn was reduced about 34.2% of total Mn concentration.

Introduction of earthworms for vermicomposting tend to increase the DTPA extractability of Mn in all trials expect trial 2, this behaviour was attributed to a greater rate of degradation in the presence of higher concentrations of different microorganisms within earthworm intestine (Bhattacharya and Chattopadhyay, 2006). Metabolic conversion of highly toxic form Cr (VI) to nontoxic form Cr (III) through mitochondrial and cytoplasmic fractions has also been demonstrated in *E. fetida* (Jain et al., 2004). However, metal reduction was comparatively higher in trials, showed the maximum earthworm activities, e.g. trials 2, 3, 4 and 5. On the other hand, trial 1 exhibited the minimum mineralization rate and even earthworm biomass production, showed the least metal decrease or increase during the vermicomposting process. The reduction of DTPA extractable metals during vermicomposting is also reported by other researchers (Maity et al., 2008; Suthar, 2009). Vermicomposting of organic wastes accelerates organic matter stabilization and gives chelating and phyto-hormonal elements which have a high content of microbial matter and stabilized humic substances (Gupta and Garg, 2008; Suthar, 2009, Hait and Tare, 2012).

The interaction of the humic acid with metals is one of the main factors affecting the partitioning of heavy metals during the process. It has a stronger sorption effect on heavy metals, particularly Cu and Zn (Hait and Tare, 2012). The variation in DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr concentrations in different trials were significant ($F = 46.2$, $p < 0.001$ for Zn, $F = 26.8$, $p < 0.001$ for Cu, $F = 88$, $p < 0.001$ for Mn, $F = 48$, $p < 0.001$ for Fe, $F = 292$, $p < 0.001$ for Ni, $F = 3.7$, $p = 0.012$ for Cr). DTPA extractable fractions of heavy metals (Zn, Cu, Mn, Fe, Ni and Cr) were significantly correlated to pH, organic matter, electrical conductivity, ash content and total metal concentration (Table 4.14).

4.3.4 LEACHABILITY OF HEAVY METALS

The leachability of the contaminants from the solid samples to the liquid phase might be influenced by several physicochemical factors such as type of the leaching medium, particle size, pH and complexing agents that may be present in the solid sample (Skodras et al., 2009). Table 4.15 illustrates the changes in leachable Zn, Cu, Mn, Fe, Ni, Cd, Pb and Cr concentration during 45 days of vermicomposting period. The leachable concentration of Zn, Fe, Pb and Cr was reduced (% of total metal) in all trials in the ranges of 12.8-49.1%, 31-48%, 1.1-64.7% and 19.5-56.75% of total Zn, Fe, Pb and Cr respectively during the vermicomposting period. The leachability of Mn was reduced in trials 4 and 5; however it was increased in trials 1, 2 and 3 (% of the total Mn) during the vermicomposting process.

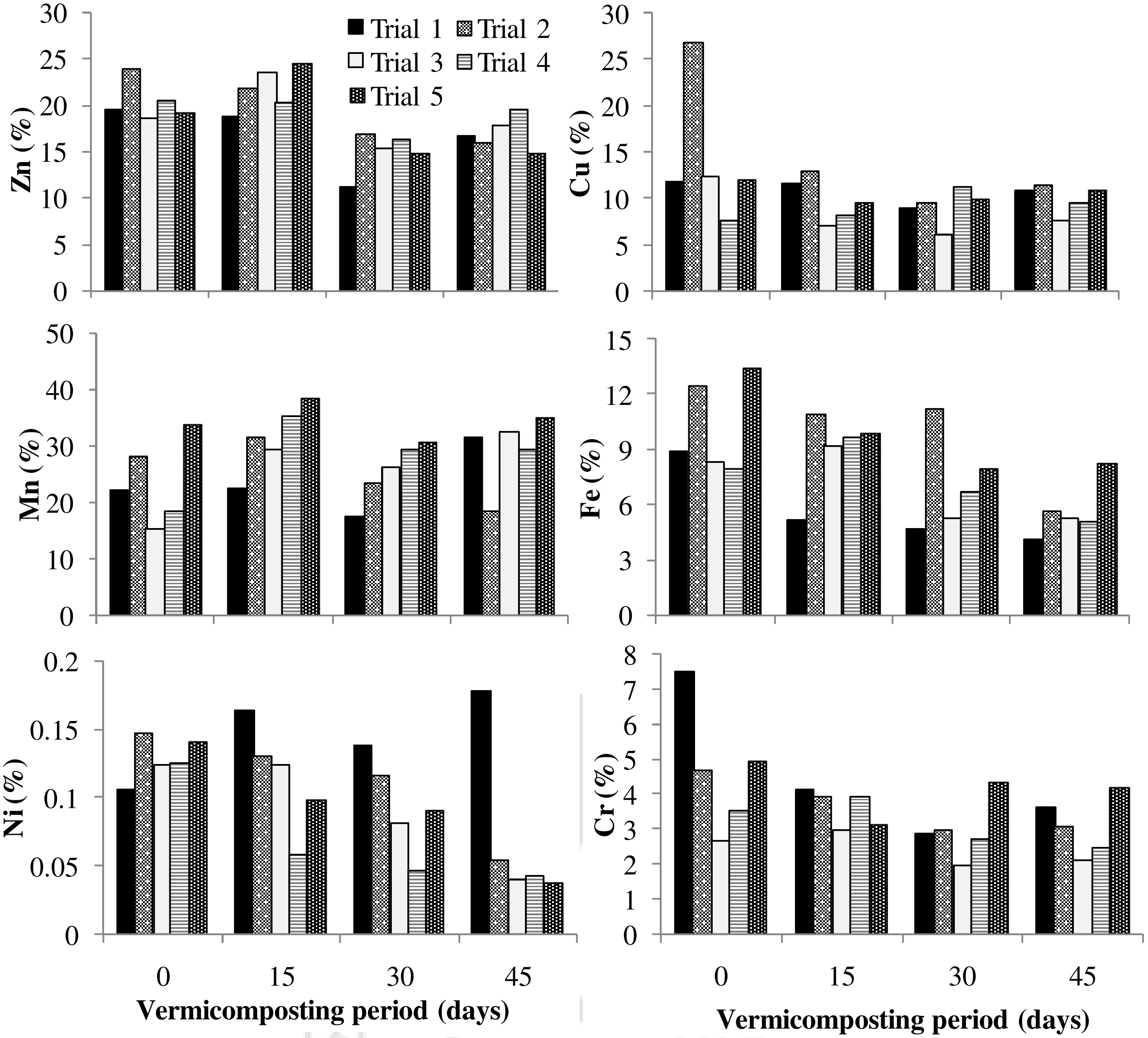


Fig. 4.16 Variation of DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr during vermicomposting process

The leachability of Cu, Ni and Cd was reduced in the range of 13.4- 62.3%, 28.2-71.2% and 30.6-51.9% of total Cu, Ni and Cd respectively in all trials except trial 1. In trial 1 leachability of these metals was increased due to partial degradation and less growth of biomass (Gupta et al., 2007).

Table 4.13 Linear correlation coefficients (R^2) of water soluble heavy metal fractions with pH, organic matter contents (OM), electrical conductivity (EC), ash content and total heavy metals during vermicomposting process

Trials	Water soluble heavy metals									
	Zn					**Cu**				
	pH	**OM**	**EC**	**Ash content**	**Total metal**	**pH**	**OM**	**EC**	**Ash content**	**Total metal**
Trial 1	0.718	0.509	0.824	0.578	0.743	0.211	0.111	0.977	0.186	0.770
Trial 2	0.420	0.989	0.145	0.989	0.959	0.284	0.503	0.015	0.503	0.679
Trial 3	0.619	0.605	0.113	0.605	0.545	0.102	0.014	0.225	0.014	0.098
Trial 4	0.344	0.322	0.242	0.344	0.000	0.163	0.008	0.105	0.021	0.201
Trial 5	0.865	0.722	0.996	0.722	0.000	0.175	0.0446	0.518	0.046	0.005
	Mn					**Fe**				
Trial 1	0.964	0.698	0.490	0.695	0.771	0.732	0.287	0.504	0.273	0.503
Trial 2	0.326	0.902	0.049	0.902	0.989	0.698	0.177	0.349	0.177	0.851
Trial 3	0.708	0.912	0.262	0.912	0.341	0.166	0.024	0.564	0.024	0.166
Trial 4	0.389	0.363	0.282	0.386	0.166	0.144	0.032	0.147	0.039	0.975
Trial 5	0.827	0.827	0.806	0.953	0.628	0.068	0.000	0.028	0.358	0.086
	Cr					**Ni, Cd and Pb**				
Trial 1	0.690	0.237	0.339	0.205	0.270	ND	ND	ND	ND	ND
Trial 2	0.920	0.256	0.624	0.256	0.078	ND	ND	ND	ND	ND
Trial 3	0.034	0.398	0.991	0.398	0.110	ND	ND	ND	ND	ND
Trial 4	0.307	0.247	0.197	0.273	0.001	ND	ND	ND	ND	ND
Trial 5	0.125	0.182	0.004	0.182	0.168	ND	ND	ND	ND	ND

ND= not detectable metals

Table 4.14 Linear correlation coefficients (R^2) of DTPA extractable heavy metal fractions with pH, organic matter contents (OM), electrical conductivity (EC), ash content and total metals during vermicomposting process

Trials	DTPA extractable heavy metals									
	Zn					**Cu**				
	pH	**OM**	**EC**	**Ash content**	**Total metal**	**pH**	**OM**	**EC**	**Ash content**	**Total metal**
Trial 1	0.046	0.000	0.174	0.024	0.105	0.331	0.740	0.010	0.687	0.175
Trial 2	0.011	0.306	0.004	0.304	0.304	0.047	0.797	0.015	0.797	0.935
Trial 3	0.055	0.151	0.016	0.151	0.166	0.447	0.106	0.253	0.106	0.000
Trial 4	0.284	0.283	0.399	0.276	0.527	0.424	0.713	0.565	0.660	0.040
Trial 5	0.276	0.541	0.063	0.541	0.930	0.201	0.191	0.002	0.191	0.630
	Mn					**Fe**				
Trial 1	0.796	0.649	0.580	0.714	0.486	0.499	0.476	0.848	0.588	0.072
Trial 2	0.013	0.010	0.187	0.010	0.016	0.000	0.324	0.002	0.324	0.372
Trial 3	0.715	0.556	0.001	0.556	0.530	0.727	0.588	0.149	0.588	0.527
Trial 4	0.895	0.664	0.720	0.782	0.471	0.258	0.440	0.422	0.238	0.301
Trial 5	0.750	0.460	0.646	0.460	0.559	0.725	0.933	0.581	0.933	0.036
	Cr					**Ni**				
Trial 1	0.954	0.642	0.214	0.642	0.225	0.579	0.174	0.648	0.181	0.333
Trial 2	0.361	0.169	0.634	0.169	0.017	0.080	0.894	0.000	0.0894	0.034
Trial 3	0.644	0.810	0.469	0.810	0.158	0.547	0.405	0.108	0.405	0.665
Trial 4	0.360	0.325	0.184	0.184	0.870	0.157	0.078	0.064	0.057	0.105
Trial 5	0.933	0.987	0.660	0.987	0.454	0.949	0.001	0.288	0.001	0.001

Table 4.15 Leachable heavy metals concentration during vermicomposting (mean ± SD, n=3)

	Vermireactors									
Days	**Zn (mg/kg)**					**Zn (mg/kg)**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	
0	75.3±9.0a	73.1±0.1a	55.6±0.7b	54.8±0.5b	66.9±1.8a	3.5±0.1a	5.4±0.4b	4.4±0.2c	3.5±0.2a	3.4±0.4a
15	76.3±1.9a	66.9±1.5b	67.8±2.0b	62.6±2.6bc	55.5±4.9c	3.2±0.1a	3.1±0.3a	3.4±0.2a	3.0±0.2a	2.4±0.2b
30	55.9±0.2a	49.5±0.04b	43.4±2.6bc	41.0±0.8c	46.9±4.5bc	3.0±0.6a	3.7±0.5a	4.3±1.1a	2.8±0.2a	2.8±0a
45	62.2±10.8a	51.5±1.1a	60.9±1.2a	52.8±5.5a	48.9±3.4a	3.9±0.7a	3.3±0.1a	4.0±0.2a	2.7±0.9a	2.7±0.1a
Days	**Mn (mg/kg)**					**Fe (mg/kg)**				
0	276.0±2.4a	251.7±0.9a	342.1±3.3b	609.9±43.3c	452.8±3.6d	148.7±5.5a	180.0±0.6b	129.9±0.7c	173.5±1.9b	158.1±0.5d
15	318.7±14.3a	279.0±20ab	349.9±48a	443.4±9.8b	383.5±42abc	141.8±18a	123.8±38ab	125.9±8.3ab	86.5±5.9b	82.1±1.7b
30	294.6±16a	283.5±19.3a	297.8±34a	364.9±25.5b	393.4±15.8b	152.2±2a	137.0±68a	137.4±40a	69.4±4.2a	122.0±44a
45	345.8±15.4a	300.7±29ab	336.9±32a	368.9±10.3ac	394.3±16.3ac	149.6±9.2a	157.6±1.4a	79.2±15.6b	74.6±31b	73.4±4.6b
Days	**Ni (mg/kg)**					**Pb (mg/kg)**				
0	12.0±0.1a	12.4±1.1a	11.5±1.0a	11.2±1.2a	11.3±0.4a	21.5±0.9a	21.9±1.5a	23.0±1.6a	21.9±1.3a	20.8±0.3a
15	9.0±0.13a	9.9±0.4a	9.0±0.5a	9.2±0.8a	9.0±0.2a	16.7±1.1a	21.0±1.2a	19.7±0.7a	15.3±2.7a	15.1±4.9a
30	9.6±0.2a	9.0±1.8ab	5.9±1.3ab	5.4±1.0b	4.0±3.2b	16.0±2.0a	17.7±1.7ab	21.0±3.0a	13.2±3.2ab	11.7±3.7b
45	10.8±0.4a	8.5±0.5bc	3.9±2.7b	7.4±0.8b	3.7±1.5bd	17.2±0.9a	17.0±9.0ab	21.2±0.8a	10.0±2.0ab	8.0±2.0b
Days	**Cd (mg/kg)**					**Cr (mg/kg)**				
0	2.4±0.1a	2.5±0.08ab	2.4±0.05a	2.3±0.09ac	2.5±0.06ab	6.6±0.3a	9.9±0.7b	11.0±0.4b	12±1.2b	5.7±0.7a
15	1.9±0.1a	2.2±0.11a	2.2±0.07a	2.2±0.08a	2.0±0a	5.9±0.5a	8.0±0.6a	8.3±0.3a	6.8±1.2a	7.8±3.8a
30	1.1±0.5a	2.1±0.69a	1.9±0.5a	1.8±0.4a	2.3±0.9a	5.8±0.6a	8.6±2.1a	6.2±2.0a	7.5±1.3a	8.3±0.7a
45	1.9±0.1a	1.6±0.4b	1.0±0.2b	1.5±0.5b	1.8±0.4b	6.4±0.6a	5.8±0.4a	5.4±1.9a	6.4±0.6a	5.1±1.3a

Mean value followed by different letters in columns is statistically different (ANOVA; Tukey's test, $p < 0.001$)

Table 4.16a Linear correlation coefficients (R^2) of TCLP extractable heavy metal fractions with pH, organic matter contents (OM), electrical conductivity (EC), ash content and total metals during vermicomposting process

Trials	Leachable heavy metals (Zn, Cu, Mn and Fe)									
	Zn					**Cu**				
	pH	**OM**	**EC**	**Ash content**	**Total metal**	**pH**	**OM**	**EC**	**Ash content**	**Total metal**
Trial 1	0.366	0.367	0.843	0.367	0.723	0.010	0.129	0.144	0.129	0.062
Trial 2	0.057	0.812	0.005	0.812	0.748	0.319	0.798	0.0.34	0.795	0.916
Trial 3	0.041	0.017	0.099	0.017	0.000	0.002	0.013	0.023	0.013	0.079
Trial 4	0.064	0.001	0.046	0.001	0.056	0.453	0.507	0.372	0.519	0.413
Trial 5	0.612	0.814	0.626	0.814	0.292	0.537	0.382	0.898	0.382	0.147
	Mn					**Fe**				
Trial 1	0.932	0.657	0.143	0.572	0.986	0.018	0.003	0.712	0.000	0.180
Trial 2	0.480	0.977	0.196	0.977	0.927	0.006	0.256	0.123	0.256	0.020
Trial 3	0.253	0.009	0.149	0.009	0.758	0.285	0.772	0.134	0.772	0.907
Trial 4	0.324	0.364	0.243	0.376	0.180	0.300	0.297	0.153	0.276	0.156
Trial 5	0.622	0.577	0.923	0.824	0.376	0.871	0.4330	0.927	0.641	0.000

Table 4.16b Linear correlation coefficients (R^2) of TCLP extractable heavy metal fractions with pH, organic matter contents (OM), electrical conductivity (EC), ash content and total metals during vermicomposting process

Trials	Leachable heavy metals (Ni, Pb Cd and Cr)									
	Ni					**Pb**				
	pH	**OM**	**EC**	**Ash content**	**Total metal**	**pH**	**OM**	**EC**	**Ash content**	**Total metal**
Trial 1	0.217	0.004	0.651	0.009	0.167	0.136	0.001	0.015	0.001	0.255
Trial 2	0.280	0.965	0.044	0.965	0.820	0.149	0.834	0.070	0.834	0.139
Trial 3	0.980	0.852	0.121	0.852	0.009	0.226	0.089	0.142	0.089	0.153
Trial 4	0.059	0.143	0.036	0.135	0.515	0.587	0.635	0.507	0.649	0.030
Trial 5	0.585	0.852	0.414	0.852	0.096	0.864	0.994	0.663	0.994	0.490
	Cd					**Cr**				
Trial 1	0.492	0.144	0.655	0.122	0.253	0.986	0.746	0.230	0.685	0.639
Trial 2	0.609	0.848	0.419	0.848	0.145	0.809	0.762	0.531	0.762	0.301
Trial 3	0.711	0.980	0.499	0.980	0.471	0.997	0.750	0.037	0.750	0.417
Trial 4	0.481	0.753	0.518	0.718	0.922	0.491	0.374	0.356	0.412	0.001
Trial 5	0.900	0.659	0.854	0.659	0.044	0.014	0.002	0.065	0.002	0.616

Reduction of leachable concentration of metals during vermicomposting might be due to accumulation in earthworms. Similar results also reported by Jain et al. (2004). This study revealed that the leachability of all metals decreases with vermicomposting time; it might be due to increase in pH and complexity of metals humic substances. According to Maity et al. (2008) pH may influence leachability of metals by following mechanism: an increase in compost pH causes an increase in surface negative charge which can raise cationic adsorption, formation of metal hydroxy ionic species that have a greater affinity for adsorption sites than the metal cations and precipitation of metal as metal hydroxides.

In addition, during vermicomposting metals interact with many chemicals and participate in detoxification processes, as part of the enzymes of the antioxidant systems, such as superoxide dismutase and in metallothioneins (Li et al., 2010). Moreover, the cutaneous absorption of metals was also evidenced in earthworms (Suthar, 2009). The reduction of Cu during vermicomposting might be due to high affinity of Cu for functional groups -OH or -COO of humic substances (Kang et al., 2011). The order of leachable heavy metal content in the vermicomposted water hyacinth was Mn > Fe > Zn > Pb > Ni > Cr > Cu > Cd. The variation in leachable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in different trials were significant ($F = 50.2$, $p < 0.001$ for Zn, $F = 8.4$, $p < 0.001$ for Cu, $F = 12.3$, $p < 0.001$ for Mn, $F = 30.4$, $p < 0.001$ for Fe, $F = 20.9$, $p < 0.001$ for Ni, $F = 4.9$, $p = 0.003$ for Pb, $F = 8$, $p < 0.001$ for Cd, $F = 685$, $p < 0.001$ for Cr). TCLP extractable fraction of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) was significantly correlated to pH, organic matter, electrical conductivity, ash content and total metal concentration (Table 4.16a and b).

4.3.5. SPECIATION OF HEAVY METALS

- **Speciation of Zn, Cu, Mn, Fe and Ni**

Speciation of Zn in trials 1 and 2 is given in Table 4.17a, and speciation in trials 3 and 4 is given in Table 4.18a. Fig. 4.17 shows speciation of Zn in trial 5 during the process. The F1 fraction of Zn was reduced in the range of 32.5-86% of total fraction in all trials. The F2 fraction was reduced in the range of 13.3-27.8% of total fraction in trials 1, 3, 4 and 5 but increased in trial 2. The F3 fraction of Zn was reduced in all trials in the range of 2.9-71.3% of total fraction.

Table 4.17a Speciation of heavy metals (Zn, Cu, Mn and Fe) in trials 1 and 2 during 45 days of vermicomposting period (Mean ± SD, n = 3)

Days	Vermireactors									
	Trial 1					Trial 2				
	Zn (mg/kg)					Zn (mg/kg)				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
0	12.0±0.3 a	35.0±0.6a	39.02±0.2a	31.0±0.2a	51.8±11a	28.0±0.2b	5.8±0.07b	36.2±0.3b	31.0±0.44a	46.0±0.8ad
15	9.0±1.8a	29.2±0.3a	51.38±3.9a	34.8±4.7a	80.5±4.9a	12.1±1.5ac	20.8±2.2b	35.8±5.3b	33.4±3.1a	69.9±5.6a
30	10.9±2.5a	28.5±4.0a	59.19±12.9a	41.2±5.3ac	95.1±2.7a	6.4±0.45b	26.7±1.7a	53.3±2.8a	29.8±2.5a	68.8±5.7b
45	11.7±1.0a	34.9±0.1a	73.79±9.6a	55.6±11.0a	71.0±4.8a	5.6±0.5b	25.9±0.5bd	52.8±6.7b	53.6±3.04a	70.6±7.9a
Days	**Cu (mg/kg)**					**Cu (mg/kg)**				
0	1.8±0.03a	1.1±0.08a	1.2±0.02a	13.8±0.12a	27.9±4.7a	3.9±0.1b	3.2±0.1b	4.4±0.05b	10.3±0.2b	6.8±1.4b
15	2.0±0.1a	1.6±0.1a	1.4±0.4a	13.5±0.64a	17.5±1.0a	1.6±0.3a	1.0±0.1a	1.5±0.3a	15.6±1.0a	14.4±3.0a
±30	1.8±0.08a	1.8±0.2a	1.3±0.2a	20.5±1.1a	20.0±4.5a	2.0±0.0ba	1.3±0.05b	0.9±0.2a	19.6±0.5a	11.1±1.0bd
45	1.9±0.3a	1.9±0.2a	1.8±0.1a	21.8±1.6a	17.0±4.5a	1.7±0.2a	2.2±0.08ac	1.5±0.2ac	23±0.07ad	10.5±0.5a
Days	**Mn (mg/kg)**					**Mn (mg/kg)**				
0	62.7±0.8a	59.0±0.3a	83.0±0.6a	64.7±0.7a	138.2±12.9a	151.6±1.7b	52.1±0.2b	58.3±0.4b	48.2±0.4b	55.0±1.8b
15	150.4±35.1a	78.0±16.7a	80.5±5.0a	65.6±1.9a	156.5±2.0a	154.0±10.6a	71.5±5.9a	69.8±4.7ab	57.4±0.6a	79.4±5.7bc
30	122.5±12.5a	116.7±9.9a	108.7±30.7a	70.7±4.2a	87.8±6.3a	151.3±14.2b	79.7±6.2b	94.1±0.2a	56±1.7abc	63.0±3.0b
45	116.7±23.4a	151.8±31.2a	153.1±4.3a	98.5±11.3a	59.6±4.2a	95.0±5.0a	168.5±2.6ab	114.8±2.5b	81.6±6.0a	46.3±2.3ab
Days	**Fe (mg/kg)**					**Fe (mg/kg)**				
0	86.0±0.3a	52.4±0.4a	1874.0±18a	6094.4±490a	1851.5±11.2a	22.8±0.3b	45.1±0.2b	1372±7.0b	5242.3±25b	2069±1.0a
15	63.9±1.7a	39.6±5.4a	2959.5±204a	7068.0±828a	5400.0±350a	23.8±0.22b	26.3±1.4b	3051±64.5a	6450±854a	2723±88b
30	73.3±5.6a	53.4±1.3a	3478.5±603a	7168.6±244a	2575.0±380a	20.5±3.3b	29.3±1.5b	2369±420a	6342±8.8ab	2095±45a
45	74.0±4.0a	48.8±6.0a	3495.0±118a	8082.6±937a	3755.0±170a	23.6±2.7b	34.1±3.6b	1997±347a	7529±430ab	2205±15b

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$)

Table 4.17b Speciation of heavy metals (Cr, Ni, Cd and Pb) in trials 1 and 2 during 45 days of vermicomposting period (Mean ± SD, n = 3)

Days	Vermireactors									
	Trial 1					**Trial 2**				
	Ni (mg/kg)					**Ni (mg/kg)**				
	F1	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
0	6.2±0.7a	4.8±0.7a	ND	ND	227.0±19a	5.9±0.4a	5.1±0.3a	ND	ND	147.4±11b
15	7.6±0.05a	5.6±0.7a	ND	ND	229.5±14ac	5.6±0.03bc	4.8±0.1b	ND	ND	231.8±8.3ac
30	4.6±0.1a	4.8±0.3a	ND	ND	238.8±8.8a	4.8±0.2a	4.7±0.1a	ND	ND	212.3±7.3a
45	6.2±0.4a	4.3±0.08a	ND	ND	229.5±11.0a	4.2±0.1b	4.6±0.3a	ND	ND	211.3±3.4b
Days	**Pb (mg/kg)**					**Pb (mg/kg)**				
0	12.1±1.1a	4.9±0.4a	ND	ND	894±9.0a	9.8±0.2b	7.8±0.2b	ND	ND	642±53a
15	17.0±0.0a	6.5±2.0a	ND	ND	905±35a	11.0±0.5b	7.5±0.5ab	ND	ND	945±25ab
±30	11.3±0.3ac	12.0±1.0a	ND	ND	980±15a	18.0±0.5b	6.5±1b	ND	ND	858±37.5b
45	12.0±1.5a	10.0±0.5a	ND	ND	905±30a	11.5±1.5a	7.3±1.3a	ND	ND	900±45a
Days	**Cd (mg/kg)**					**Cd (mg/kg)**				
0	0.6±0.05a	0.3±0.03a	ND	ND	56.2±0.9a	0.7±0.1a	0.7±0.2b	ND	ND	40.3±7.9b
15	0.7±0.1a	0.4±0.06a	ND	ND	56.0±3.0a	0.5±0.05a	0.6±0.03b	ND	ND	55.3±1.8a
30	0.4±0.2a	0.6±0.05a	ND	ND	56.0±3.5a	0.8±0.1b	0.6±0.1a	ND	ND	53.1±11.9a
45	0.6±0.07a	0.7±0.03a	ND	ND	55.8±4.8a	0.0+0.0b	0.5±0.2a	ND	ND	51.9±0.6a
Days	**Cr (mg/kg)**					**Cr (mg/kg)**				
0	6.3±0.5a	2.0±0.08a	0.4±0.06a	6.0±0.4a	17.6±4.4a	7.1±0.4a	2.4±0.2a	1.2±0.2b	8.6±0.02b	36.8±4.8b
15	6.5±0.2a	2.2±0.3a	0.4±0.04a	8.8±1.0a	39.5±1.5a	6.0±0.08a	2.3±0.08a	0.8±0.2b	8.8±1.0a	38.0±3.0a
30	7.9±0.5a	2.1±0.3a	1.8±1.6a	15.1±4.6a	60.5±1.5a	6.7±0.05b	2.0±0.03a	0.6±0.1a	8.6±1.3b	38.8±3.3b
45	8.1±1.0a	2.4±0.5a	1.5±0.7a	7.2±1.4a	38.8±3.8a	3.1±0.3b	0.9±0.08b	0.3±0.1a	9.6±1.1b	40.3±1.3a

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$); ND - Not detected

The reduction of F1, F2 and F3 fractions in all trials might be due to cation exchange and complexation by organic ligends and in addition the formation of Zn complex with humic substances formed at the end of vermicomposting. Humic substances contain various organic functional groups that can adsorb metal ions through ionic force (Cai et al., 2007; Kumpiene et al., 2008). Furthermore when the organic matter was passes through the gut of earthworm, some part of its digested; pH and microbial activity of the gut increased. As a result, the possibilities of metals binding to the ions and carbonates increased in ingested material (Hait and Tare, 2012). The F4 fraction of Zn was increases in the range of 21.7-101.6% of total fraction in all trials except trial 5.

The F5 fraction of Zn was enhanced in the range of 4.9-8.4% of total fraction in trials 1, 2, 3 and 5; however it was reduced in trial 4 during the vermicomposting process. The F4 and F5 fractions of Zn were increased in most trials during the vermicomposting process, it might be due to synergism effect of earthworms and microbes increased the decomposition of organic matter (Liu et al., 2005). The variation in F1, F2, F3, F4 and F5 fractions of Zn in different trials were significant ($F = 104.57$, $p < 0.001$ for F1, $F = 91.62$, $p < 0.001$ for F2, $F = 109.4$, $p < 0.001$ for F3, $F = 147.7$, $p < 0.001$ for F4, $F = 20.0$, $p < 0.001$ for F5). The BF of Zn in trials 1 and 4 was increased from 0.69 and 0.5 (initial) to 0.71 and 0.65 (final); however, in trials 2, 3 and 5 it was decreased from 0.68, 0.65 and 0.62 (initial) to 0.66, 0.63 and 0.59 (final) respectively during the vermicomposting process. The precipitation of Zn with hydroxides, carbonates, phosphates, sulfides and several other anions in addition to form complexes with organic legends (Kumpiene et al., 2008).

Speciation of Cu in trials 1 and 2 is given in Table 4.17a, and speciation in trials 3 and 4 is given in Table 4.18a. Fig. 4.17 shows speciation of Cu in trial 5 during the process. The F1 fraction of Cu was reduced in the range of 11.4-67% of total fraction in all trials except trial 1 in which F1 fraction was enhanced. The F2 fraction was reduced in the range of 20.6-48.8% of total fraction in trials 2, 3, 4 and 5 but increased in trial 1. The F3 fraction of Cu was reduced in trials 2, 3 and 4; in the range of 17.5-74.4% of total fraction; however this fraction was increased in the range of 11-72% of total fraction in trials 1 and 5. The F4 fraction of Cu was increased (percentage of total Cu fraction) in all trials. The reduction of F1 and F2 fractions of Cu in all trials could be attributed the interaction of these fractions with stabilized organic matter formed during vermicomposting of water hyacinth. The stabilized organic matter furnished chelating and phyto-hormonal elements which contain a high content of microbial matter and stabilized humic substances (Suthar, 2009: Hait and Tare, 2012). In addition reduction of these fractions might be due to accumulation of these fractions by earthworm, and transformation into F4 and F5 fraction. The F5 fraction of Cu was enhanced in the range of 8.8-14.5% of total Cu fraction in trials 2 and 5 however reduced in trials1, 3 and 4 during the vermicomposting process. The Cu was mainly present in F4 and F5 fraction in the water hyacinth vermicompost. The BF of Cu in trials 1, 3 and 4 was increased from 0.39, 0.25 and 0.23 (initial) to 0.62, 0.39 and 0.0.67 (final); however, in trials 2 and 5 it was decreased from 0.76 and 0.59 (initial) to 0.72, 0.55 (final) respectively during the vermicomposting process. The variation in F1, F2, F3, F4 and F5 fractions of Cu in different trials were significant ($F = 81.8$, $p < 0.001$ for F1, $F = 36.33$, $p < 0.001$ for F2, $F = 40.8$, $p < 0.001$ for F3, $F = 106.4$, $p < 0.001$ for F4, $F = 17.4$, $p < 0.001$ for F5).

Speciation of Mn in trials 1 and 2 is given in Table 4.17a, and speciation in trials 3 and 4 is given in Table 4.18a. Fig. 4.17 shows speciation of Mn in trial 5 during the process. The F1 fraction of Mn was reduced in the range of 18-51.4% of total fraction in all trials except trial 1 in which F1 fraction was enhanced. The F2 fraction was reduced in the range of 0.6-11.3% of total fraction in trials 4 and 5 but in trials 1, 2 and 3 it was increased about 25.7- 80.8% of the total fraction. The epithelial layer of gut absorbs most available fractions (F1 and F2 fractions) of Mn during the transiting of wastes through it as the bioaccumulation of a high concentration of metals in earthworm tissues is well documented (Suthar and Singh, 2008; Hait and Tare, 2012). The F3 fraction of Mn was increased in all trials; in the range of 29.8-158.8% of total fraction. The F4 fraction of Mn was increased in the range of 5.0-76.6% of total fraction in trials 1, 3, 4 and 5; however this fraction was reduced in trial 2.

The F5 fraction of Mn was reduced in the range of 2.9-69.7% of total Mn fraction in all trials during the vermicomposting process. The F3 and F4 fractions of Mn was increased in most trials, it might be due to conversion of F5 fraction into the F3 and F4 fraction during vermicomposting process. The Mn was mainly present in F1 fraction in the water hyacinth vermicompost. The rise in pH during vermicomposting can decrease Mn mobility by precipitate formation, by this means increasing the number of adsorption sites and decreasing the competition of H^+ for adsorption, followed by increase in metal stability with humic substances (Achiba et al., 2009). There was no study found for comparison with the present study. BF of Mn in trials 1, 2, 3 and 5 was increased from 0.66, 0.85, 0.82 and 0.84 (initial) to 0.89, 0.9, 0.87 and 0.89 (final) respectively; however, in trial 4 very little change was observed during the vermicomposting process. The variation in F1, F2, F3, F4 and F5 fractions of Mn in different trials were significant ($F = 44.8$, $p < 0.001$ for F1, $F = 536.8$, $p < 0.001$ for F2, $F = 48.8$, $p < 0.001$ for F3, $F = 34.9$, $p < 0.001$ for F4, $F = 84.0$, $p < 0.001$ for F5).

Speciation of Fe in trials 1 and 2 is given in Table 4.17a, and speciation in trials 3 and 4 is given in Table 4.18a. Fig. 4.17 shows speciation of Fe in trial 5 during vermicomposting. The F1 fraction of Fe was reduced in the range of 23.3-44.5% of total fraction in trials 1 and 2, however in trials 3, 4 and 5; it was increased in the range of 3-140% of total fraction. The F1 fraction accounted for a low percentage (0.2-0.9%) of the total fraction of Fe in the water hyacinth vermicompost in different trials. The F2 fraction was reduced in all trials in the range of 3.9-43.9% of total fraction it could be explained by increase in pH at end of the vermicomposting. The F2 fraction is susceptible to changes in pH and this can become soluble and mobilized when the pH is lowered (Liu et al., 2007). The F3 fraction of Fe was increased in all trials in the range of 8-39% of total fraction. The F4 fraction of Fe was increased in the range of 5.2-40.8% of total fraction in trials 2, 3, 4 and 5; however this fraction was reduced in trial 1. The F5 fraction of Fe was reduced in the range of 20.9-63.2% of total Fe fraction in all trials except trial 1 in which it was increased about 31% of total fraction of Fe during the vermicomposting process. The F3 and F4 fractions of Fe was increased in most of trials, it might be due to conversion of F5 fraction into the F3 and F4 fraction during vermicomposting process. The Fe was mainly present in F4 fraction in the water hyacinth vermicompost. The BF of Fe in trials 2, 3, 4 and 5 was increased from 0.76, 0.73, 0.61 and 0.76 (initial) to 0.8, 0.87, 0.86 and 0.86 (final) respectively; however in trial 1, BF was reduced slightly during the vermicomposting process. The increase in BF of Fe might be due to poor organometallic complexing property of Fe. The increase in bioavailability of Fe during vermicomposting can be attributed to a greater rate of degradation in the presence of higher concentrations of different microorganisms within earthworm intestine (Hait and Tare, 2012). The variation in F1, F2, F3, F4 and F5 fractions of Fe in different trials were significant ($F = 29.7$, $p < 0.001$ for F1, F = 88.0, $p < 0.001$ for F2, F = 133.3, $p < 0.001$ for F3, $F = 5.9$, $p < 0.001$ for F4, $F = 3334$, $p < 0.001$ for F5).

Speciation of Ni in trials 1 and 2 is given in Table 4.17b, and speciation in trials 3 and 4 is given in Table 4.18b. Fig. 4.17 shows speciation of Ni in trial 5 during vermicomposting. The F1 fraction of Ni was reduced in the range of 0.8-56% of total fraction in all trials. The minimum reduction of F1 fraction was observed in trial 1. The F2 fraction was reduced in the range of 11.1-47.8% of total fraction in all trials. The reduction of F2 fraction might be due to increase in pH during vermicomposting process. Due to increase in pH during vermicomposting process caused an increase in surface negative charge, resulting in an increase the reduction of F1 fraction might be due to interaction of Ni with many chemicals

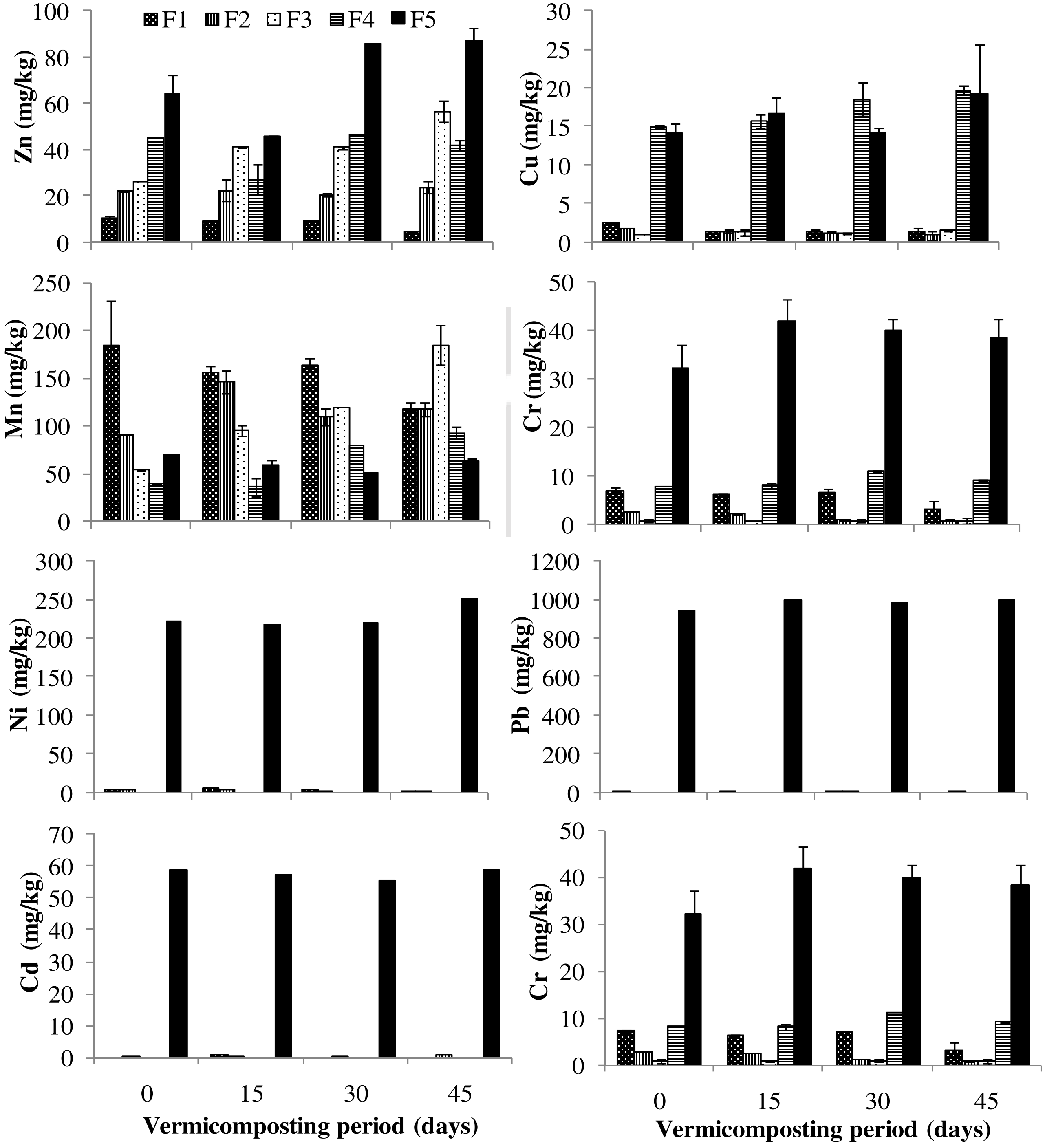


Fig. 4.17 speciation of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) in trial 5

and participated in detoxification processes, as part of the enzymes of the antioxidant systems, such as superoxide dismutase and in metallothioneins. In addition Ni adsorbed on stabilized organic matter and formed precipitate as nickel hydroxides (Maity et al., 2008).

Furthermore, the cutaneous absorption of metals was also affirmation in earthworms (Suthar, 2009). Both F1 and F2 fractions contributed <5% of the total fraction of Ni in all trials. About 15-30% in F1 and F2 fractions can cause environmental toxicity during mobility. The F3 and F4 fractions of Ni were not detected during the vermicomposting process. The F5 fraction of Ni was increased (percentage of total fraction) in all trials during the vermicomposting process. The F5 fraction of Ni was dominant from initial to final vermicompost. In present study F5 fraction of Ni was about 94-97%; however, Wong and Selvam (2006) reported about 75% of Ni bounded with F5 fraction during co-composting of sewage sludge and lime. The proportions of F1 and F2 fractions were decreased while the proportion of F5 fraction Ni was increased. The results demonstrate that these F1 and F2 fractions of Ni wre transformed during vermicomposting to the F5 fraction. Zheng et al. (2007) also repoterted similar results during sewage sludge composting. During vermicomposting, the stabilization of Ni offers the potential to reduce the risks of the heavy metals in the final vermicompost. Ni occurred predominantly in the F5 fraction, which agreed with the report of Zheng et al. (2007) and Wang et al. (2008). Fuentes et al. (2004) also reported maximum amount of Ni bound with F5 fraction. The significant increase in the level of Ni with F5 fraction may be due to alkaline stabilization process (Gupta and Sinha, 2007). The BF of Ni in all trials 1, 2, 3, 4 and 5 was decreased from 0.046, 0.069, 0.054, 0.049 and 0.048 (initial) to 0.044, 0.039, 0.042, 0.031 and 0.027 (final) respectively during the vermicomposting process. The variation in F1, F2 and F5 fractions of Ni in different trials were significant ($F = 38.9$, $p < 0.001$ for F1, $F = 3.3$, $p < 0.001$ for F2, $F = 36.9$, $p < 0.001$ for F5).

- **Speciation of Pb, Cd and Cr**

Speciation of Pb in trials 1 and 2 is given in Table 4.17b, and speciation in trials 3 and 4 is given in Table 4.18b. Fig. 4.17 shows speciation of Pb in trial 5 during vermicomposting. The F1 fraction of Pb was reduced in the range of 0.8-15.7% of total fraction in all trials. The higher reduction of F1 fraction was observed in trials 2 and 5. In the present study F1 fraction of Pb was about 0.8-1.5% of total fraction while Li et al. (2010) reported about 15% F1 fraction during vermicomposting of pig manure. The F2 fraction was increased in all trials; however in trial 2 this fraction was reduced about 33.3% of total fraction. The F3 and F4 fractions of Pb were not detected during the vermicomposting process. The F5 fraction was reduced in all trials except trial 2 in which this fraction was increased slightly during the vermicomposting process. The reduction of F1, F2 and F5 fraction of Pb might be due to accumulation of Pb by *E. fetida*. It has been considered that the adult earthworm to have an ability to store high concentrations of heavy metals in the non-toxic forms and formation of Pb phosphate nodules in earthworms was a Pb storage mechanism (Li et al., 2010). The F5 fraction of Pb was dominant from initial to final vermicompost. The variation in F1, F2 and F5 fractions of Pb in different trials were significant ($F = 21.2$, $p < 0.001$ for F1, $F = 149.4$, $p < 0.001$ for F2, $F = 23.9$, $p < 0.001$ for F5). BF of Pb in all trials 1, 3 and 4 was increased from 0.019, 0.015 and 0.015 (initial) to 0.024, 0.020 and 0.019 (final) respectively; however in trials 2 and 5, BF was reduced slightly during the vermicomposting process.

Speciation of Cd in trials 1 and 2 is given in Table 4.17b, and speciation in trials 3 and 4 is given in Table 4.18b. Fig. 4.17 shows speciation of Cd in trial 5 during vermicomposting. The F1 fraction of Cd was reduced in the range of 5.0-100% of total fraction in all trials. The 100% reduction was observed in all trials except trial 1 during the vermicomposting process. The reduction in F1 fraction of Cd was in all trials might be due to accumulation of this fraction by earthworm in their tissues (Suthar and Singh, 2008). The F1 fraction of Cd tended to decrease in concentration during the vermicomposting process in response to the increase of pH in the range of 6-6.8 (initial) to7.5-7.7 (final) in all trials. Similar finding was also reported by Hanc et al. (2009) during composting of sewage sludge and after soil application. The F2 fraction was increased in all trials; however in trial 2, this fraction was reduced about 40.1% of total fraction. The F3 and F4 fractions of Cd were not detected during the vermicomposting process. The F5 fraction was increased in the range of 0.4-2.5% in all trials except trial 1 in which this fraction was reduced about 0.8% of total fraction during the vermicomposting process. The F5 fraction of Cd was dominant from initial to final vermicompost. The Cd in the F5 fraction is generally considered permanently bound in the crystal lattice and as a result not capable of entering the food chain under normal conditions (Ciba et al., 2003).

Table 4.18a Speciation of heavy metals (Zn, Cu, Mn and Fe) in trials 1 and 2 during 45 days of vermicomposting period (Mean ± SD, n = 3)

Days	Vermireactors									
	Trial 1					**Trial 2**				
	Zn (mg/kg)					**Zn (mg/kg)**				
	F1	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
0	13.7±0.2c	21.9±0.3c	31.7±0.12c	25.1±0.3b	49.2±1.0ad	10.1±0.1d	21.9±0.4c	32.4±0.2c	19.4±0.2b	85.4±0.1ad
15	10.1±1.1a	22.9±2.5ab	41.7±0.9bc	27.6±0.14a	68.6±8.2a	7.3±0.9ab	22.2±2.1ab	37.9±2.03bc	29.1±5.02a	59.9±5.3a
30	5.4±0.1b	24.7±3.8a	50.0±4.6a	36.1±5.0ab	71.7±5.9b	6.3±0.11b	28.9±1.3ac	45.2±2.2a	28.6±2.0ab	53.2±2.0b
45	4.8±0.05b	22.8±0.7b	53.3±6.0b	44.7±0.5a	72.0±10.7a	5.0±0.4b	22.4±1.1cb	56.8±5.5b	46.2±2.1a	69.6±4.1a
Days	**Cu (mg/kg)**					**Cu (mg/kg)**				
0	1.6±0.04c	1.6±0.05c	1.8±0.05c	6.9±0.1c	35.0±0.3c	2.1±0.03d	1.6±0.03c	1.0±0.04d	6.0±0.4d	37.1±0.8c
15	1.6±0.2a	1.5±0.5a	1.4±0.0a	14.9±0.9a	28.0±5.3b	1.6±0.13a	1.3±0.2a	1.0±0.1a	6.6±2.0b	30.9±0.1cb
30	1.8±0.1ac	1.3±0.03b	1.0±0.2a	16.1±3.0a	31.1±0.3c	2.2±0.1b	1.6±0.03ab	0.8±0.2a	11.2±0.8b	19.3±0.4ab
45	1.6±0.2a	1.3±0.05ab	0.8±0.1b	17.4±0.3b	32.3±1.5b	1.3±0.03b	1.3±0.2b	1.3±0.2c	27.8±1.4c	15.2±4.5a
Days	**Mn (mg/kg)**					**Mn (mg/kg)**				
0	182±0.6bc	93.7±0.6c	79.7±0.5a	52.1±0.5c	92.0±6.5c	154.0±3.8bcd	141.7±0.6d	63.8±5.5bc	53.9±0.4d	55.6±4.1b
15	180.3±1.7a	82±16.3ab	94.6±3.8adc	54.8±0.7a	91.3±2.8c	141.6±1.0a	113.6±0.9b	87.0±13.3a	54.3±4.1a	68.3±1.8b
30	141±1.5abc	137±24.3a	115.0±5.3a	62.3±11.3abc	78.5±3.0a	132.5±2.5ab	119.1±4.8a	97.4±5.5a	52.5±1.5bc	67.0±2.5b
45	105.8±5.8a	143.3±9.7a	129.7±2.7ab	54.5±2.3b	63.5±3.5ac	117.5±2.5a	158.6±12.1a	166.3±0.0ac	81.0±0.5ac	68.0±11.0ac
Days	**Fe (mg/kg)**					**Fe (mg/kg)**				
0	15.9±0.4c	35.6±0.1c	2243±5.5c	5306.1±15b	2709±196bb	22.0±0.3b	32.6±1.13c	2154±9d	4877±7.9b	4541.0±1.0c
15	15.4±4bc	25.1±2b	2407±2161b	5645±93ab	2798±782.5b	47±25ab	27.7±0.5ab	1867±72c	4608±398b	1360.0±65c
30	22.7±0.3b	26.6±4b	2425±180ab	6292±210b	2303±423a	43.3±5.3c	23.4±7.4ab	1689±160b	4760±432c	1120.0±40b
45	22.9±3bd	31.7±3b	2376±239bc	6131±491b	1258±13c	43.3±7.1c	25.2±1.2b	2397±276abc	5491±177bc	1337.5±48c

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$)

Table 4.18b Speciation of heavy metals (Cr, Ni, Cd and Pb) in trials 1 and 2 during 45 days of vermicomposting period (Mean ± SD, n = 3)

Days	Vermireactors									
	Trial 1					**Trial 2**				
	Ni (mg/kg)					**Ni (mg/kg)**				
	F1	**F2**	**F3**	**F4**	**F5**	**F1**	**F2**	**F3**	**F4**	**F5**
0	6.0±0.6a	5.5±0.1a	ND	ND	198.5±9.5a	6.4±0.4a	5.2±0.4a	ND	ND	226.3±7.8a
15	7.1±0.8ab	4.2±0.05bc	ND	ND	213.3±1.8ab	6.5±0.3b	5.3±0.1a	ND	ND	200.8±5.8b
30	5.6±1.2a	4.3±0.7ab	ND	ND	225.8±2.3a	4.2±0.4a	4.6±0.4a	ND	ND	209.5±7.5a
45	4.6±0.2b	4.7±0.1a	ND	ND	212.3±6.8b	2.6±0.5c	4.2±0.4a	ND	ND	213.3±3.3b
Days	**Pb (mg/kg)**					**Pb (mg/kg)**				
0	9.6±0.6b	4.6±0.6a	ND	ND	910.5±9.5a	9.85±0.4b	5.3±0.3a	ND	ND	1010±15c
15	9.7±1.4bd	3.3±0.8ac	ND	ND	942.5±27.5ab	13.8±0.8c	3.5±1.5ac	ND	ND	920.0±30a
30	8.5±1.5c	6.5±0.5b	ND	ND	871.0±34b	9.5±1.5c	7.8±1.3ab	ND	ND	852.5±27.5b
45	9.8±0.8ab	10.0±0.5a	ND	ND	937.5±17.5a	8.0±0.5b	10.0±0.5a	ND	ND	922.5±57.5a
Days	**Cd (mg/kg)**					**Cd (mg/kg)**				
0	0.7±0.2a	0.4±0.005a	ND	ND	53.7±4.7a	0.8±0.2a	0.5±0.08ab	ND	ND	53.9±1.8a
15	0.6±0.05a	0.3±0.05ac	ND	ND	54.0±6.0a	0.6±0.08a	0.6±0.08b	ND	ND	52.8±3.3a
30	0.4±0.2a	0.2±0.05b	ND	ND	47.0±0.5a	0.4±0.06a	0.7±0.05ac	ND	ND	53.8±0.8a
45	0.0+0.0b	0.7±0.05a	ND	ND	48.8±0.3a	0.0+0.0b	0.7±0.03a	ND	ND	52.3±2.2a
Days	**Cr (mg/kg)**					**Cr (mg/kg)**				
0	6.6±0.05a	3.1±0.4b	0.6±0.5b	8.5±0.06b	39.5±9.5b	7.1±0.4a	2.5±0.1a	0.5±0.08b	7.6±0.06c	35.1±0.6b
15	6.0±0.5a	2.3±0.2a	0.3±0.04a	8.8±1.0a	38.3±3.3a	6.4±0.3a	2.5±0.22a	0.3±0.02a	8.6±0.24a	23.0±6.5b
30	4.5±0.03c	1.8±0.5a	0.4±0.05a	9.3±2.0b	46.0±1.0b	6.9±0.7b	1.8±0.05a	0.5±0.2a	10.4±0.6b	40.3±7.8b
45	2.8±0.6b	1.2±0.05b	0.1±0.04b	10.8±0.6b	42.0±1.0a	3.0±0.1b	0.9±0.0b	0.3±0.05b	10.0±0.23b	51.0±4.0b

Mean value followed by different letters in columns is statistically different (ANOVA; $p < 0.05$); ND - Not detected

In the present study, F5 fraction of Cd was in the range of 96-99% of total fraction during vermicomposting; while Hanc et al. (2009) reported that the highest part of Cd was present in the F5 fraction (about 60%). BF of Cd was increased in trial 1; however, in trials 2, 3, 4 and 5, it was reduced from 0.034, 0.02, 0.022 and 0.015 (initial) to 0.01, 0.014, 0.013 and 0.012 (final) during the vermicomposting process. The pH and transformation of organic matter to humic substances during vermincomposting process might influence the transformation of Cd and as a result the speciation distribution. The variation in F1, F2 and F5 fractions of Cd in different trials were significant ($F = 13.6$, $p < 0.001$ for F1, $F = 24.0$, $p < 0.001$ for F2, $F = 7.9$, $p < 0.001$ for F5).

Cr is an essential nutrient for human, there is no uncertainty that Cr (V1) compounds are both acutely and chronically toxic, in contrast to Cr (VI), Cr (III) is less toxic to mammalian, aquatic organism and plants due to its low solubility, mobility and bioavailability (Zhou et al., 2006). Speciation of Cr in trials 1 and 2 is given in Table 4.17b, and speciation in trials 3 and 4 is given in Table 4.18b. Fig. 4.17 shows speciation of Cr in trial 5 during the process. The F1 fraction of Cr was reduced in the range of 27.7-66.3% of total fraction in all trials. Higher reduction was observed in all trials except trial 1. The F2 fraction of Cr was reduced in the range of 40.3-72.4% of total fraction in all trials. The higher reduction in F1 and F2 fractions were observed about 66.3 and 72.4% of total fraction respectively in trials 4.

The F3 fraction of Cr was increased in trials 1 and 5; on the other hand, this fraction was reduced in trials 2, 3 and 4 in the range of 48-81.8% of total fraction at the end of vermicomposting. The decline in F1, F2 and F3 fraction of Cr during vermicomposting probably caused by the reduction of Cr(VI) to Cr(III) by biological organisms, followed by the combination of Cr(III) with decomposed organic materials (Hait and Tare, 2012). The F4 fraction of Cr was increased in the range of 0.8-23.2% of total fraction in trials 2, 3, 4 and 5; however this fraction was reduced in trial 1. The F5 fraction of Cr was increased in the range of 6.4-24.7% of total Cr fraction in all trials during the vermicomposting process. Cr was distributed in all five fractions but it was maximum present in F5 fraction followed by F4 fraction. Gupta and Sinha (2007) also reported that maximum level of Cr was associated with F5 fraction followed by the F4 fraction. The organic matter is one of the factors that may reduce the ability of these metals to be phytotoxic in the soil due to metal-organic complexation. BF of Cr in all trials 1, 2, 3, 4 and 5 was increased from 0.46, 0.34, 0.33, 0.34 and 0.37 (initial) to 0.33, 0.26, 0.26, 0.22 and 0.27 (final) respectively during the vermicomposting process. The reduction of BF of Cr during vermicomposting process, it might be the due to bioaccumulation of Cr by *E.*

fetida and organometallic complex formation. The variation in F1, F2, F3, F4 and F5 fractions of Cr in different trials were significant ($F = 17.5$, $p < 0.001$ for F1, $F = 12.2$, $p < 0.001$ for F2, $F = 12.7$, $p < 0.001$ for F3, $F = 60.6$, $p < 0.001$ for F4, $F = 40.03$, $p < 0.001$ for F5).

4.3.6 CONCLUSION OF PHASE 3

A higher proportion of water hyacinth in the feed mixture hindered the growth and productiveness of the earthworms. An addition of cattle manure in the feed mixture enhanced the growth and productiveness of the earthworms. The reduction of bioavailability and leachability of heavy metals were not correlated with earthworm productiveness during the process. The pH was increased significantly during the process which was main factor for reducing bioavailability of heavy metals. The total concentration of heavy metals (Cu, Mn, Fe, Ni and Cd) was decreased in the in some trials. Water solubility of Zn, Cu, Mn and Cr was reduced significantly in all trials except trial 1. DTPA extractability of Cu, Fe, Ni and Cr was reduced significantly. Leachability of Zn, Cu, Fe, Ni, Pb, Cd and Cr was reduced significantly during the vermicomposting process. Leachable concentration of heavy metals in all trials was under the threshold limits. Water soluble, DTPA extractable and leachable heavy metals were positively correlated with pH, organic matter, electrical conductivity, ash content and total metal concentration.

The F1 fraction of Mn was dominant in initial feed mixture but in the final vermicompost it was converted into less mobile fractions. The F1 fraction of Cd was reduced 100% during the vermicompostng of water hyacinth. The order of BF of different metals as follow: Mn (0.9) > Fe (0.86) > Zn (0.7) > Cu (0.6) > Cr (0.5) > Ni (0.07) > Cd (0.03) > Pb (0.02). The order of BF indicates that toxicity of metals does not depend on total metal concentration. The total concentration of Pb was higher than Zn, Cu, Mn, Ni, Cd and Cr but its BF was lowest among the all eight metals. The F3 and F4 fractions of Ni, Pb and Cd were not detected during vermicomposting of water hyacinth. Vermicomposting of water hyacinth by using *Eisenia fetida* was very effective for reduction of bioavailability of heavy metals in comparison to agitated pile and rotary drum composting. This study suggests that the need for continuous monitoring of bioavailability, leachability and speciation of heavy metals is essential rather than total metal concentration in the final vermicompost.

4.4 SUMMARY

This study was carried out on bioavailability, leachability and speciation of heavy metals during composting with different proportions of water hyacinth, cattle manure and sawdust using different composting techniques i.c. agitated pile, rotary drum and vermicomposting. Some key observations are given as follow:

- Composting and vermicomposting was successful for conversion of water hyacinth into stabilizes organic matter. It can be suggested that water hyacinth, cattle manure and sawdust ratio (6:3:1, Trial 4) was better in agitated pile and rotary drum composting with respect to organic matter degradation, reduction of bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate fractions), however in the vermicomposting these parameters were not depending on water hyacinth, cattle manure and sawdust ratio. Therefore, it can be suggested that in trial 4 majorities of results were found better than other trials for both agitated pile and rotary drum composting.
- Higher degradation of organic matter was observed in vermicomposting followed by rotary drum and agitated pile composting. Nutrients such as Na, K, Ca and Mg were increased during all three types of composting process.
- Total metal concentration was increased in agitated pile and rotary drum composting, but concentration of some metals such as Cu, Mn, Fe, Ni and Cd was reduced during vermicomosting process.
- Water solubility of Fe was increased in all trials of agitated pile composting; however its solubility reduced in most trials of rotary drum and vermicomposting. It can be suggested that water solubility, DTPA extractability and leachability of heavy metals reduced significantly during vermicomposting in comparison to traditional composting.
- Water solubility of Ni, Pb and Cd, and DTPA extractability of Pb and Cd were not found in all three types of composting processess. Leachability of Ni, Pb and Cd was not found with 0.1M $CaCl_2$ extraction (phase 1); however leachability of all selected heavy metals was observed with TCLP extraction (phase 2 and 3).
- The reducible and oxidizable fractions were not detected in all three types of composing processess. Bioavailability factor was reduced higher in rotary drum composting in comparison to agitated pile and vermicomposting; however exchangeable and carbonate fractions were reduced higher in vermicomposting in comparison to agitated pile and rotary drum composting.

- Bioavailability and leachability of heavy metals were not only reduced but total concentration of heavy metals was also reduced during the vermicomposting process using *Eisenia fetida.*
- Finally, this study concluded that the performance of vermicomposting was better than agitated pile and rotary drum composting of water hyacinth mixed with cattle manure and sawdust.

Chapter 5

EFFECTS OF LIME AND NATURAL ZEOLITE DURING COMPOSTING PROCESS

This chapter explained phase 4 as mentioned in chapter 3 (3.1 Experimental design section). The effects of lime and natural zeolite on the bioavailability, leachability and speciation of heavy metals were studied during the agitated pile and rotary drum composting process. The study was conducted in four sections given as follows:

1. Effects of lime on bioavailability, leachability and speciation of heavy metals during agitated pile composting
2. Effects of lime on bioavailability, leachability and speciation of heavy metals during rotary drum composting
3. Effects of natural zeolite on bioavailability, leachability and speciation of heavy metals during agitated pile composting
4. Effects of natural zeolite on bioavailability, leachability and speciation of heavy metals during rotary drum composting

5.1 EFFECTS OF LIME DURING AGITATED PILE COMPOSTING

Various studies have been carried out on the enhancement of the composting process to minimize the bioavailability of heavy metals to plants using various alkaline materials such as lime, coal fly ash etc. (Fang and Wong, 1999; Wong and Selvam, 2006; Chiang et al., 2007; Wang et al., 2008). In India, about 0.75 million tones of lime sludge generated per year during acetylene gas production and expected to increase annually due to very limited utilization of this lime sludge (CPCB, 2006). Phase 1 study suggested that mixture of 90 kg water hyacinth, 45 kg cattle manure and 15 kg sawdust (Trial 4) was best combination for reduction of bioavailability of heavy metals during agitated pile composting of water hyacinth. Therefore, same combination (Trial 4) was selected for lime 1 treatment (1%), 2 treatment (2%) and 3 treatment (3%) studies. Trial 4 was considered as control with respect to lime addition.

5.1.1 PHYSICO-CHEMICAL ANALYSIS

Fig. 5.1 shows the temperature profiles of agitated pile composting with lime. The reaction temperature during composting process was increased rapidly up to the thermophilic phase (about 57°C) as a result of the intense microbial activity. Fang and Wong (1999) also reported more than 50°C temperature during thermophilic phase in sewage sludge composting. Long duration (about 15 days) of thermophilic stage was observed in lime added composting process in comparison to control (one week). In lime 2 treatment, very quick development of thermophilic stage was observed and subsequently the temperature decreased at the final stage. After 14 days, the temperature decreased to about 30°C, indicating that the speed of biodegradation was diminished. Similar trend of temperature was also observed by Fang and Wong (1999) during sewage sludge composting with lime. The duration of thermophilic phase was increased with lime amendment percentage in the water hyacinth composting mixture. Higher percentage of lime addition caused slow down the metabolic activity of microbes due to high alkalinity (Wong and Fang, 2000).

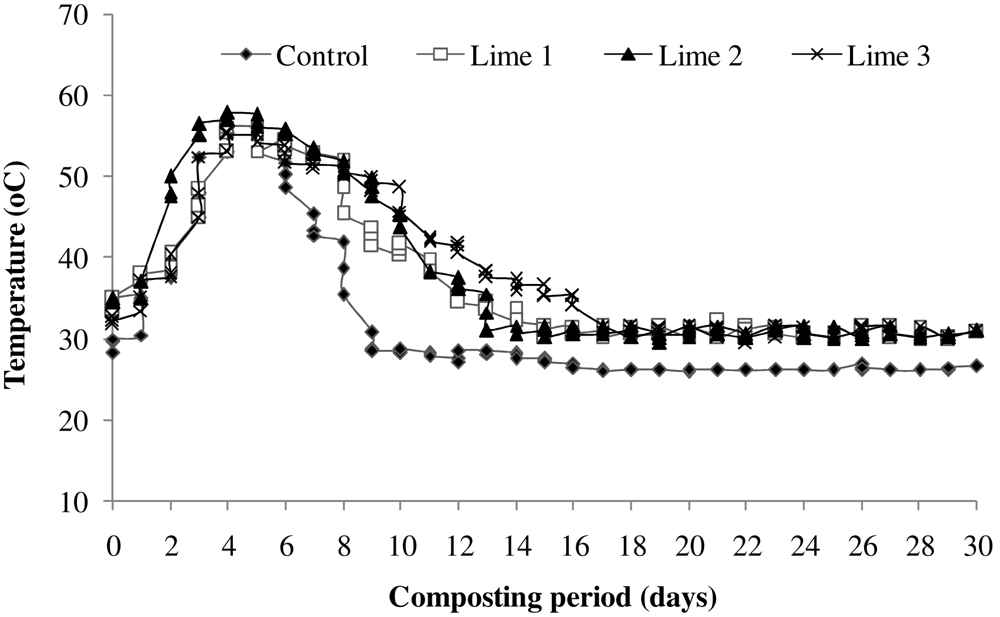


Fig. 5.1 Variation of temperature during pile composting with lime

The moisture content of the compost was reduced significantly ($F = 115.3$, $p < 0.05$) from 83.9, 86.8, 83.8 and 82.4% to 37.6, 43.2, 40.2 and 38.3% in control, lime 1, 2 and 3 treatments respectively during composting process (Fig. 5.2a). Lower reduction of moisture was observed in lime addition compost in comparison to control. This indicated that the lime could supply better moisture holding capacity and promote the optimum microbial degradation rate (Chiang et al., 2007). Due to addition of lime initial pH of the compost mixtures was higher than control compost mixture but it decreased gradually thereafter to nearly neutral. Similar results were also observed with the control. Initially maximum pH value was observed about 10.4 in lime 3 treatment; however in the final compost pH values were in the range of 7.3-7.7 (Fig. 5.2b). The similar trends of pH change were also reported by other researchers (Fang and Wong, 1999, 2000; Wong and Selvam, 2006; Chiang et al., 2007). Alkaline lime could neutralize the organic acids released during composting, and thus reduce the formation of metal-organic matter complexes during lime-sludge co-composting (Wong and Selvam, 2006). The changes in pH during composting was significant ($F = 28.1$, $p < 0.001$). Fig. 5.2c shows the decreasing trend of organic matter degradation during composting process. The maximum reduction of organic matter was observed in lime 2 treatment (38.5%) followed by lime 1 treatment (35.1%), lime 3 treatment (27.8%) and control (23%). The decrease of organic matter might be due to the reduction of the substrate carbon resulting in CO_2 loss (Zheng et al., 2007). During the composting process organic matter is decomposed and transformed to stable humic substances which have a capacity to interact with metal ions (Cai et al., 2007). The organic matter was reduced significantly ($F = 23.6$, $p < 0.001$) during the composting process.

High electrical conductivity (EC) in the final compost of water hyacinth is unwanted since it will slow down plant rooting and reduce the transportation of water and nutrients into the plants (Chiang et al., 2007). As shown in Fig. 5.2d, the EC values were decreased with the increase in composting time in control and all lime added composting process. The EC values were lower in the lime treated compost in comparison to control. The final EC values of the all compost were about 4.7, 1.9, 1.7 and 1.9 dS/m in control, lime 1, 2 and 3 treatments respectively. Similar values of EC in lime amended compost were also monitored by Fang and Wong (1999). The EC was decreased significantly ($F = 46.6$, $p < 0.05$) in control and lime amended compost. The total nitrogen (TN) was increased about 33.5, 31.6, 29.1 and 12.44% in control, lime 1, 2 and 3 treatments respectively during the process. It might be due to net loss of organic matter in terms of CO_2 and water vapour. Nitrogen fixing bacteria might also accountable for increasing TN in the later stage of composting (Kalamdhad et al., 2009). Fig. 5.3a shows the significant changes in TN ($F = 4.6$, $p < 0.001$) during the composting process.

The NH_4-N transform into the organic-N due to growth of the microbial population (Bernal et al., 1993). Odour emission was not observed during the composting process. The NH_4-N was decreased significantly ($F = 251$, $p < 0.05$) about 63, 56.2, 57.5 and 51.9% in control, lime 1, 2 and 3 treatments respectively during the composting process. According to Kalamdhad et al. (2009) the absence or decrease in NH_4-N is an indicator of high-quality composting process, similarly the decrease in the NH_4-N content shown in

Fig. 5.3b. The NH_4-N concentration was sharply decreased during themophilic phase in all lime treatments in comparison to control due to shifting of the ammonia-ammonium ion equilibrium at high pH (Wong et al., 2009).

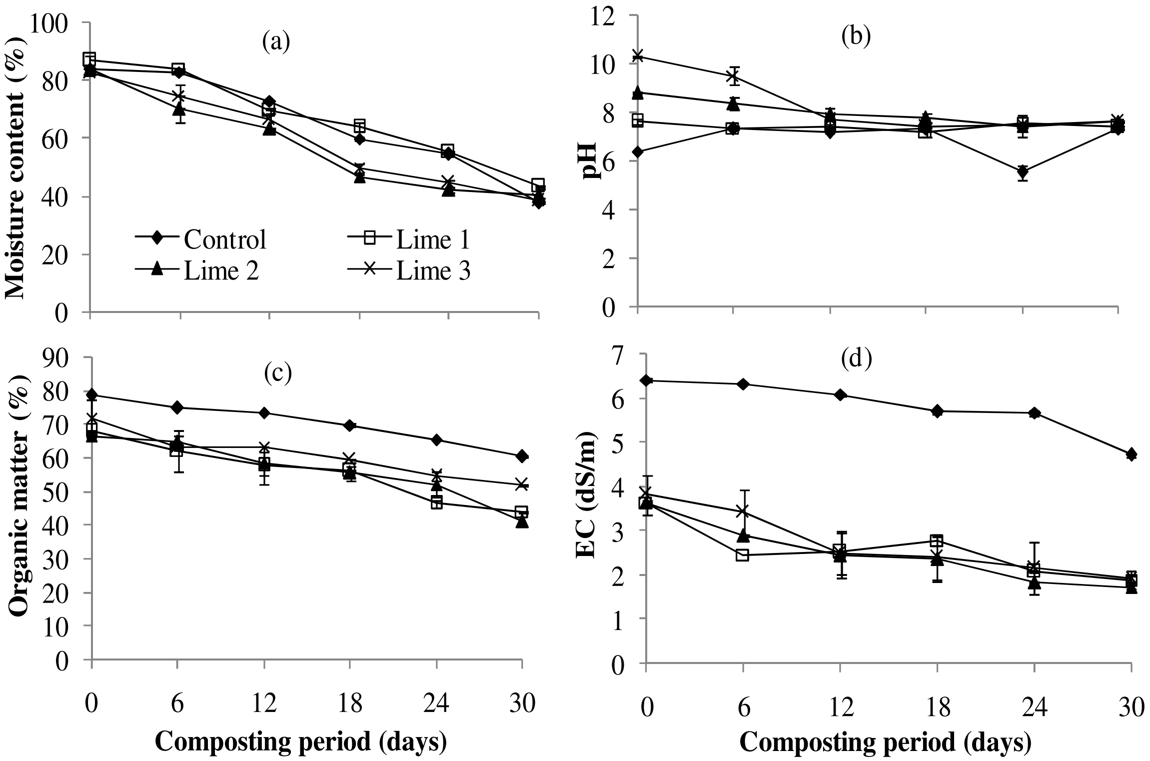


Fig. 5.2 Variation of physico-chemical parameters: (a) moisture content (b) pH (c) organic matter and (d) electrical conductivity (EC) during pile composting with lime (bars denoted as ± standard deviation)

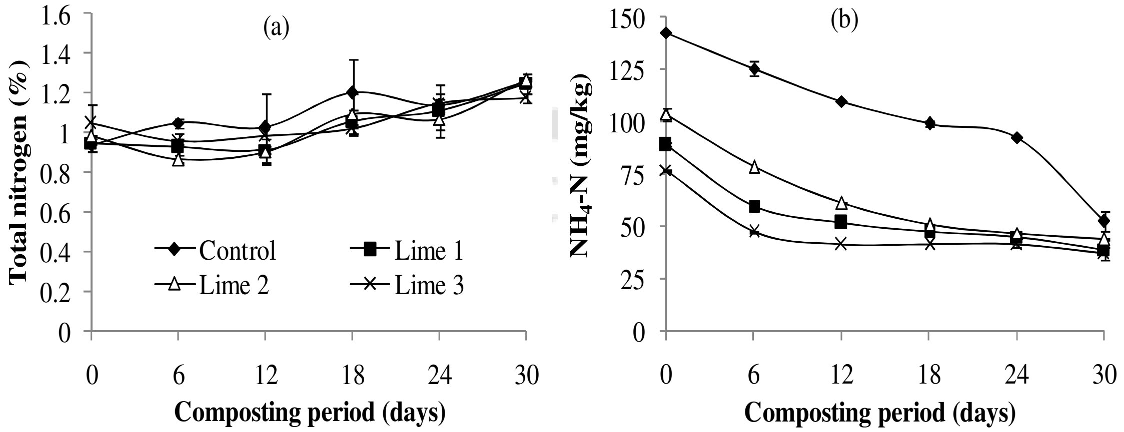


Fig. 5.3 Variation of total nitrogen (a) and NH_4-N (b) during pile composting with lime (bars denoted as ± standard deviation)

However after themophilic phase its concentration was reduced gradually till the end of composting process, it might be due to change in highly alkaline to neutral or slightly alkaline condition. In the present study, the final NH_4-N concentrations of all these treatments were below 500 mg/kg, considered as a characteristic of mature compost (Wong et al., 2009). Ammonia volatilization from composting system depends on the available carbon source for the microorganisms. When an easily available carbon source (cattle manure) is being degraded by microorganisms, the NH_4-N transform into the organic-N due to growth of the microbial population (Bernal et al., 1993). Odour emission was not observed during the composting process.

Fig. 5.4 illustrates the significant variation in concentration of the nutrients as Na ($F = 8.26$, $p < 0.001$), K ($F = 23.11$, $p < 0.001$), Ca ($F = 32.3$, $p < 0.001$) and Mg ($F = 39.87$, $p < 0.001$) which increased significantly in control and all lime treatments. The concentration of Na, K, Ca and Mg was increased from its initial concentration at the end of the composting process due to the net loss of dry mass (Zorpas et al., 2000). The concentration of Ca was increased about 5-6 times than control due to addition of lime.

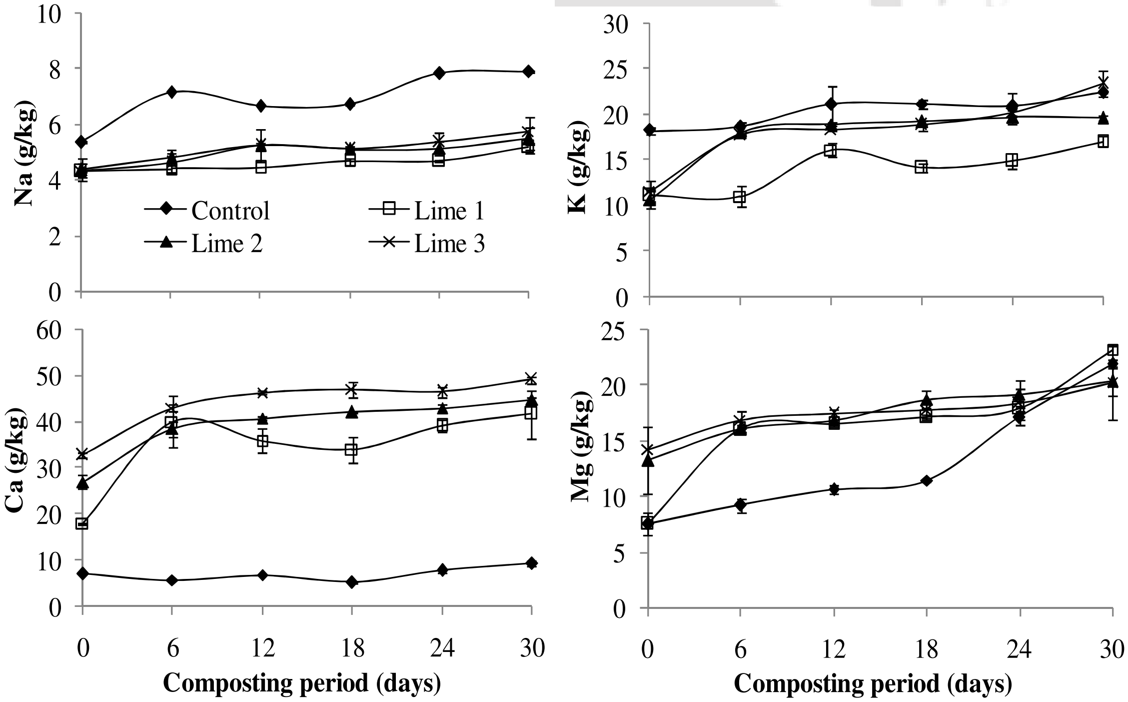


Fig. 5.4 Variation of nutrients (Na, K, Ca and Mg) during pile composting with lime (bars denoted as ± standard deviation)

Fig. 5.5 illustrates the variation in total concentration of metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) in control and lime added compost during 30 days of composting period.

The total concentration of metals was increased due to reduction of organic matter and release of CO_2 during the mineralization processes (Zorpas et al., 2000).

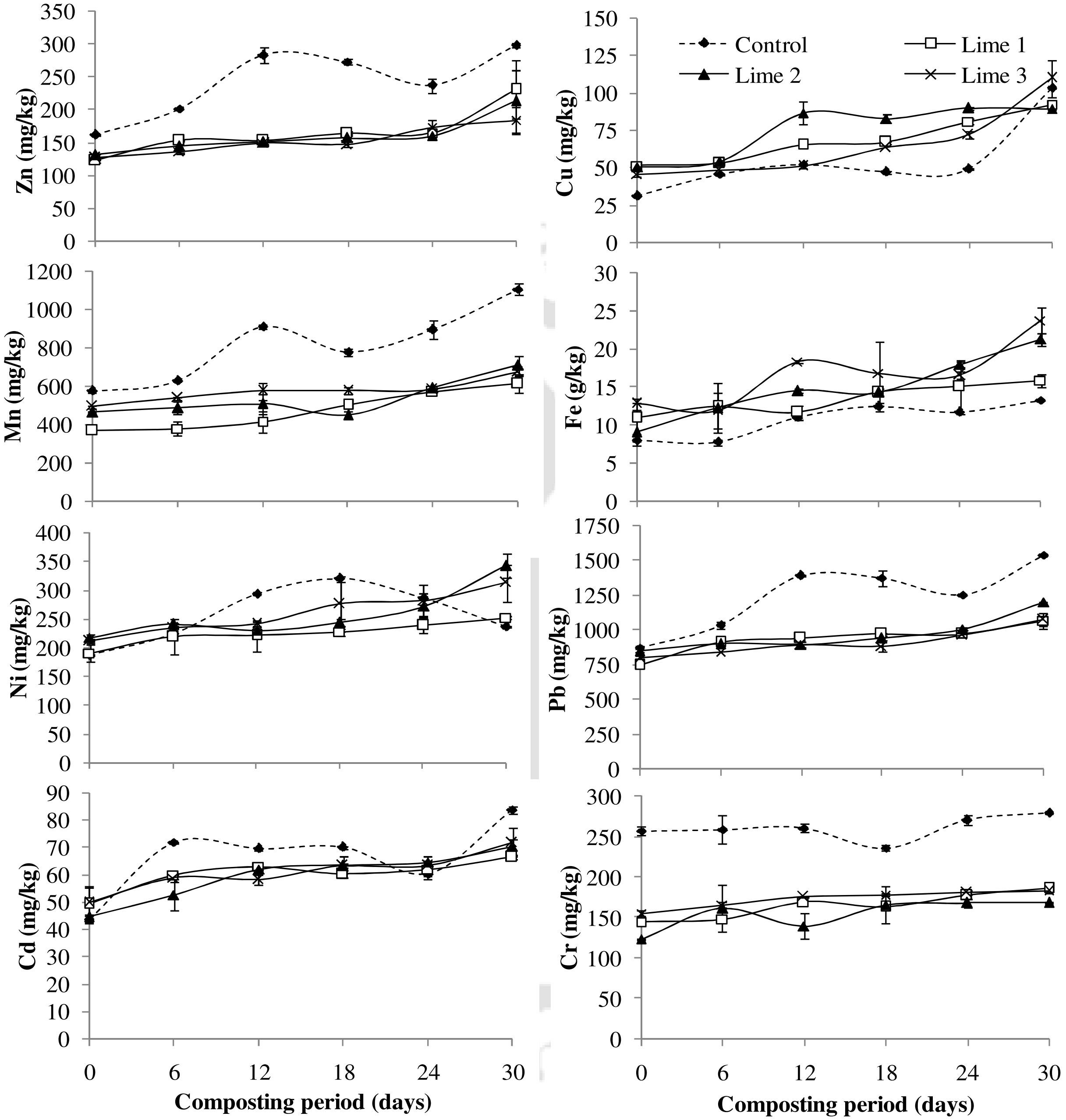


Fig. 5.5 Variation of total heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) concentration during pile composting with lime

The variation in Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in control and lime amended compost were significant ($F = 34.8$, $p < 0.001$ for Zn, $F = 179.02$, $p < 0.05$ for

Cu, $F = 21.94$, $p < 0.001$ for Mn, $F = 9.14$, $p < 0.001$ for Fe, $F = 30.13$, $p < 0.001$ for Ni, $F = 45.24$, $p < 0.05$ for Pb, $F = 27.5$, $p < 0.001$ for Cd, $F = 8.46$, $p < 0.001$ for Cr).

5.1.2 BIOAVAILABILITY OF HEAVY METALS

- **Water soluble heavy metals**

Fig. 5.6 shows the changes in water-soluble concentration of Zn, Cu, Mn, Fe and Cr during water hyacinth composting process. The quantity of water soluble heavy metals in compost was low as compared to their total contents; it is believed that water-soluble heavy metals belong to the most toxic fraction in sludge compost to plants (Fang and Wong, 1999). Addition of lime caused a significant reduction in water-soluble Zn, Cu, Fe and Cr contents at the end of composting process. Fang and Wong (1999) also reported that water soluble concentration of Zn and Cu was reduced in the sewage sludge compost. Furthermore, Zn occurs predominantly as Zn^{2+} species at pH < 7.5 and is expected to form hydroxides at pH 7.5-11.5; the dominant species of Zn(II) are $Zn(OH)_2$. In addition to Zn hydroxy-complexes, a calcium-Zn complex hydrated compound ($CaZn_2\ (OH)_6.2H_2O$) may also form at pH 7-12, consequently the solubility of Zn was reduced due to complex formation (Chen et al., 2009).

The water solubility of Mn was increased in control, lime 2 and 3 treatments; however it was reduced very slightly in lime 1 (Fig. 5.6). Although the water soluble percentage of total Mn was reduced in control and lime 1 treatment, and increased in lime 2 and 3 treatments during the composting process. However, Fang and Wong (1999) reported that water soluble content of Mn reduced during sewage sludge composting process. This has been clarified by the various sources of composting materials and chemical additions that affected the water solubility of Mn (Fang and Wong, 1999; Li et al., 2012). Concentration of water soluble Fe was increased in the control but even lowest percentage of lime addition effectively reduced the water solubility of Fe during composting process. The higher reduction of Cu, Fe and Cr was observed in lime 2 treatment about 85, 61 and 88% of total metal respectively. It might be due to higher reduction of organic matter and formation of organo-metallic complexes during the composting process (Fang and Wong, 1999). Addition of lime shows a strong resistance against the increasing trend of Cu, Fe and Cr concentrations. Therefore, lime amendment was very efficient in reducing bioavailability of heavy metals during water hyacinth composting by forming less soluble carbonate salts (Fang and Wong, 1999). Calcium hydroxide breaks into calcium ion and hydroxide ion in the presence of water (Montes-Hernandez et al., 2009). Hydroxide ion combined with metal ion (M^{2+}) and form metal hydroxides. These metal hydroxides might be adsorbed on charged colloids such as degraded organic matter, consequently reduces the metal solubility (Garau et al., 2007). Lime increased the pH which reduces the bioavailability of most heavy metals (Baker et al., 2011). Furthermore, the water solubility of heavy metals reduced due to biosorption by the microbial biomass or metal complexation with the newly formed humic substances (Garcia et al., 1995; Castaldi et al., 2006; Cai et al., 2007).

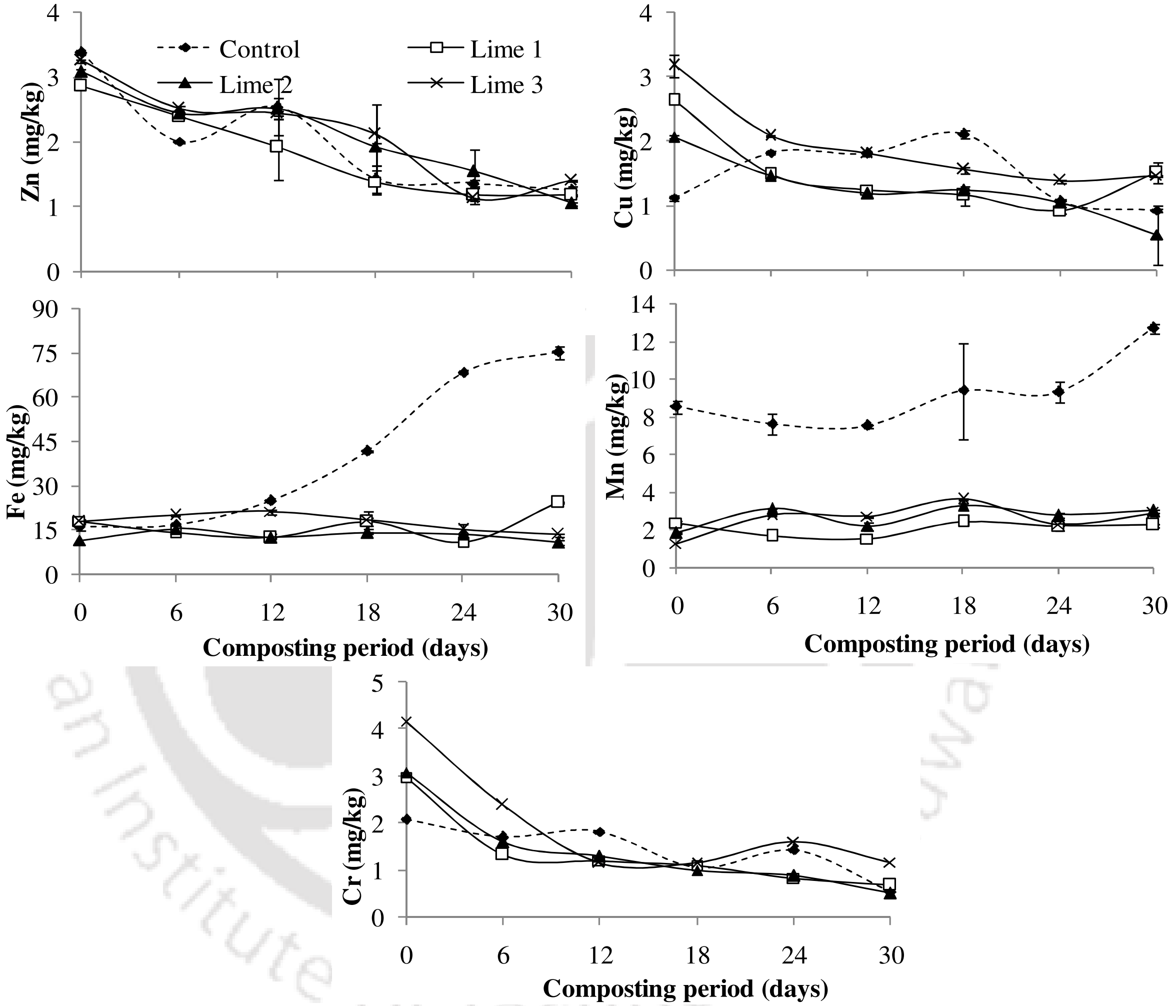


Fig. 5.6 Variation of water soluble metals (Zn, Cu, Mn, Fe and Cr) during pile composting with lime (bars denoted as ± standard deviation)

Fuentes et al. (2004) reported that the quantity of water-soluble Ni was lower than 1 mg/kg for all sludges, however in the present study water soluble Ni was not observed throughout composting process. The reduction in water soluble fraction of metals might be due to formation of humic substances, which has the higher affinity to complex with metals due to high contents of carboxyl groups (Cai et al., 2007). Water-soluble Ni, Pb and Cd contents in all treatments were not detected throughout composting process. The variation in water soluble Zn, Cu, Mn, Fe and Cr concentrations in control and lime amended compost were significant ($F = 36.86$, $p < 0.001$ for Zn, $F = 82$, $p < 0.001$ for Cu, $F = 91.13$, $p < 0.05$ for Mn, $F = 103.5$, $p < 0.001$ for Fe, $F = 33.53$, $p < 0.001$ for Cr).

- **Plant availability of heavy metals (extraction with DTPA)**

Fig. 5.7 shows the DTPA extraction efficiency of heavy metals during the composting process. DTPA extractable Zn, Cu, Fe, Ni and Cr were decreased significantly with lime amendment. However reduction of Mn concentration was not significant in lime treated composts in comparison to control. DTPA extractable Pb and Cd were not detected in all treatments throughout composting process. The higher reduction of Zn content was observed in lime 2 treatment (52.6%) followed by lime 3 treatment (38%), lime 1 treatment (34%) and control (2.0%) during the composting process. Khan and Jones (2009) also reported that lime was the very effective for reducing bioavailability of Zn by raising its immobilization in Cu mine tailing soil. The higher reduction of Fe content was observed in lime 2 treatment (56.6%) followed by lime 1 treatment (12%), lime 3 treatment (11%); however it was increased in control. The higher reduction of Ni and Cr about 66% and 79% of total metal respectively was also observed in lime 2 treatment. The reduction in plant availability of metals is probably due to the formation of less soluble metal carbonates and hydroxides with lime (Wong and Selvam, 2006). The order of DTPA extractable metals (percentage of total metal) was Mn > Zn > Cu > Fe > Cr > Ni in the final water hyacinth compost. The results obtained in our study were similar to the other finding (Fang and Wong, 1999; Wong and Selvam, 2006). The increase in pH by liming was expressed to be successful in reducing the DTPA-extractable contents of Zn, Cu, Mn, Fe, Ni and Cr during the composting process.

Fang and Wong (1999) reported that Zn was the highest DTPA extractable percentage of the total metal content during sewage sludge composting; but in present study, Mn was the highest extractable percentage of the total metal content. The reduction in bioavailability of heavy metals might be due to formation of insoluble carbonates during the thermophilic phase, adsorption of metals onto fine particles of lime or the formation of organo-metallic compound with organic matter in insoluble fractions during the water hyacinth composting process (Wong and Fang, 2000).

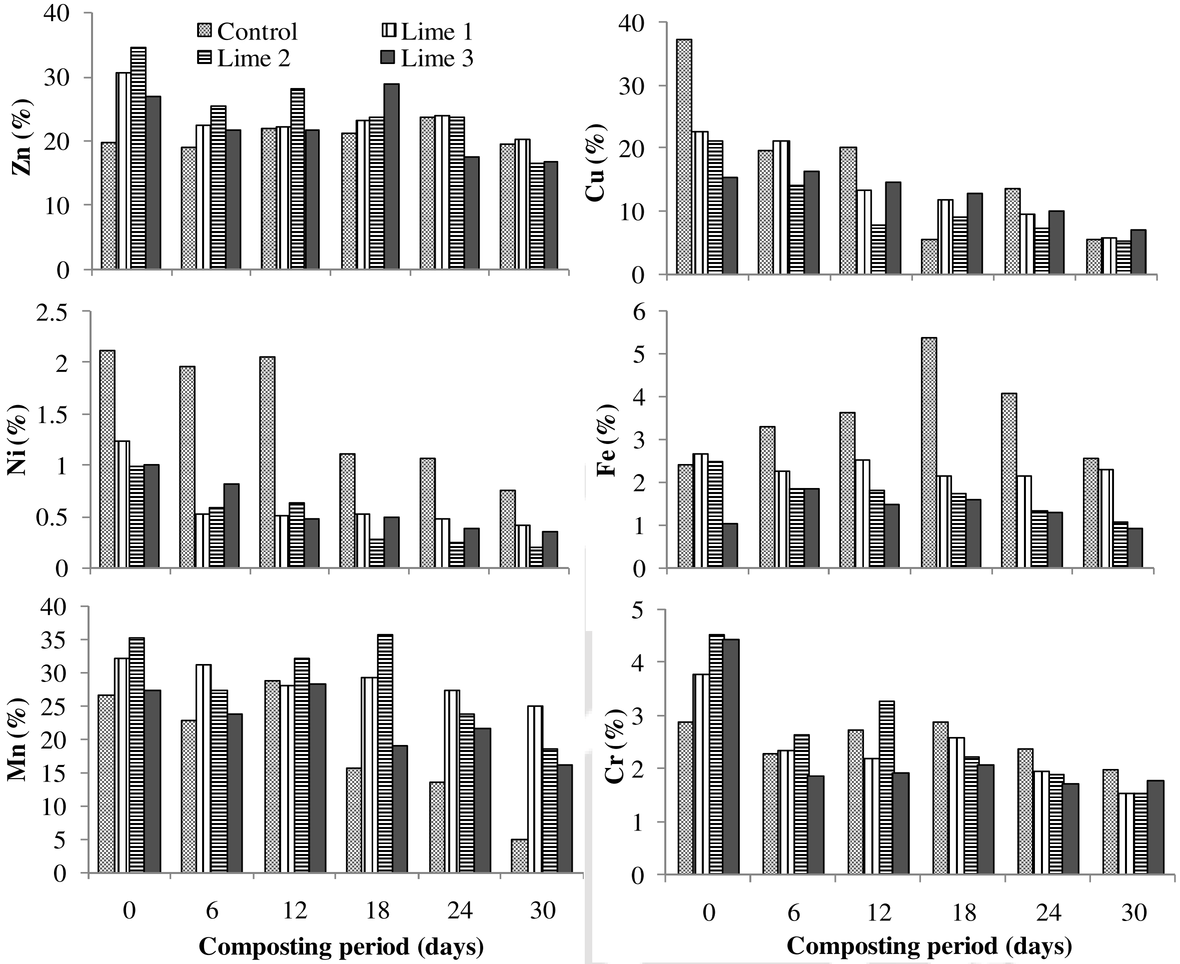


Fig. 5.7 Variation of DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr during pile composting with lime (bars denoted as ± standard deviation)

Fuentes et al. (2004) reported DTPA extractable Ni, Zn and Cd about 14-37%, 11-37% and 17-30% of total Ni, Zn and Cd respectively in four different sludges; however in the present study, these metals were about 0.21-0.75%, 16.4-20.2% and 0% of total Ni, Zn and Cd respectively in the final compost of control and all lime treatments. The variation in DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr concentrations in control and lime treatments were significant (F = 297.6, $p < 0.05$ for Zn, F = 38.84, $p < 0.001$ for Cu, F = 74.3, $p < 0.05$ for Mn, F = 45.97, $p < 0.05$ for Fe, F = 199.34, $p < 0.05$ for Ni, F = 104.4, $p < 0.05$ for Cr).

5.1.3 LEACHABILITY OF HEAVY METALS

The heavy metal leached fraction concept, defined as the ratio of the amount of a heavy metal liberated from TCLP to its total content, is generally used to appraise the leachability of heavy metals in the compost (Chiang et al., 2007). Table 5.1a and b illustrates the changes in leachable Zn, Cu, Mn, Fe, Ni, Cd, Pb and Cr concentration during 30 days of composting period. Concentrations of these metals were very much less than threshold limits. Results confirmed that this compost is not hazardous for land applications. Chiang et al. (2007) reported that Pb, Cd and Cr were not detected in the sewage sludge composting with different additives; however in this study these metals were obtained. Based on the experimental results obtained in this study, the leachable fraction of Zn, Fe, Ni, Cr and Cd (percentage of total metal) was reduced significantly in control and lime amended compost. The higher reduction of Zn, Fe, Ni, Cr and Cd was observed in lime 2 treatment, about 67.5, 77.8, 48.9, 53.7 and 85.7% of the total metal respectively during the composting process. The reduction might be due to higher degradation of organic matter resulting formation of humic substances, which had a capacity to complex with metals resulting formation of insoluble organometallic complexes (Wong and Fang, 2000). The pH of the water hyacinth compost also affected the solubility of the metal hydroxides and carbonates, and the lower pH values increased the solubility of heavy metals resulting enhanced leachability of metals (Qiao and Ho, 1997).

Leachable fraction of Cu and Pb was not reduced significantly in lime amended compost in comparison to control during composting process. Higher reduction of Mn content (percentage of Mn) was observed in lime 3 treatments during the composting process. In the lime amended water hyacinth compost, order of leachable metal contents (percentage of total metal) was: Mn (34.3%) > Zn (24.4%) > Cu (13.14%) > Cr (12.3%) > Pb (0.9%) > Ni (0.7%) > Fe (0.6%) > Cd (0.5%). The variation in leachable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in control and lime amended compost were significant ($F = 154.2$, $p < 0.05$ for Zn, $F = 32.4$, $p < 0.001$ for Cu, $F = 280.3$, $p < 0.05$ for Mn, $F = 421$, $p < 0.05$ for Fe, $F = 46.6$, $p < 0.05$ for Ni, $F = 35.64$, $p = 0.003$ for Pb, $F = 20.8$, $p < 0.001$ for Cd, $F = 48.4$, $p < 0.05$ for Cr).

5.1.4 SPECIATION OF HEAVY METALS

- **Zn, Cu, Mn, Fe and Ni**

Speciation of Zn in control, lime 1, 2 and 3 treatments are given in Table 4.6a, 5.2a, 5.3a and 5.4a respectively during the composting process. The F1, F2, F3 and F4 fractions of Zn were reduced in the control and lime amended compost. Higher reduction of F1 (69.7%) and F3 (36.5%) fractions was observed in control but higher reduction of F2 (61.3%) and F4 (60.2%) fractions was observed in lime 1 treatment.

Table 5.1 a Changes in leachable heavy metal concentration (Zn, Cu, Mn and Fe) during pile composting with lime (mean ± SD, n=3)

	Leachable heavy metal concentration							
Days	**Zn (mg/kg)**				**Cu (mg/kg)**			
	Control	**Lime 1**	**Lime 2**	**Lime 3**	**Control**	**Lime 1**	**Lime 2**	**Lime 3**
0	30.39±0.2	59.5±0.5	64.7±0.3	58.1±0.3	3.5±0.5	7.5±0.1	11.8±0.2	10.0±0.6
6	31.37±4.9	41.2±1.2	47.0±1.4	43.6±1.7	2.9±0.1	9.8±0.6	9.0±0.2	10.9±0.1
12	32.09±0.1	46.5±1.2	62.5±0.2	45.6±0.6	3.0±0.04	9.3±0.3	8.1±0.5	9.0±0.2
18	36.96±5.3	49.1±2.8	55.2±0.4	54.0±0.3	2.8±0.7	7.8±0.2	8.2±0.6	9.4±0.2
24	32.62±6.7	53.1±1.8	52.2±0.5	44.4±1.2	3.3±0.3	13.1±0.3	8.3±0.4	9.2±0.4
30	28.62±8.9	56.1±0.8	34.4±1.4	36.7±0.14	3.5±0.06	12.1±0.5	6.8±0.2	9.3±0.3
Days	**Mn (mg/kg)**				**Fe (mg/kg)**			
0	155.1±18	137.8±1	258.4±1.6	258.0±2	141.5±1.9	187.5±2.5	124.1±0.6	125.8±0.3
6	214.7±2.3	145.9±0.6	199.5±1.5	209.0±1	136.7±7.3	75.9±0.7	78.0±2	94.8±0.5
12	280.2±58	136.9±2.9	217.5±4.5	233.8±2.5	134.1±6.1	62.5±0.5	67.0±0.1	73.8±0.7
18	256.7±9.9	146.3±1.3	200.9±0.9	217.9±0.7	127.1±2.9	57.3±0.5	67.6±0.8	66.9±1.4
24	234.3±12.5	193.0±7	229.7±1.3	164.3±0.8	158.6±2.6	55.1±0.6	66.8±0.9	66.9±0.7
30	233.8±1	210.0±2	205.0±1.6	163.9±0.5	136.7±14.5	94.6±1.8	64.6±0.4	70.8±0.6

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.1b Changes in leachable heavy metal concentration (Ni, Pb, Cd and Cr) during pile composting with lime (mean ± SD, n=3)

Days	Leachable heavy metal concentration							
	Ni (mg/kg)				Pb (mg/kg)			
	Control	Lime 1	Lime 2	Lime 3	Control	Lime 1	Lime 2	Lime 3
0	10.9±0.9	1.90±0.01	1.90±0.02	2.12±0.08	36.5±1.5	6.2±0.2	14.6±0.6	9.6±0.6
6	10.7±0.7	1.58±0.04	1.73±0.01	1.94±0.02	41.0±1.0	7.8±0.3	12.9±0.2	9.1±0.1
12	10.6±1.2	1.38±0.01	1.60±0.02	1.68±0.07	27.0±1.0	8.6±0.6	16.0±0.1	8.9±0.1
18	7.7±0.1	1.47±0.03	1.77±0.01	1.82±0.07	36.0±2.0	6.8±0.8	13.9±0.2	8.3±1.3
24	10.5±1.1	1.46±0.02	1.77±0.05	2.04±0.04	32.2±4.2	5.7±0.7	13.2±0.8	7.7±0.6
30	9.5±0.01	1.69±0.01	1.53±0.03	1.68±0.06	32.0±2.0	5.6±0.4	10.7±0.3	7.1±0.1
Days	Cd (mg/kg)				Cr (mg/kg)			
0	1.50±0.1	1.00±0.1	1.12±0.22	0.99±0.09	4.6±0.02	10.7±0.3	11.2±0.2	10.8±0.15
6	1.41±0.4	0.70±0.12	0.86±0.04	0.95±0.01	4.7±0.04	11.6±0.6	10.1±1.0	10.0±1.0
12	1.74±0.1	0.77±0.03	0.72±0.04	0.90±0.02	4.3±0.09	11.0±0.7	10.5±0.12	10.0±0.35
18	1.33±0.1	0.72±0.04	0.43±0.01	0.83±0.02	4.5±0.22	10.6±0	9.8±0.15	9.6±1.0
24	1.32±0.1	0.39±0.19	0.33±0.01	0.75±0.03	4.0±0.78	9.0±0.6	8.5±0.05	8.0±0.45
30	1.21±0.11	0.36±0.02	0.26±0.10	0.74±0.03	3.8±0.06	7.5±0.5	7.1±0.10	7.6±0.40

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

The F5 fraction of Zn was enhanced in control and lime amended compost. The order of different fractions of Zn in the final compost of control was: F5 > F4 >F3 >F2 >F1, but in the lime amended compost order was: F5 > F3 >F4 >F2 >F1. In the lime amended compost F4 fraction was transform into F3 and F5 fractions. The Zn was mainly present in residual fraction in the control and lime amended compost but higher percentage of Zn in residual fraction was present in lime amended compost in comparison to control. These results confirm that mobile fractions of Zn were converted in to stable fraction due to lime addition. Wong and Selvam (2006) also observed that the Zn was found in the residual fraction in the lime amended sewage sludge compost. The BF of Zn was decreased from 0.75 to 0.48, 0.61 to 0.35, 0.67 to 0.43 and 0.52 to 0.38 in control and lime 1, 2 and 3 treatments respectively (Fig. 5.8). The higher reduction of BF was observed in lime 1 treatment (43.4%) followed by control (36.37%), lime 2 treatment (35.1%) and lime 3 treatment (27.3%) during the composting process. Fig. 4 illustrates the variation in BF of Zn from initial to final compost. The variation in F1, F2, F3, F4 and F5 fractions of Zn in control and lime treatments were significant ($F = 28.89$, $p < 0.001$ for F1, $F = 47.78$, $p < 0.001$ for F2, $F = 139.77$, $p < 0.05$ for F3, $F = 73.23$, $p < 0.05$ for F4, $F = 17.76$, $p < 0.001$ for F5).

Speciation of Cu in control, lime 1, 2 and 3 treatments are given in Table 4.6a, 5.2a, 5.3a and 5.4a respectively during the composting process. The F1, F2, F3 and F4 fractions of Cu were reduced in the control and lime amended compost. Higher reduction of F1 (77%) and F3 (75%) fractions (percentage of total fraction) was observed in control but higher reduction of F2 (80.5%) and F4 (58.6%) fractions were observed in lime 2 and 1 treatment respectively. The F5 fraction of Cu was enhanced in control and lime amended compost. The BF of Cu was decreased from 0.63 to 0.28, 0.57 to 0.23, 0.53 to 0.20 and 0.44 to 0.18 in control and lime 1, 2 and 3 treatments respectively (Fig. 5.8). The higher reduction of BF was observed in lime 2 treatment (62.1%) followed by lime 1 treatment (59%), lime 3 treatment (58.2%) and control (55.5%) during the composting process. The higher reduction in BF of Cu was observed in lime added compost which could be explained as, the conversion of easily available fractions into residual fraction and formation of Cu ion complex with two or more organic functional groups mainly carboxylic, carbonyl and phenolic, so that the ion is immobilized in a rigid inner-sphere complex (Qiao and Ho, 1997). The complex formation of Cu with humic substances generally higher than Zn due to water solubility of humic substances, which have a high content of carboxyl groups, and the stability constant humic complexes depend on the nature of organics, ion strength and pH in environment (Liu et al., 2008).

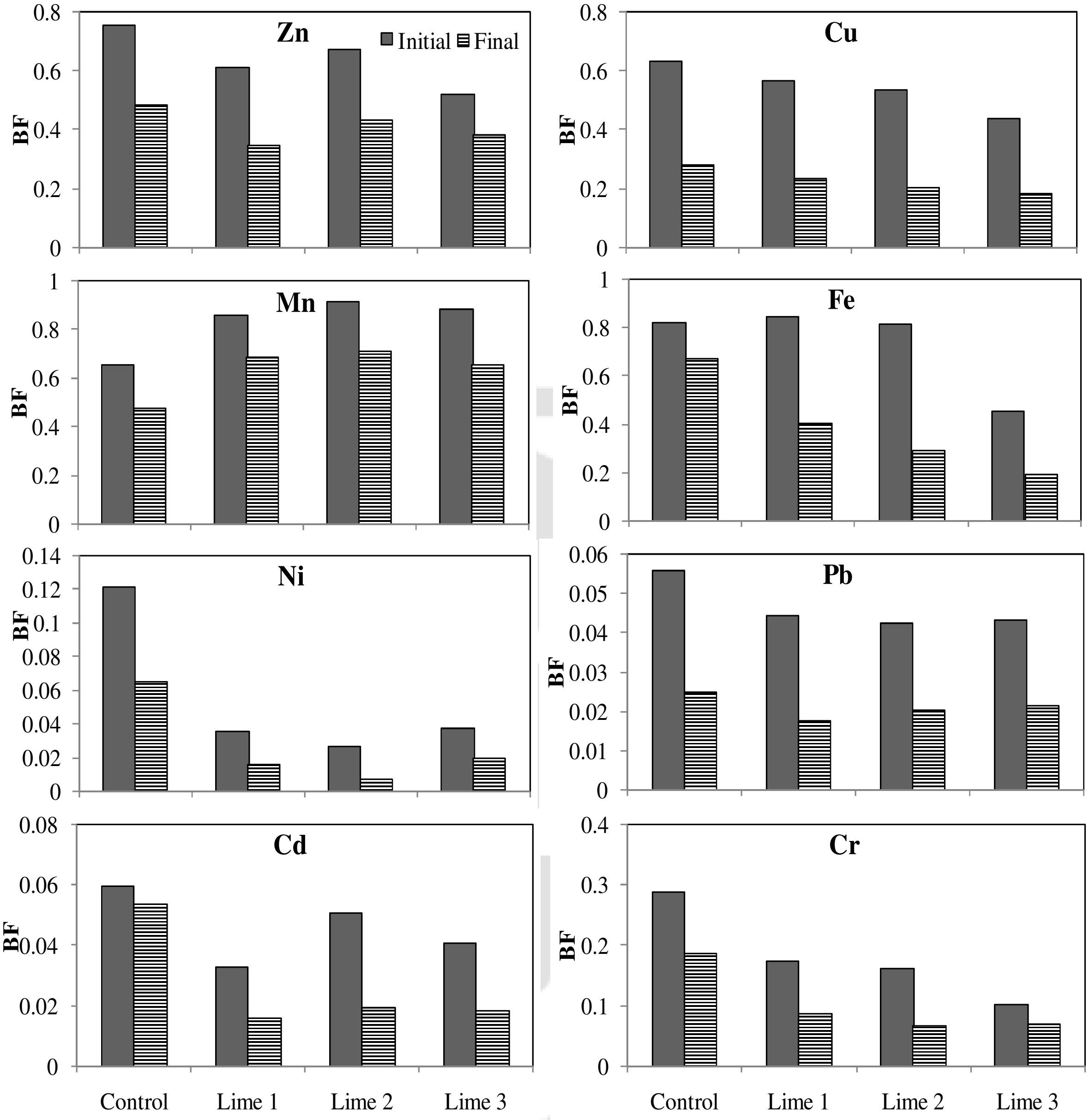


Fig. 5.8 Changes in bioavailability factor (BF) of heavy metals in control and lime treatments during pile composting process

The variation in F1, F2, F3, F4 and F5 fractions of Cu in control and lime treatments were significant (F = 104.94, $p < 0.05$ for F1, F = 36.22, $p < 0.001$ for F2, F = 11.84, $p < 0.001$ for F3, F = 59.69, $p < 0.001$ for F4, F = 83.25, $p < 0.05$ for F5).

Speciation of Mn in control, lime 1, 2, and 3 treatments are given in Table 4.6a, 5.2a, 5.3a and 5.4a respectively during the composting process. The F1 fraction of Mn was reduced about 63.8 and 50.9% of total fraction in control and lime 1 treatment respectively; however this fraction was increased in lime 2 and 3 treatments. The F2 and F4 fractions of Mn were reduced in all lime treatments; however these fractions were increased in the control. The F3 fraction of Mn was reduced about 38.14, 2.9 and 36.1% of total fraction in control, lime 2 and 3 treatments respectively. The F5 fraction of Mn was increased in the control and all lime treatments during the composting process. The F2 and F4 fractions of Mn decreased in all lime treated compost; however these fractions were increased in control. Wong and Selvam (2006) reported that initially Mn was dominated by the F5 fraction but when the composting progressed, the F3 fraction increased and became the largest fraction. However in the present study, the F3 fraction was predominated throughout composting process. The BF of Mn was decreased from 0.65 to 0.47, 0.86 to 0.68, 0.91 to 0.71 and 0.88 to 0.65 in control and lime 1, 2 and 3 treatments respectively (Fig. 5.8). The higher reduction of BF was observed in control (27.2%) followed by lime 3 (26%), lime 2 (22.6%) and lime 1 (20.8%) during the composting process. The variation in F1, F2, F3, F4 and F5 fractions of Mn in control and lime treatments were significant ($F = 90.71$, $p < 0.05$ for F1, $F = 437$, $p < 0.05$ for F2, $F = 38.27$, $p < 0.001$ for F3, $F = 205.1$, $p < 0.05$ for F4, $F = 74.34$, $p < 0.001$ for F5).

Speciation of Fe in control, lime 1, 2, and 3 treatments are given in Table 4.6a, 5.2a, 5.3a and 5.4a respectively during the composting process. The F1, F2, F3 and F4 fractions of Fe were reduced in the control and lime treatments during the composting process. Higher reduction of F1 and F2 fractions were observed about 75 and 84% of the total fraction respectively in lime 1 treatment. Higher reduction of F3 and F4 fractions were observed about 68 and 64.5% of the total fraction respectively in lime 1 treatment. The F5 fraction of Fe was enhanced in control and lime amended compost. An extremely significant decrease in F1 fraction was observed in all lime treatments, which suggest that the addition of lime could prevent the mobility of Fe. The BF of Fe was decreased from 0.82 to 0.67, 0.85 to 0.40, 0.82 to 0.29 and 0.46 to 0.19 in the control and lime 1, 2 and 3 treatments respectively. The higher reduction of BF was observed in lime 2 treatment (64.4%) followed by lime 3 treatment (58.1%), lime 1 treatment (52.6) and control (18.8%) during the composting process. It has been observed that in control and all lime treatments, BF of Fe was decreased; it could be explained as humic substances formed a complex compound with Fe. Fig. 5.8 illustrates the variation in BF of Fe from initial to the final compost. The variation in F1, F2, F3, F4 and F5 fractions of Fe in control and lime treatments were significant ($F = 41.73$, $p < 0.001$ for F1, $F = 186$, $p < 0.05$ for F2, $F = 1988$, $p < 0.05$ for F3, $F = 1199.9$, $p < 0.05$ for F4, $F = 37$, $p < 0.001$ for F5).

Table 5.2a Speciation of heavy metals (Zn, Cu, Mn and Fe) in lime 1 treatment during 30 days of composting period

	Lime 1 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	5.19±0.013	6.41±0.41	26.30±0.7	31.0±0.47	44.0±0.68	4.68±0.03	1.88±0.03	0.90±0.10	20.65±4.5	21.65±5.7
6	4.460.06	6.07±0.23	37.10±1.40	27.28±0.52	76.5±4.22	2.65±0.05	1.67±0.03	0.70±0.20	18.92±0.1	25.9±0.6
12	3.14±0.033	5.74±0.11	40.22±0.02	22.61±0.29	77.75±5.75	1.95±0.05	1.45±0.05	0.50±0.10	14.17±0	43.0±4.5
18	4.03±0.23	5.51±0.013	37.92±0.32	23.54±0.84	76.35±2.25	1.69±0.02	1.50±0.20	0.35±0.05	14.46±0.2	45.0±1.0
24	3.84±0.058	4.81±0.60	35.16±0.36	22.91±0.11	82.58±0.58	1.40±0.06	1.10±0.10	0.36±0.05	13.53±0.3	58.08±4.7
30	3.88±0.025	4.50±3.4	40.30±3.4	23.33±0.58	136.45±2.25	2.60±0.10	1.40±0.10	0.80±0.20	15.38±0.02	66.85±15.9
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	36.1±0.68	106.3±0.7	86.9±2.4	78.7±0.39	50.5±4.05	27.7±0.7	21.7±0.7	2789.5±3.5	6260.9±141	1671.0±139
6	30.9±0.33	99.0±1.0	125.6±1.0	56.1±0.25	62.5±12.5	18.2±0.2	16.8±0.1	2693.5±3.5	5011.3±1.7	3915.0±480
12	26.9±0.35	86.0±3.0	121.3±0.7	47.2±1.16	95.9±0.35	12.8±0.7	12.0±0.3	2270.5±9.5	3978.1±43	5264.5±510
18	30.9±0.45	88.2±1.9	136.8±1.3	46.8±0.05	161.5±1.5	14.5±0.8	11.1±0.3	2712.0±1.0	4538.0±25	5990.5±150
24	47.5±1.9	112.7±0.4	166.5±0.9	49.0±0.37	182.3±1.8	9.3±0.6	5.9±0.5	2647.5±0.5	4361.5±6.5	7571.5±503
30	30.0±0.38	93.4±0.03	244.2±0.9	45.4±0.30	194.1±4.6	16.3±0.7	9.6±0.4	2123.0±3.0	3725.0±20	8785.0±475

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.2b Speciation of heavy metals (Ni, Pb, Cd and Cr) in lime 1 treatment during 30 days of composting period

	Lime 1 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	2.98±0.03	3.25±0.05	ND	ND	166.9±6.4	22.9±2.9	9.9±0.4	ND	ND	708±2
6	2.03±0.08	2.85±0.15	ND	ND	209.3±0.8	22.6±2.4	8.6±0.05	ND	ND	833±51
12	1.91±0.06	3.18±0.22	ND	ND	211.5±3.5	17.2±0.4	8.4±0.1	ND	ND	847±34
18	1.73±0.03	2.28±0.03	ND	ND	217.8±16.8	17.9±0.4	8.2±0.4	ND	ND	873±3
24	2.08±0.08	2.90±0.10	ND	ND	211.6±52.4	12.9±0.6	6.7±0.2	ND	ND	920±5
30	1.43±0.03	2.18±0.08	ND	ND	228.9±50.4	11.2±0.7	6.7±0.3	ND	ND	1016±21
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	0.73±0.03	0.84±0.01	ND	ND	45.9±0.4	5.45±0.15	1.33±0.03	1.55±0.05	15.85±0.45	115.8±0.25
6	0.61±0.01	0.83±0.02	ND	ND	52.7±4.9	4.75±0.05	1.67±0.03	1.80±0.2	13.03±0.43	114.8±0.25
12	0.62±0.02	0.82±0.04	ND	ND	57.1±1.6	4.05±0.15	1.25±0.05	2.55±0.05	12.56±0.14	142.6±12.1
18	0.67±0.07	0.81±0.05	ND	ND	55.0±0.1	4.13±0.18	1.27±0.08	2.0±0.6	12.12±1.7	128.5±26.5
24	0.53±0.03	0.77±0.08	ND	ND	58.1±4.6	3.68±0.08	1.15±0.05	1.55±0.4	8.92±0.23	151.3±3.3
30	0.46±0.01	0.58±0.02	ND	ND	64.9±4.4	3.65±0.15	1.05±0.05	1.45±0.2	8.85±0.45	160.5±5.95

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

Speciation of Ni in control, lime 1, 2 and 3 treatments are given in Table 4.6b, 5.2b, 5.3b and 5.4b respectively during the composting process. The F1 and F2 fractions of Ni were reduced in the control and lime treatments but the higher reduction of these fractions were observed about 69 and 63% of the total fraction in lime 2 treatment respectively. The F5 fraction of Ni was enhanced in control and lime treatment during the composting process. The F1 fractions contribute about 5% of the total fraction in final compost of control, however this fraction contribute < 1.5% of total fraction in lime treated compost. The F2 fractions contribute about 1.5% of the total fraction in final compost of control, however this fraction contribute < 1% of the total fraction in lime treated compost. Wong and Selvam (2006) reported that both F1 and F2 fractions contribute about 2% of the total Ni content. The F5 fraction of Ni was about 92% of total fraction in the final compost of control as compared to 98% in the lime treated compost. The BF of Ni was decreased from 0.12 to 0.064, 0.04 to 0.016, 0.027 to 0.007 and 0.038 to 0.02 in control and lime 1, 2 and 3 treatments respectively (Fig. 5.8). The highest reduction of BF was observed in lime 2 treatment (71.9%) followed by lime 1 treatment (56.9%), control (52.6%) and lime 3 treatment (46.6%) during the composting process. A significant decrease in BF was observed in lime 2 treatment, which suggests that the addition of lime could prevent the bioavailability of Ni during the process. The significant decreases in BF of Ni might be due to alkaline stabilization process. Su and Wong (2003) reported that the F5 fraction contributes 52% of the total Ni content; followed by F3 fraction in sewage sludge; whereas, in present study F5 fraction contributed about 90-98%. The variation in F1, F2 and F5 fractions of Ni in control and lime treatments were significant ($F = 18.78$, $p < 0.001$ for F1, $F = 15$, $p < 0.001$ for F2, $F = 9.6$, $p < 0.001$ for F5).

- **Speciation Pb, Cd and Cr**

Speciation of Pb in control, lime 1, 2, and 3 treatments are given in Table 4.6b, 5.2b, 5.3b and 5.4b respectively during the composting process. The F1 and F2 fractions of Pb were reduced in the control and lime treated compost but the higher reduction of these fractions was observed about 55.2 and 56.8% respectively in control. Wong and Selvam (2006) reported that there is no marked difference in F1 and F2 fractions during sewage sludge composting. The F5 fraction of Pb was increased in all lime treatments; however this fraction was reduced in control about 2.5% of total fraction during the composting process. The BF of Pb was decreased from 0.056 to 0.025, 0.044 to 0.017, 0.042 to 0.020 and 0.043 to 0.021 in control and lime 1, 2 and 3 treatments respectively (Fig. 5.8).

Table 5.3a Speciation of heavy metals (Zn, Cu, Mn and Fe) in lime 2 treatment during 30 days of composting period

	Lime 2 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	7.61±0.49	7.56±1.06	42.07±0.47	24.66±0.64	40.28±0.28	6.26±0.05	3.65±0.05	0.65±0.05	15.69±0.5	23.0±0.07
6	6.64±0.44	6.08±0.73	49.75±0.25	24.34±0.26	53.15±0.35	2.48±0.03	1.52±0.02	0.80±0.1	12.57±5.6	31.6±0.08
12	4.45±1.45	3.32±0.24	57.09±0.79	28.95±0.35	56.13±0.73	2.38±0.48	1.20±0.05	0.75±0.05	12.90±1.1	62.45±0.1
18	3.17±0.02	2.70±0.06	49.17±0.44	23.11±0.29	64.90±14.3	2.49±0.04	1.36±0.01	0.88±0.03	16.72±7.7	60.35±0.2
24	3.64±0.10	2.81±0.54	54.1±0.70	23.69±0.59	68.55±3.55	2.05±0.05	1.50±0.20	0.91±0.01	12.59±5.5	63.95±0.05
30	3.02±0.02	4.11±0.51	54.55±0.75	21.62±0.22	109.0±3.0	1.99±0.01	1.14±0.07	0.88±0.20	12.41±0.8	64.8±0.04
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	13.0±0.2	126.2±0.9	194.2±2.1	58.7±0.6	36.5±5.5	27.4±0.3	26.9±1.3	2788±20	4477±21	1655±445
6	20.3±5.7	81.5±0.9	238.8±48.7	51.3±0.6	50.8±4.8	17.7±0.02	22.1±0.6	1990±5	3571±4	5840±500
12	29.4±0.8	108.5±0.5	242.8±51.7	57.4±0.7	47.4±4.9	15.8±0.2	13.9±0.5	2062±15	4623±18	7307±443
18	27.2±0.1	83.1±0.6	214.8±50.3	50.6±1.1	58.8±4.8	15.0±0.5	12.0±0.4	1805±60	3564±89	7643±497
24	35.8±1.5	110.9±0.7	287.1±9.1	50.0±0.3	98.0±12	16.0±0.2	11.9±0.3	2482±19	4244±12	10418±882
30	33.2±0.8	97.7±0.7	285.1±2.9	43.1±0.4	189.0±6	13.1±0.5	11.9±0.7	1929±4.5	3741±3.8	13925±625

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.3b Speciation of heavy metals (Ni, Pb, Cd and Cr) in lime 2 treatment during 30 days of composting period

	Lime 2 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	2.58±0.03	3.25±0.7	ND	ND	210.8±50.8	24.9±0.4	10.8±1.4	ND	ND	803±3
6	2.05±0.05	2.18±0.3	ND	ND	218.3±34.8	21.1±3.5	9.9±0.2	ND	ND	829±1
12	1.91±0.05	2.0±0.3	ND	ND	214.6±5.6	15.4±2.4	9.7±0.7	ND	ND	781±76
18	1.88±0.5	1.80±0.6	ND	ND	234.8±59.8	15.9±0.6	9.9±0.4	ND	ND	838±28
24	1.74±0.5	1.75±0.05	ND	ND	247.2±0.4	15.4±0.7	7.8±0.2	ND	ND	913±2.5
30	1.15±0.2	1.20±0.06	ND	ND	337.8±12.3	16.0±0.6	7.5±0.03	ND	ND	1153±8
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	0.94±0.02	1.25±0.25	ND	ND	40.85±0.7	3.75±0.05	2.29±0.47	1.75±0.05	11.0±2.0	97.9±0.4
6	0.74±0.01	0.93±0.02	ND	ND	47.6±0.6	4.28±0.08	1.90±0.1	2.10±0.1	8.8±0.45	136.5±1.5
12	0.72±0.04	0.88±0.03	ND	ND	55.3±3.7	3.55±0.05	1.77±0.03	1.35±0.05	8.4±0.2	117.5±5.5
18	0.73±0.03	0.83±0.03	ND	ND	56.0±2.3	3.53±0.13	1.38±0.02	1.25±0.05	8.9±0.57	136.8±6.3
24	0.72±0.12	0.82±0.01	ND	ND	57.1±0.4	2.67±0.96	1.68±0.03	1.27±0.03	8.6±0.22	148.6±22.3
30	0.48±0.03	0.79±0.07	ND	ND	64.8±0.8	1.80±0.05	1.18±0.03	1.15±0.05	7.0±0.20	157.5±26.5

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

The higher reduction of BF was observed in lime 1 treatment (61%) followed by control (55.6%), lime 2 treatment (51%) and lime 3 treatment (48.7%) during the composting process. The decrease in Pb mobility during sewage sludge composting was also observed by Qiao and Ho (1997). Development of slightly alkaline medium by lime addition can reduce mobility of Pb by forming Pb-organic matter complex, but higher alkaline conditions can have a reverse effect on Pb stability due to the amphoteric nature of Pb (Kumpiene et al., 2008). The variation in F1, F2 and F5 fractions of Pb in control and lime treatments were significant ($F = 29.94$, $p < 0.001$ for F1, $F = 13.25$, $p < 0.001$ for F2, $F = 58.76$, $p < 0.001$ for F5).

Speciation of Cd in control, lime 1, 2 and 3 treatments are given in Table 4.6b, 5.2b, 5.3b and 5.4b respectively during the composting process. The F1 and F2 fractions of Cd were reduced in the control and lime treatments. The higher reduction of these fractions were observed about 66.6 and 58.8% of total fraction respectively in lime 2 treatment during the composting process. The F1 and F2 fractions were reduced and F5 fraction was increased might be due to formation of strong chemical bond between Cd and degraded organic matter (Haroun et al., 2007). A similar observation was also reported by Hanc et al. (2009) during composting of sewage sludge. The F5 fraction of Cd was enhanced in all lime treatments; however this fraction was reduced in control about 1.3% of total fraction during the composting process. The BF of Cd was decreased from 0.06 to 0.053, 0.033 to 0.016, 0.051 to 0.019 and 0.041 to 0.018 in control and lime 1, 2 and 3 treatments respectively (Fig. 5.8). The higher reduction of BF was observed in lime 2 treatment (62.1%) followed by lime 3 treatment (51.3%), lime 1 treatment (48.5%) and control (10.5%) during the composting process. The decrease in BF of Cd could be due to maintaining neutral pH (7 to 7.6) in lime treatments throughout the composting process. An enormously significant decrease in BF of Cd was observed in all lime treatments, which suggest that the addition of lime could prevent the mobility of Cd. The variation in F1, F2 and F5 fractions of Cd in control and lime treatments were significant ($F = 70.5$, $p < 0.05$ for F1, $F = 13.55$, $p < 0.001$ for F2, $F = 8.73$, $p < 0.001$ for F5).

Speciation of Cr in control, lime 1, 2, and 3 treatments are given in Table 4.6b, 5.2b, 5.3b and 5.4b respectively during the composting process. The F1, F2, F3 and F4 fractions of Cr were reduced in the control and lime treatments. Higher reduction of F1, F2 and F3 fractions were observed about 67.9, 65.7 and 54.6% of the total fraction in lime 2 treatment; however the maximum reduction of F4 fraction was observed about 42.2% of total fraction in control during the composting process.

Table 5.4a Speciation of heavy metals (Zn, Cu, Mn and Fe) in lime 3 treatment during 30 days of composting period

	Lime 3 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	4.46±1.05	3.83±0.48	36.26±0.66	19.28±0.04	59.1±0.04	2.82±0.07	2.17±0.07	0.65±0.15	13.48±0.02	24.65±0.7
6	2.69±0.02	2.62±0.63	36.59±0.39	16.43±0.07	77.5±0.07	3.72±0.05	1.60±0.05	0.76±0.04	12.19±0.04	24.65±0.2
12	2.71±0.01	3.10±0.10	44.05±0.25	19.17±0.57	67.3±0.57	2.40±0.03	1.78±0.03	0.85±0.05	13.88±0.10	28.90±4.6
18	2.87±0.03	3.70±0.16	50.91±0.61	20.93±0.53	60.9±0.53	2.05±0.04	1.64±0.04	1.00±0.1	13.48±0.23	40.25±4.5
24	2.49±0.03	3.75±0.05	43.15±0.77	16.74±0.04	91.8±0.04	2.50±0.03	1.63±0.03	1.65±0.05	12.29±0.65	50.55±4.6
30	2.73±0.06	2.48±0.03	42.39±0.02	16.33±0.53	105.4±0.53	2.02±0.04	1.67±0.04	1.25±0.05	13.97±0.08	84.57±2.1
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	9.4±0.2	92.0±0.5	280.1±6.0	49.2±1.1	56.8±3.8	21.9±3.3	21.6±0.5	2061.0±2	3569.5±5.5	6791±259
6	14.2±0.7	96.0±0.7	272.8±33	47.5±1.9	61.8±1.8	16.2±0.3	15.2±0.7	2472.0±14	3804.5±20	5255±355
12	26.2±0.4	122.2±1.8	267.0±1.6	59.9±1.2	73.5±14.5	15.2±0.6	15.8±0.4	2936.5±31	4547.2±22	9170±630
18	27.7±0.3	111.0±1.0	271.2±0.4	49.8±1.4	91.0±1.0	13.1±0.5	13.1±0.3	2311.5±3.5	3649.0±9	10645±855
24	22.8±0.3	72.0±4.0	264.5±0.5	33.5±0.9	175.5±1.5	14.8±0.3	10.8±0.2	1949.5±85	2784.7±46	11273±472
30	22.1±0.27	72.0±3.0	251.7±1.7	33.9±0.8	202.0±48	14.1±0.4	11.1±0.5	1819.5±23	2426.4±1.4	18101±101

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.4b Speciation of heavy metals (Ni, Pb, Cd and Cr) in lime 3 treatment during 30 days of composting period

	Lime 3 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	4.09±0.04	3.48±0.22	ND	ND	193±1.3	20.6±1.1	12.7±0.4	ND	ND	737.5±7.5
6	3.23±1.0	3.20±0.4	ND	ND	223±41	13.4±1.9	14.0±1.5	ND	ND	762.5±43
12	2.70±0.4	3.33±0.03	ND	ND	216±14.3	12.7±0.7	14.8±0.2	ND	ND	841.5±3.5
18	2.55±0.05	2.51±0.06	ND	ND	265±29.8	12.0±0.5	13.0±0.6	ND	ND	828.5±3.5
24	2.57±0.03	2.44±0.4	ND	ND	255±51	11.9±0.7	11.2±0.8	ND	ND	911.5±11.5
30	3.36±0.05	2.40±0.05	ND	ND	300±21.5	12.9±0.2	8.9±0.2	ND	ND	1004.5±10.5
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	0.93±0.03	0.98±0.03	ND	ND	44.9±4.6	4.08±0.13	1.53±0.03	1.50±0.1	7.40±0.05	127.8±0.3
6	0.85±0.01	0.94±0.04	ND	ND	52.6±3.6	3.53±0.08	1.90±0.05	2.25±0.05	7.13±0.17	136.0±4.0
12	0.63±0.03	0.88±0.03	ND	ND	55.0±1.0	3.25±0.15	2.13±0.03	1.55±0.05	7.11±0.11	148.8±3.3
18	0.59±0.04	0.82±0.02	ND	ND	57.2±1.2	2.93±0.08	1.83±0.47	1.81±0.01	7.31±0.11	150.5±2.5
24	0.58±0.08	0.74±0.04	ND	ND	59.9±4.6	3.0±0.2	1.48±0.08	1.65±0.45	6.60±0.10	153.8±1.8
30	0.48±0.03	0.76±0.06	ND	ND	66.2±2.4	3.15±0.05	1.57±0.04	1.15±0.05	6.07±0.03	164.3±18.3

Mean values in columns are statistically different (ANOVA; $P < 0.05$); ND- not detected

The F5 fraction of Cr was enhanced in control and lime amended compost. The BF of Cr was decreased from 0.29 to 0.18, 0.17 to 0.09, 0.16 to 0.07 and 0.10 to 0.07 in control and lime 1, 2 and 3 treatments respectively (Fig. 5.8). The higher reduction of BF was observed in lime 2 treatment (58.9%) followed by lime 1 treatment (50.5%), control (36.3%) and lime 3 treatment (33.5%) during the composting process. The significant reduction in BF was observed in all lime treatments in comparison to control. It could be explained that F1 and F2 forms may bound with various organic functional groups present in the humic substances, while F3 and F4 fractions might be converted into F5 fraction during the process. The F5 fraction of Cr was dominant throughout composting process. Smith (2009) also reported that Cr was mainly present in F5 fraction. The variation in F1, F2, F3, F4 and F5 fractions of Cr in control and lime treatments were significant ($F = 29.29$, $p < 0.001$ for F1, $F = 56.64$, $p < 0.05$ for F2, $F = 20.36$, $p < 0.001$ for F3, $F = 16.20$, $p < 0.001$ for F4, $F = 12.66$, $p < 0.001$ for F5).

5.1.5 CONCLUSION

The lime amendment was significant in reducing water soluble metals (Zn, Cu, Fe and Cr), DTPA extractable metals (Zn, Cu, Fe, Ni and Cr) and leachable metals (Zn, Fe, Ni, Cr and Cd) during agitated pile composting. TCLP test confirm that the heavy metals concentrations in control and lime amended compost were under the threshold limits. The maximum reduction of water soluble, DTPA extractable and leachable heavy metals were observed in lime 2 treatment, which indicated optimum percentage of lime can enhance organic matter degradation and humification process; consequently it reduced the toxicity of the metals. The bioavailability factor of Zn, Cu, Fe, Ni, Pb, Cd and Cr was reduced extremely significant in lime 2 treatment in comparison to control, lime 1 and 3 treatments. This study suggests that the addition of lime could not reduce the bioavailability of Mn. The order of BF of different metals in the water hyacinth compost was: Mn (0.70) > Fe (0.67) > Zn (0.48) > Cu (0.28) > Cr (0.18) > Ni (0.06) > Cd (0.05) > Pb (0.03). The order of BF indicates that toxicity of metals does not depend on its total concentration. The total concentration of Pb was higher than Zn, Cu, Mn, Ni, Cd and Cr but its BF was lowest among the all eight metals. The total concentration of Cu was much less than Pb and Ni but its BF about 5 times of Ni and 10 times of Pb. Therefore this study concluded that the addition of lime sludge was successful for reducing bioavailability of heavy metals during agitated pile composting of water hyacinth mixed with cattle manure and sawdust.

5.2 EFFECTS OF LIME DURING ROTARY DRUM COMPOSTING

Bioavailability of heavy metals during the composting process minimized by addition various alkaline materials such as lime, coal fly ash etc. (Fang and Wong, 1999; Wong and Selvam, 2006; Chiang et al., 2007; Wang et al., 2008). Phase 2 study suggested that that mixture of 90 kg water hyacinth, 45 kg cattle manure and 15 kg sawdust (Trial 4) was best combination for reduction of bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate) of heavy metals during rotary drum composting of water hyacinth. Therefore, same combination (Trial 4) was selected for lime 1 treatment (1%), 2 treatment (2%) and 3 treatment (3%) studies. Trial 4 was considered as control with respect to lime addition.

5.2.1 PHYSICO-CHEMICAL ANALYSIS

The deviations of composting temperatures in control and lime treatments were generally seen to follow the typical patterns of aerobic composting reactions (Fig. 5.9). The temperature was increased rapidly after 3 h of the mixture feeding, the maximum temperature (59.9°C) was observed in lime 2 treatment during the thermophilc phase. Long duration (about 12 days) of thermophilic stage was observed in lime added composting process in comparison to control (one week). The addition of small amount of lime provided a buffering against the decrease in pH and an appropriate amount of Ca, which would improve the metabolic activity during composting process (Fang and Wong, 1999). The temperature rising were observed in all treatments after every turning during thermophilic stage. It might be due to the degradation of partially decomposed materials which were shifted from the anaerobic to the aerobic conditions (Jiang et al., 2011).

The moisture content of the compost was reduced significantly (F = 51.3, $p < 0.05$) from 89.9, 77.2, 76.9 and 77.0% to 65.3, 56.1, 56.6 and 58.5% in control, lime 1, 2 and 3 treatments respectively during the composting process (Fig. 5.10a). Lower reduction of moisture was observed in lime treatments in comparison to control; it might be due to lime supply better moisture holding capacity and promote the optimum microbial degradation rate (Chiang et al., 2007). Initial pH was higher in lime added compost mixture in comparison to control, but it gradually decreased and become nearly neutral. Initially maximum pH value was observed about 10.2 in lime 3 treatment. The final compost pH values were 7.3, 7.38, 7.64 and 7.61 in control, lime 1, 2 and 3 treatments respectively (Fig. 5.10b).

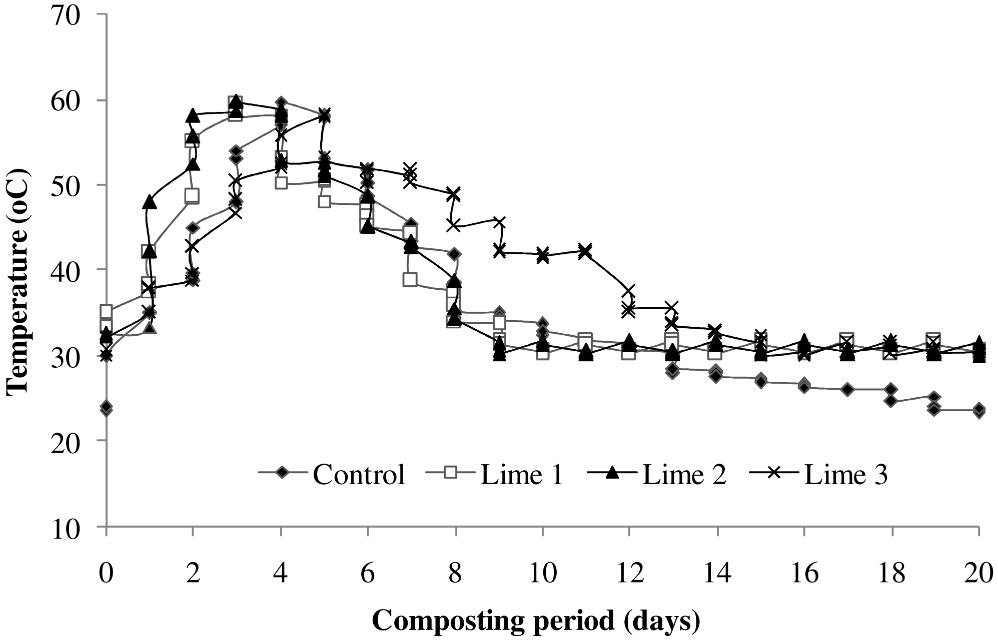


Fig. 5.9 Variation of temperature during drum composting with lime

These results were corroborate with other finding (Fang and Wong, 1999, 2000; Wong and Selvam, 2006; Chiang et al., 2007). The changes in pH values during composting were significant ($F = 14.5$, $p < 0.001$).

The organic matter was reduced significantly ($F = 10.4$, $p < 0.001$) in control and all lime treatments during the process (Fig. 5.10c). The maximum reduction of organic matter was observed in lime 2 treatment (46.1%) followed by lime 1 treatment (44.6%) and control (32.6%) lime 3 treatment (28.1%). The decrease of organic matter might be due to the reduction of the readily available carbon resulting in CO_2 loss during the composting process (Zheng et al., 2007). Fig. 5.10d shows the EC values decreased significantly ($F = 3.4$, $p < 0.001$) during the composting process. The EC values were lower in the lime treated compost in comparison to control. The final EC values of the all compost were about 2.3, 1.8, 1.83 and 3.3 dS/m in control, lime 1, 2 and 3 treatments respectively. The concentration of Na, K, Ca and Mg was increased significantly ($F = 8.26$, $p < 0.001$ for Na, ($F = 23.11$, $p < 0.001$ for K), ($F = 32.3$, $p < 0.001$ for Ca, $F = 39.87$, $p < 0.001$ for Mg) during the composting process (Fig. 5.11).

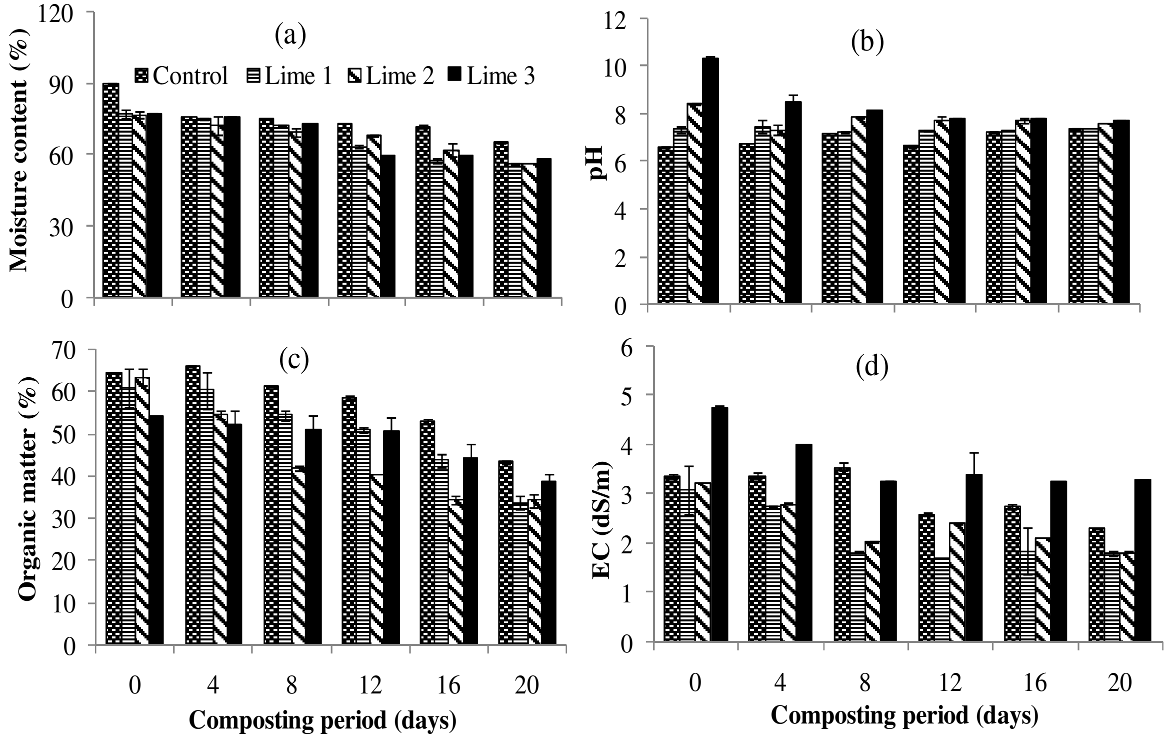


Fig. 5.10 Variation of physico-chemical parameters: (a) moisture content (b) pH (c) organic matter and (d) electrical conductivity (EC) during drum composting with lime (bars denoted as ± standard deviation)

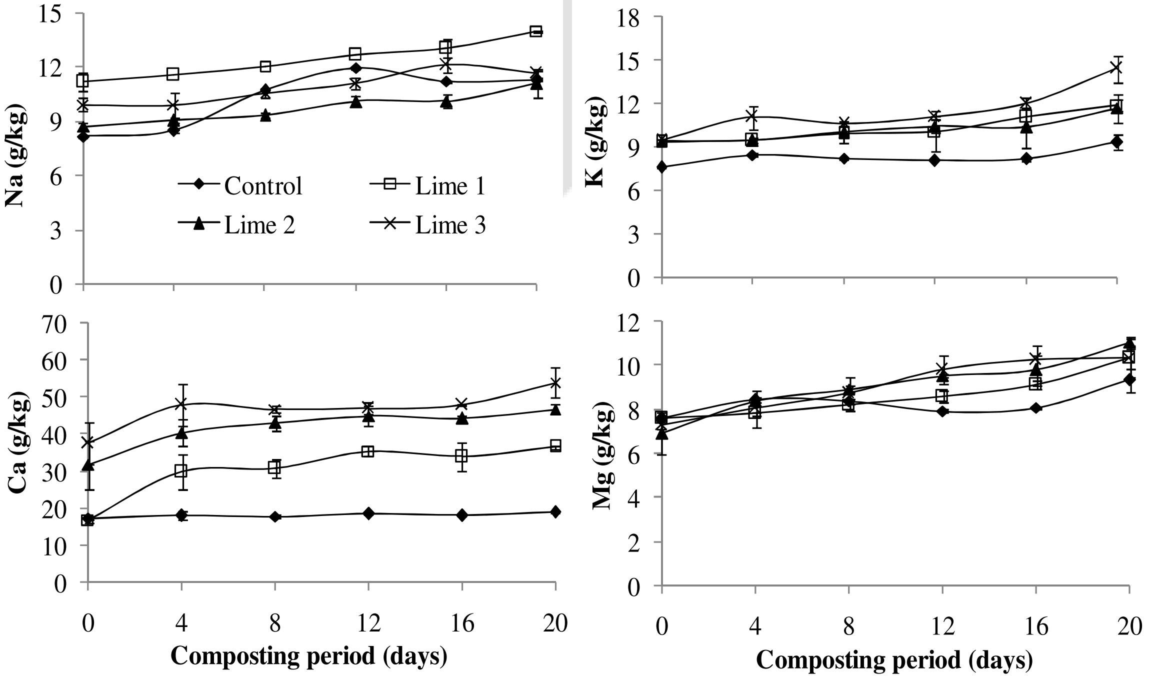


Fig. 5.11 Variation of nutrients (Na, K, Ca and Mg) during drum composting with lime (bars denoted as ± standard deviation)

The concentration of Ca was increased about 2-3 times of control due to addition of lime. The total concentration of metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) was also increased (Table 5.5a and b). An increase in nutrient and heavy metals might be due to net loss of dry mass and release of CO_2 during the composting process (Zorpas et al., 2000). The variation in total metals concentration were significant ($F = 9.73$, $p < 0.001$ for Zn, $F = 16.43$, $p < 0.001$ for Cu, $F = 15$, $p < 0.001$ for Mn, $F = 4.9$, $p < 0.001$ for Fe, $F = 9.03$, $p < 0.001$ for Ni, $F = 10.43$, $p < 0.001$ for Pb, $F = 2.95$, $p = 0.0034$ for Cd, $F = 6.46$, $p < 0.001$ for Cr) in control and lime treatment during 20 days of composting period.

5.2.2 BIOAVAILABILITY OF HEAVY METALS

- **Water soluble heavy metals**

Table 5.6 shows the changes in water-soluble Zn, Cu, Mn, Fe and Cr contents during rotary drum composting process. The changes in water soluble Zn, Cu, Mn, Fe and Cr concentrations in control and lime amended compost were significant ($F = 100.11$, $p < 0.05$ for Zn, $F = 16.7$, $p < 0.001$ for Cu, $F = 32.5$, $p < 0.001$ for Mn, $F = 38.3$, $p < 0.001$ for Fe, $F = 19.4$, $p < 0.001$ for Cr). Water-soluble Ni, Pb and Cd were not detected in control and all lime treatments during the composting process.

Higher reduction (percentage of total Zn) of water soluble Zn was observed in lime 2 treatment (68.3%) followed by lime 1 treatment (64.3%), control (47.4%) and lime 3 treatment (45.4%) during the composting process. Higher reduction of Cu was observed in lime 1 (78.98%) followed by lime 2 (78.8%), lime 3 (71.7% of) and control (63.2% total Cu) during the composting process. Higher reduction of Mn was observed in lime 2 treatment (74.3%) followed by control (72.5%), lime 1 treatment (65.6%) and lime 3 treatment (28.9% of total Mn) during the process. Lime was very effective for reduction of water soluble Fe in comparison to control. Solubility of Fe was reduced about 17.9%, 61.2%, 64.8% and 51.3% of total Fe in control, lime 1, 2 and 3 treatments respectively during the composting process. Water solubility of Cr was reduced very significantly in control, lime 1 and 2 treatments; however its reduction was lower in lime 3 treatment during the process. Added lime was very effective for reduction of water-soluble Zn, Cu, Fe and Cr concentration during the composting process; it might be due to higher reduction of organic matter, formation of soluble carbonates and the formation of organo-metallic complexes (Fang and Wong, 1999). Calcium hydroxide breaks into calcium ion and hydroxide ion in the presence of water (Montes-Hernandez et al., 2009).

Table 5.5a Variation of total metal concentration (Zn, Cu, Mn and Fe) in control and lime treatments during 20 days of composting period (mean ± SD, n=3)

	Total heavy metal concentration							
Days	**Zn (mg/kg)**				**Cu (mg/kg)**			
	Control	**Lime 1**	**Lime 2**	**Lime 3**	**Control**	**Lime 1**	**Lime 2**	**Lime 3**
0	147.3±1.9	136.5±2.5	135.3±0.4	137.9±5.4	70.5±1	33.6±2	37.2±1.8	32.1±1.1
4	155.4±1.2	137.1±1.1	143.4±8	136.1±1.1	71.7±1.15	42.4±2.8	47.1±1.4	35.4±0.4
8	177.6±2.2	141.9±8.2	135.6±1	134.2±3.5	71.3±0.5	47.2±1.3	49.9±3.15	43.2±0.7
12	153.9±3	142.1±4.4	136.7±3.4	137.9±0.3	73.0±0.4	54.5±4	52.1±3.1	45.9±0.9
16	141.0±12.7	146.7±1.4	139.6±1.6	142.4±1.7	76.9±1.6	59.5±2.9	57.7±1.6	50.2±6.3
20	155.1±2.9	154.0±0.2	154.9±1.8	163.6±3.8	91.6±1.9	63.2±3.3	64.9±4.1	67.6±6.1
Days	**Mn (mg/kg)**				**Fe (g/kg)**			
0	538±18	468.5±16	361.8±22.3	426.5±4.8	11.0±0.34	8.2±0	6.6±0.48	6.5±0.008
4	700±95	470.0±6.3	461.0±15.5	441.0±4.5	13.4±0.76	10.6±1	10.0±0	6.7±0.01
8	705±40	476.5±9.5	481.5±44.5	441.5±5	12.8±0.21	11.2±0	9.8±0	7.5±0.06
12	550±85	479.0±2.5	484.8±0.8	451.5±10	13.2±0.2	11.6±1	9.5±0.42	9.2±0.40
16	670±190	525.0±28	518.8±34.3	471.0±15	13.4±0.35	11.91±1	10.5±0.48	9.5±0.2
20	658±13	535.0±23	522.5±14.5	533.5±24.8	14.1±0.41	12.7±0.5	11.0±0.94	10.3±0.11

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.5b Variation of total metal concentration (Ni, Pb, Cd and Cr) in control and lime treatments during 20 days of composting period (mean ± SD, n=3)

Days	Total heavy metal concentration							
	Ni (mg/kg)				**Pb (mg/kg)**			
	Control	**Lime 1**	**Lime 2**	**Lime 3**	**Control**	**Lime 1**	**Lime 2**	**Lime 3**
0	239.5±10.5	198.5±4.0	215.8±21.3	216.5±20	957.5±7.5	676.5±23.5	764.0±11.0	751.5±11.5
4	232.8±3.3	213.8±13.7	233.0±15	258.5±0.5	930.0±35	750.3±4.7	692.8±47.3	775.3±19.5
8	240.0±3.5	236.3±0.8	241.8±4.8	262.5±4.5	950.0±20	787.5±57.5	777.5±32.5	812.7±57.0
12	223.5±9.0	247.0±9.4	250.2±6.2	264.5±1.9	919.9±4.9	799.5±15.5	781.0±3.0	812.4±37.6
16	233.5±4.5	256.2±11.6	259.9±7.9	265.4±2.4	980.0±35	855.0±10	857.5±7.5	910.4±44.7
20	243.5±2.5	265.2±5.1	263.7±6.7	271.9±1.6	1111.0±53	904.5±49.5	917.0±37	934.5±19.5
Days	**Cd (mg/kg)**				**Cr (mg/kg)**			
0	51.45±1.0	48.7±2.1	42.2±3.4	40.9±8.1	59.3±3.2	101±5	99.5±0	82±11.5
4	54.3±1.3	49.6±5.1	49.6±6.0	45.6±1	66.3±2.3	114±10	114.0±10	84.5±6.5
8	56.6±2.1	53.9±3.8	52.6±4.1	53.6±4.1	78.4±4.1	119.5±5	125.0±4	99.0±0.1
12	56.9±2.8	58.1±0.6	57.9±0.9	53.9±5.8	65.5±2.5	129±3.5	133.5±5	95.5±5
16	57.3±3.7	59.0±0.5	58.3±0.8	55.3±2.25	77.0±2.0	132.5±3	136.5±1.5	101±5.5
20	60.7±1.7	62.0±6.9	60.2±4.7	65.0±0.95	76.3±2.4	141.5±3	147.0±5	106.5±4

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Hydroxide ion combined with metal ion (M^{2+}) and form metal hydroxides. These metal hydroxides might be adsorbed on charged colloids such as degraded organic matter, consequently reduces the metal solubility (Garau et al., 2007). The water solubility of heavy metals was reduced might be due to biosorption by the microbial biomass or metal complexation with the newly formed humic substances and raises in pH during the composting process (Baker et al., 2011). However, Fang and Wong (1999) reported that water soluble content of Mn reduced during sewage sludge composting process. The order of water extractable metals (percentage of total metal) was Cu (2.3%) > Zn (1.4%) > Mn (0.7%) > Cr (0.5%) > Fe (0.2%) in the final water hyacinth compost.

- **Plant availability of heavy metals (extraction with DTPA)**

Fig. 5.12 shows the significant reduction of DTPA extraction efficiency of Zn, Cu, Mn, Fe, Ni and Cr during the composting process. The higher reduction (percentage of total Zn) of Zn content was observed in lime 2 treatment (61%) treatment followed by lime 1 treatment (55.8%) and lime 3 treatment (31.5%), however it was increased in control during the process. The concentration of Cu was reduced in control and all lime treatments; however higher reduction was observed in lime 1 treatment (71.1%) and lime 2 treatment (79.8% of the total Cu) during the rotary drum composting process. The concentration of Mn was reduced about 45.5, 48.4, and 44.8% of total Mn in lime 1, 2 and 3 treatments respectively; however it was enhanced in control during the rotary drum composting. The higher reduction of Fe content was observed in lime 2 treatment (58.4%) treatment followed by lime 3 treatment (50.2%), lime 1 treatment (49.7% of total Fe); however it was increased in control. The concentration of Ni and Cr was reduced in control and all lime treatments. The higher reduction of these metals was observed in lime 1 and 2 treatments during the rotary drum composting. The order of DTPA extractable metals (percentage of total metal) was: Mn (48.4%) > Zn (33%) > Cu (7.8%) > Fe (4%) > Cr (3.5%) > Ni (0.4%) in the final water hyacinth compost. DTPA extractable Pb and Cd were not detected in control and all treatments throughout composting process.

The reduction in bioavailability of heavy metals might be due to formation of insoluble carbonates, adsorption of metals onto fine particles of lime or the formation of organo-metallic compound during the composting process (Wong and Fang, 2000). The addition of lime was very effective for reduction in plant availability of metals probably due to formation of less soluble metal carbonates and hydroxides at higher pH (Wong and Selvam, 2006).

Table 5.6 Changes in water soluble heavy metal concentration during drum composting with lime (mean ± SD, n=3)

Days	Water soluble metal concentration							
	Zn (mg/kg)				**Cu (mg/kg)**			
	Control	**Lime 1**	**Lime 2**	**Lime 3**	**Control**	**Lime 1**	**Lime 2**	**Lime 3**
0	2.28±0.01	3.48±0.12	3.70±0.04	3.48±0.022	2.28±0.22	2.55±0.25	2.97±0.61	2.59±0.21
4	2.38±0.01	2.6±0.01	3.45±0.05	3.48±0.28	1.71±0.03	1.77±0.17	1.69±0.31	2.25±0.05
8	3.0±0.03	2.26±0.04	2.70±0.09	2.71±0.21	1.69±0.01	1.68±0.12	1.67±0.20	1.74±0.36
12	1.84±0.04	2.1±0.08	2.61±0.39	2.13±0.07	1.31±0.01	1.56±0.16	1.53±0.22	1.67±0.19
16	1.41±0.01	2.06±0.04	2.48±0.32	2.55±0.24	1.12±0.04	1.59±0.07	1.50±0.12	1.56±0.18
20	1.26±0.02	1.40±0.2	1.32±0.10	2.25±0.55	1.09±0.01	1.01±0.11	1.10±0.06	1.54±0.02
Days	**Mn (mg/kg)**				**Fe (mg/kg)**			
0	9.99±0.01	4.095±0.84	3.04±1.0	4.23±0.65	19.32±0.26	12.09±0.41	19.96±0.38	20.73±0.51
4	9.55±0.15	3.06±0.18	2.86±0.5	2.82±0.03	23.43±0.25	12.78±0.44	13.44±1.1	20.35±0.04
8	6.86±0.06	2.48±0.18	2.22±0.0	3.72±1.5	21.64±0.1	12.19±0.95	17.32±1.22	22.54±2.26
12	5.37±0.03	3.28±0.58	2.10±0.5	3.40±0.0	22.2±0.14	9.73±0.62	15.91±2.7	19.51±1.21
16	4.0±0.04	1.69±0.17	1.51±0.25	3.68±0.0	14.25±0.19	9.75±0.09	14.22±1.8	17.64±2.24
20	3.36±0.04	1.61±0.05	1.13±0.05	3.76±0.5	20.28±0.16	7.26±01.28	11.77±0.23	16.10±3.30
Days	**Cr (mg/kg)**				**Ni, Pb, Cd, Cr (mg/kg)**			
0	1.69±0.21	1.76±0.11	2.21±0.37	1.67±0.54	ND	ND	ND	ND
4	1.60±0.06	1.36±0.35	0.94±0.32	1.035±0.21	ND	ND	ND	ND
8	1.42±0.16	0.89±0.31	0.93±0.28	0.89±0.31	ND	ND	ND	ND
12	1.32±0.08	0.74±0.31	0.99±0.19	0.78±0.32	ND	ND	ND	ND
16	0.61±0.03	0.75±0.2	0.59±0.07	0.71±0.04	ND	ND	ND	ND
20	0.40±0.14	0.41±0.01	0.35±0.01	0.55±0.01	ND	ND	ND	ND

Mean values in columns are statistically different (ANOVA; $p < 0.05$), ND-not detected

Fuentes et al. (2004) reported DTPA extractable Ni, Zn and Cd about 14-37%, 11-37% and 17-30% of total Ni, Zn and Cd respectively in four different sludges; on the other hand, in the present study these metals were found about 0.15-0.40%, 12-33% and 0% of total Ni, Zn and Cd respectively in the final compost of control and all lime treatments. Fang and Wong (1999) reported DTPA extractable Zn (percentage of the total Zn) in the sewage sludge compost; however in the present study, Mn was the highest DTPA extractable. The variation in DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr concentrations in control and lime treatments were significant ($F = 33.6$, $p < 0.001$ for Zn, $F = 24.7$, $p < 0.001$ for Cu, $F = 22.4$, $p < 0.001$ for Mn, $F = 36.21$, $p < 0.001$ for Fe, $F = 19.4$, $p < 0.001$ for Ni, $F = 15.6$, $p < 0.001$ for Cr).

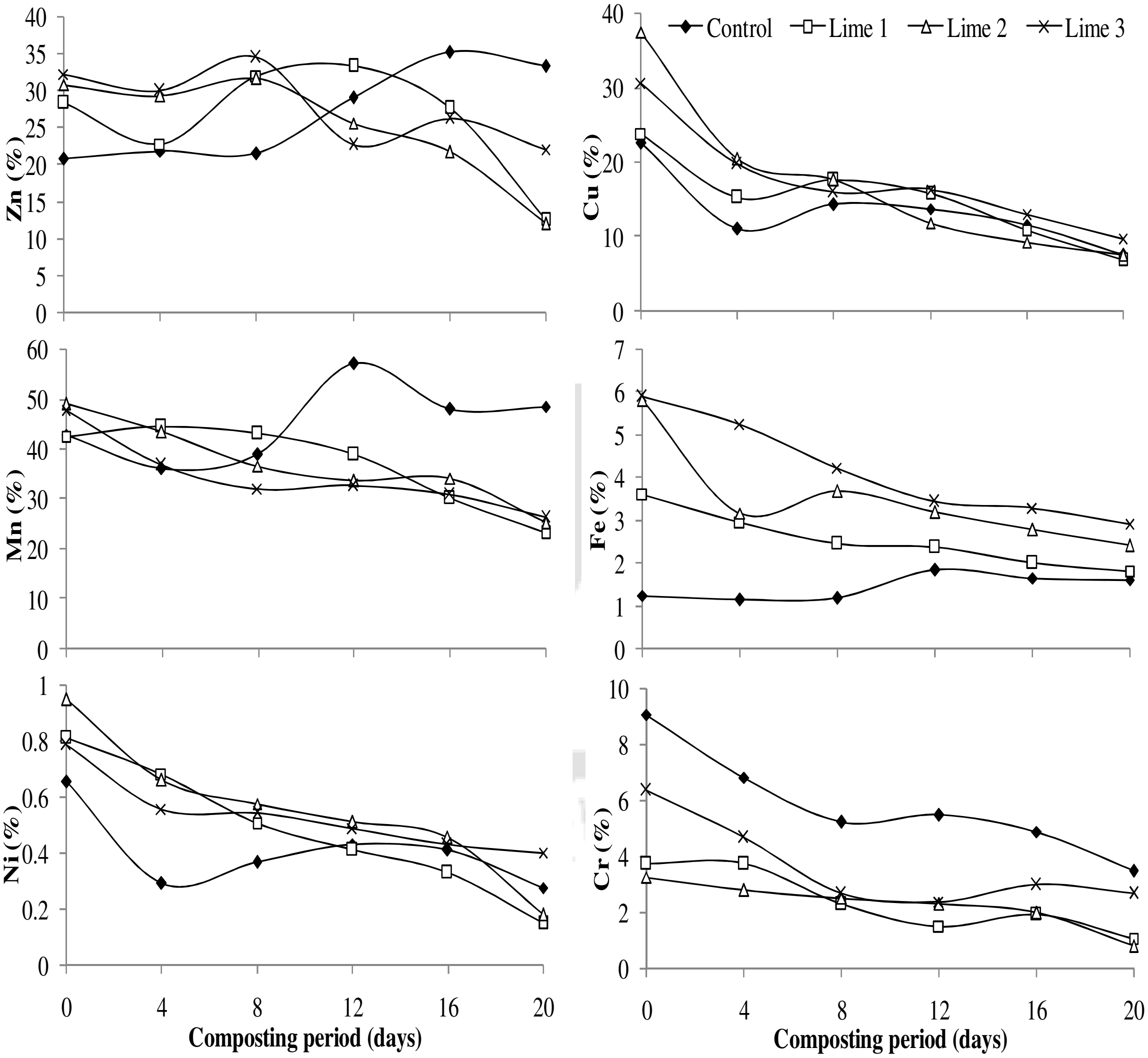


Fig. 5.12 Variation of DTPA extractable heavy metals concentration during drum composting with lime

5.2.3 LEACHABILITY OF HEAVY METALS

Fig. 5.13 illustrates the variations in leachable Zn, Cu, Mn, Fe, Ni, Cd, Pb and Cr concentration during 20 days of composting period. In the present study these metal concentrations (mg/kg) in the lime amended compost were in the range of 0.5-1.8, 3.4-5.1 and 4.9-17 for Cd, Cr and Pb respectively. Results of TCLP test confirmed that this compost is suitable for the land applications.

The concentration of Zn was reduced (% of total Zn) in control and all lime treatments; however it's higher reduction was observed in lime 1 treatment (61.4%) and lime 2 treatment (61.0%) treatments. The higher concentration of Cu was reduced in lime 1 treatment (79.3%) followed by lime 2 treatment (78.6%), control (73.2%) and lime 3 treatment (64.1%) during the process. The higher reduction of Mn was observed in lime 2 treatment (54.6%) followed by lime 3 treatment (40.9%), lime 1 treatment (35.6%) and control (30.1%). The higher reduction of Fe was observed in lime 1 treatment (74.6%) and 2 treatment (76.1%) followed by lime 3 treatment (68.8%) and control (59.3%). The higher content of Ni was reduced in lime 1 treatment (62.3%) followed by lime 2 treatment (59.9%), control (57.1%) and lime 3 treatment (40.8%) during the composting process. Addition of lime was not much effective for reduction of Pb concentration but lime 1and 2 treatments were very effective for reduction of Cd and Cr in comparison to control and lime 3 treatment during the process.

The leachable concentration of Zn, Cu, Fe, Ni, Cr and Cd (percentage of total metal) was reduced significantly in control and lime amended compost, it might be due to higher degradation of organic matter resulting formation of humic substances, which had a capacity to complex with metals resulting formation of insoluble organometallic complexes (Wong and Fang, 2000). The order of TCLP extractable metal concentration (percentage of total metal) was: Mn (27.7%) > Zn (26.4%) > Cu (9.54%) > Cr (4.8%) > Ni (4.2%) > Cd (2.8%) > Pb (1.5%) > Fe (0.6%) in the final compost. The CO_2 produced during mineralization of the organic matter, which enters the carbonate/bicarbonate equilibrium, thus affecting metal precipitation by the formation of metal carbonate, oxides or hydroxides, consequently reduced concentration of soluble metals (Garau et al., 2007; Pardo et al., 2011). The variation in leachable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in the control and lime amended compost were significant ($F = 53.2$, $p < 0.001$ for Zn, $F = 32.4$, $p < 0.001$ for Cu, $F = 19.3$, $p < 0.001$ for Mn, $F = 11.4$, $p < 0.001$ for Fe, $F = 37.9$, $p < 0.001$ for Ni, F = 4.7, $p < 0.001$ for Pb, $F = 19.8$, $p < 0.001$ for Cd, $F = 12.6$, $p < 0.001$ for Cr).

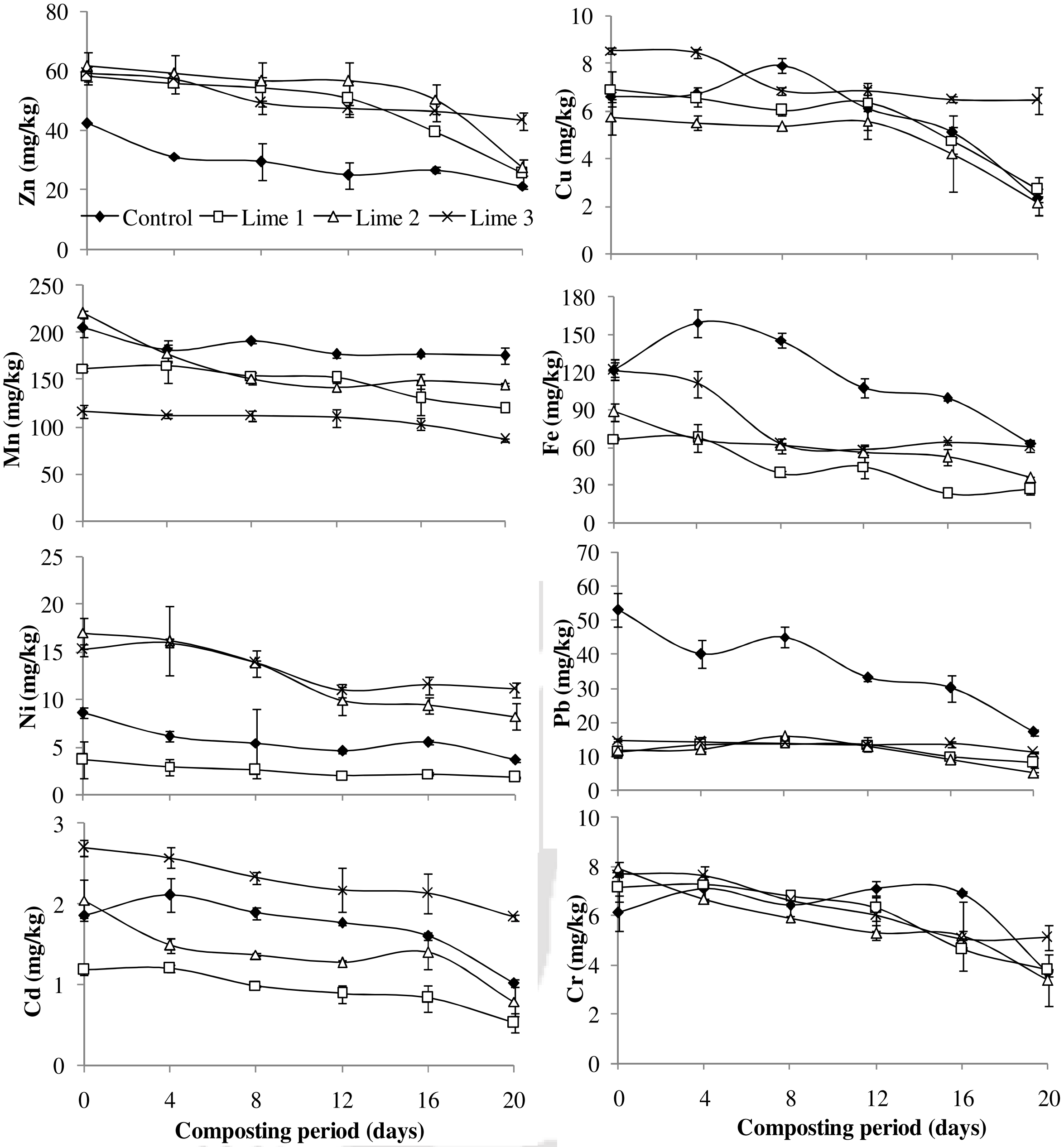


Fig. 5.13 Variation of leachable heavy metal concentration during drum composting with lime (bars denoted as ± standard deviation)

5.2.5 SPECIATION OF HEAVY METALS

- **Speciation of Zn, Cu, Mn, Fe and Ni**

Speciation of Zn in control and lime 2 and 3 treatments are given in table 4.11, 5.7a and 5.8a respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. The F1, F2, F3 and F4 fractions of Zn were reduced in the control and all lime treated compost. Higher reduction of F1 (73.9%) and F4 (67.6%) fractions was observed in lime 1 treatment. higher reduction of F2 (77.7%) and F3 (56.9% of total fraction) fractions were observed in lime 2 treatment and control respectively. The F5 fraction of Zn was enhanced in control and all lime amended compost. Significant reduction of F4 fraction might be due to conversion of this fraction into F3 and F5 fractions during the composting process. The F5 fraction of Zn was dominant fraction in control and all lime treatments. Higher enhancement of F5 fraction was observed in lime treatment in comparison to control. These results confirm that bioavailable fractions of Zn were converted into stable inert fraction due to the addition of lime. Zhu et al. (2011) reported Zn mainly found in F3 and F4 fractions in sewage sludge and humus soil. However in our study it was mainly found in F5 fraction in control and lime treated compost. The reduction of F1, F2, F3 and F4 fractions (percentage of total fraction) in control and all lime treated compost might be due to cation exchange and complexation by decomposed organic matter and in addition the formation of Zn complex with humic substances (Cai et al., 2007; Kumpiene et al., 2008). The most mobile fractions of Zn were transformed into stable fraction (F5 fraction) with stabilization of organic matter during the process (Smith, 2009). Furthermore, at higher pH Zn can precipitate with hydroxides, carbonates, phosphates, sulfides and several other anions as well as form complexes with stabilized organic matter (Kumpiene et al., 2008).

The BF of Zn was decreased from 0.37 to 0.23, 0.59 to 0.36, 0.61 to 0.36 and 0.66 to 0.44 in control and lime 1, 2 and 3 treatments respectively during the process (Fig. 5.15). Higher reduction of BF was observed in lime 2 treatment (41.3%) followed by lime 1 treatment (35.1%), control (37.5%) and lime 3 treatment (33.15%) during the composting process. The reduction of BF in control and all lime treatments might be due to formation of Zn complex with humic substances at the final stage of composting process (Cai et al., 2007). The variation in F1, F2, F3, F4 and F5 fractions of Zn in control and lime treatments were significant ($F = 8.4$, $p < 0.001$ for F1, $F = 42.56$, $p < 0.001$ for F2, $F = 20.19$, $p < 0.001$ for F3, $F = 19.63$, $p < 0.001$ for F4, $F = 9.7$, $p < 0.001$ for F5).

Speciation of Cu in control and lime 2 and 3 treatments are given in table 4.11, 5.7a and 5.8a respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. Speciation of Cu in control and lime 1 treatment is given in Fig. 3 and 4 respectively. Speciation of Cu in lime 2 and 3 treatments is given in table 3 and 4 respectively. Similar as Zn; F1, F2, F3 and F4 fractions of Cu were decreased in the control and all lime treatments. Reduction of F1 (88.6%), F2 (76.7%) and F3 (89.4%) fractions (percentage of total fraction) was observed in lime 2 treatment but higher reduction of F4 (60.2%) was observed in lime 3 treatment. The F1 and F2 fraction of Cu was easily released during the composting process and ions were bound to one or more organic functional groups, mainly carboxylic, carbonyl and phenolic groups (Nomeda et al., 2008; Zhu et al., 2011). The F5 fraction of Cu was enhanced in control and all lime treatments. Therefore it can be concluded that addition of lime could effectively prevent bioavailability of Cu during rotary drum composting. The F4 and F5 fractions of Cu were dominant in the final compost of control and lime treatments. Similar results also reported by other researchers (Zhu et al., 2011; Wong and Selvam, 2006)).

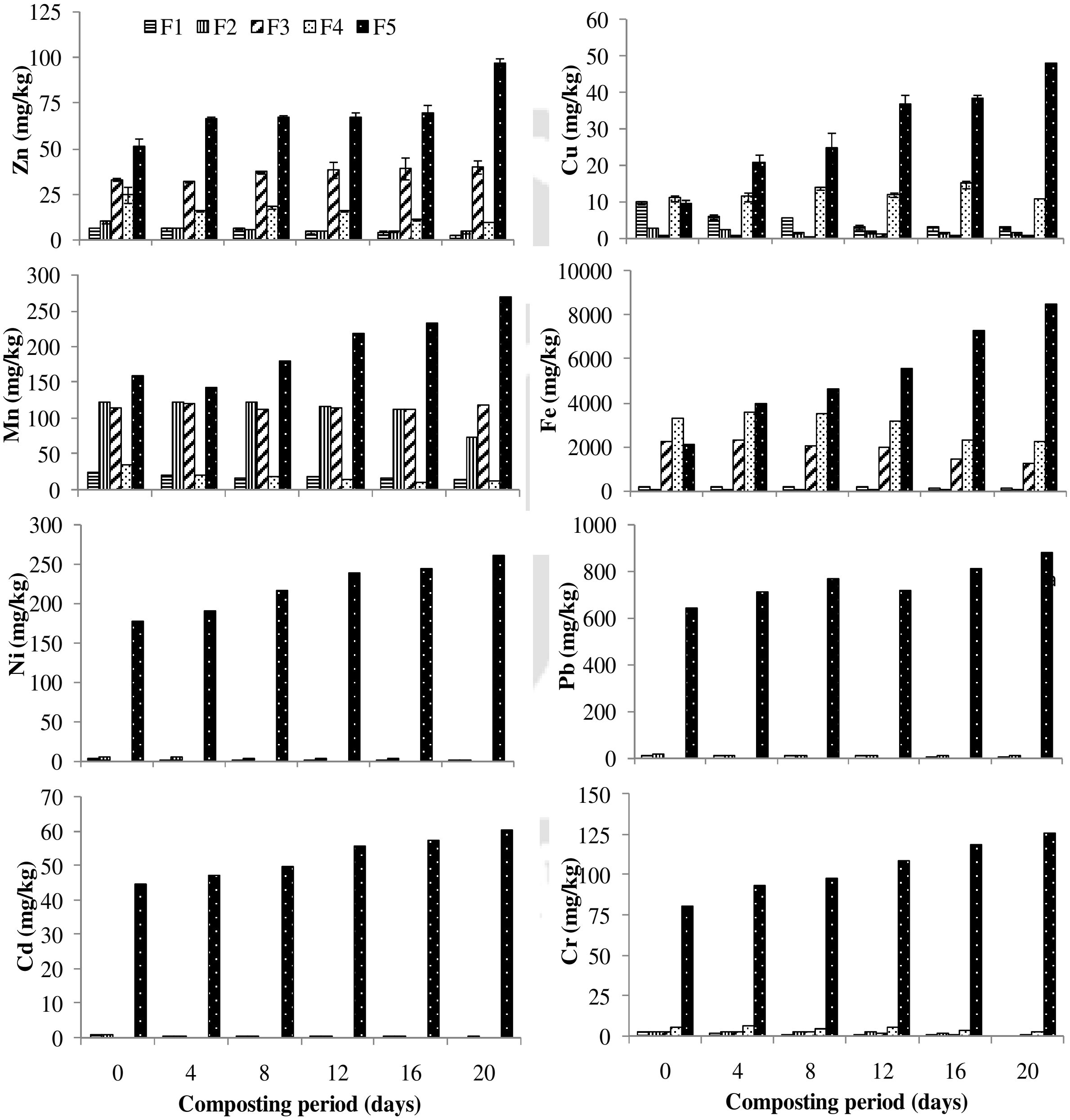


Fig. 5.14 Speciation of Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr in lime 1 treatment during drum composting (bars denoted as ± standard deviation)

The BF of Cu was decreased from 0.52 to 0.22, 0.72 to 0.27, 0.64 to 0.20 and 0.65 to 0.21 in control and lime 1, 2 and 3 treatments respectively during the process (Fig. 5.15). Higher reduction of BF was observed in lime 2 treatment (69.4%) followed by lime 1 treatment (67.15%), lime 3 treatment (64.6%) and control (58.7%) during the composting process. The higher reduction in BF of Cu was observed in lime treatments which might be due to conversion of most bioavailable fractions into more stable fraction (F5 fraction) and formation of Cu ion complex with two or more organic functional groups mainly carboxylic, carbonyl and phenolic, so that the ion is immobilized in a rigid inner-sphere complex (Qiao and Ho 1997). Wong and Selvam (2006) reported that the order of Cu fractions in control and lime amended sewage sludge compost was: F4 > F5 > F3 > F1 > F2. In the present study order in control was: F5 > F4 > F3 > F2 > F1 and in lime amended compost was: F5 > F4 > F1 > F2 > F3. The variation in F1, F2, F3, F4 and F5 fractions of Cu in control and lime treatments were significant ($F = 29.56$, $p < 0.001$ for F1, $F = 11.76$, $p < 0.001$ for F2, $F = 46.26$, $p < 0.001$ for F3, $F = 8.31$, $p < 0.001$ for F4, $\mathrm{F} = 14.1$, $p < 0.001$ for F5).

Speciation of Mn in control and lime 2 and 3 treatments are given in table 4.11, 5.7a and 5.8a respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. The F1 and F4 fractions of Mn were reduced in control and all lime treatments. Higher reduction of F1 fraction was observed in control (66.12%) followed by lime 2 treatment (64.7%), lime 3 treatment (48.4%) and lime 1 treatment (44.3% of total fraction) during the process. The F2 fraction was reduced in all lime treatments; however these fractions were increased in the control. The F3 fraction of Mn was reduced in lime 1 treatment; however it was increased in control, lime 2 and 3 treatments. The F5 fraction was increased in the control and all lime treatments but higher enhancement was observed in lime 2 treatment during the process. Wong and Selvam (2006) reported that the order of different fractions of Mn in control and lime amended sewage sludge compost was: F3 > F5 > F1 > F2 > F4. However in the present study this order was: F5 > F2 > F3 > F1 > F4 in control and F5 > F3 > F2 > F1 > F4 in all lime treatments. These orders reveal that F2 fraction was dominant after F5 fraction in control, but in the lime treated compost F3 fraction dominated after F5 fraction. It can be concluded that F2 fraction converted into F3 and F5 fraction due lime addition.

The BF of Mn was decreased from 0.73 to 0.51, 0.64 to 0.45, 0.86 to 0.55 and 0.62 to 0.50 in control and lime 1, 2 and 3 treatments respectively during the process (Fig. 5.15).

Table 5.7a Speciation of heavy metals (Zn, Cu, Mn and Fe) in lime 2 treatment during 20 days of composting period (mean ± SD, n=3)

	Lime 2 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	6.44±0.40	16.64±0.2	35.1±0.35	21.34±0.80	50.95±0.95	6.03±0.32	2.80±0.46	1.74±0.14	12.45±0.09	12.78±2.58
4	5.17±0.14	11.82±0.28	36.66±0.42	19.93±0.17	58.75±6.25	4.80±0.40	1.65±0.10	0.96±0.06	14.98±0.63	24.49±3.99
8	3.97±0.17	6.91±0.11	43.54±1.04	18.80±0.44	60.0±6.00	4.86±0.54	1.55±0.10	0.79±0.19	12.57±1.78	25.52±2.02
12	3.86±0.22	6.88±0.13	44.44±0.77	16.83±0.63	61.75±2.25	4.44±0.43	1.43±0.03	0.47±0.07	11.45±0.92	30.43±6.07
16	2.52±0.02	4.85±0.2	38.73±3.3	13.10±0.85	68.0±6.00	2.20±0.30	1.20±0.15	0.34±0.00	11.15±0.77	34.61±0.11
20	2.12±0.76	4.15±0.61	35.95±0.75	9.90±0.31	93.5±1.50	1.13±0.07	1.08±0.03	0.31±0.07	9.10±0.74	47.45±2.05
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	41.38±3.98	130.90±1.4	119.0±6	19.87±1.4	49.2±5.8	121.5±11.5	23.1±0.48	2126±186	2517±357	1567±33
4	32.97±2.3	131.50±3.9	133.1±21	17.5±0.98	122.5±12.5	157.5±13.5	23.8±2.4	2367±52	2967±457	3851±54
8	27.55±3.7	130.53±2.8	141.7±3.6	16.0±0.37	123.7±3.7	157.9±12.1	20.2±0.97	2242±128	2490±150	4209±659
12	28.70±3.0	122.99±2.4	153.2±3.2	15.5±0.03	155.5±19	142.9±7.1	19.6±0.89	1892±82	2124±120	5237±737
16	24.78±0.22	99.90±2.4	159.0±2.6	11.0±0.79	218.5±8.5	136.5±1.5	14.2±1.8	1775±255	2006±508	5872±628
20	20.60±0.43	72.45±5.8	173.2±15	10.94±0.9	230.0±15	148.9±11	15.3±30	1208±161	1737±507	7433±66

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.7b Speciation of heavy metals (Ni, Pb, Cd and Cr) in lime 2 treatment during 20 days of composting period (mean ± SD, n=3)

	Lime 2 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	1.50±0.05	3.97±0.48	ND	ND	191.4±2.6	16.15±0.15	14.20±0.8	ND	ND	683.4±52.4
4	1.60±0.5	3.17±0.03	ND	ND	209.8±14.3	15.10±3.1	11.65±0.7	ND	ND	664.9±14.8
8	1.98±0.2	2.70±0.00	ND	ND	234.0±1.00	9.05±0.44	13.60±1.6	ND	ND	732.5±7.5
12	1.70±0.1	2.67±0.28	ND	ND	237.3±4.7	8.17±0.33	9.07±0.4	ND	ND	750.7±25.7
16	1.33±0.03	1.95±0.20	ND	ND	254±3.00	6.83±0.82	8.82±1.3	ND	ND	820.0±15.0
20	1.00±0.05	1.00±0.04	ND	ND	252.5±3.5	5.43±0.93	8.07±1.2	ND	ND	897.5±1.5
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	0.87±0.02	0.86±0.01	ND	ND	37.5±5	1.5±0.30	2.57±0.52	3.20±0.10	4.68±0.40	78.5±3.5
4	0.80±0.05	0.69±0.10	ND	ND	48.0±1.5	1.27±0.17	2.23±0.07	2.25±0.05	6.31±0.58	94.85±1.6
8	0.75±0.05	0.65±0.05	ND	ND	47.0±5.5	1.29±0.41	2.75±0.35	1.95±0.45	4.73±0.04	98.55±3.0
12	0.63±0.03	0.68±0.02	ND	ND	54.8±3.2	1.05±0.15	2.35±0.05	1.60±0.70	2.79±0.20	112.65±3.8
16	0.56±0.06	0.63±0.08	ND	ND	55.0±2.5	0.91±0.04	1.95±0.15	1.20±0.10	2.68±0.20	124.4±0.9
20	0.33±0.03	0.53±0.17	ND	ND	56.8±1.7	0.79±0.25	1.50±0.2	1.55±0.25	1.75±0.20	139.35±0.8

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

The higher reduction of BF was observed in lime 2 treatment (36.7%) followed by lime 1 treatment (31.4%), control (29.6%) and lime 3 treatment (21.3% of total fraction) during the composting process. The variation in F1, F2, F3, F4 and F5 fractions of Mn in control and lime treatments were significant ($F = 21.13$, $p < 0.001$ for F1, $F = 75.13$, $p < 0.001$ for F2, $F = 28.0$, $p < 0.001$ for F3, $F = 47.32$, $p < 0.001$ for F4, $F = 26.74$, $p < 0.001$ for F5).

Speciation of Fe in control and lime 2 and 3 treatments are given in table 4.11, 5.7a and 5.8a respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. F1 fraction of Fe was reduced in control, lime 1 and 2 treatments; however this fraction enhanced in lime 3 treatment during the process. Higher reduction of F1 was observed about 55.4% in lime 1 treatment. The F2, F3 and F4 fractions of Fe were decreased in the control and all lime treatments in the process. Reduction of F3 and F4 fractions were observed about 65.4 and 57% of the total fraction in lime 2 treatment, higher reduction of F2 fraction was observed in lime 3 treatment during the process. The F5 fraction of Fe was enhanced in control and all lime amended compost; however higher enhancement was observed in lime treatments in comparison to control. At initial stage F4 fraction was dominant but at end of composting process F5 fraction was dominant, it might be due to addition of lime F4 fraction was converted into F5 fraction. In order of different fractions of Fe was: F5 (67.6%) > F4 (15.8%) > F3 (11.5%) > F1 (1.4%) > F2 (0.15%).

The BF of Fe was decreased from 0.82 to 0.56, 0.73 to 0.29, 0.75 to 0.30 and 0.77 to 0.31 in the control and lime 1, 2 and 3 treatments respectively during the process (Fig. 5.15). Higher reduction of BF was observed in lime 2 treatment (60.85%) followed by lime 1 (60.2%), lime 3 (59.3%) and control (32.15%) during the composting process. The variation in F1, F2, F3, F4 and F5 fractions of Fe in control and lime treatments were significant ($F = 126.73$, $p < 0.001$ for F1, $F = 8.72$, $p < 0.001$ for F2, $F = 54.7$, $p < 0.05$ for F3, $F = 15.96$, $p < 0.001$ for F4, $F = 39.41$, $p < 0.001$ for F5).

Speciation of Ni in control and lime 2 and 3 treatments are given in table 4.11, 5.7b and 5.8b respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. The F1 and F2 fractions of Ni were reduced in control and all lime treatments during the process. Reduction of F1 fraction was observed about 64.6% of the total fraction in control and higher reduction of F2 fraction was observed in lime treatments. The F5 fraction of Ni was enhanced in control and all lime treatments but it was higher increased in lime 1 and 2 treatments in comparison to control and lime 3 treatment during the rotary drum composting. Wong and Selvam (2006) reported that the order of Ni fractions in control and lime amended sewage sludge compost was: F5 > F3 > F4 > F2 > F1. However in the present study this order was: F5 > F1 > F2 in control and F5 > F2 > F1 in all lime treated compost. These results confirmed that F1 fraction was converted into F2 and F5 fractions due to lime addition. Zhu et al. (2011) reported, Ni mainly found in F3 and F4 fractions in sewage sludge and humus soil. However in the present study F5 fraction of Ni was dominant. The F3 and F4 fractions of Ni were not detected throughout composting process.

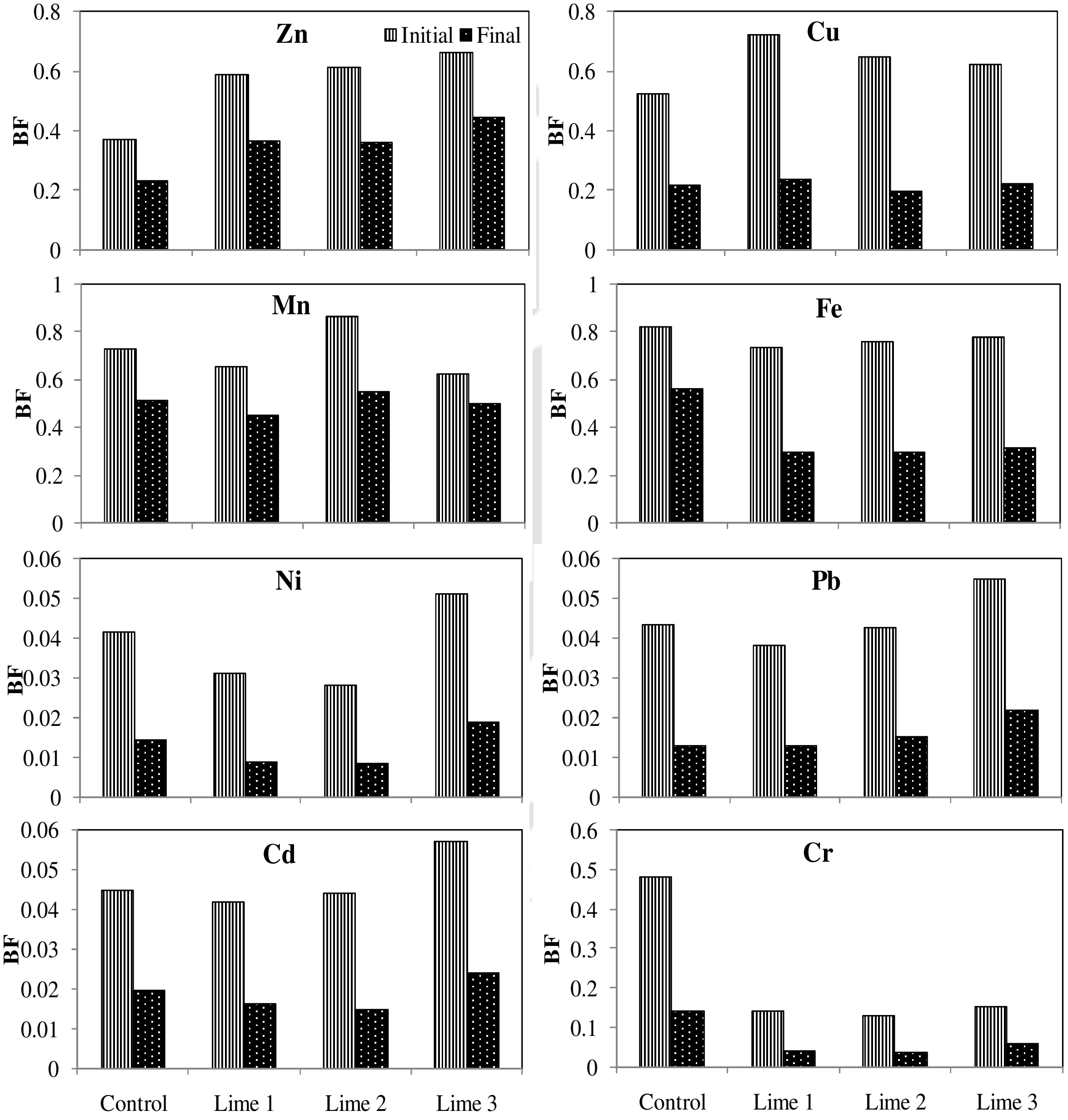


Fig. 5.15 Variation of bioavailability factor (BF) of heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during drum composting with lime

The BF of Ni was decreased from 0.041 to 0.014, 0.031 to 0.009, 0.028 to 0.008 and 0.051 to 0.019 in control and lime 1, 2 and 3 treatments during the process (Fig. 5.15). Highest reduction of BF was observed in lime 1 treatment (71.5%) followed by lime 2 treatment (70.4%), control (65.0%) and lime 3 treatment (63.2%) during the composting process. A significant decrease in BF was observed in lime 1 and 2 treatments, which suggests that the addition of optimum percentage of lime could prevent the bioavailability of Ni during the process. The alkaline stabilization could also accountable for reduction of BF. Su and Wong (2003) reported that the F5 fraction of Ni contributed 52% of the total Ni content; followed by F3 fraction in sewage sludge; however in the present study F5 fraction contributed about 96-98% of total fraction. The variation in F1, F2 and F5 fractions of Ni in control and lime treatments were significant ($F = 9.63$, $p < 0.001$ for F1, $F = 14.82$, $p < 0.001$ for F2, $F = 12.01$, $p < 0.001$ for F5).

- **Speciation of Pb, Cd and Cr**

Speciation of Pb in control and lime 2 and 3 treatments are given in table 4.11, 5.7b and 5.8b respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. The F1 and F2 fractions of Pb were decreased in the control and all lime treatments during the process. Higher reduction of F1 (77.4%) and F2 (60.1%) fractions were observed in control and lime 1 treatment respectively during the process. The F5 fraction of Pb was increased in control and all lime treatments. Higher percentage of this fraction was increased in lime 1 and 2 treatments when compared with control and lime 3 during the process. The F5 fraction of Pb was dominant fraction in the final compost of control and lime. Zhu et al. (2011) also reported that the F5 fraction of Pb was dominant in sewage sludge and humus soil.

Zorpas et al. (2008) reported the order of Pb fractions in sewage sludge compost with natural zeolite was: F5 > F3 > F4 > F2 > F1. However in the present study this order was: F5 > F2 > F1 in control and lime treated compost. The F3 and F4 fractions of Pb were not detected during the composting process. Wong and Selvam (2006) reported that the F1 and F2 fractions of Pb were remain same during the sewage sludge composting with lime; but in the present study significant reduction was observed during the process. Appropriate amount of lime addition could reduce the bioavailabilty of Pb by forming Pb-organic matter complex, but higher lime addition can have a reverse effect on Pb stability due to the amphoteric nature of Pb (Kumpiene et al., 2008).

Table 5.8a Speciation of heavy metals (Zn, Cu, Mn and Fe) in lime 3 treatment during 20 days of composting period (mean ± SD, n=3)

	Lime 3 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	5.83±0.41	6.16±0.18	44.97±0.26	26.63±0.27	42.98±0.58	3.60±0.60	2.40±0.20	1.33±0.03	11.31±1.06	10.09±0.41
4	6.80±0.56	3.78±0.42	48.41±3.19	17.33±0.09	46.90±1.50	3.45±0.45	1.70±0.14	1.29±0.01	10.49±0.17	17.77±4.77
8	5.60±0.39	3.71±0.39	51.34±6.96	17.15±0.79	48.25±6.25	2.28±0.22	1.52±0.02	1.97±0.13	10.41±0.14	22.80±2.70
12	4.40±0.90	3.71±0.20	44.79±1.59	22.53±2.25	58.00±6.00	1.89±0.09	1.40±0.05	1.77±0.13	11.53±0.18	26.32±5.82
16	4.45±1.05	3.06±0.44	52.24±6.46	16.84±6.56	54.50±0.50	1.52±0.28	1.17±0.07	1.30±0.06	11.94±4.39	34.27±0.73
20	4.74±1.04	3.34±0.24	48.05±0.25	12.25±2.55	86.50±7.50	1.30±0.56	1.49±0.43	1.26±0.22	9.47±0.82	49.70±7.70
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	14.2±0.46	77.10±5.2	140.7±1.6	9.68±0.18	147.8±20	27.3±5.75	41.0±7.4	1936±376	2795±78	1417±183
4	11.41±0.96	59.44±6.4	151.0±1.0	10.45±0.21	168.7±23	60.0±16	41.7±10	1953±362	2538±114	1798±401
8	11.98±0.48	57.43±4.9	151.8±46	12.70±0.35	187.6±58	122.9±22	26.5±2.8	1867±53	2300±40	3184±315
12	11.70±0.55	57.69±2.4	152.8±48	10.59±0.14	184.0±30	105.6±30	30.2±1.4	1638±337	2262±82	4312±338
16	10.90±0.59	47.91±5.5	156.5±56	12.26±0.62	233.5±2.5	133.3±1.7	23±0.67	1140±109	2087±72	5387±143
20	9.20±0.05	45.15±0.2	178.0±46	11.83±0.52	244.6±1.0	121.4±16	17.3±5	1083±36	1842±202	6683±316

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.8b Speciation of heavy metals (Ni, Pb, Cd and Cr) in lime 3 treatment during 20 days of composting period (mean ± SD, n=3)

	Lime 3 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	2.70±0.25	7.67±1.2	ND	ND	193.1±25.4	16.25±0.25	23.0±0.5	ND	ND	680.0±40
4	2.43±0.32	6.47±1.3	ND	ND	243.7±4.7	11.25±2.25	19.5±0.5	ND	ND	712.5±27
8	2.30±0.15	6.30±1.6	ND	ND	238.75±1.7	11.0±1.50	15.0±0.5	ND	ND	780.0±15
12	2.10±0.27	5.85±0.9	ND	ND	253.75±1.2	10.75±1.25	14.5±0.5	ND	ND	767.5±12.5
16	1.85±0.15	4.02±0.8	ND	ND	254.25±1.7	9.15±0.85	13.1±0.1	ND	ND	817.5±37
20	1.40±0.35	3.60±0.6	ND	ND	266.5±2.5	7.0±2.50	10.8±0.2	ND	ND	851.0±91
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	0.82±0.04	1.38±0.18	ND	ND	38.0±0.5	1.08±0.13	3.45±0.35	3.0±0.15	4.16±0.13	63.5±1.5
4	0.93±0.17	1.10±0.40	ND	ND	43.0±0.5	1.20±0.10	2.35±0.05	2.7±0.40	2.40±0.32	70.6±2.6
8	0.77±0.03	0.95±0.10	ND	ND	47.0±5.5	1.15±0.45	2.25±0.15	2.4±0.05	1.75±0.07	83.1±1.4
12	0.60±0.05	0.91±0.14	ND	ND	49.5±1.5	0.98±0.22	2.48±0.17	2.5±0.15	1.84±0.15	85.2±3.6
16	0.59±0.04	0.90±0.15	ND	ND	51.8±1.2	0.90±0.05	2.40±0.30	2.0±2.6	1.47±0.0	89.2±3.8
20	0.53±0.07	0.87±0.03	ND	ND	58.3±6.7	0.91±0.15	1.88±0.22	2.0±0.3	1.54±0.3	93.1±2.9

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

The BF of Pb was decreased from 0.043 to 0.013, 0.038 to 0.013, 0.042 to 0.015 and 0.055 to 0.022 in control and lime 1, 2 and 3 treatments respectively during the process (Fig. 5.15). Higher reduction of BF was observed in control (70.22%) followed by lime 1 treatment (66.1%), lime 2 treatment (65.15%) and lime 3 treatment (60.4%) during the composting process. Qiao and Ho (1997) also reported that the bioavailability of Pb decrease during sewage composting. The variation in F1, F2 and F5 fractions of Pb in control and lime treatments were significant ($F = 16.19$, $p < 0.001$ for F1, $F = 27.31$, $p < 0.001$ for $F2$, $F = 8.54$, $p < 0.001$ for F5).

Speciation of Cd in control and lime 2 and 3 treatments are given in table 4.11, 5.7b and 5.8b respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. The F1 and F2 fractions of Cd were decreased in the control and all lime treatments during the process. The higher reduction of F1 (74.4%) and F2 (58.5% of total fraction) fractions were observed in lime 2 treatment during the composting process. The F5 fraction was increased in all lime treatments; however this fraction was reduced in control during the process. Liu et al. (2007) reported that the order of Cd fraction in sewage sludge composting was: F3 > F2 > F4 > F1 > F5. However in the present study, this order was: F5 > F2 > F1 in control and all lime treated compost. The F3 and F4 fractions of Cd were not detected during the composting process. The F1 and F2 fractions were reduced during the process might be due to formation of strong chemical bond between Cd and humic substances (Haroun et al., 2007). The F5 fraction of Cd was increased in all lime treatments; however this fraction was reduced in control during the rotary drum composting process.

The BF of Cd was decreased from 0.045 to 0.019, 0.042 to 0.016, 0.044 to 0.015 and 0.057 to 0.024 in control and lime 1, 2 and 3 treatments respectively during the process (Fig. 5.15). Higher reduction of BF was observed in lime 2 treatment (66.44%) followed by lime 1 treatment (61.1%), lime 3 treatment (57.8%) and control (56.7%) during the process. The variation in F1, F2 and F5 fractions of Cd in different trials were significant ($F = 28.63$, $p < 0.001$ for F1, $F = 8.5$, $p < 0.001$ for F2, $F = 6.59$, $p < 0.001$ for F5). The decrease in BF of Cd could be due to maintaining neutral or alkaline pH in lime treatments during the composting process. A significant decrease in BF of Cd was observed in lime 1 and 2 treatments, which suggest that the addition of lime could prevent the bioavailability of Cd during the process.

Speciation of Cr in control and lime 2 and 3 treatments are given in table 4.11, 5.7b and 5.8b respectively, and speciation in lime 1 treatment is given in Fig. 5.14 during the composting process. The F1, F2, F3 and F4 fractions of Cr were reduced in the control and all lime treatments during the process. Higher reduction of F1 and F2 fractions were observed about 88.5 and 66.6% of the total fraction in lime 2 treatment during the process. Higher reduction of F3 and F4 fractions were observed in control. The F5 fraction of Cr was enhanced in control and all lime treatments in the rotary drum composting. Liu et al. (2007) reported that the order of Cr fraction in sewage sludge composting was: F4 > F5 > F1 = F3 > F2. However in the present study this order was: F5 > F4 > F1> F2 > F3 in control, F5 > F4 > F2> F3 > F1 in lime 1, F5 > F4 > F3 > F2 > F1 in lime 2 treatment and F5 > F3 > F2 > F4 > F1 lime 3 treatment during the process.

The BF of Cr was decreased from 0.48 to 0.14, 0.14 to 0.04, 0.13 to 0.04 and 0.16 to 0.06 in control and lime 1, 2 and 3 treatments respectively during the process (Fig. 5.15). Higher reduction of BF was observed in lime 2 treatment (72.2%) followed by lime 1 treatment (71.3%), control (70.5%) and lime 3 treatment (60.8%) during the composting process. A significant reduction in BF of Cr was observed in lime 1 and 2 treatments in comparison to control. It could be explained that at pH 6.6-8.5 dominant species of Cr is $Cr(OH)_2^+$ bound with various organic functional groups present in the humic substances (Chen et al., 2009). The variation in F1, F2, F3, F4 and F5 fractions of Cr in control and lime treatments were significant ($F = 12.92$, $p < 0.001$ for F1, $F = 14.62$, $p < 0.001$ for F2, $F = 20.22$, $p < 0.001$ for F3, $F = 50.8$, $p < 0.001$ for F4, $F = 15.42$, $p < 0.001$ for F5).

5.2.5 CONCLUSION

The quick development of thermophilic stage was observed in lime 2 treatment due to intense microbial growth. Organic matter reduced significantly during the composting process. The water soluble metals (Zn, Cu, Fe and Cr), DTPA extractable metals (Zn, Cu, Fe, Ni and Cr) and leachable metals (Zn, Cu, Mn, Fe, Ni, Cr and Cd) were reduced significantly due to lime addition during rotary drum composting of water hyacinth. Total concentration of Cu was lowest among five water soluble metals but its water solubility was highest. It indicates that the Cu is more toxic than Zn, Fe, Mn and Cr with respect to water solubility. The lowest water solubility and leachability of Fe was observed even its total concentration was highest among eight metals during the process. Leachable fraction of Pb was not reduced significantly in lime amended compost in comparison to control. The maximum reduction of water soluble, DTPA extractable and leachable metals were observed in lime 1 and 2 treatments, which indicated optimum percentage of lime can enhance organic matter degradation and humification process in rotary drum; consequently it reduced the toxicity of the metals. The TCLP concentrations of the targeted metals in the compost were completely in agreement with the thresholds limits.

Speciation of heavy metals in rotary drum composting was influenced by physico-chemical parameters of the compost such as temperature, organic matter and pH. The bioavailability factor (BF) of Zn, Mn, Ni, and Cr was reduced significantly in lime 1 and 2 treatment in comparison to control and lime 3 treatment. However; BF of Cu, Fe and Cd was reduced in all lime treatments in comparison to control. The order of BF of different metals in the water hyacinth compost was: Fe (0.56) > Mn (0.55) > Zn (0.44) > Cu (0.24) > Cr (0.14) > Cd (0.024) > Pb (0.022) > Ni (0.019). The total concentration of Ni was higher than Zn, Cu, Cd and Cr but its BF was lowest among these metals. Therefore this study concluded that the addition of appropriate proportion of lime sludge was successful for reducing bioavailability and leachability of heavy metals during rotary drum composting of water hyacinth mixed with cattle manure and sawdust.

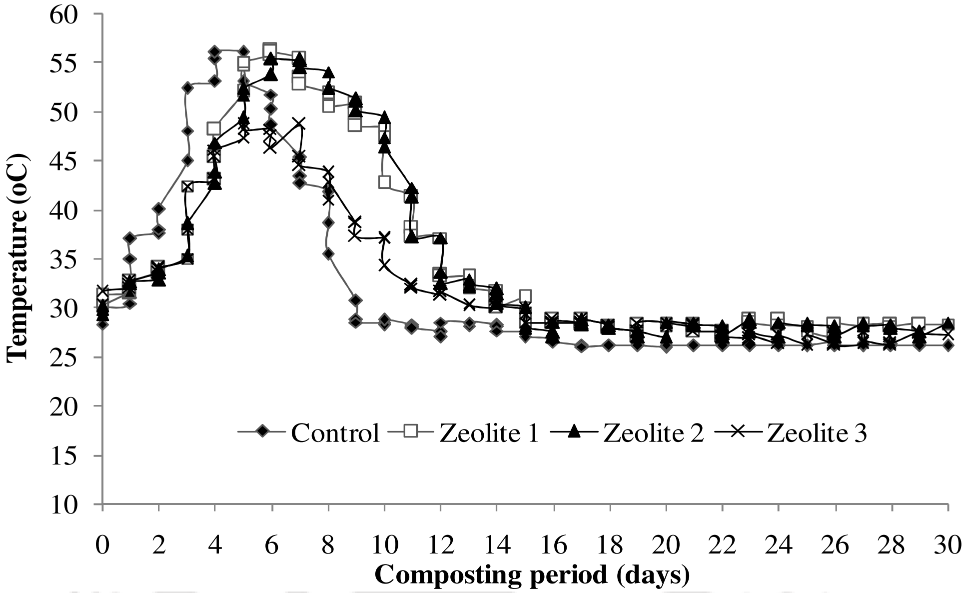
5.3 EFFECTS OF NATURAL ZEOLITE DURING AGITATED PILE COMPOSTING

Natural zeolite improves the composting process and the biodegradability of the organic matter due to its ability to increase the porosity of the substrate (Zorpas et al., 2000). It has the ability to uptake of heavy metals which are in easily available fractions, and exchange of sodium and potassium (Zorpas et al., 2000). Phase 1 study suggested that that mixture of 90 kg water hyacinth, 45 kg cattle manure and 15 kg sawdust (Trial 4) was best combination for reduction of bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate) of heavy metals during agitated pile composting of water hyacinth. Therefore, same combination (Trial 4) was selected for zeolite 1 treatment (5%), 2 treatment (10%) and 3 treatment (15%) studies. Trial 4 was considered as control with respect to zeolite addition.

5.3.1 PHYSICO-CHEMICAL ANALYSIS

Fig. 5.16 shows the changes in temperature profiles in control and all zeolite treatments during the composting process. The temperature was observed in the range of 48.6-56.2^{o}C in control and zeolite treatments during the thermophilic phase as results of the extreme microbial activity (Chiang et al., 2007). The thermophilic phase was started slightly later in zeolite treatments in comparison to control but highest thermophilic temperature was similar as control except zeolite 3. It could be explained as microbes take slightly more time for acclimatization with zeolite compost mixture than control. Addition of zeolites might be reduced the self heating property of composting process due to the bulking effect, which increased the transport of mass and energy to the atmosphere. A similar effect also reported by Villasenor et al. (2011) during sewage sludge composting with natural zeolite.

Fig. 5.17a shows the significant variation in pH values from 6.38 to 7.85 during the composting process ($F = 18.1$, $p < 0.001$). The initial pH values of compost mixture were increased a little in all zeolite treatments in comparison to control. The pH decreased slightly in the beginning of the composting process may be due to acid formation during the decomposition of organic matter, a similar results also observed by Zorpas et al. (2000) during sewage sludge composting with zeolite. The pH in initial feed mixtures of zeolite higher than control, but at end of composting pH reduced and became similar as control. It might be due to buffering capacity of composting process (Garg and Gupta, 2011).



5.16 Variation of temperature during pile composting with zeolite

Fig. 5.17b shows the significant reduction in moisture content in control and all zeolite treatments during the process ($F = 92.8$, $p < 0.05$). The higher reduction of moisture content was observed in control (55.25%) followed by zeolite 1 treatment (52.38%), zeolite 2 treatment (50.66%) and zeolite 3 treatment (47.18%). Lower reduction of moisture content in all zeolite treatments might be due to moisture retained by zeolite (Villasenor et al., 2011). Fig. 5.17c shows the significant reduction in organic matter in control and all zeolite treatments during the process ($F = 25.4$, $p < 0.001$). The higher reduction of organic matter was observed in zeolite 1 treatment (47.4%) followed by zeolite 2 treatment (43.5%), zeolite 3 treatment (31.2%) and control (23.0%). It could be explained as the biodegradation of organic matter was progressed by the addition of zeolite (Villasenor et al., 2011). Lower organic matter was observed in all zeolite treatments as compared to the control during composting period. Zeolite has the ability to increase the porosity of the composting materials due to its bulking property, consequently enhanced the biodegradability of the organic matter (Zorpas et al., 2000). The EC was increased from 3.13 to 3.35 dS/m, 3.39 to 3.52 dS/m and 3.40 to 3.57 dS/m in zeolite 1, 2 and 3 treatments; however it was reduced in control from 6.42 to 4.71dS/m during the composting process (Fig. 5.17d). An increase in EC during composting process also reported by other researcher (Zorpas and Loizidou, 2008; Chiang et al., 2007). The EC value was lower in the all zeolite treatments in comparison to control due to the unique properties of natural zeolite (Chiang et al., 2007). The significant variation in EC was observed in control and all zeolite treatments during the process ($F = 165.1$, $p < 0.05$).

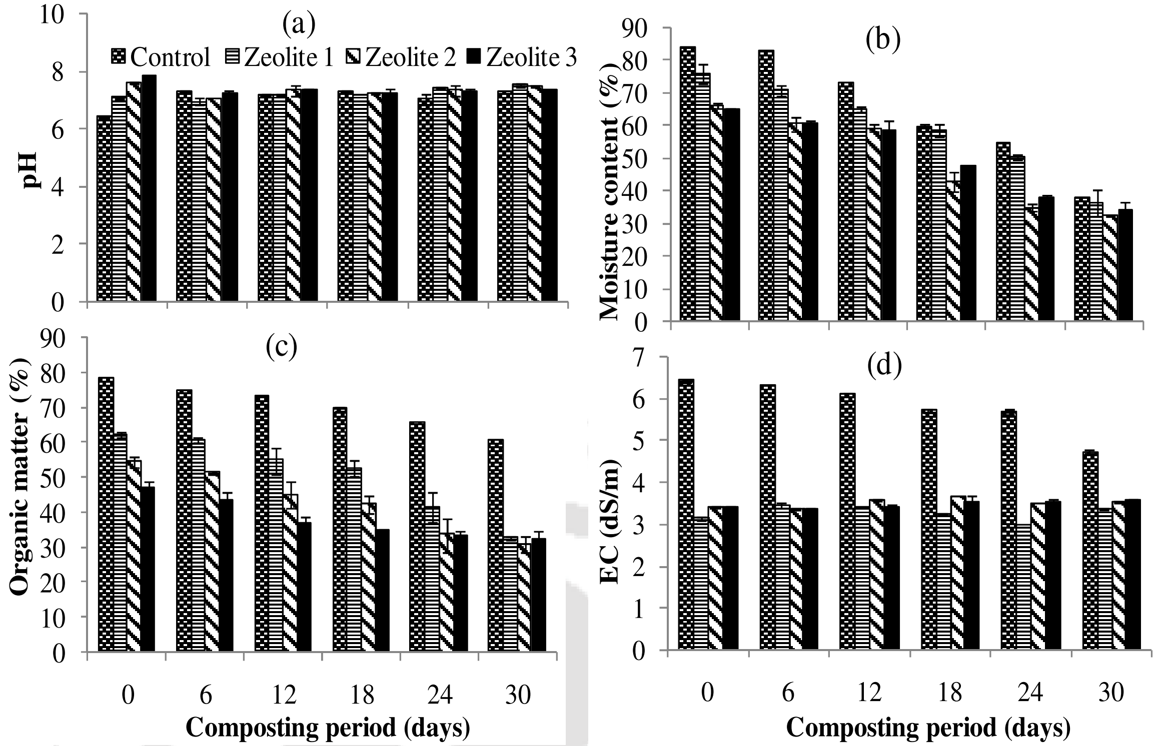


Fig. 5.17 Variation of physico-chemical parameters: (a) pH (b) moisture content (c) organic matter and (d) electrical conductivity (EC) during pile composting with zeolite (bars denoted as ± standard deviation)

Fig. 5.18 illustrates the significant variation in concentration of the nutrients as Na (F =4.12, $p < 0.001$), K ($F = 7.27$, $p < 0.001$), Ca ($F = 29.47$, $p < 0.001$) and Mg ($F = 19.77$, $p < 0.001$) which increased significantly in control and zeolite treatments throughout the composting process. The concentration of Na, K, Ca and Mg was increased during composting process due to the net loss of dry mass (Zorpas et al., 2000).

Fig. 5.19 illustrates the variation in total concentration of metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) in control and all zeolite treatment during 30 days of composting period. The total concentration of metals was increased due to reduction of organic matter and release of CO_2 during the mineralization processes (Zorpas et al., 2000). The total concentration of heavy metals obtained after strong acid digestion is indicator of pollution but unable to provide useful information of bioavailability of heavy metals in the compost and compost amended soil (Cai et al., 2007). The variation in Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in control and zeolite amended compost were significant ($F = 7.36$, $p < 0.001$ for Zn, $F = 42.72$, $p < 0.05$ for Cu, $F = 45.15$, $p < 0.05$ for Mn, $F = 50.59$, $p < 0.05$ for Fe, $F = 42.31$, $p < 0.05$ for Ni, $F = 8.96$, $p < 0.001$ for Pb, $F = 26.49$, $p < 0.001$ for Cd, $F = 4.37$, $p < 0.001$ for Cr).

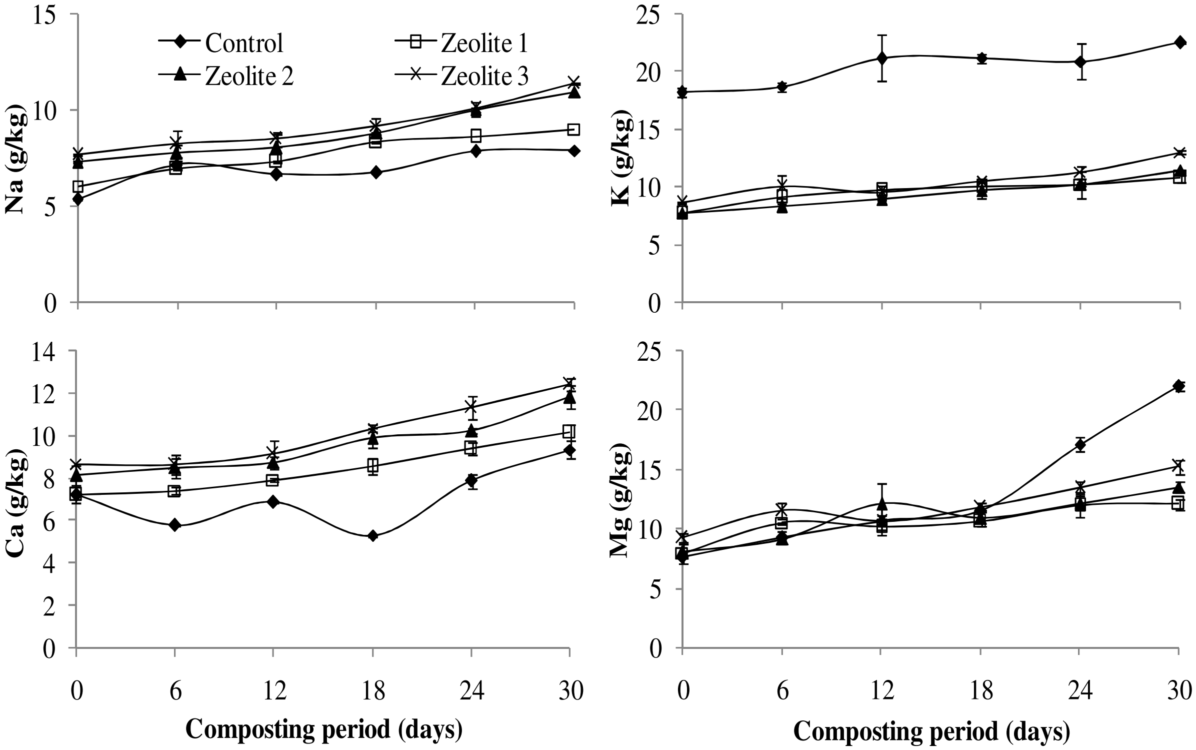


Fig. 5.18 Variation of nutrients (Na, K, Ca and Mg) during pile composting with zeolite (bars denoted as ± standard deviation)

5.3.2 BIOAVAILABILITY OF HEAVY METALS

- **Water solubility of heavy metals**

The water soluble fraction of heavy metals in the compost was lower as compared to their total concentration which is most toxic fraction in the compost to plants (Fang and Wong, 1999). Table 5.9 shows the changes in water-soluble Zn, Cu, Mn, Fe and Cr concentration in contol and all zeolite treatments during the composting process. The water solubility of metals (percentage of total metal) was reduced in the ranges: 66.5-80.0% for Zn, 45.2-76.7% for Cu, 22.7-83.1% for Mn and 61.1-100% for Cr in control and all zeolite treatments. Higher reduction of water soluble concentration of Zn was observed in control followed by zeolite 1, 2 and 3 treatments. Higher reduction in water soluble concentration of Cu and Mn was observed in zeolite 1 and 2 treatments respectively during the process. Higher reduction (percentage of total Fe) of water soluble concentration of Fe was observed in zeolite 2 treatment (70.50%) followed by zeolite 1 treatment (65.64%), zeolite 3 treatment (47.76%), however its concentration was increased in control during the process.

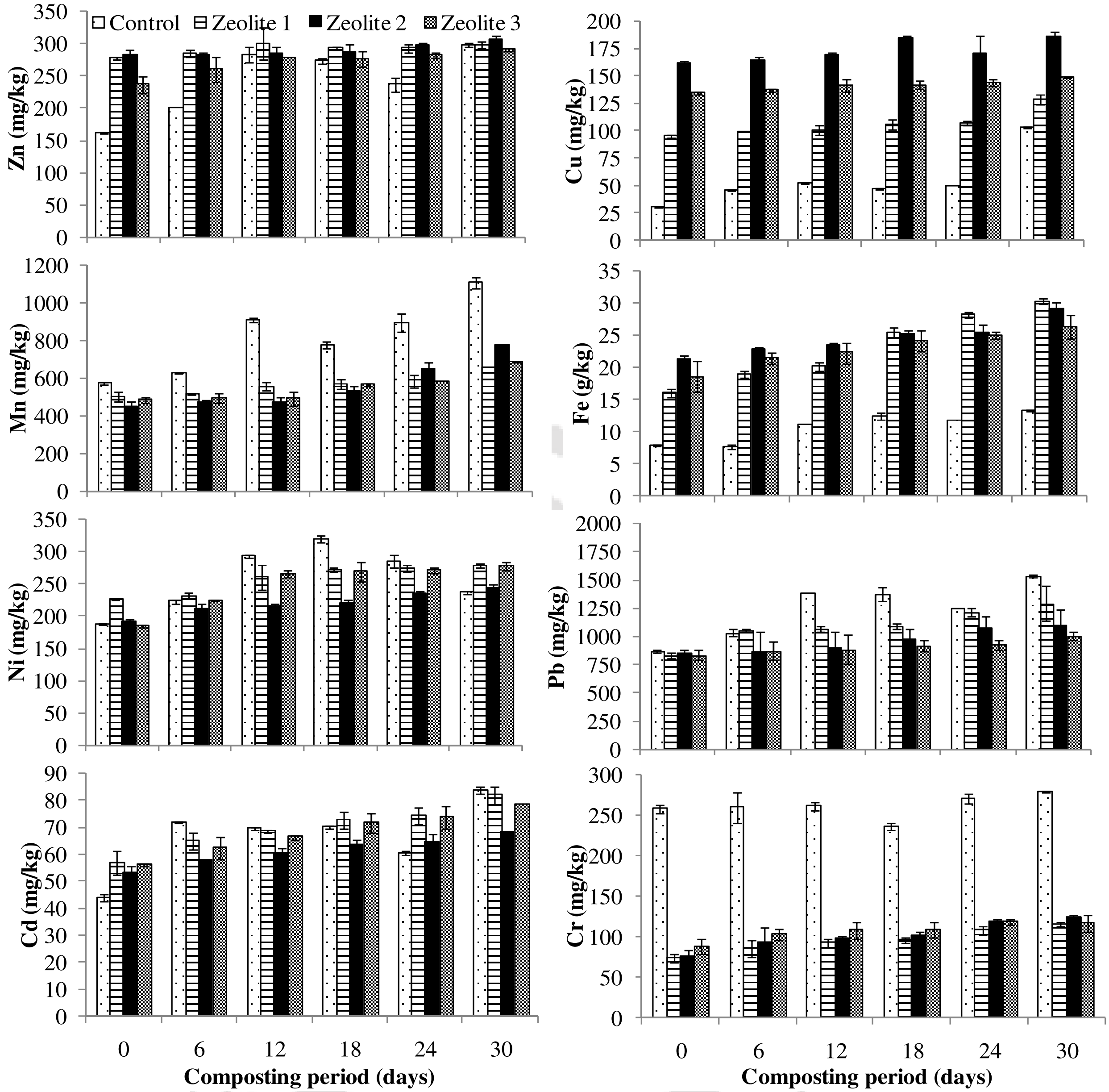


Fig. 5.19 Variation of total metals concentration (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during pile composting with zeolite (bars denoted as ± standard deviation)

Water soluble concentration of Cr was reduced 100% of total Cr in zeolite 1 and 2 treatments. It was reduced about 75.99% and 61.12% of total Cr in control and zeolite 3 treatment respectively. Water soluble concentration of Ni, Pb and Cd were not detected in all treatments during the composting process. Addition of zeolite caused a significant reduction in water soluble Cu, Mn, Fe and Cr contents during the composting process.

Table 5.9 Changes in water soluble metals concentration during pile composting with zeolite (mean ± SD, n=3)

Days	Water soluble metals concentration							
	Zn (mg/kg)				**Cu (mg/kg)**			
	Control	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**	**Control**	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**
0	3.38±0.04	3.59±0.05	1.49±0.05	1.42±0.10	1.12±0.04	1.73±0.06	1.77±0.195	1.60±0.30
6	2.00±0.01	2.42±0.06	1.07±0.15	1.24±0.05	1.82±0.02	1.46±0.02	1.38±0.06	1.55±0.21
12	1.53±0.44	1.43±0.07	0.49±0.05	0.72±0.08	1.80±0.04	1.02±0.12	1.06±0.06	1.15±0.03
18	1.42±0.22	1.24±0.02	0.48±0.01	0.77±0.11	1.10±0.06	0.98±0.13	1.13±0.10	1.04±0.04
24	1.36±0.06	0.95±0.01	0.46±0.0	0.57±0.01	1.06±0.04	0.82±0.40	1.03±0.05	1.00±0.13
30	1.25±0.07	0.87±0.00	0.46±0.01	0.59±0.04	0.92±0.03	0.55±0.04	0.49±0.016	0.97±0.22
Days	**Mn (mg/kg)**				**Fe (mg/kg)**			
0	8.50±0.30	10.89±0.32	3.80±0.80	2.90±0.34	16.14±1.10	15.28±0.0	9.11±0.44	8.89±0.65
6	7.60±0.52	8.08±0.56	1.98±0.36	2.07±0.03	16.68±0.52	13.08±0.0	8.87±0.63	9.66±0.16
12	7.50±0.10	5.16±0.44	1.47±0.10	1.26±0.02	24.58±0.06	9.59±0.41	7.95±0.09	8.59±0.45
18	9.36±2.52	4.02±0.28	1.29±0.01	1.25±0.05	41.46±0.21	11.22±0.0	5.06±0.62	7.94±0.50
24	9.30±0.54	3.55±0.25	1.25±0.05	1.21±0.08	68.10±0.17	11.43±1.02	3.37±0.11	5.58±1.32
30	12.68±0.24	3.65±0.24	1.11±0.06	1.29±0.06	75.08±2.20	9.92±0.48	3.65±0.15	6.59±2.10
Days	**Cr (mg/kg)**				**Ni, Pb and Cd (mg/kg)**			
0	2.07±0.07	0.43±0.05	0.62±0.05	0.73±0.05	ND	ND	ND	ND
6	1.71±0.07	0.28±0.04	0.49±0.05	0.59±0.05	ND	ND	ND	ND
12	1.82±0.02	0.22±0.03	0.34±0.10	0.54±0.11	ND	ND	ND	ND
18	1.05±0.11	0.20±0.01	0.26±0.07	0.47±0.03	ND	ND	ND	ND
24	1.42±0.06	0.14±0.04	0.23±0.06	0.45±0.03	ND	ND	ND	ND
30	0.54±0.04	ND	ND	0.38±0.08	ND	ND	ND	ND

Mean values in columns are statistically different (ANOVA; $p < 0.05$), ND- not detected

Similar results reported by Stylianou et al. (2007) during the sewage sludge composting with natural zeolite. Furthermore, the water soluble concentration of heavy metals was reduced during the process could be explained as higher reduction of organic matter and the formation of organo-metallic complexes (Fang and Wong, 1999). Stylianou et al. (2007) reported that the organic matter in soluble and insoluble forms plays important role in water soluble metals reduction. Decomposition of organic matter during composting process enhanced the conversion of stable form of metals into exchangeable form, as results of binding with natural zeolite through ion exchange process (Stylianou et al., 2007). The variation in water soluble Zn, Cu, Mn, Fe and Cr concentrations in control and zeolite treated compost were significant ($F = 39.69$, $p < 0.001$ for Zn, $F = 27.04$, $p < 0.001$ for Cu, $F = 23.18$, $p < 0.05$ for Mn, $F = 58.55$, $p < 0.05$ for Fe, $F = 23.75$, $p < 0.001$ for Cr).

- **Plant availability of heavy metals (extraction with DTPA)**

The DTPA extraction efficiency of metals were reduced in the ranges: 2.8-56.6% for Zn, 26.8-85.4% for Cu, 43.4-81.7% for Mn, 63.8-78.5% for Ni and 31.1-75.5% for Cr (Fig. 5.20) in control and all zeolite treatments. The maximum DTPA extraction efficiency of Cu and Mn were reduced in control when compared with zeolite treatments. The maximum DTPA extraction efficiency of Zn and Cr were reduced in all zeolite treatments in comparison to the control. Higher reduction of DTPA extraction efficiency of Ni was observed in zeolite 2 treatment followed by zeolite 1 treatment and control. Higher extraction efficiency of Fe was reduced in zeolite 1 treatment (65.0%) followed by zeolite 2 treatment (62.2%) and Zeolite 3 treatmens (43.2%); however it was enhanced in control.

The reduction of DTPA extractability of Zn, Cu, Ni and Cr was also observed by Chiang et al. (2007) during the sewage sludge composting with natural zeolite. DTPA extractable heavy metal reduction attributed to mechanism of ion-exchange processes, where processes metal cations exchanged with mainly Na, K and Ca during the composting process (Erdem et al., 2004; Zorpas et al., 2000). Furthermore, reduction in DTPA extractable metals at the end of the composting process might be due to transformation of organic matter leads to the formation of metal-humus complexes, which make the metals insoluble and therefore less easily extractable (Garcia et al., 1995). DTPA extractable concentration of Pb and Cd were not detected in all treatments during the composting process. The variation in DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr concentrations in control and zeolite treated compost were significant ($F = 67.5$, $p < 0.05$

for Zn, $F = 11.97$, $p < 0.001$ for Cu, $F = 87.9$, $p < 0.05$ for Mn, $F = 5.24$, $p < 0.001$ for Fe, $F = 29.08$, $p < 0.001$ for Ni, $F = 12.24$, $p < 0.05$ for Cr).

5.3.3 LEACHABILITY OF HEAVY METALS

The TCLP extraction is an acetic acid digestion at pH of 4.93 to dissolve carbonates and few soluble oxides of organic matter and as a result these fractions of metals released from organic matter along with the soluble and exchangeable fractions (Nair et al., 2008). The TCLP concentration of heavy metals (mg/kg) was in the range of 0.43-1.20, 2.78-4.38 and 27-32 for Cd, Cr and Pb respectively.

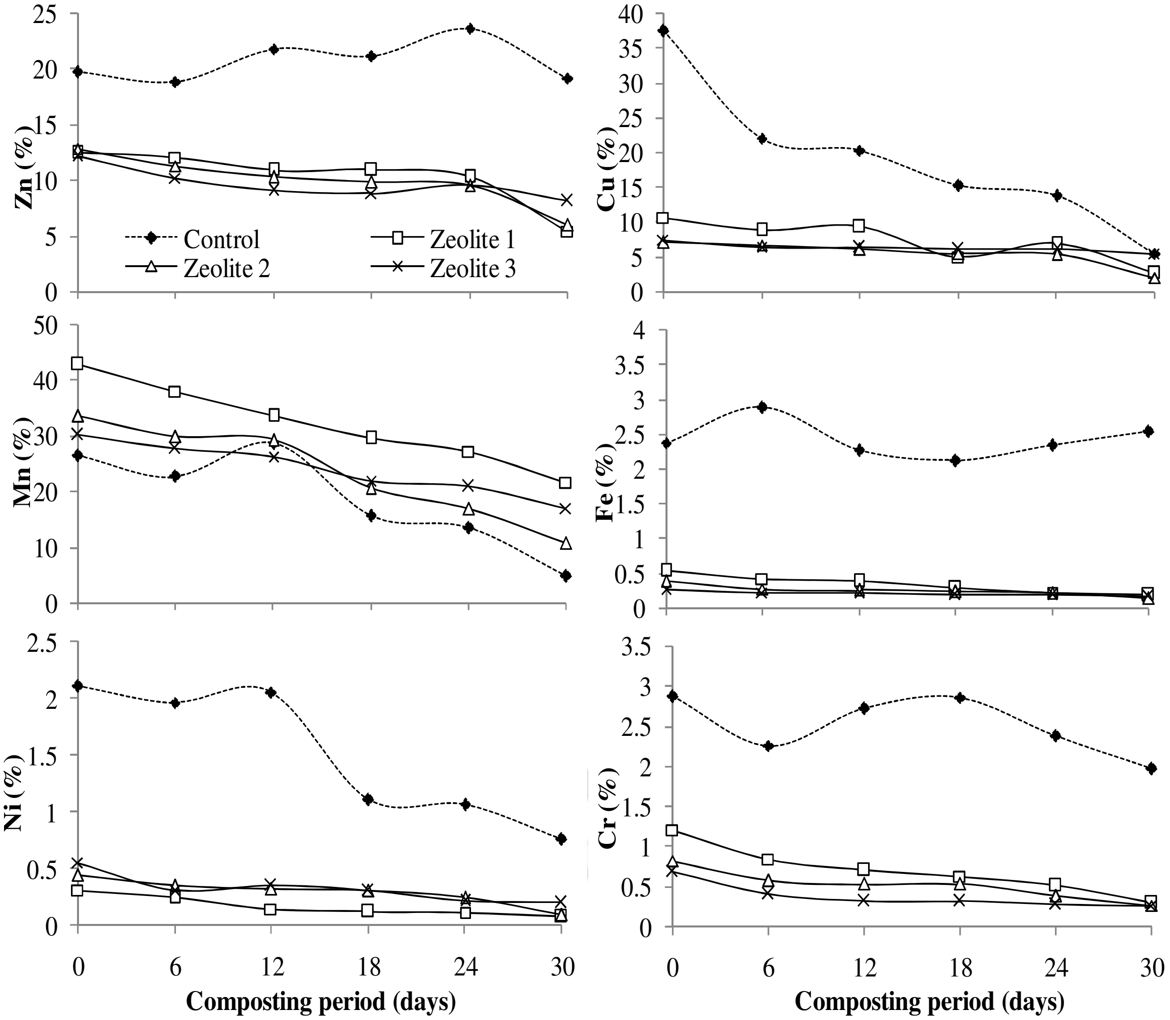


Fig. 5.20 Variation of DTPA extractable metals (Zn, Cu, Mn, Fe, Ni and Cr) during pile composting with zeolite

The TCLP concentration of metals were reduced in the ranges: 43.0-61.4% for Zn, 59.7-72.0% for Cu, 21.9-51.4% for Mn, 42.3-73.9% for Fe, 42.6-64.6% for Ni, 34.7-

53.3% for Pb, 58.2-82.8% for Cd and 23.9-59.7% for Cr in control and all zeolite treatments (Fig. 5.21). The TCLP concentration of Mn, Fe, Ni, Cd and Cr were reduced significantly in all zeolite treatments in comparison to the control. The higher reduction in TCLP concentration of Zn was observed in zeolite 1 treatment followed by zeolite 2 treatment, control and zeolite 3 treatment. The higher reduction in TCLP concentration of Cu and Pb were observed in zeolite 1 treatment followed by control, zeolite 2 and 3 treatments during the composting process. The TCLP concentrations of the selected metals were completely in compliance with the EPA regulatory thresholds.

Moirou et al. (2001) studied stabilization of Pb, Zn, and Cd-contaminated soils by natural zeolite, and reported that TCLP extractability of metals reduced moderately about 38% for Pb, 33% for Zn and 32% for Cd. However in the present study, reduction of these metals was observed about 53% for Pb, 61.4% for Zn and 82.8% for Cd at the end of composting process. The reduction could be attributed as the higher degradation of organic matter due to zeolite addition resulting formation of humic substances, which had a capacity to complex with metals, resulting formation of insoluble organometallic complexes (Wong and Fang, 2000; Villasenor et al., 2011). The initial pH values were enhanced slightly by zeolite addition, which reduced the solubility of heavy metals, consequentially reduction in TCLP extractability of heavy metals. The variation in leachable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in control and zeolite treatments were significant ($F = 5.45$, $p < 0.001$ for Zn, $F = 9.50$, $p < 0.001$ for Cu, $F = 5.56$, $p < 0.001$ for Mn, $F = 7.97$, $p < 0.001$ for Fe, $F = 39.89$, $p < 0.001$ for Ni, $F = 7.33$, $p < 0.001$ for Pb, $F = 19.4$, $p < 0.001$ for Cd, $F = 3.45$, $p = 0.001$ for Cr).

5.3.4 SPECIATION OF HEAVY METALS

- **Speciation of Zn, Cu, Mn, Fe and Ni**

Speciation of Zn in control, zeolite 2 and 3 treatments are given in table 4.6a, 5.10a and 5.11a respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1, F2 and F3 fractions of Zn were reduced in the control and zeolite 1 and 3 treatments. The F4 fraction was reduced in all treatments except zeolite 2 treatment during the process. It was increased in zeolite 2 treatment. Higher reduction of F1 (72.8%) and F4 (79.6%) fractions was observed in zeolite 1 treatment but higher reduction of F2 (70.0%) and F3 (37.0%) fractions was observed in zeolite 2 treatment. The F5 fraction of Zn was enhanced in all treatments. The order of different fractions of Zn in the final compost of control was: F5 > F4 >F3 >F2 >F1. In the zeolite 1 and 2 treatments order was: F5 > F3 >F4 >F2 >F1; however in zeolite 3 treatment order was: F5 > F3 >F4

>F1 >F2. The F4 fraction of Zn was transform into F3 and F5 fractions in all zeolite treatments.

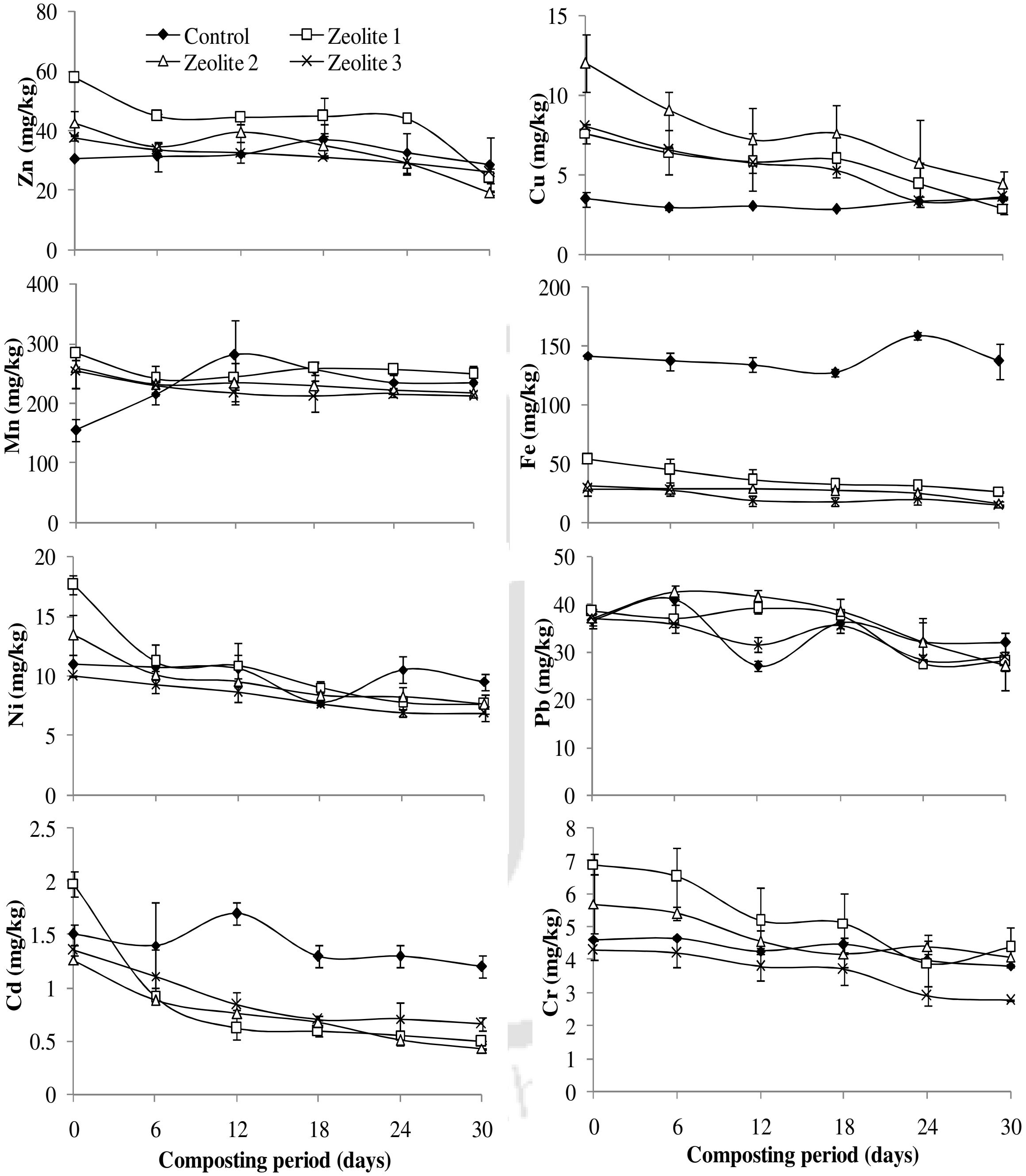


Fig. 5.21 Variation of TCLP extractable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr during pile composting with zeolite (bars denoted as ± standard deviation)

The BF of Zn was decreased from 0.75 to 0.48, 0.28 to 0.15, 0.21 to 0.13 and 0.20 to 0.15 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.23). The higher reduction of BF was observed in Zeolite 1 treatment (45.57%) followed by zeolite 2 treatment (38.41%), control (36.37%) and zeolite 3 treatment (27.30%). Zorpas et al. (2008) reported that Zn was mainly found in F3 and F4 fraction in the sewage sludge compost with natural zeolite; however in the present study it was mainly associated with F5 fraction. The reduction of BF in zeolite 1 and 2 treatments might be due to the formation of Zn complex with various organic functional groups of humic substances formed during maturity of compost (Cai et al., 2007). Kumpiene et al. (2008) suggested that cation exchange and complexation by organic legends are the main Zn bioavailability controlling mechanisms. The variation in F1, F2, F3, F4 and F5 fractions of Zn in control and zeolite treatments were significant ($F = 20.7$, $p < 0.001$ for F1, $F = 15.0$, $p < 0.001$ for F2, $F = 12.3$, $p < 0.001$ for F3, $F = 45.6$, $p < 0.05$ for F4, $F = 5.04$, $p < 0.001$ for F5).

Speciation of Cu in control, zeolite 2 and 3 treatments are given in table 4.6a, 5.10a and 5.11a respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1, F2, F3 and F4 fractions of Cu were reduced in the control and all zeolite treatments. Higher reduction of F1 (78.59%) and F2 (83.54% of total fraction) fractions were observed in zeolite 1 treatment. Higher reduction of F3 (77.88%) and F4 (54.0%) fractions were observed in zeolite 2 treatment. The F5 fraction of Cu was increased in all treatments during the process. The BF of Cu was decreased from 0.63 to 0.28, 0.15 to 0.06, 0.11 to 0.04 and 0.10 to 0.05 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.23). Higher reduction of BF was observed in zeolite 2 treatment (66.4%) followed by zeolite 1 treatment (63.0%), control (55.5%) and zeolite 3 treatment (52.6%). The higher reduction in BF of Cu in zeolite 1 and 2 treatment might be due to higher degradation of organic matter and conversion into humic substances resulting reduction in mobile fractions fractions (F1, F2, F3 and F4) of Cu during the process. The lower BF values were observed in all zeolite treatments in comparison to control.

Zorpas et al. (2000) reported that a maximum amount of Cu was bound to F4 and F5 fractions while less than 2% in the F1 and F2 fractions during sewage sludge composting with natural zeolite. In the present study, Cu was mostly bound with F5 fraction, but F1 and F2 fractions contributed less that 2% in all zeolite treatments. In control these fractions were found more than 2% of total fractions. Results reveal that addition of zeolite significantly reduced F1 and F2 fractions of Cu during the composting process. The maximum reduction of movable fractions (from F1 to F4) of Cu was observed in zeolite 2 and 3 treatments, it might be due to maximum organic matter loss resulting conversion of organic matter into humic substances which have high contents of carboxyl groups (Liu et al., 2008). The variation in F1, F2, F3, F4 and F5 fractions of Cu in control and zeolite treatments were significant ($F = 44.9$, $p < 0.001$ for F1, $F = 10.2$, $p < 0.001$ for F2, $F = 29.3$, $p < 0.001$ for F3, $F = 40.31$, $p < 0.001$ for F4, $F = 26.7$, $p < 0.001$ for F5).

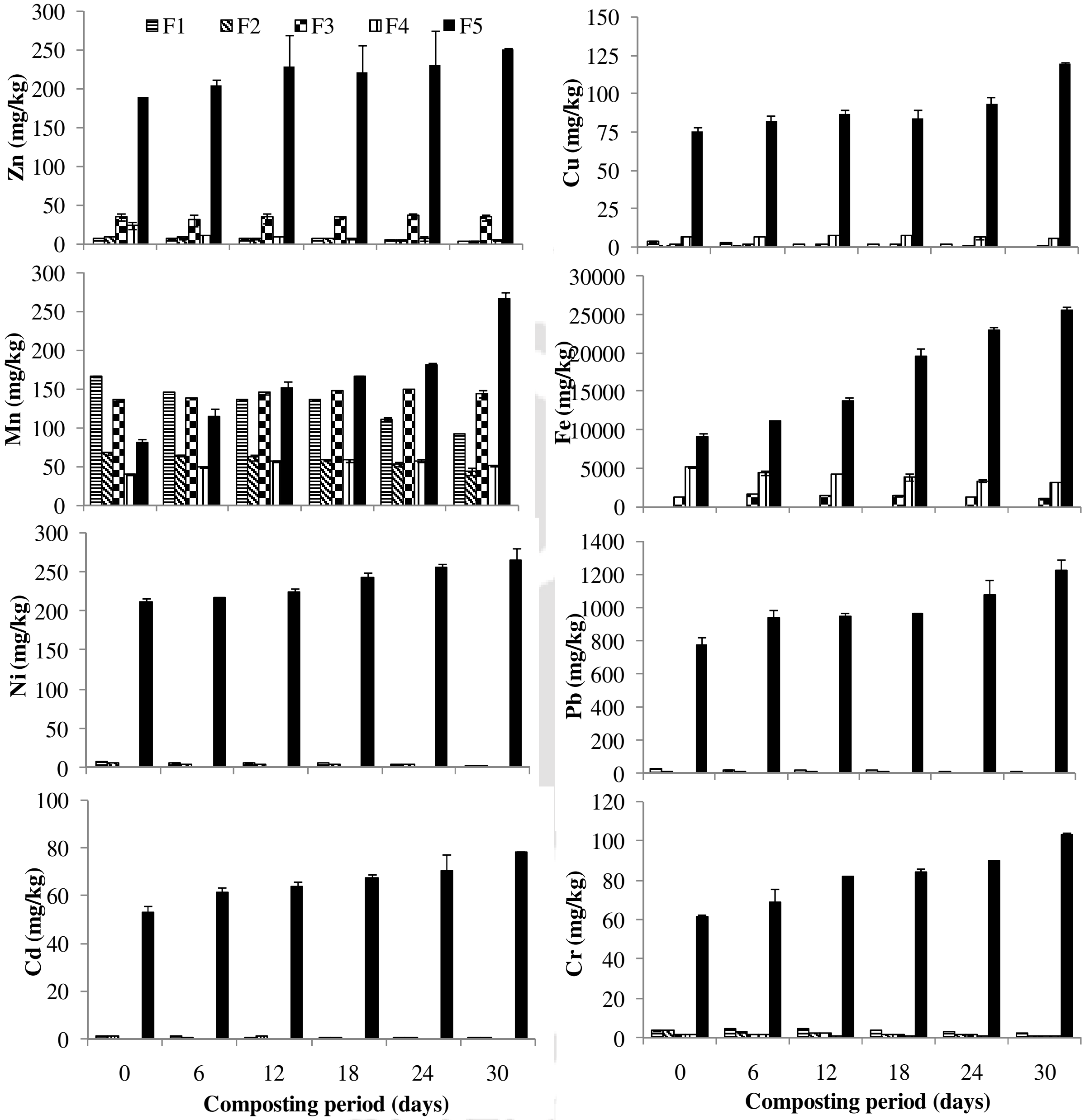


Fig. 5.22 Speciation of heavy metals in zeolite 1 treatment during pile composting with zeolite (bars denoted as ± standard deviation)

Speciation of Mn in control, zeolite 2 and 3 treatments are given in table 4.6a, 5.10a and 5.11a respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1, F2, F3 and F4 fractions of Mn were reduced in the all zeolite treatments in comparison to control. The F1 and F3 fractions were reduced; however remaining mobile fractions (F2 and F4) were increased in the mature compost.

The F5 fraction was increased in control and all zeolite treatments. The maximum reduction of F1 (70%) and F2 (73.4% of total fraction) fractions were observed in zeolite 2 treatment; however higher reduction of F3 and F4 fractions were observed in control and zeolite 3 treatment. The BF of Mn was decreased from 0.65 to 0.47, 0.84 to 0.60, 0.78 to 0.35 and 0.71 to 0.44 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.23). Higher reduction of BF was observed in zeolite 2 treatment (55.0%) followed by zeolite 3 treatment (38.7%), zeolite 1 treatment (33.79) and control (26.5%). Zorpas et al. (2000) reported approximately 60% of Mn was bound to the F3 fraction during sewage sludge composting with natural zeolite. However in the present study, F5 fraction of Mn was dominant fraction and it was about 40-60% of total fraction in control and zeolite treatments. It has been observed that F5 fraction was increased in zeolite added compost in comparison to control; it indicates that mobile fractions (F1 to F4) of Mn were transformed into most stable F5 fraction due to zeolite addition. The variation in F1, F2, F3, F4 and F5 fractions of Mn in control and zeolite treatments were significant ($F = 77.7$, $p < 0.05$ for F1, $F = 19.1$, $p < 0.001$ for F2, $F = 21.0$, $p < 0.001$ for F3, $F = 62.7$, $p < 0.05$ for F4, $F = 23.8$, $p < 0.001$ for F5).

Speciation of Fe in control, zeolite 2 and 3 treatments are given in table 4.6a, 5.10a and 5.11a respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1, F2, F3 and F4 fractions of Fe were reduced in the control and all zeolite treatments. Higher reduction of F1 and F2 fractions were observed about 70.9 and 74.5 % of the total fraction in zeolite 2 treatment and control respectively; however higher reduction of F3 and F4 fractions were observed about 58.4 and 68.4% of the total fraction respectively in zeolte 1 treatment. The F5 fraction of Fe was increased in control and all zeolite treatments. The F1 fraction reduced significantly in all zeolite treatments compared to control. The BF of Fe was decreased from 0.82 to 0.67, 0.41 to 0.14, 0.37 to 0.15 and 0.42 to 0.20 in the control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.23). Higher reduction of BF was observed in zeolite 1 treatment (63.4%) followed by zeolite 2 (61.1%), zeolite 3 (53.1%) and control (18.8%). It has been observed that higher BF was reduced in all zeolite treatments in comparison to control, which suggested that the addition of natural zeolite could check the mobility and bioavailability of Fe during the composting process. The F5 fraction of Fe was dominant fraction (about 84 to 91% of total fraction) in all zeolite amended compost; however in the control F4 fraction was dominant. Zorpas et al. (2000) reported that Fe was bound in the F3 and F5 fractions (about 95% for the Fe).

Table 5.10a Speciation of heavy metals (Zn, Cu, Mn and Fe) in zeolite 2 treatment during 30 days of composting period

	Zeolite 2 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	8.35±0.47	7.65±0.24	28.96±0.27	10.08±0.13	202.97±11.72	4.81±0.56	1.75±0.15	1.74±0.04	7.27±0.38	131.18±5.82
6	6.24±0.55	7.67±0.88	21.89±1.55	10.79±0.14	232.83±8.22	4.45±0.45	1.69±0.09	1.37±0.14	5.58±1.29	138.85±0.16
12	6.28±0.08	7.14±0.94	26.15±0.85	11.00±0.75	233.18±5.55	3.59±0.19	1.47±0.12	1.58±0.02	5.54±0.19	152.40±7.1
18	5.78±0.55	5.13±0.84	22.03±0.55	12.45±0.13	224.88±17.63	1.58±0.02	0.86±0.34	1.83±0.18	6.02±0.68	151.75±5.3
24	5.53±0.20	4.43±0.10	22.50±0.21	15.05±0.63	243.13±28.88	1.66±0.19	0.84±0.36	1.07±0.23	4.54±0.44	151.00±3.0
30	2.45±0.24	2.63±0.35	20.90±0.68	12.86±0.82	256.73±11.33	1.29±0.01	0.38±0.07	0.44±0.16	3.87±0.09	161.80±0.8
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	133.28±2.33	76.84±1.84	76.79±1.11	46.01±0.57	92.18±2.8	10.65±0.30	7.80±0.20	1210.0±36	6156±46	12450±200
6	102.95±0.15	67.87±0.87	86.17±1.92	49.27±0.58	121.95±3.4	8.73±1.83	6.98±0.33	1003.5±118	5288±215	15832±242
12	98.11±0.26	54.21±1.16	94.44±4.77	65.02±0.44	139.70±15.7	8.84±0.69	5.03±1.33	1057.5±128	5052±19	16638±637
18	84.24±1.44	47.63±1.08	97.54±1.97	76.97±1.29	210.30±39.3	6.83±1.43	4.80±0.95	1247.5±272	5042±12	17800±200
24	71.70±0.45	40.48±0.78	97.10±1.50	69.29±0.62	310.84±16.7	6.33±0.42	4.10±0.95	1116.5±120	4827±143	18203±202
30	67.49±0.16	35.25±0.45	101.34±0.47	54.26±2.13	474.65±21.9	4.45±1.1	3.60±0.05	892.5±7.5	3089±7.5	23528±528

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.10b Speciation of heavy metals (Ni, Pb, Cd and Cr) in zeolite 2 treatment during 30 days of composting period

	Zeolite 2 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	8.17±0.28	4.92±0.07	ND	ND	172.5±14.5	22.9±0.35	10.3±0.08	ND	ND	792.5±38
6	7.14±0.38	3.64±0.14	ND	ND	198.7±1.4	19.6±0.95	9.5±0.20	ND	ND	785.0±20
12	6.95±0.25	3.45±0.25	ND	ND	198.2±9.8	16.9±0.90	9.7±0.70	ND	ND	855.0±10
18	5.95±0.35	3.45±0.15	ND	ND	208.7±1.7	15.9±0.40	8.9±0.60	ND	ND	866.0±4
24	5.32±0.01	3.32±0.02	ND	ND	219.8±20	14.4±0.35	7.8±0.20	ND	ND	941.5±42
30	3.96±0.27	1.96±0.30	ND	ND	219.8±9.8	10.0±0.55	4.0±0.48	ND	ND	1044.0±24
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	0.93±0.03	0.74±0.01	ND	ND	48.20±1.8	3.65±0.40	1.65±0.15	2.10±0.20	0.63±0.05	65.1±2.42
6	0.61±0.01	0.73±0.08	ND	ND	54.35±5.7	3.55±0.25	1.68±0.08	1.90±0.30	0.73±0.05	83.6±15.10
12	0.62±0.02	0.78±0.02	ND	ND	58.79±7.2	3.50±0.10	1.50±0.10	1.55±0.05	0.81±0.03	90.0±4.0
18	0.67±0.07	0.71±0.06	ND	ND	58.93±3.6	3.13±0.58	1.58±0.03	1.40±0.20	1.82±0.14	91.0±2.45
24	0.53±0.03	0.67±0.03	ND	ND	61.60±6.9	2.95±0.35	1.09±0.11	1.27±0.03	2.32±0.27	101.8±3.25
30	0.41±0.06	0.53±0.06	ND	ND	67.45±0.9	2.35±0.10	0.88±0.13	1.05±0.05	2.71±0.21	112.8±5.25

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

Zorpas et al. (2008) reported that the order of Fe fraction was: F5 (64.19%) > F3 (27.21%) > F4 (7.95%) > F2 (0.38%) > F1 (0.27%) in sewage sludge composting with natural zeiolite. In the present study this order was: F5 (86.0%) > F4 (10.5%) > F3 (3.5%) > F1 (0.020%) > F2 (0.017%). The variation in F1, F2, F3, F4 and F5 fractions of Fe in control and zeolite treatments were significant ($F = 20.6$, $p < 0.001$ for F1, $F = 19.4$, $p < 0.001$ for F2, $F = 30.6$, $p < 0.001$ for F3, $F = 42.4$, $p < 0.001$ for F4, $F = 11.7$, $p < 0.001$ for F5).

Speciation of Ni in control, zeolite 2 and 3 treatments are given in table 4.6b, 5.10b and 5.11b respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1 and F2 fractions of Ni were reduced in the control and all zeolite treatments. Higher reduction of these fractions was observed about 64 and 67% of the total fraction in zeolite 1 and 2 treatments respectively; it could be explained as zeolite has taken up the Ni bound in the F1 and F2 fractions (Zorpas et al., 2008). The F5 fraction of Ni was increased in control and all zeolite treatments. The F1 fractions contribute about 5% of the total fraction in final compost of control, however this fraction contribute less than 2% of total fraction in zeolite treatments. The F2 fraction contributed about 1.5% of the total fraction in final compost of control; however this fraction contributed less than 1% of the total fraction in zeolite treatments. Similar results also reported by Zorpas et al. (2000) in the sewage sludge compost with natural zeolite.

The BF of Ni was decreased from 0.12 to 0.06, 0.06 to 0.02, 0.07 to 0.03 and 0.033 to 0.015 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.23). Highest reduction of BF was observed in zeolite 1 treatment (64.5%) followed by zeolite 2 treatment (62.8%), zeolite 3 treatment (54.2%) and control (46.9%). Higher reduction of BF in all zeolite treatments in comparison to control indicated that zeolite could inhibit the bioavailability of Ni during the composting process. Zorpas et al. (2008) reported that Ni was found to be associated with F3 fraction (36%) and F5 fraction (23%) during the sewage sludge composting with natural zeolite. While in the present study Ni was mostly bound in F5 fraction (about 93-98% of total fraction); however F3 and F4 fractions were not detected throughout the composting process. The variation in F1, F2 and F5 fractions of Ni in control and zeolite treatments were significant ($F = 18.6$, $p < 0.001$ for F1, $F = 19.0$, $p < 0.001$ for F2, $F = 20.1$, $p < 0.001$ for F5).

- **Speciation of Pb, Cd and Cr**

Speciation of Pb in control, zeolite 2 and 3 treatments are given in table 4.6b, 5.10b and 5.11b respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1 and F2 fractions of Pb were reduced in the control and all zeolite treatments.

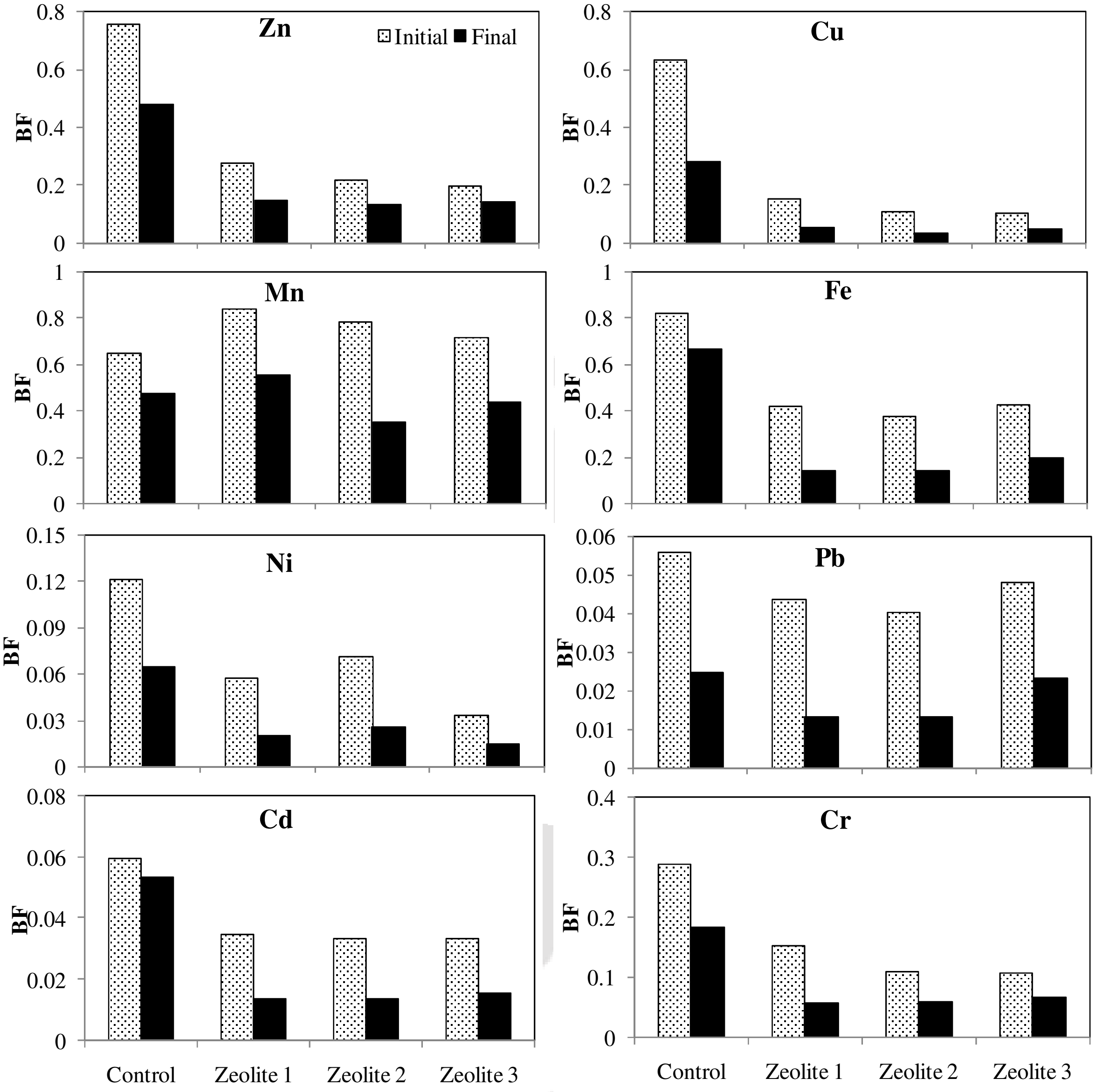


Fig. 5.23 Changes in bioavailability factor (BF) of heavy metals in control and zeolite treatments during pile composting process

Higher reduction of F1 (71.0%) and F2 (67.8% of total fraction) fractions was in zeolite 1 and 2 treatments respectively during the composting process; it might be due to these fractions taken up from the zeolite and transformed into the more stable fractions (Zorpas et al., 2008). The F5 fraction of Pb was increased (percentage of total fraction) in all zeolite treatments; however it was reduced in control. Zorpas et al. (2000) reported that Pb bound in the F3 and F5 fractions (83% of total fraction) during sewage sludge composting with lime. However in the present study Pb was mainly bound in F5 fraction (about 97-98% of total fraction). The F3 and F4 fractions were not found during the composting process. The BF of Pb was decreased from 0.056 to 0.025, 0.044 to 0.013, 0.040 to 0.013 and 0.048 to 0.023 in control and zeolite 1, 2 and 3 treatments respectively during the process (Fig. 5.23). Higher reduction of BF was observed in zeolite 1 treatment (69.6%) followed by zeolite 2 (67.2%), control (51%) and zeolite 3 treatment (51.6%) during the composting process. The lower values of BF were observed in all zeolite treatments when compared with control, confirmed that zeolite was efficient for reducing bioavailability of Pb in the mature compost. The variation in F1, F2 and F5 fractions of Pb in control and zeolite treatments were significant ($F = 56.3$, $p < 0.05$ for F1, $F = 13.2$, $p < 0.001$ for F2, $F = 16.4$, $p < 0.001$ for F5).

Speciation of Cd in control, zeolite 2 and 3 treatments are given in table 4.6b, 5.10b and 5.11b respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1 and F2 fractions of Cd were reduced in the control and all zeolite treatments during the process. Higher reduction of F1 and F2 fractions were observed about 72.2% and 69.8% of total fraction in zeolite 1 and 2 treatments respectively; it could attribute since ion-exchange reactions occurring in the microporous minerals of the zeolite (Erdem et al., 2004). The F5 fraction of Cd was increased (percentage of total fraction) in all zeolite treatments; but it was reduced in control. The BF of Cd was decreased from 0.06 to 0.053, 0.035 to 0.014, 0.033 to 0.014 and 0.033 to 0.015 in control and zeolite 1, 2 and 3 treatments respectively during the process (Fig. 5.23). Higher reduction of BF was observed in zeolite 1 treatment (61.3%) followed by zeolite 2 treatment (59.1%), zeolite 3 treatment (53.9%) and control (10.5%). The higher reduction in BF of Cd in zeolite 1 and 2 treatment might be due to higher degradation of organic matter and conversion into humic substances, resulting reduction in mobile fractions (F1, F2, F3 and F4) of Cd during the process. The lower values of BF were observed in all zeolite treatments when compared with control, proved that zeolite treatment could effective for reducing bioavailability of Cd during the composting process. According to Zorpas et al. (2000), about 60% of Cd was bound to the F3 fraction during sewage sludge composting with natural zeolite, while in the present study, F5 fraction of Cd was dominant and contributed about 98% of total fraction. The F1 and F2 fractions of Cd was contributed about 3.4 and 1.9% of total fraction respectively in the final compost of control; however these fractions were assessed less than 1% of total fraction each in the final compost of zeolite treatments.

Table 5.11a Speciation of heavy metals (Zn, Cu, Mn and Fe) in zeolite 3 treatment during 30 days of composting period

	Zeolite 3 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	6.67±0.22	7.26±0.16	23.28±4.05	8.07±0.23	185.07±3.57	4.36±0.01	0.80±0.05	1.29±0.01	6.44±0.14	113.1±2.6
6	6.64±0.14	7.37±0.18	24.24±0.80	13.83±0.29	202.40±2.20	2.08±0.08	0.68±0.80	1.23±0.03	9.83±0.18	112.3±6.7
12	6.45±0.25	7.16±0.91	26.76±0.23	13.59±0.05	220.30±15.0	1.79±0.01	0.55±0.10	1.95±0.06	8.12±0.52	122.4±2.9
18	6.45±0.15	7.39±0.44	25.06±2.50	10.64±0.02	217.23±8.93	1.61±0.05	0.69±0.01	1.59±0.09	7.40±0.15	126.2±5.3
24	4.32±0.02	4.84±1.69	25.33±3.04	8.45±0.48	228.15±10.65	1.51±0.04	0.61±0.05	0.98±0.48	6.32±1.0	131.0±2.5
30	3.46±0.24	3.26±0.73	26.43±3.20	8.31±00.63	245.12±42.42	1.54±0.26	0.48±0.08	0.83±0.43	4.19±0.85	138.2±1.3
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	114.53±0.78	42.47±1.28	88.05±0.15	93.88±2.70	137.5±17.5	7.05±0.6	6.70±0.1	1146±132	6188±178	10125±125
6	105.43±0.43	43.43±2.43	101.65±3.56	84.85±3.84	140.0±25.5	7.93±0.6	6.28±1.0	1125±104	5683±20	13065±525
12	95.60±1.15	46.29±1.84	101.80±2.60	76.36±1.78	166.0±8.0	7.25±0.9	5.58±0.8	1212±24	5640±32	13398±397
18	91.93±2.18	45.35±4.40	105.60±6.1	73.32±2.37	226.0±65.0	6.80±1.1	4.98±0.8	1162±136	4940±486	15585±415
24	88.80±4.70	46.44±2.34	105.85±10.3	68.72±0.05	232.3±54.8	5.58±0.5	4.58±0.5	1137±107	4341±121	19128±128
30	81.73±1.93	38.83±0.98	106.75±4.95	57.12±0.74	367.3±0.75	6.35±0.8	4.65±0.1	997±8.5	4135±38	20950±950

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.11b Speciation of heavy metals (Ni, Pb, Cd and Cr) in zeolite 3 treatment during 30 days of composting period

	Zeolite 3 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	3.73±0.17	2.26±0.31	ND	ND	175.3±8	23.55±1.95	16.15±0.85	ND	ND	785.0±20
6	3.85±0.01	2.43±0.28	ND	ND	210.3±30	17.40±2.10	13.95±1.45	ND	ND	837.5±18
12	3.70±0.05	1.88±0.03	ND	ND	251.0±23	15.65±2.35	14.80±0.20	ND	ND	845.0±20
18	3.69±0.82	1.88±0.02	ND	ND	260.5±7	15.95±1.45	12.95±0.55	ND	ND	853.5±9
24	2.51±0.10	1.68±0.03	ND	ND	265.9±21	12.35±1.15	11.20±0.80	ND	ND	889.5±35
30	2.54±0.36	1.65±0.10	ND	ND	273.0±37	13.35±0.35	9.35±0.35	ND	ND	951.5±17
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	0.97±0.02	0.86±0.01	ND	ND	52.94±6.1	4.05±0.80	1.54±0.14	1.53±0.07	1.64±0.05	72.94±6.1
6	0.90±0.06	0.79±0.01	ND	ND	57.18±4.8	3.25±0.05	1.17±0.38	2.25±0.05	1.60±0.02	93.05±1.6
12	0.68±0.03	0.73±0.03	ND	ND	62.82±3.2	3.33±0.08	0.71±0.06	1.55±0.04	2.20±0.33	98.00±6.0
18	0.64±0.01	0.67±0.04	ND	ND	66.89±2.6	3.05±0.05	0.97±0.08	1.56±0.06	1.76±0.13	100.10±3.6
24	0.58±0.08	0.59±0.01	ND	ND	66.85±4.2	2.38±0.22	1.02±0.04	1.85±0.25	2.05±0.75	104.45±4.1
30	0.53±0.03	0.66±0.06	ND	ND	75.70±3.2	2.55±0.30	0.99±0.04	1.25±0.05	2.47±0.13	102.95±1.5

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

The reduction in most bioavailable fractions of Cd attributed as enhancement of initial pH in all zeolte treatments. The variation in F1, F2 and F5 fractions of Cd in control and zeolite treatments were significant ($F = 50.3$, $p < 0.05$ for F1, $F = 13.0$, $p < 0.001$ for F2, $F = 3.1$, $p = 0.002$ for F5).

Speciation of Cr in control, zeolite 2 and 3 treatments are given in table 4.6b, 5.10b and 5.11b respectively, and speciation in zeolite 1 treatment is given in Fig. 5.22 during the composting process. The F1 and F2 fractions of Cr were reduced in the control and all zeolite treatments. The F3 and F4 fractions were reduced in control and zeolite 1 treatment; however these fractions were increased in zeolite 2 and 3 treatments during the composting process. Higher reduction of F1 and F3 fractions were observed about 60.7 and 69.5% of the total fraction in zeolite 2 treatment. The maximum reduction of F2 fraction was observed about 76.1% of total fraction in zeolite 1 treatment during the composting process. The F5 fraction of Cr was increased in control and all zeolite treatments. The BF of Cr was decreased from 0.29 to 0.18, 0.15 to 0.06, 0.11 to 0.06 and 0.11 to 0.07 in control and zeolite 1, 2 and 3 treatments respectively during the process (Fig. 5.23). Higher reduction of BF was observed in zeolite 1 treatment (62.7%) followed by zeolite 2 treatment (46.9%), zeolite 3 treatment (38.6%) and control (36.4%) during the composting process. The higher reduction of BF was observed in all zeolite treatments in comparison to control. The higher reduction in BF of Cr in zeolite 1 and 2 treatment might be due to higher degradation of organic matter and reduction in readily available fractions (F1 and F2) of Cr during process. Furthermore, it could be attributed as zeolite has the ability to absorb and exchange of Cr with other cations (Na, K and Ca) (Zorpas et al., 2008). Zorpas et al. (2000) reported that a maximum amount of Cr was bound to the F4 and F5 fractions while less than 2% with F1 and F2 fractions in the zeolite amended sewage sludge compost. However in the present study, Cr was mainly associated with F5 fraction. The F1 and F2 fractions of Cr was observed about 9.1 and 4.0% of total fraction respectively in the final compost of control; however these fractions were measured less than 2.5 and 1.5% of total fraction respectively in the final compost of zeolite treatments. The variation in F1, F2, F3, F4 and F5 fractions of Cr in control and zeolite treatments were significant ($F = 8.0$, $p < 0.001$ for F1, $F = 48.8$, $p < 0.001$ for F2, $F = 16.1$, $p < 0.001$ for F3, $F = 17.3$, $p < 0.001$ for F4, $F = 19.2$, $p < 0.001$ for F5).

5.3.6 CONCLUSION

Natural zeolite addition enhanced the initial pH and organic matter degradation resulting speciation of heavy metals was affected during the composting process. Addition of the natural zeolite (clinoptilolite) during composting process led to increased in concentration of Na, Ca and K concentration, and significantly reduced water solubility, DTPA and TCLP extractability of heavy metals. TCLP test proved that the all selected heavy metals concentrations in control and zeolite treated compost were below the threshold limits. The maximum reduction of bioavailability and leachability of heavy metals were observed in zeolite 1 and 2 treatments, which indicated optimum percentage of zeolite could enhance organic matter degradation; therefore it decreased the toxicity of the heavy metals during water hyacinth composting with cattle manure and sawdust.

The bioavailability factor (BF) of all selected heavy metals was reduced significantly in zeolite 1 and 2 treatments in comparison to control and zeolite 3 treatment. The order of BF of different metals in the compost of control was: Fe (0.66) > Zn (0.55) > Mn (0.47) > Cu (0.28) > Cr (0.18) > Ni (0.06) > Cd (0.053) > Pb (0.025); however, order of these metals in zeolite treatments was: Mn (0.35) > Fe (0.19) > Zn (0.15) > Cr (0.066) > Cu (0.060) > Ni (0.026) > Cd (0.023) = Pb (0.023). The total concentration of Pb was higher than Zn, Cu, Mn, Ni and Cr but its BF was lowest among the all these metals. The total concentration of Cu and Cr was much lower than Pb and Ni but their BF were higher than Pb and Ni in the mature compost. The total concentration of Pb was eighteen times of Cd but BF of both metals was found to be equal. Therefore this study concluded that the use of appropriate proportion of natural zeolite was successful for reducing bioavailability of heavy metals during agitated pile composting of water hyacinth mixed with cattle manure and sawdust.

5.4 EFFECTS OF NATURAL ZEOLITE DURING ROTARY DRUM COMPOSTING

Natural zeolite can be used as a bulking material in the composting process. It enhanced the composting process and the biodegradability of the organic matter due to its ability to increase the porosity of the substrate (Zorpas et al., 2000). It has the ability to uptake of heavy metals which are in mobile and available fractions (Zorpas et al., 2000). Phase 2 study suggested that that mixture of 90 kg water hyacinth, 45 kg cattle manure and 15 kg sawdust (Trial 4) was best combination for reduction of bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate) of heavy metals during rotary drum composting of water hyacinth. Therefore, same combination (Trial 4) was selected for zeolite 1 treatment (5%), 2 treatment (10%) and 3 treatment (15%) studies. Trial 4 was considered as control with respect to zeolite addition.

5.4.1 PHYSICO-CHEMICAL ANALYSIS

The maximum thermophilic temperatures were observed in the range of 44.4-59.7^{o}C in control and zeolite treatments due to intense microbial activity (Chiang et al., 2007). Lowest thermophilic temperature was observed in zeolite 3 treatment might be due to poor degradation of organic matter. Higher addition of zeolite may absorbed water (Villasenor et al., 2011) caused food scarcity and appropriate oxygen for composting microbs. Fig. 5.24 shows the changes in temperature profiles in control and all zeolite treatments during the rotary drum composting process.

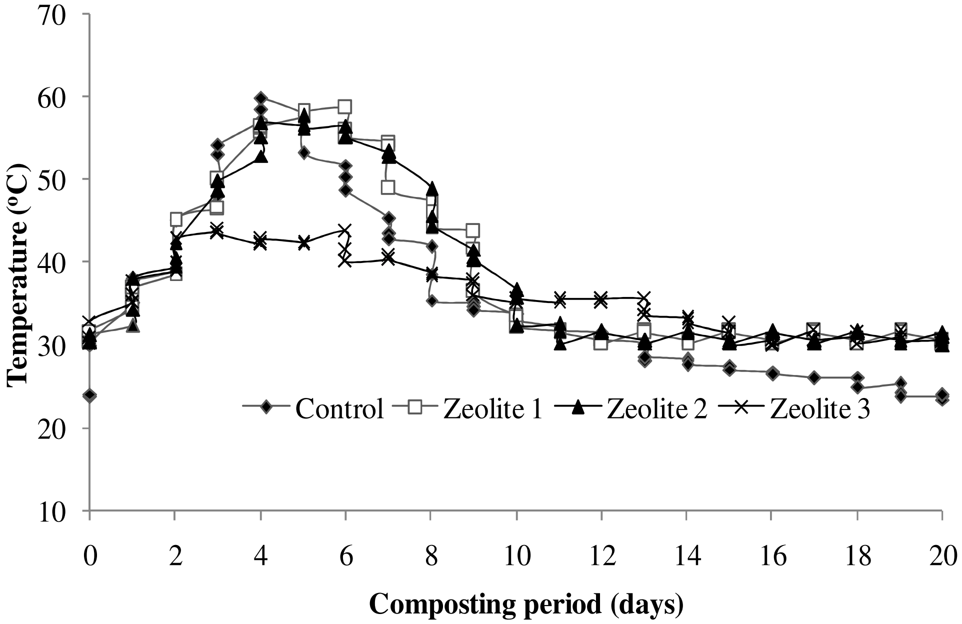


Fig. 5.24 Variation of temperature during drum composting with zeolite

Fig. 5.25a shows the significant reduction in moisture content in control and all zeolite treatments during the process (F = 39.9, $p < 0.001$). The higher reduction of moisture content was observed in control (27.4%) followed by zeolite 1 treatment (26.5%), zeolite 2 treatment (23.4%) and zeolite 3 treatment (10.2%). Lower reduction of moisture content in all zeolite treatments might be due to moisture hold by zeolite (Villasenor et al., 2011). Fig. 5.25b shows the significant variation in pH values from 6.7 to 7.7 during the composting process (F =38.5, $p < 0.001$). The pH of initial compost mixture was increased a slightly in all zeolite treatments in comparison to control. The pH decreased in the initial phase of the composting might be due to acid formation during the decomposition of organic matter, a similar results also observed by (Zorpas et al., 2000) during sewage sludge composting with natural zeolite.

Fig. 5.25c shows the significant reduction in organic matter in control and all zeolite treatments (F = 61.3, $p < 0.05$). The higher reduction of organic matter was observed in zeolite 1 treatment (45.6%) followed by zeolite 2 treatment (44.8%), control (32.6%) and zeolite 3 treatment (16.4%). Lower values of organic matter were observed in all zeolite treatments in comparison to control. Natural zeolite has the ability to increase the porosity of the composting materials due to its bulking property, consequently fasten the composting process (Zorpas et al., 2000).

The EC was increased from 3.52 to 4.06 dS/m and 3.0 to 3.7 dS/m in zeolite 1 and 2 treatments; however it was reduced in control from 3.3 to 2.3 dS/m (Fig. 5.25d). In zeolite 3 treatment, EC was increased in thermophilic stage but in the mature compost there was no change observed in comparison to control. The reduction of EC in control might be due to volatilization of ammonia and the precipitation of mineral salts at the later phase of composting (Kalamdhad et al., 2009). An increase in EC during composting process with zeolite due to the weight loss undergone by the mineralization of organic matter (Garcia et al., 1995). The significant variation in EC was observed in control and all zeolite treatments during the process (F = 334.2, $p < 0.05$). Fig. 5.26 illustrates the concentration of Na, K, Ca and Mg was increased significantly (F =8.59, $p < 0.001$ for Na, F = 18.4, $p < 0.001$ for K, F = 7.1, $p < 0.001$ for Ca and F = 8.07, $p < 0.001$ for Mg) during composting process due to the net loss of dry mass (Zorpas et al., 2000). Table 5.12a and b illustrates the variation in total concentration of metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) in control and all zeolite treatments during 20 days of composting period. During the composting process total heavy metals concentration was increased due to reduction of organic matter and release of CO_2 (Zorpas et al., 2000).

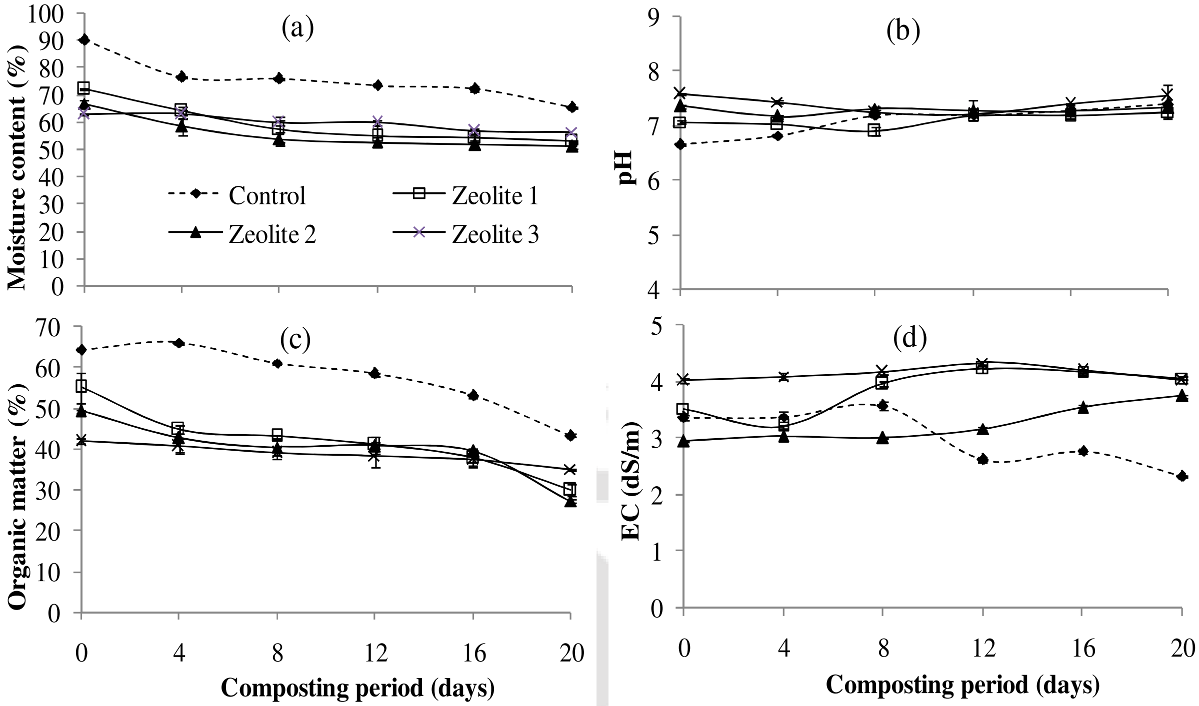


Fig. 5.25 Variation of physico-chemical parameters: (a) moisture content (b) pH (c) organic matter and (d) electrical conductivity (EC) during drum composting with zeolite (bars denoted as ± standard deviation)

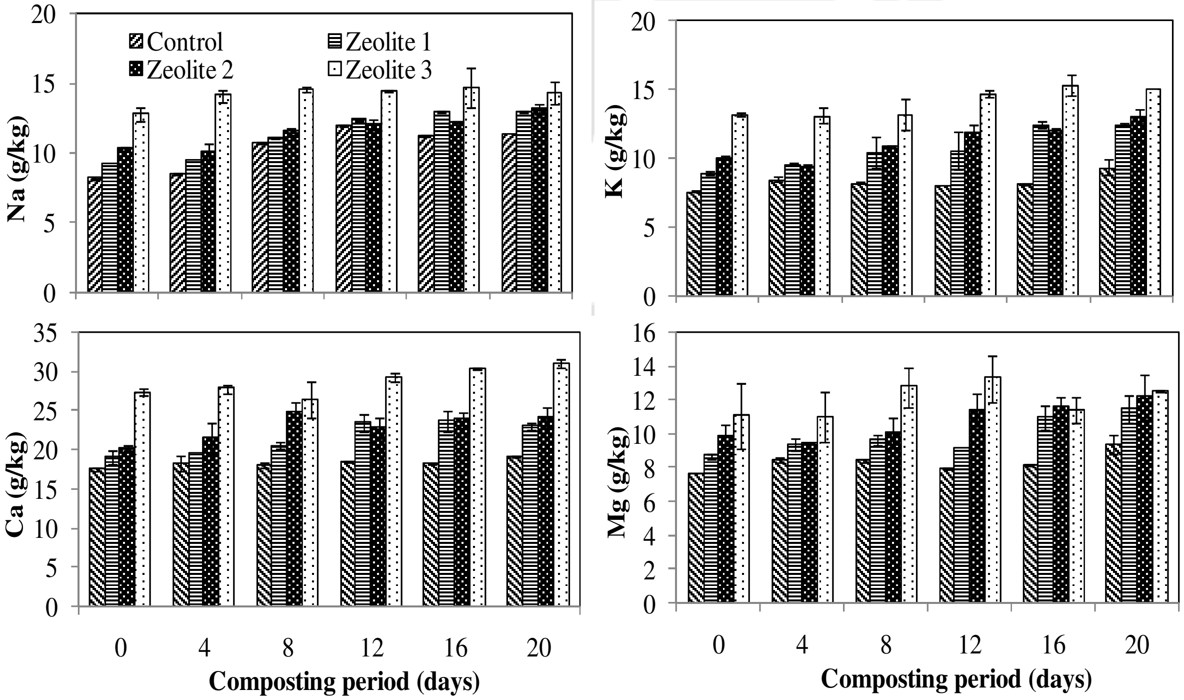


Fig. 5.26 Variation of nutrients (Na, K, Ca and Mg) during drum composting with zeolite (bars denoted as ± standard deviation)

Table 5.12a Changes in total heavy metals concentration (Zn, Cu, Mn and Fe) during drum composting with zeolite (mean ± SD, n=3)

	Total heavy metal concentration							
Days	**Zn (mg/kg)**				**Cu (mg/kg)**			
	Control	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**	**Control**	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**
0	147.3±1.9	213.9±1.5	218.9±0.02	240.0±9	70.5±1.0	80.1±0.4	88.1±0.5	108.8±10
4	155.4±1.2	222.5±1.7	225.7±0.3	244.4±13	71.7±1.1	85.9±0.4	93.9±3.6	108.6±9.4
8	177.6±2.3	235.4±10	227.5±2.4	248.4±12	71.3±0.5	87.2±0.4	96.8±0.2	118.0±20
12	153.9±3.0	254.5±3.6	232.2±4.7	250.1±7	73.0±0.4	88.3±0.3	96.2±1.6	110.9±13
16	141.0±13	261.5±3.2	244.1±9.6	272.2±18	76.9±1.6	94.4±4.6	99.8±1.7	112.3±14
20	155.1±2.9	290.1±2.1	276.0±24	273.9±22	91.6±1.9	96.7±0.8	100.4±1.9	115.4±13
Days	**Mn (mg/kg)**				**Fe (g/kg)**			
0	538±18	475±11	492±2.5	531±7	11.03±0.3	19.90±0.4	21.10±0.6	21.12±0.3
4	650±45	484±6	494±14	546±3	13.40±0.8	24.58±0.1	24.02±0.6	21.85±0.5
8	655±10	509±11	505±13	551±25	12.80±0.2	26.15±1.3	28.49±3.2	22.70±0.6
12	650±15	519±18	526±2.3	553±24	13.15±0.2	28.67±0.2	29.86±1.1	23.23±1.2
16	670±10	554±10	573±4.7	563±5	13.34±0.4	29.08±0.3	29.63±0.9	23.90±0.4
20	668±3	652±26	588±13	620±44	14.10±0.4	31.30±1.2	31.51±10.0	23.95±0.0

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.12b Changes in total heavy metals (Ni, Pb, Cd and Cr) concentration during drum composting with zeolite (mean ± SD, n=3)

Days	Total heavy metal concentration							
	Ni (mg/kg)				**Pb (mg/kg)**			
	Control	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**	**Control**	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**
0	239.5±10.0	222.3±2.2	244.0±3.0	267.3±2.3	958±8	977±47	1037±1.5	1032±7
4	232.8±3.2	232.0±4.0	259.6±7.1	290.4±3.7	930±35	1055±29	1088±33	1058±63
8	240.0±3.5	246.3±2.7	258.8±0.2	294.1±4.6	950±20	1139±41	1099±39	1062±23
12	223.5±9.0	249.5±5.0	261.8±2.2	293.8±0.8	920±5	1140±15	1144±23	1044±29
16	233.5±4.5	251.8±7.3	273.7±4.2	296.2±1.7	980±35	1190±15	1188±57	1055±10
20	243.5±2.5	256.3±8.2	285.0±3.0	300.7±7.2	1111±53	1209±76	1268±0.5	1100±30
Days	**Cd (mg/kg)**				**Cr (mg/kg)**			
0	51.5±1.0	72.4±0.1	75.7±1.7	75.8±0.2	59.3±3.2	60.5±4.5	76.2±0.2	93.9±1.5
4	54.3±1.3	75.1±3.6	77.6±1.1	75.2±1.7	66.3±2.2	63.5±0.5	78.0±0.5	94.8±6.1
8	56.6±2.1	78.9±2.5	80.4±0.9	76.5±1.0	78.4±4.1	64.9±0.4	77.6±4.9	97.5±1.0
12	56.9±2.8	78.4±3.1	82.4±2.9	76.7±1.2	65.5±2.5	66.8±1.2	84.3±0.3	96.5±2.0
16	57.3±3.7	85.5±1.0	85.0±0.5	78.6±0.4	77.0±2.1	73.8±2.2	86.6±0.1	97.7±1.7
20	60.7±1.7	91.2±1.7	93.9±4.1	78.7±0.7	76.3±2.5	83.5±0.0	91.2±1.8	105.1±3.6

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

The variation in total concentration of Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in control and zeolite amended compost were significant (F = 14.3, $p < 0.001$ for Zn, F = 15.3, $p < 0.001$ for Cu, F = 9.2, $p < 0.001$ for Mn, F = 6.1, $p < 0.001$ for Fe, F = 8.6, $p < 0.001$ for Ni, F = 10.3, $p < 0.001$ for Pb, F = 7.01, $p < 0.001$ for Cd, F = 10.74, $p < 0.001$ for Cr).

5.4.2 BIOAVAILABILITY OF HEAVY METALS

- **Water solubility of heavy metals**

The contribution of water soluble fraction of heavy metals in the compost is mainly bioavailable and toxic fraction to the plants (Fang and Wong, 1999). Fig. 5.27 shows the changes in water-soluble Zn, Cu, Mn, Fe and Cr concentration in contol and all zeolite treatments during the composting process. The water soluble fraction of heavy metals were reduced (percentage of total metal) in the ranges: 41-71.3% for Zn, 36.5-79.1% for Cu, 45.5-78.3% for Mn, 17.9-76.8% for Fe and 24.7-89.4% for Cr (Fig. 5) in control and all zeolite treatments. Highest reduction of water soluble Zn, Cu, Mn and Cr was observed in zeolite 1 treatment; however higher reduction of Fe was observed in zeolite 2 treatment it can attributed as higher reduction of organic matter and metals taken up by zeolite (Zorpas et al., 2000). The reduction in water soluble fraction of metals during the composting process probably due to transformed into a more stable fraction (Castaldi et al., 2006). The poor reduction of water solubility of metals was observed in zeolite 3 treatment, it could be explained as higher addition of zeolite hindered the composting process by absorbing water content (Villasenor et al., 2011) resulting clumping of compost biomass. It may cause scarcity of food and appropriate oxygen for microbial survival. The water solubility of Ni, Pb and Cd were not found in all treatments throughout the composting process. The variation in water soluble Zn, Cu, Mn, Fe and Cr in control and zeolite treated compost were significant (F = 121.5, $p < 0.05$ for Zn, F = 15.01, $p < 0.001$ for Cu, F = 107.3, $p < 0.05$ for Mn, F = 135.9, $p < 0.05$ for Fe, F = 28.4, $p < 0.001$ for Cr).

- **Plant availability of heavy metals (extraction with DTPA)**

The DTPA extraction efficiency of metals were reduced in the ranges: 12.2-58.6% for Zn, 33.6-81.1% for Cu, 27-48% for Mn, 10.4-52.1% for Fe, 50.0-93.2% for Ni and 31-77% for Cr (Fig. 5.28) in control and all zeolite treatments. The DTPA extraction efficiency of Zn, Mn and Fe was increased in control. The maximum reduction of DTPA extraction efficiency of Zn, Cu, Mn, Fe and Cr was observed in zeolite 1 treatment; however maximum reduction of Ni was observed in zeolite 2 treatments. The decrease in

DTPA extractability of heavy metals was also reported by Chiang et al. (2007) during the sewage sludge composting with natural zeolite. DTPA extractable concentration of Pb and Cd were not found in control and all zeolite treatments throughout the composting process.

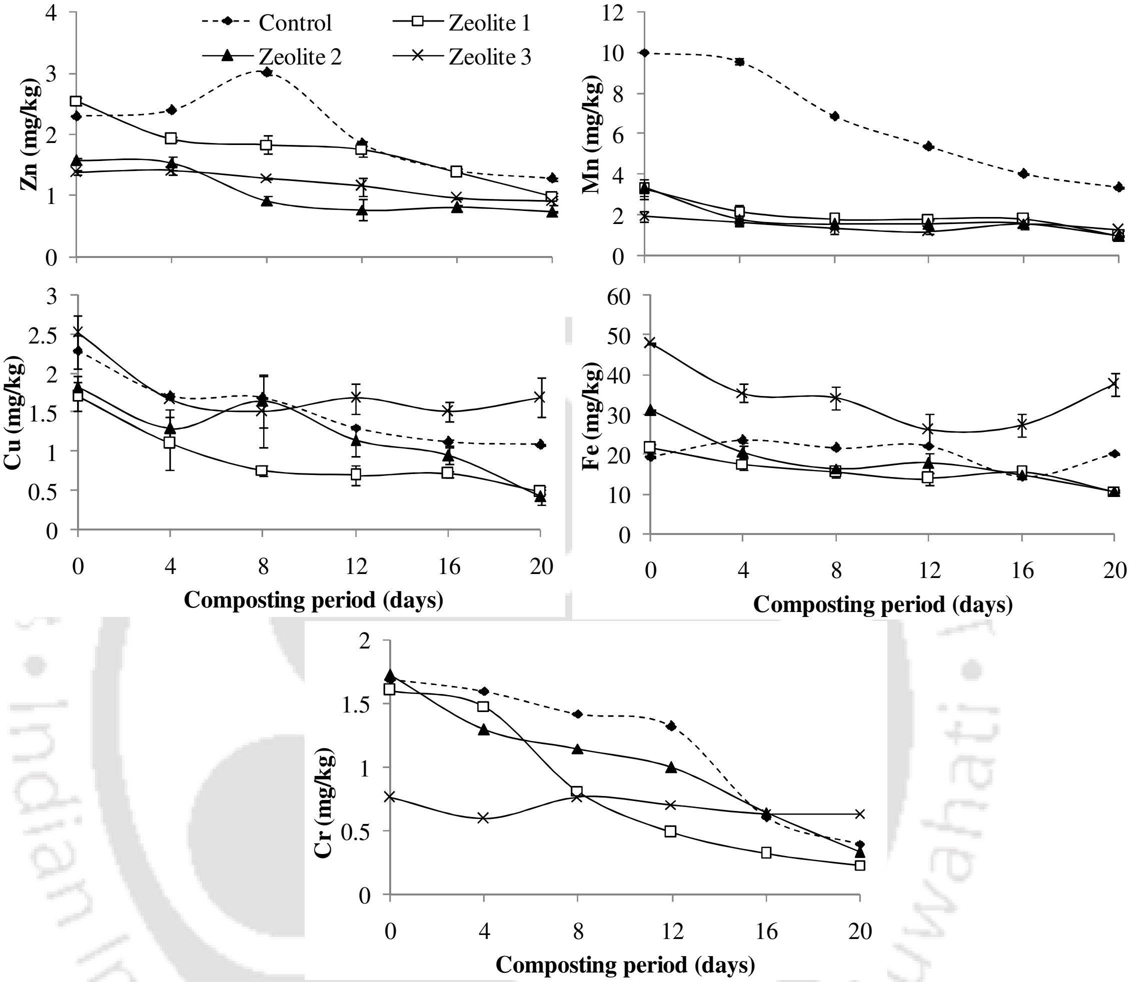


Fig. 5.27 Variation of water soluble heavy metals (Zn, Cu, Mn, Fe and Cr) during drum composting with zeolite (bars denoted as ± standard deviation)

The reduction in DTPA extractable heavy metal can be attributed as ion-exchange process where metal cations exchanged with mainly Na, K and Ca (Erdem et al., 2004; Zorpas et al., 2000). Additionally, decrease in DTPA extractable metals in the mature compost could be explained as transformation of organic matter leads to the formation of metal-humus complexes, which make the metals insoluble, consequently less easily DTPA extractable (Garcia et al., 1995). Xiong et al. (2010) concluded that ligneous bulking agents especially wood sawdust promote both the formation and complexation ability of humic acid which can reduce bioavailability of heavy metals, resulting reduce the pollution risk of heavy metals in compost applied for land-application. The variation in

DTPA extractable Zn, Cu, Mn, Fe, Ni and Cr concentrations in control and zeolite treated compost were significant ($F = 9.6$, $p < 0.001$ for Zn, $F = 18.2$, $p < 0.001$ for Cu, $F = 21.3$, $p < 0.001$ for Mn, $F = 29.6$, $p < 0.001$ for Fe, $F = 43.6$, $p < 0.001$ for Ni, $F = 8.96$, $p < 0.001$ for Cr).

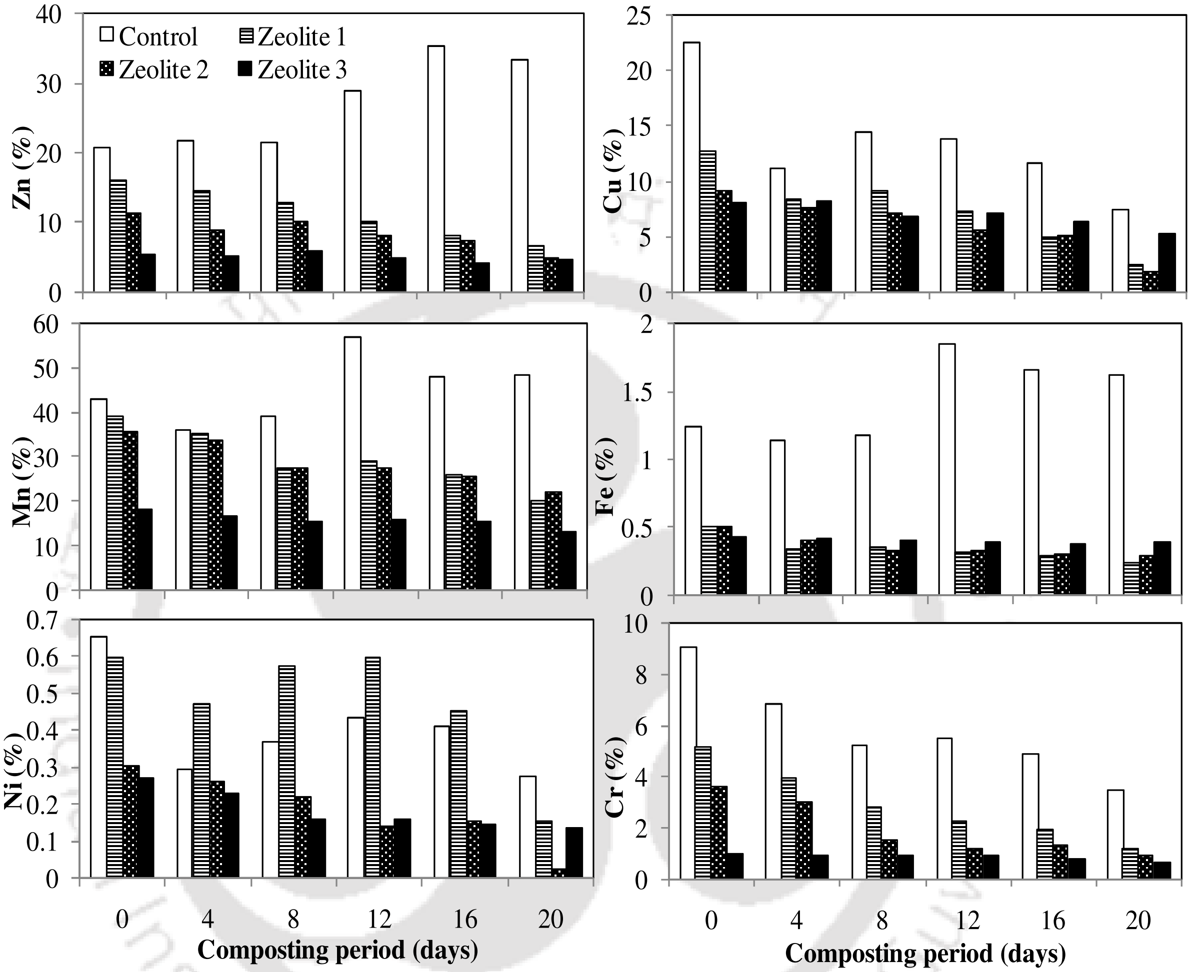


Fig. 5.28 Variation of DTPA extractable heavy metals (Zn, Cu, Mn, Fe, Ni and Cr) during drum composting with zeolite

5.4.4 LEACHABILITY OF HEAVY METALS

The TCLP extractable heavy metals concentration (mg/kg) were in the range of 0.82-3.0, 1.2-3.7 and 12.7-17.9 for Cd, Cr and Pb respectively in the mature compost, confirmed that water hyacinth compost is not hazardous to the environment. The TCLP extractable heavy metals were in compliance with the EPA regulatory thresholds limit. Table 5.13a and b shows the variation of leachable heavy metals (Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr) during the composting the process.

Leachability of heavy metals were reduced (percentage of total metal) in the ranges: 25.7-67.4% for Zn, 31.6-73.2% for Cu, 18.2-52.7% for Mn, 35.4-67.0% for Fe, 44.3-67.9% for Ni, 32.8-72.4% for Pb, 46.7-71.0% for Cd and 42.2-72.6% for Cr in control and all zeolite treatments. Higher reduction in leachability of Zn, Mn, Fe and Ni was observed in zeolite 1 treatment; however reduction of Cd and Cr was observed in zeolite 2 treatment during the composting process. Due to addition of natural zeoite, pH of initial feed mixture was enhanced in comparison to control; resulting leachability of heavy metals was reduced (Su and Wong, 2003). Furthermore, the reduction in leachable concentration of heavy metals might be due to humic substances formed at the end of composting process which had a capacity to form complex with metals (Wong and Fang, 2000; Villasenor et al., 2011). The reduction in leachability of Cu and Pb was not significant in all zeolite treatments in comparison to control. It has been observed that higher percentage of zeolite addition unable to reduced leachability of metals. Very less reduction in leachability of heavy metals was observed in zeolite 3 treatment in comparison to control and zeolite 1 and 2 treatments during rotary drum composting. Table 3 shows variation in leachable Zn, Cu, Mn, Fe, Ni, Pb, Cd and Cr concentrations in control and zeolite treatments were significant ($F = 7.9$, $p < 0.001$ for Zn, $F = 931.6$, $p < 0.001$ for Cu, $F = 13.8$, $p < 0.001$ for Mn, $F = 14.7$, $p < 0.001$ for Fe, $F = 8.6$, $p < 0.001$ for Ni, $F = 8.5$, $p < 0.001$ for Pb, $F = 17.6$, $p < 0.001$ for Cd, $F = 18.3$, $p = 0.001$ for Cr).

5.4.4 SPECIATION OF HEAVY METALS

- **Speciation of Zn, Cu, Mn and Fe and Ni**

Speciation of Zn in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14a, 5.15a and 5.16a respectively during the composting process. The F1, F2, F3 and F4 fractions of Zn were reduced in the control and all zeolite treatments. Higher reduction of F1 and F3 fractions was observed about 57.8 and 56.9% of total fraction respectively in zeolite 1 treatment but higher reduction of F2 and F4 fractions was observed in zeolite 1 and 2 treatments respectively. The F5 fraction of Zn was increased in all treatments. The F1 and F2 fractions contributed about 5% of total fraction in the final compost of control; however these fractions were reduced and became about 4.1, 3.2 and 4.93% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction contributed about 77% of total fraction in the mature compost of control; however this fraction was about 82.8, 85.8 and 83.5% of total fraction in zeolite 1, 2 and 3 treatments respectively.

Table 5.13a Changes in TCLP extractable heavy metals concentration (Zn, Cu, Mn and Fe) during drum composting with zeolite (mean ± SD, n=3)

Days	Leachability heavy metal concentration							
	Zn (mg/kg)				**Cu (mg/kg)**			
	Control	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**	**Control**	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**
0	42.22±0.3	50.08±2.3	38.15±3.6	24.77±0.3	6.60±0.2	13.18±0.8	7.31±0.5	6.13±0.8
4	31.05±0.2	48.24±5.6	39.96±4.0	24.21±1.8	6.70±0.3	10.19±1.3	6.47±0.1	5.70±0.5
8	29.53±6.3	38.50±0.1	32.20±6.3	17.53±1.8	7.90±0.3	9.30±1.0	5.24±0.23	5.90±0.9
12	24.79±4.6	33.20±1.6	28.85±2.2	21.42±4.8	6.10±0.9	8.95±0.6	4.03±0.2	5.57±0.4
16	26.62±1.0	34.75±1.1	29.00±6.2	23.00±0.2	5.10±0.2	7.83±0.5	3.30±0.3	5.85±0.1
20	21.27±0.1	22.20±2.0	21.28±1.2	20.99±1.5	2.30±0.1	7.40±0.2	3.60±0.2	4.45±0.2
Days	**Mn (mg/kg)**				**Fe (mg/kg)**			
0	205.1±9.7	184.1±5.2	180.4±3.6	140.2±2.2	121.7±8.1	48.3±4.5	27.1±1.0	32.8±1.8
4	181.8±11.0	160.4±15	175.0±4.0	141.8±1.7	159.4±11	39.7±2.8	22.5±0.1	35.0±1.1
8	191±1.6	167.6±0.4	155.9±0.9	137.0±2.2	145.5±6.0	34.3±3.0	23.2±3.9	24.1±1.7
12	177.0±3.0	172.2±6.8	162.5±5.0	140.5±5.5	107.4±7.8	33.0±1.7	23.9±2.6	22.0±5.4
16	176.7±2.1	160.6±1.2	164.4±5.0	136.1±1.9	98.9±1.5	30.9±2.8	23.8±0.6	24.1±1.3
20	175.5±8.5	119.6±1.2	130.0±6.0	133.9±3.1	63.3±2.3	27.4±6.8	23.4±2.8	24.0±1.5

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.13b Changes in TCLP extractable heavy metals concentration (Ni, Pb, Cd and Cr) during drum composting with zeolite (mean ± SD, n=3)

Days	Leachability heavy metal concentration							
	Ni (mg/kg)				**Pb (mg/kg)**			
	Control	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**	**Control**	**Zeolite 1**	**Zeolite 2**	**Zeolite 3**
0	8.60±0.6	7.69±0.2	5.50±0.3	5.33±0.0	53.2±5.2	26.05±0.5	22.05±0.6	21.40±0.9
4	6.10±0.5	6.86±0.4	4.65±0.4	4.23±0.3	40.0±4.0	20.40±2.0	19.45±4.0	20.90±0.1
8	5.40±3.6	6.45±0.1	4.25±0.2	3.69±0.3	45.1±3.1	18.70±2.7	22.85±1.2	19.25±0.3
12	4.60±0.2	5.75±0.6	3.65±0.1	3.16±0.6	33.3±10	16.95±3.5	23.95±1.6	17.80±0.8
16	5.50±0.3	5.30±0.5	2.70±0.5	3.40±0.2	30.1±4.2	17.55±1.1	25.05±0.1	17.32±2.2
20	3.60±0.2	2.80±0.1	2.10±0.3	3.29±0.5	17.0±1.1	12.87±0.4	12.65±0.2	17.80±0.6
Days	**Cd (mg/kg)**				**Cr (mg/kg)**			
0	1.86±0.02	1.75±0.03	2.95±0.15	4.55±0.25	6.10±0.70	2.53±0.33	4.30±0.30	2.55±0.25
4	2.11±0.21	1.52±0.04	2.30±0.10	4.25±0.15	7.10±0.50	2.90±0.52	2.80±0.40	2.50±0.10
8	1.89±0.07	1.45±0.05	1.72±0.12	4.10±0.30	6.40±0.40	1.50±0.10	2.70±0.10	2.00±0.40
12	1.76±0.02	1.44±0.04	1.30±0.30	3.50±0.30	7.10±0.30	1.44±0.03	1.71±0.50	1.87±0.33
16	1.60±0.04	1.27±0.07	1.45±0.15	3.20±0.40	6.90±0.10	1.40±0.20	1.60±0.30	1.85±0.35
20	1.02±0.04	0.82±0.22	1.22±0.02	3.01±0.45	3.65±0.15	1.18±0.02	1.41±0.39	1.65±0.15

Mean values in columns are statistically different (ANOVA; $p < 0.05$), The F1 and F2 fractions are most movable and bioavailable fractions which were reduced in zeolite treatments in comparison to control. These fractions were bound with zeolite through ion exchange and converted into F5 fraction which is most inert fraction (Zorpas et al., 2000; Venkateaswaran et al., 2007). Zorpas et al. (2008) reported that Zn was mainly found in F3 and F4 fraction in the sewage sludge compost with natural zeolite; but in the present study, it was mainly bound in F5 fraction throughout composting process. The BF of Zn was decreased from 0.37 to 0.23, 0.31 to 0.17, 0.28 to 0.14 and 0.19 to 0.17 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). The higher reduction of BF was observed in Zeolite 2 treatment (49.36%) followed by zeolite 1 (43.76%), control (37.5%) and zeolite 3 treatment (14.4%). The higher reduction of BF in zeolite 1 and 2 treatments might be due to transformation of organic matter into humic substances which formed complexes with Zn through its various organic functional groups (Cai et al., 2007). The variation in F1, F2, F3, F4 and F5 fractions of Zn in control and zeolite treatments were significant ($F = 9.57$, $p < 0.001$ for F1, $F = 12.54$, $p < 0.001$ for F2, $F = 34.79$, $p < 0.001$ for F3, $F = 22.84$, $p < 0.001$ for F4, $F = 16.94$, $p < 0.001$ for F5).

Speciation of Cu in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14a, 5.15a and 5.16a respectively during the composting process. The F1, F2 and F3 fractions of Cu were reduced in the control and all zeolite treatments. The F4 fraction was increased in zeolite 3 treatment while it was reduced in control, zeolite 1 and 2 treatments. Higher reduction of F1, F2 and F3 fractions were observed about 81.3, 80.6 and 74.1% of total fraction respectively in zeolite 1 treatment; however the higher reduction of F4 fraction was observed in control. The F5 fraction of Cu was increased in all treatments during the composting process. The F1 and F2 fractions contributed about 2.2% of total fraction in the final compost of control; however these fractions were reduced and became about 1.46, 2.1 and 2.0% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction contributed about 78.4% of total fraction in the compost of control; however this fraction was about 90.6, 87.3 and 86.7% of total fraction in zeolite 1, 2 and 3 treatments respectively. Cu was mainly bound in F5 fraction in control and all zeolite treatments; while Zorpas et al. (2000) reported that Cu was mainly bound to the F4 and F5 fractions during sewage sludge composting with natural zeolite. The BF of Cu was decreased from 0.52 to 0.22, 0.26 to 0.09, 0.22 to 0.13 and 0.15 to 0.13 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). Higher reduction of BF was observed in zeolite 1 treatment (64.2%) followed by control (58.69%), zeolite 2 treatment (40.4%) and zeolite 3 treatment (8.5%).

Table 5.14a Speciation of heavy metals (Zn, Cu, Mn and Fe) in zeolite 1 treatment during 20 days of composting period

Days	Zeolite 1 treatment									
	Zn (mg/kg dry matter)					Cu (mg/kg dry matter)				
	F1	F2	F3	F 4	F5	F1	F2	F3	F 4	F5
0	7.78±0.08	13.47±1.30	24.63±0.97	14.38±0.64	136.5±1.50	2.70±0.55	2.88±0.08	1.70±0.10	11.87±0.49	53.80±0.80
4	6.82±0.20	13.22±0.13	24.84±1.16	15.38±1.80	152.2±3.80	1.73±0.63	2.04±0.24	1.28±0.33	11.59±0.86	61.35±3.15
8	6.88±0.01	11.80±0.31	21.98±3.78	17.05±0.78	174.4±11.6	1.63±0.23	1.33±0.18	1.20±0.30	10.63±0.62	64.55±1.75
12	6.19±0.14	11.76±0.11	19.50±0.30	20.11±1.58	178.2±3.20	1.51±0.35	1.43±0.18	1.10±0.20	10.87±0.60	68.30±0.30
16	5.71±0.40	7.62±1.51	21.13±1.43	22.30±0.41	187.9±1.95	1.33±0.03	1.65±0.05	1.25±0.05	9.68±0.56	74.35±4.75
20	5.18±0.18	6.11±0.05	16.10±0.80	20.14±0.52	228.5±1.50	0.67±0.07	0.74±0.02	0.58±0.02	7.09±0.05	87.05±1.15
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	96.5±3.2	76.4±2.0	115.0±2.5	70.9±2.0	106.5±1.7	19.43±0.48	34.25±0.45	2353±128	2901±12	13282±1757
4	90.6±5.4	75.4±1.9	108.0±3.2	62.6±2.7	129.4±4.0	23.90±0.10	24.33±0.93	2296±96	2544±17	17984±946
8	91.0±0.9	71.7±3.7	97.5±6.5	62.5±9.5	158.1±3.3	17.43±4.23	23.43±1.93	2071±81	2452±85	19821±154
12	88.8±6.8	71.7±1.1	89.7±0.4	58.1±3.9	172.7±1.3	16.75±1.90	20.94±0.68	1963±63	2424±139	23850±380
16	73.6±4.0	66.4±1.6	85.6±4.2	56.6±1.0	264.8±2.8	11.93±0.33	17.90±0.75	1931±181	2237±66	24168±1732
20	52.8±5.2	50.5±4.3	55.5±3.5	49.9±2.3	407.0±3.4	11.28±0.33	17.88±0.03	1720±130	2207±54	25289±8635

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.14b Speciation of heavy metals (Ni, Pb, Cd and Cr) in zeolite 1 treatment during 20 days of composting period

	Zeolite 1 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	3.58±0.18	2.83±0.13	ND	ND	214.5±4.0	12.75±0.25	10.94±0.29	ND	ND	879.5±20.5
4	2.45±0.05	2.60±0.05	ND	ND	216.7±2.2	12.50±0.50	10.84±0.16	ND	ND	953.0±33.0
8	1.83±0.58	2.23±0.38	ND	ND	234.5±11	10.25±0.25	9.47±0.53	ND	ND	1053.5±21.5
12	1.60±0.10	2.20±0.40	ND	ND	242.8±35	9.70±0.80	8.41±0.10	ND	ND	1094.1±5.90
16	1.25±0.15	2.03±0.08	ND	ND	240.0±0.1	9.75±0.75	8.18±0.18	ND	ND	1127.5±12.5
20	1.20±0.10	0.89±0.09	ND	ND	242.3±0.8	3.55±0.05	4.79±0.21	ND	ND	1157.5±42.5
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	3.58±0.18	2.83±0.13	ND	ND	214.5±4.0	12.75±0.25	10.94±0.29	ND	ND	879.5±20.5
4	2.45±0.05	2.60±0.05	ND	ND	216.7±2.2	12.50±0.50	10.84±0.16	ND	ND	953.0±33.0
8	1.83±0.58	2.23±0.38	ND	ND	234.5±11	10.25±0.25	9.47±0.53	ND	ND	1053.5±21.5
12	1.60±0.10	2.20±0.40	ND	ND	242.8±35	9.70±0.80	8.41±0.10	ND	ND	1094.1±5.90
16	1.25±0.15	2.03±0.08	ND	ND	240.0±0.1	9.75±0.75	8.18±0.18	ND	ND	1127.5±12.5
20	1.20±0.10	0.89±0.09	ND	ND	242.3±0.8	3.55±0.05	4.79±0.21	ND	ND	1157.5±42.5

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

The maximum reduction of movable fractions (from F1 to F4) of Cu was observed in zeolite 1 treatment could be explained as higher degradation of organic matter followed by conversion of organic matter into humic substances which have high contents of carboxyl groups (Liu et al., 2008). The variation in F1, F2, F3, F4 and F5 fractions of Cu in control and zeolite treatments were significant (F = 14.09, $p < 0.001$ for F1, F = 14.10, $p < 0.001$ for F2, F = 28.63, $p < 0.001$ for F3, F = 29.03, $p < 0.001$ for F4, F = 12.81, $p < 0.001$ for F5).

Speciation of Mn in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14a, 5.15a and 5.16a respectively during the composting process. The F1 and F4 fractions of Mn were reduced in control and all zeolite treatments. The F2 fraction was increased in control; however, it was reduced in all zeolite treatments. The F3 fraction was increased in control and zeolite 3 treatment but it was reduced in zeolite 1 and 2 treatments. The F5 fraction was increased in control and all zeolite treatments. The higher reduction of F1 fractions was observed about 66% in control; however higher reduction of F2, F3 and F4 fractions were observed about 49.8, 63.6 and 46.8% of total fraction respectively in zeolite 1 treatment. The F5 fraction of Mn was increased in control and all zeolite treatments during the composting process. The F1 and F2 fractions contributed about 28.1% of total fraction in the final compost of control; while these fractions were reduced and became about 16.8, 23.9 and 20.0% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction contributed about 48.9% of total fraction in the compost of control while this fraction was about 66.1, 49.2 and 36.7% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction of Mn was dominant fraction and it was about 36.7-66% of total fraction in control and zeolite treatments while Zorpas et al. (2000) reported about 60% of Mn was bound to the F3 fraction during sewage sludge composting with natural zeolite.

The BF of Mn was decreased from 0.58 to 0.51, 0.72 to 0.34, 0.81 to 0.50 and 0.80 to 0.63 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). Higher reduction of BF was observed in zeolite 1 (56.0%) followed by zeolite 2 treatment (40.6%), control (26.5%) and zeolite 3 treatment (22.5%). It has been observed that BF of Mn was reduced higher in zeolite 1 and 2 treatments in comparison to control and zeolite 3 treatment; it indicated that mobile fractions (F1 to F4) of Mn were bound with zeolite. The variation in F1, F2, F3, F4 and F5 fractions of Mn in control and zeolite treatments were significant (F = 30.30, $p < 0.001$ for F1, F = 22.91, $p < 0.001$ for F2, F = 28.17, $p < 0.001$ for F3, F = 12.77, $p < 0.001$ for F4, F = 151.74, $p < 0.001$ for F5).

Speciation of Fe in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14a, 5.15a and 5.16a respectively during the composting process. The F1, F2, F3 and F4 fractions of Fe were reduced in the control and all zeolite treatments. Higher reduction of these fractions was observed about 63.1, 66.8, 53.5 and 51.6% of the total fraction respectively in zeolite 1 treatment. The F5 fraction of Fe was enhanced in control and all zeolite treatments. The F1 and F2 fractions contributed about 0.54% of total fraction in the final compost of control; however these fractions were reduced and became about 0.10, 0.10 and 0.19% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction contributed about 44.3% of total fraction in the compost of control while this fraction was about 86.5, 87.2 and 85.5% of total fraction in zeolite 1, 2 and 3 treatments respectively. Zorpas et al. (2000) reported that Fe was bound in the F3 and F5 fractions (about 95% for the Fe); however in the present study, Fe was mainly bound in F5 fraction.

The BF of Fe was decreased from 0.82 to 0.56, 0.29 to 0.14, 0.24 to 0.13 and 0.21 to 0.15 in the control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). Higher reduction of BF was observed in zeolite 1 treatment (52.6%) followed by zeolite 2 (47.4%), control (32.2%) and zeolite 3 (29.5%). It was observed that higher BF was reduced in zeolite 1 and 2 treatments in comparison to control and zeolite 3 treatment, which recommended that the addition of natural zeolite could prevent the mobility and bioavailability of Fe during the composting process. The variation in F1, F2, F3, F4 and F5 fractions of Fe in control and zeolite treatments were significant ($F = 70.82$, $p < 0.05$ for F1, $F = 33.29$, $p < 0.001$ for F2, $F = 42.4$, $p < 0.05$ for F3, $F = 22.86$, $p < 0.001$ for F4, $F = 9.56$, $p < 0.001$ for F5).

Speciation of Ni in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14b, 5.15b and 5.16b respectively during the composting process. The F1 and F2 fractions of Ni were reduced in the control and all zeolite treatments. Higher reduction of these fractions were observed about 69.7 and 71.5% of the total fraction in zeolite 1 treatments; it could be explained as zeolite has taken up the Ni bound in the F1 and F2 fractions (Zorpas et al., 2008). The F5 fraction of Ni was enhanced in control and all zeolite treatments. The F1 and F2 fractions contributed about 1.44% of total fraction in the final compost of control; however these fractions were reduced and became about 0.85, 1.81 and 2.36% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction contributed about 98.56% of total fraction in the compost of control while this fraction contributed about 99.15, 98.20 and 97.64% of total fraction in zeolite 1, 2 and 3 treatments respectively.

Table 5.15a Speciation of heavy metals (Zn, Cu, Mn and Fe) in zeolite 2 treatment during 20 days of composting period

	Zeolite 2 treatment									
Days	**Zn (mg/kg dry matter)**					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	8.22±0.15	7.58±0.22	26.09±0.52	15.71±2.65	147.2±6.8	2.28±0.53	2.10±0.30	2.45±0.35	10.87±0.21	65.05±0.45
4	7.22±0.33	7.40±0.06	24.16±1.14	13.28±2.08	157.2±23	1.70±0.10	1.33±0.13	1.45±0.35	10.04±0.68	71.35±1.85
8	7.16±0.06	6.81±1.56	22.80±1.35	13.29±0.96	160.0±4.7	1.68±0.18	1.16±0.20	2.05±0.55	10.47±0.33	74.45±1.55
12	6.58±0.01	7.34±0.02	21.93±1.53	12.38±1.10	182.3±2.3	1.70±0.10	1.17±0.24	1.60±0.0	9.33±0.94	77.25±3.75
16	6.20±0.05	6.34±0.51	19.53±1.17	11.54±0.92	197.3±8.3	1.48±0.23	1.18±0.03	1.55±0.15	10.24±0.81	80.30±1.80
20	5.23±0.09	3.24±0.12	18.00±2.10	11.24±0.02	226.8±12.0	1.13±0.03	0.93±0.13	1.29±0.31	9.24±0.06	86.20±2.30
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	121.7±1.65	83.2±5.13	129.4±3.2	83.1±4.8	71.3±6.3	19.23±0.28	31.45±1.25	1953±28	2686±216	14563±963
4	94.0±4.00	81.0±2.73	126.3±1.5	73.0.±7.5	87.8±6.8	22.85±0.85	28.10±3.10	1835±65	2503±58	18265±665
8	86.8±3.28	83.8±4.20	121.7±0.7	71.2±1.97	101.8±6.8	22.43±0.28	24.03±2.53	1706±16	2439±99	21738±763
12	94.3±1.30	74.4±6.22	127.4±1.9	66.1±11.0	124.9±4.2	16.75±1.90	25.70±1.90	1720±20	2449±114	22875±1595
16	72.4±2.83	75.2±0.40	120.3±1.1	66.3±10.7	222.5±1.1	11.93±0.33	18.63±0.23	1750±0	2301±101	24958±3942
20	64.6±3.40	71.4±3.48	83.5±8.6	69.2±2.1	280.0±2.0	13.28±1.68	17.50±1.35	1440±50	2361±200	26048±123

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.15b Speciation of heavy metals (Ni, Pb, Cd and Cr) in zeolite 2 treatment during 20 days of composting period

	Zeolite 2 treatment									
Days	**Ni (mg/kg dry matter)**					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	6.68±0.28	3.50±0.10	ND	ND	210.3±1.8	12.0±0.50	11.93±0.58	ND	ND	940±35
4	5.28±0.23	2.93±0.03	ND	ND	230.8±7.8	12.9±0.15	12.18±0.18	ND	ND	993±73
8	5.37±0.03	2.83±0.43	ND	ND	232.5±3.0	12.0±2.00	11.49±0.02	ND	ND	1068±7.5
12	4.15±0.35	2.70±0.20	ND	ND	228.4±9.8	10.7±0.02	10.13±0.13	ND	ND	1100±100
16	3.38±0.28	2.43±0.27	ND	ND	244.0±4.0	8.0±0.50	8.69±0.83	ND	ND	1115±25
20	2.50±0.40	2.18±0.08	ND	ND	254.5±3.0	5.0±0.38	4.88±0.38	ND	ND	1193±13
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	1.31±0.06	0.94±0.05	ND	ND	71.25±5.25	2.43±0.53	0±0	2.43±0.17	11.31±1.05	59.5±4.0
4	1.13±0.13	0.88±0.08	ND	ND	73.50±0.50	1.83±0.52	0.43±0.03	2.10±0.30	10.99±0.54	62.3±2.25
8	0.98±0.23	0.64±0.07	ND	ND	73.15±0.35	1.88±0.18	0.70±0.05	2.00±0.80	10.80±0.7	62.0±0.50
12	0.90±0.05	0.50±0.05	ND	ND	75.25±3.25	1.60±0.10	0.30±0.05	1.84±0.56	9.70±0.31	66.0±1.0
16	0.86±0.01	0.40±0.05	ND	ND	79.00±4.50	1.49±0.01	0.23±0.02	1.09±0.11	8.48±0.12	72.3±6.25
20	0.73±0.03	0.23±0.03	ND	ND	85.50±2.50	1.33±0.03	0±0	0.95±0.05	3.80±0.45	86.0±1.0

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

Zorpas et al. (2008) reported that Ni was found to be associated with the F3 fraction (36%) and the F5 fraction (23%) during the sewage sludge composting with natural zeolite; while in the present study Ni was mostly bound in F5 fraction (about 97.6-99.15% of total fraction); however F3 and F4 fractions were not detected during the composting process, similar results also reported by Fuentes et al. (2004) in different sewage sludges. The significant increase in the F5 fraction of Ni may be due to alkaline stabilization process (Gupta and Sinha, 2007). The BF of Ni was decreased from 0.041 to 0.014, 0.029 to 0.009, 0.046 to 0.018 and 0.039 to 0.024 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). Highest reduction of BF was observed in zeolite 1 treatment (70.5%) followed by control (65.04%), zeolite 2 treatment (60.9%) and control (38.1%). Higher reduction of BF was observed in zeolite 1 treatment in comparison to control, zeolite 2 and 3 treatments. Higher reduction of BF of Ni may be due to the effect of zeolite, which has the ability to absorb and exchange easily available fractions (Zorpas et al., 2008). The variation in F1, F2 and F5 fractions of Ni in control and zeolite treatments were significant ($F = 49.61$, $p < 0.05$ for F1, $F = 18.88$, $p < 0.001$ for F2, F = 4.11, $p < 0.001$ for F5).

- **Speciation of Pb, Cd and Cr**

Speciation of Pb in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14b, 5.15b and 5.16b respectively during the composting process. The F1 and F2 fractions of Pb were reduced in the control and all zeolite treatments. Higher reduction (percentage of total fraction) of F1 fractions was observed in zeolite 1 treatment (78.4%) followed by control (77.4%), zeolite 2 treatment (66.6%) and zeolite 3 treatment (20.7%). Higher reduction (percentage of total fraction) of F2 fraction was observed in zeolite 2 treatment (67.2%) followed by zeolite 1 treatment (66.1%), control (58.7%) and zeolite 3 treatment (35.5%). The F5 fraction of Pb was increased in control and all zeolite treatments. Zorpas et al. (2000) reported that F3 and F5 fractions of Pb contributed about 83% of total Pb. However in the present study, Pb was mainly bound in F5 fraction (about 97-98% of total fraction); however F3 and F4 fractions were not detected during the composting process.

The BF of Pb was decreased from 0.043 to 0.013, 0.026 to 0.007, 0.025 to 0.008 and 0.032 to 0.023 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). Higher reduction of BF was observed in zeolite 1 treatment (72.7%) followed by control (70.2%), zeolite 2 treatment (66.9%), and zeolite 3 treatment (28.5%). The maximum reduction of BF in zeolite 1 treatment attributed as higher degradation of organic matter and available fractions were taken up from the zeolite (Zorpas et al., 2008). The variation in F1, F2 and

F5 fractions of Pb in control and zeolite treatments were significant ($F = 41.54$, $p < 0.05$ for F1, $F = 25.29$, $p < 0.001$ for F2, $F = 6.19$, $p < 0.001$ for F5).

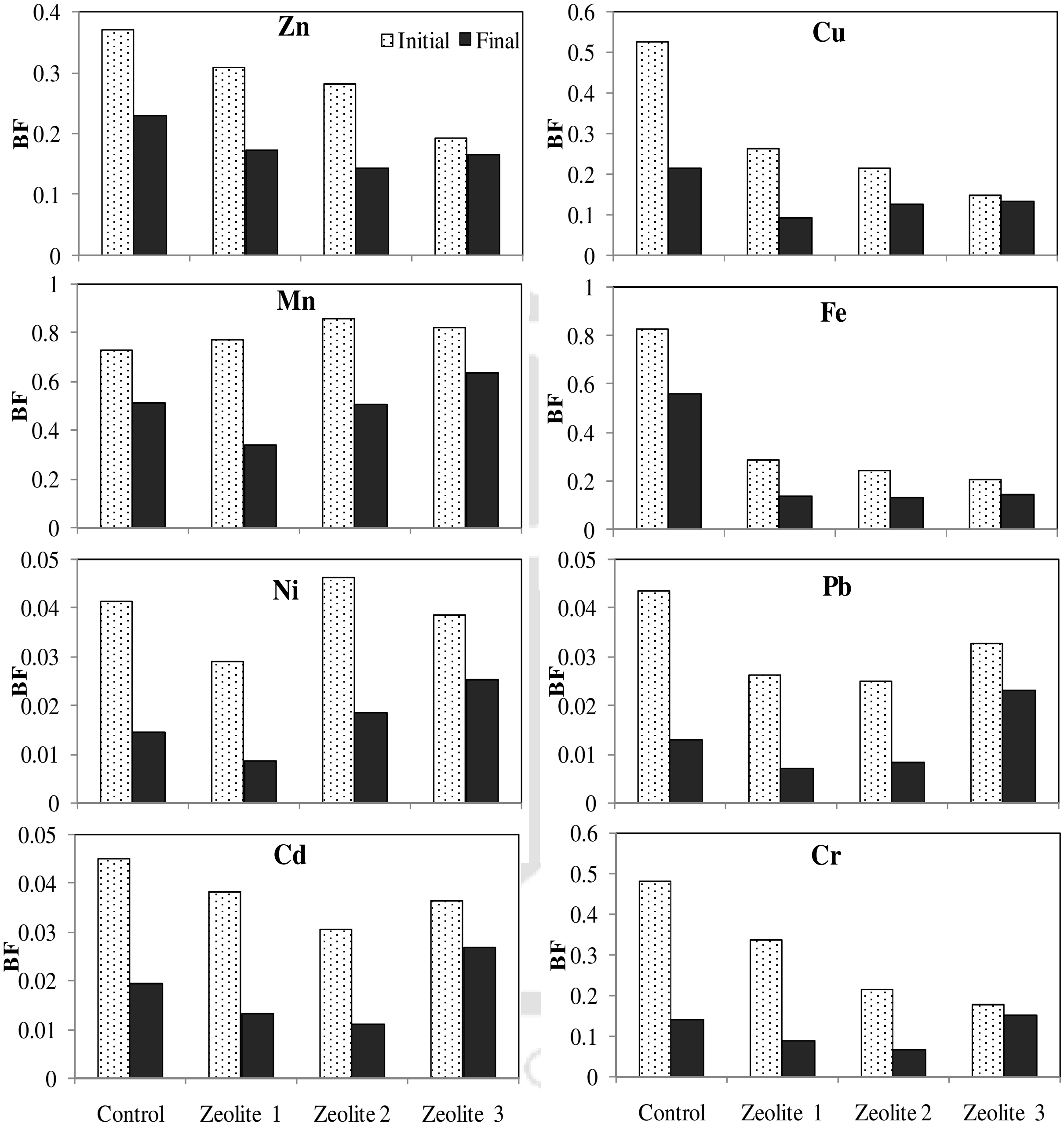


Fig. 5.29 Changes in bioavailability factor (BF) of heavy metals in control and zeolite treatments during drum composting

Speciation of Cd in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14b, 5.15b and 5.16b respectively during the composting process. The F1 and F2 fractions of Cd were reduced in the control and all zeolite treatments. Higher reduction of F1 and F2 fractions were observed about 68.4% and 79.1% of total fraction in control and zeolite 2 treatment respectively. The F5 fraction of Cd was increased in control and all zeolite treatments. The F1 and F2 fractions of Cd contributed about 2.0% of total fraction in the final compost of control; however these fractions were reduced and became about 1.32, 1.11 and 2.69% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction contributed about 98.1% of total fraction in the compost of control while this fraction was about 98.7, 98.9 and 97.3% of total fraction in zeolite 1, 2 and 3 treatments respectively. The BF of Cd was decreased from 0.045 to 0.019, 0.038 to 0.013, 0.030 to 0.011 and 0.036 to 0.027 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). Higher reduction of BF was observed in zeolite 1 treatment (65.4%) followed by zeolite 2 treatment (63.8%), control (56.7%) and zeolite 3 treatment (25.9%). Maximum reduction of BF in zeolite 1 treatment might be due to higher degradation of organic matter, resulting conversion into humus like substances which formed metal-humus complex during composting (Tiquia et al., 1997). Reduction in F1 and F2 fractions of Cd attributed to the fact that oxygen containing functional groups with higher affinities to Cd, such as phenolic and benzene-carboxylic groups, were formed during the composting process (Xiong et al., 2010). The variation in F1, F2 and F5 fractions of Cd in control and all zeolite treatments were significant ($F = 16.60$, $p < 0.001$ for F1, $F = 21.44$, $p < 0.001$ for F2, $F = 9.9$, $p < 0.001$ for F5).

Speciation of Cr in control, zeolite 1, 2 and 3 treatments are given in table 4.11, 5.14b, 5.15b and 5.16b respectively during the composting process. The F1, F2 and F3 fractions of Cr were reduced in the control and all zeolite treatments. Higher reduction of F1 and F2 fractions were observed about 70.1 and 84.0% of the total fraction in zeolite 1 treatment, but higher reduction of F3 and F4 fractions were observed in control. The F4 fraction was increased in zeolite 3 treatment. The F5 fraction of Cr was increased in control and all zeolite treatments. The F2 fraction was not observed at initially but during thermophilic stage appeared and again it was not detected in mature compost. During thermophilic stage it was released due to higher degradation of organic matter and at final stage this fraction taken up by zeolite. The F1 and F2 fractions of Cr contributed about 7.2% of total fraction in the final compost of control; however these fractions reduced and became about 1.68, 1.44 and 1.42% of total fraction in zeolite 1, 2 and 3 treatments respectively. The F5 fraction contributed about 85.8% of total fraction in the compost of control while this fraction contributed about 91.1, 93.4 and 84.9% of total fraction in zeolite 1, 2 and 3 treatments respectively. Zorpas et al. (2000) reported that Cr was mainly associated with F4 and F5 fractions in the zeolite amended sewage sludge compost.

Table 5.16a Speciation of heavy metals (Zn, Cu, Mn and Fe) in zeolite 3 treatment during 20 days of composting period

Days	**Zeolite 3 treatment**									
	Zn (mg/kg dry matter)					**Cu (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	6.55±0.20	6.61±0.03	18.27±0.10	11.39±0.26	179.8±8.6	2.03±0.18	1.43±0.03	3.05±0.25	8.20±0.12	86.0±2.0
4	6.86±0.28	7.13±0.26	16.99±0.69	11.60±0.14	185.1±9.1	2.09±0.41	1.55±0.10	3.55±0.25	8.47±1.23	84.75±6.25
8	6.77±0.45	7.86±0.61	17.85±0.45	11.48±0.30	188.7±6.0	2.13±0.28	1.40±0.05	3.50±0.0	9.03±0.77	92.15±5.85
12	6.41±0.72	7.36±0.10	19.40±0.86	11.43±0.26	183.8±2.3	2.00±0.20	1.35±0.05	4.10±0.50	9.49±0.58	91.50±5.50
16	7.20±0.95	6.80±0.74	20.88±0.51	11.77±0.27	210.2±15.2	1.90±0.65	1.38±0.23	4.00±0.30	9.67±0.38	85.04±1.54
20	6.42±0.02	7.0±0.62	18.78±0.17	12.59±0.03	227.3±0.8	1.14±0.02	1.03±0.03	2.65±0.05	9.58±0.22	93.35±12.15
Days	**Mn (mg/kg dry matter)**					**Fe (mg/kg dry matter)**				
0	95.4±2.6	86.6±1.2	125.0±1.2	85.4±2.9	88.0±3.0	27.9±0.05	37.33±0.98	1850±50	2175±95	15813±288
4	87.6±2.4	78.2±0.1	140.7±12.9	89.3±8.5	97.0±4.0	22.4±0.40	32.40±4.10	1879±108	2098±148	17715±1215
8	72.9±9.7	72.8±4.8	154.9±2.6	91.5±2.2	123.1±5.6	20.1±2.05	27.63±4.18	1812±90	2071±69	18258±717
12	72.3±9.8	68.8±8.2	168.8±6.7	91.1±6.2	129.9±0.9	24.5±0.40	27.15±0.30	1670±42	1993±42	17735±735
16	66.5±1.1	68.5±3.8	171.3±2.1	93.9±3.1	156.5±5.0	19.6±0.65	26.65±0.60	1665±292	2308±293	19700±800
20	57.4±0.2	63.2±1.3	168.6±0.5	92.2±0.9	220.8±7.3	19.2±0.55	24.30±0.60	1213±23	2083±23	19713±1213

Mean values in columns are statistically different (ANOVA; $p < 0.05$)

Table 5.16b Speciation of heavy metals (Ni, Pb, Cd and Cr) in zeolite 3 treatment during 20 days of composting period

Days	Zeolite 3 treatment									
	Ni (mg/kg dry matter)					**Pb (mg/kg dry matter)**				
	F1	**F2**	**F3**	**F 4**	**F5**	**F1**	**F2**	**F3**	**F 4**	**F5**
0	5.70±0.10	3.78±0.18	ND	ND	236.25±5.8	15.68±0.68	17.43±0.07	ND	ND	988±13
4	5.45±0.05	3.13±0.17	ND	ND	255.75±3.8	15.18±0.82	15.43±3.07	ND	ND	993±28
8	4.68±0.28	2.73±0.48	ND	ND	263.75±5.7	14.24±2.77	13.99±2.52	ND	ND	1020±60
12	4.48±0.03	3.18±0.28	ND	ND	257.50±2.0	14.13±1.87	13.38±3.13	ND	ND	1003±3
16	4.30±0.10	3.33±0.38	ND	ND	262.50±1.5	13.54±0.96	13.29±1.29	ND	ND	1023±3
20	3.45±0.35	3.00±0.75	ND	ND	267.25±1.8	13.13±0.13	11.88±1.38	ND	ND	1053±3
Days	**Cd (mg/kg dry matter)**					**Cr (mg/kg dry matter)**				
0	1.48±0.12	1.20±0.01	ND	ND	71.0±1.0	1.73±0.03	0.60±0.05	2.36±0.05	10.39±0.26	70.0±4.5
4	1.24±0.04	1.21±0.50	ND	ND	68.2±4.65	1.60±0.05	0.46±0.015	2.39±0.09	11.10±0.16	78.3±6.3
8	1.28±0.18	1.22±0.10	ND	ND	69.3±2.25	1.58±0.03	0.38±0.020	2.04±0.17	10.97±0.72	80.8±0.8
12	1.06±0.30	1.40±0.50	ND	ND	72.0±3.50	1.52±0.07	0.34±0.010	2.07±0.07	10.72±0.85	81.4±0.1
16	0.95±0.00	1.10±0.00	ND	ND	69.5±4.0	1.41±0.06	0.36±0.005	1.26±0.28	11.69±1.44	82.5±1.5
20	0.89±0.01	1.098±0.15	ND	ND	71.8±1.25	1.22±0.04	0.27±0.03	1.07±0.14	13.26±0.90	89.0±1.0

Mean values in columns are statistically different (ANOVA; $p < 0.05$); ND- not detected

However in the present study F5 fraction of Cr was dominant fraction. The BF of Cr was decreased from 0.48 to 0.14, 0.34 to 0.09, 0.21 to 0.07 and 0.18 to 0.05 in control and zeolite 1, 2 and 3 treatments respectively (Fig. 5.29). Higher reduction of BF was observed in zeolite 1 treatment (73.3%) followed by control (70.5%), zeolite 2 treatment (69.1%) and zeolite 3 treatment (14.9%) during the composting process. The reduction in readily available fractions of Cr could be explained as zeolite has the ability to absorb and exchange metals (Zorpas et al., 2008). The variation in F1, F2, F3, F4 and F5 fractions of Cr in control and zeolite treatments were significant (F = 36.0, $p < 0.001$ for F1, F = 55.20, $p < 0.001$ for F2, F = 15.44, $p < 0.001$ for F3, F = 26.39, $p < 0.001$ for F4, F = 14.45, $p < 0.001$ for F5).

5.4.5 CONCLUSION

Addition of natural zeolite during composting process increased the pH of initial feed mixture and organic matter degradation; consequentially it reduced bioavailability, leachability and most bioavailable fractions (F1 and F2) of heavy metals were influenced. TCLP test confirmed that the all selected heavy metals concentrations were below the threshold limits and compost is not hazardous to environment. The leachability of Cu and Pb was not reduced significantly in all zeolite treatments as compared to control. The maximum reduction of bioavailability and leachability of heavy metals were observed in zeolite 1 and 2 treatments, which indicates optimum percentage of zeolite could enhance organic matter degradation.

The higher reduction of most bioavailable fractions (exchangeale and carbonate) and bioavailability factor of all selected heavy metals in zeolite 1 and 2 treatments in comparison to control and zeolite 3 treatment. The order of BF of different metals in the compost of control was: Fe (0.56) > Mn (0.51) > Zn (0.23) > Cu (0.22) > Cr (0.14) > Cd (0.019) > Ni (0.014) > Pb (0.013); however order of these metals in zeolite treatments was: Mn (0.23) > Zn (0.14) > Fe (0.13) > Cu (0.09) > Cr (0.066) > Cd (0.011) > Ni (0.009) > Pb (0.007). The total concentration of Zn, Cu, Mn, Ni, Cd and Cr was lower than Pb while BF of these metals was higher. The orders of BF in control and zeolite treatments reveal that addition of natural zeolite was successful for reducing BF of heavy metals and confirmed that the toxicity of metals associated with its bioavailable fractions rather than total metal concentration. Hence this study concluded that the utilization of suitable proportion of natural zeolite was successful for reducing bioavailability of heavy metals during rotary drum composting of water hyacinth mixed with cattle manure and sawdust.

5.5 SUMMARY

This study was carried out with different percentage of lime and zeolite addition in trial 4 of agitated pile and rotary drum composting. Some main observations are given as follow:

- Addition of appropriate percentage of lime sludge during the composting process enhanced the composting process through degradation of organic matter. Significantly increase in pH of initial feed mixture consequently, reduced bioavailability, leachability and most mobile fractions (exchangeable and carbonate) of heavy metals. Study suggested that higher addition of lime slow down the organic matter degradation during the process due to prevailing higher alkaline condition for microorganisms. Addition of 2% lime was very effective for reduction of water solubility, DTPA extractability and bioavailability factor during agitated pile; however in rotary drum composting 1 and 2% lime was very effective. Furthermore, lime addition was much effective for reduction of bioavailability of heavy metals rotay drum in comparison to agitated pile composting.
- Addition of appropriate percentage of natural zeolite during composting process could increase the organic matter degradation and nutrients such as Na, K and Ca. It was very effective for reduction of bioavailability and leachability of heavy metals. An appropriate percentage of zeolite could reduce bioavailable and leachable fractions of heavy metals during both agitated pile and rotary drum composting process. It can be suggested that excess addition of natural zeolite was not much effective for reduction of bioavailability and leachability of heavy metals during rotary drum composting as compared to agitated pile composting.
- Addition of natural zeolite was much effective for reduction of bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate) in comparison to lime addition. This study suggested that addition of excess lime and natural zeolite reduced degradation of organic matter in both agitated pile and rotary drum composting processes but rotary drum composting was much affected; resulting poor reduction of bioavailability and leachability of heavy metals.

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

This chapter dealt with conclusion achieved from the all four phases conducted with different composting methods, and effects of lime and natural zeolite addition during composting process. In addition recommendations for future works are also revealed.

6.1 CONCLUSIONS

- Agitated pile, rotary drum and vermicomposting were successful for conversion of water hyacinth into compost. Rotary drum was highly efficient for producing water hyacinth compost due to appropriate agitation, mixing and aeration. Addition of cattle manure during composting and vermicomposting enhanced the composting process. A higher proportion of cattle manure hindered the agitated pile and rotary drum composting process, however vermicomposting was not affected.
- Vermicomposting of water hyacinth by using *Eisenia fetida* was very effective for reduction in bioavailability of heavy metals in comparison to traditional composting.
- Appropriate proportion of lime and natural zeolite addition during the composting process accelerates the organic matter degradation consequently converted into most stabilizes organic matter, and reduced bioavailability and leachability of heavy metals.
- Addition of natural zeolite was much effective for reduction in bioavailability, leachability and most bioavailable fractions (exchangeable and carbonate) in comparison to lime addition. This study suggested that addition of excess lime and natural zeolite reduced performance of the both agitated pile and rotary drum composting processes; resulting poor reduction of bioavailability and leachability of heavy metals. So, addition of excess lime and natural zeolite during water hyacinth composting should not be recommended.
- The reducible and oxidizable fractions of Ni, Pb and Cd were not found; however the exchangeable (F1) and carbonate (F2) fractions of these metals were found during all three types of composting process, which are most toxic and easily bioavailable fractions.
- Chemicals addition (lime and natural zeolite) were most significant for reduction of bioavailability of metals in comparison to agitated pile (phase 1), rotary drum (phase 2) and vermicomposting (phase 3).

6.1.1 BIOAVAILABILITY, LEACHABILITY AND SPECIATION OF HEAVY METALS

- During the composting process, bioavailability and leachability of heavy metals were depends on physico-chemical properties of the compost such as decomposition of organic matter and pH; besides total metal contents.
- Total concentrations of heavy metals were increased during agitated pile and rotary drum composting, however total concentrations of few metals (Cu, Mn, Fe, Ni and Cd) were reduced during the vermicomposting process
- The total concentration of Cu and Zn was low but their percentages of water soluble, plant available (DTPA extractable) and leachable fractions were similar as Fe, Cr and Mn, which observed in high concentration. Total Cu content was less than total Cr content but water solubility of Cu was higher than Cr, which indicates that toxicity risk of Cu is higher than Cr.
- The vermicomposting by *Eisenia fetida* was very effective for reduction of bioavailability and leachability of all heavy metals (Zn, Cu, Fe, Ni, Pb, Cd and Cr) except Mn.
- The concentrations of Cu and Cd were very low as compared to the other metals, but the percentage of exchangeable and carbonate fractions were similar as other selected metals. The maximum reduction in exchangeable and carbonate fractions of all metals except Mn and Cd were found in trial 4 of both agitated pile and rotary drum composting in comparison to other trials.
- The total concentrations of Cu, Cr and Cd were very low as compared to other metals (Zn, Mn, Fe, Ni and Pb), but the percentage of exchangeable (F1) and carbonate (F2) fractions of these metals were similar. The exchangeable fraction of Mn was dominant in initial feed mixture but in the final vermicompost it was converted into less mobile fractions (reducible, oxidizable and residual). The exchangeable fraction of Cd was reduced completely (100%) during the vermicomposting of water hyacinth.
- The maximum reduction of bioavailability and leachability of heavy metals was observed in trial 4 during agitated pile and rotary drum composting, which indicated optimum proportion of cattle manure can enhance organic matter degradation and humification process; consequently it reduced the toxicity risk of metals during composting process. However during the vermicomposting process, bioavailability and leachability of heavy metals were reduced significantly in all trials except trial 1 (control).

6.1.2 EFFECTS OF LIME AND NATURAL ZEOLITE

- Lime addition raised the pH effectively at the initial stage of composting, caused a decrease in water soluble, diethylene triamine pentacetic acid (DTPA) extractable, toxicity characteristics leaching procedure (TCLP) extractable and most bioavailable fractions (exchangeable and carbonate) of heavy metals. The final pH of lime treated compost was found similar as control (without lime).
- The quick development of thermophilic stage was observed in lime 2 (2%) treatment due to intense microbial growth. The maximum reduction of water soluble, plant available and leachable metals were observed in lime 2 treatment during agitated pile composting; however its higher reduction was observed in lime 1 (1%) and 2 (2%) treatments during rotary drum composting, which indicated optimum percentage of lime can enhance organic matter degradation and humification process; consequently it reduced the toxicity of the metals during water hyacinth composting. The poor performance of agitated pile and rotary drum composting was observed in excess lime addition.
- Total concentration of Cu was lowest among five water soluble metals but its water solubility was highest. It indicates that the Cu is more toxic than Zn, Fe, Mn and Cr with respect to water solubility. The lowest water solubility and leachability of Fe was observed even its total concentration was highest among eight metals. The TCLP concentrations of the targeted metals in the compost were completely in agreement with the thresholds limits.
- The bioavailability factor (BF) of Zn, Cu, Fe, Ni, Pb, Cd and Cr was reduced significantly in lime treatments in comparison to control, whereas the addition of lime could not reduced the bioavailability of Mn during agitated pile composting. However during rotary drum composting, BF of all metals was reduced significantly in lime treatments as compared to control. The order of BF of different metals in lime treatments during agitated pile composting of water hyacinth was: Mn (0.70) > Fe (0.67) > Zn (0.48) > Cu (0.28) > Cr (0.18) > Ni (0.06) > Cd (0.05) > Pb (0.03). However, the order of BF of different metals in lime treatments during rotary drum composting was: Fe (0.56) > Mn (0.55) > Zn (0.44) > Cu (0.24) > Cr (0.14) > Cd (0.024) > Pb (0.022) > Ni (0.019). The order of BF indicates that toxicity of metals does not depend on its total metal concentration. Rotary drum composting was more efficient for reduction of BF in comparison to agitated pile composting.

- The total concentration of Pb was higher than Zn, Cu, Mn, Ni, Cd and Cr but its BF was lowest among the all eight metals; the total concentration of Cu was much less than Pb and Ni but its BF was about five times of Ni and ten times of Pb in the final compost of agitated pile. The total concentration of Ni was higher than Zn, Cu, Cd and Cr but its BF was lowest among these metals in the final compost of rotary drum.
- This study concluded that the addition of waste lime sludge was successful for reducing bioavailability of heavy metals during agitated pile and rotary drum composting of water hyacinth mixed with cattle manure and sawdust. The waste lime sludge could be minimized by utilization for heavy metals immobilization during the agitated pile and rotary drum composting process. The performance of rotary drum was better that agitated pile with excess lime addition.
- Natural zeolite addition during composting process increased the pH of initial feed mixture and organic matter degradation, resulting decrease in bioavailable and leachable fractions of metals in the final compost.
- Addition of the natural zeolite during composting process increased the Na, Ca and K concentration, and reduced water solubility, DTPA and TCLP extractability of heavy metals through ion exchange. TCLP test confirmed that all selected heavy metals concentrations were below the threshold limits and compost is not hazardous to the environment.
- The maximum reduction of bioavailability, leachability and BF of heavy metals were observed in zeolite 1 (5%) and 2 (10%) treatments in comparison to control and zeolite 3 (15%) treatment during both agitated pile and rotary drum composting, which indicated optimum percentage of zeolite could enhance organic matter degradation; therefore it decreased the toxicity of the heavy metals during water hyacinth composting with cattle manure and sawdust.
- The order of BF of different metals in the final compost of zeolite treatments during agitated pile composting was: Mn (0.35) > Fe (0.19) > Zn (0.15) > Cr (0.066) > Cu (0.060) > Ni (0.026) > Cd (0.023) = Pb (0.023). However, the order of BF of different metals in the compost of zeolite treatments during rotary drum composting was: Mn (0.23) > Zn (0.14) > Fe (0.13) > Cu (0.09) > Cr (0.066) > Cd (0.011) > Ni (0.009) > Pb (0.007). The BF of all metals except Cu and Cr was observed lower in the final compost of rotary drum as compared to agitated pile compost.
- The total concentration of Cu and Cr was much lower than Pb and Ni but their BF were higher than Pb and Ni in the mature compost of agitated pile. The total

concentration of Zn, Cu, Mn, Ni, Cd and Cr was lower than Pb while BF of these metals was higher in both agitated pile and rotary drum composting.

- Water soluble Ni, Pb and Cd contents and DTPA extractable Pb and Cd were not detected during agitated pile, rotary drum composting and vermicomposting process.

6.2 RECOMMENDATIONS FOR FUTURE WORK

Specifically more effort should be required in the following directions:

- Addition of some chemicals such as bamboo charcoal, red mud and gypsum; which are not harmful for composting process and having cation exchange capacity, consequently reduces most bioavailable fractions of metals during water hyacinth composting.
- Identification of microorganisms which plays major role in conversion of most bioavailable fractions into most inactive fraction (residual fraction) during the composting process. Addition of specific microorganisms (Bacteria, Fungi and Blue green algae) in the compost which are able to reduce bioavailability and convert easily available fractions (exchangeable and carbonate fractions) into less mobile fractions (reducible and oxidizable fractions) or inert (residual) fraction.
- Studies needed on bioavailability, leachability and speciation of Hg, As, Ba, Se and Ag and their reduction solutions during the water hyacinth composting.

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- ***International Journals (Published or Accepted)***

1. Singh, J., Kalamdhad, A.S. (2011). Effects of heavy metals on soil, plants, human health and aquatic life. ***International Journal of Research in Chemistry and Environment*** 1 (2), 15-21.
2. Singh, J., Kalamdhad, A.S. (2012). Reduction of heavy metals during composting- a review. ***International Journal of Environmental Protection*** 2 (9), 36-43.
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4. Singh, J., Kalamdhad, A.S. (2013). Assessment of bioavailability and leachability of heavy metals during rotary drum composting of green waste (Water hyacinth). ***Ecological Engineering*** 52, 59-69.
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8. Singh, J., Kalamdhad, A.S. (2013). Chemical speciation of heavy metals in compost and compost amended soil-A review. ***International Journal of Environmental Engineering and Research*** 2 (2), 27-37.
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- ***International/National conferences (Oral presentation)***

1. Singh, J., Kalamdhad, A.S. (2012). Paper presentation on "Effects of heavy metals on the environment by utilization of urban waste compost for land application", in **International Conference on Environmentally Sustainable Urban Ecosystems** held at IIT Guwahati, during 24th-26th February 2012.
2. Singh, J., Kalamdhad, A.S. (2012). Paper presentation on "Estimation of bioavailability, leachability and speciation of Zn, Cu, Cd and Pb during water hyacinth composting", in **2nd International Science congress** held at Bon Maharaj Engineering College Vrindavan, Mathura (U.P.) during 6-8 December 2012.
3. Singh, J., Kalamdhad, A.S. (2013). Paper presentation on "Study of heavy metals during water hyacinth composting", in **International Conference on Waste, Health and Wealth** held at International Institute of Waste Management, Bhopal, M.P. during 15-17 February 2013.
4. Singh, J., Kalamdhad, A.S. (2013). Paper presentation on "Assessment of bioavailability and speciation of Zn, Cu, Ni and Cr during water hyacinth composting" in **Waste Safe 2013-3rd International Conference on Solid Waste Management in Developing Countrie**s, held at Khulna University of Engineering and Technology, Khulna, Bangladesh, during 10-12 February 2013.
5. Singh, J., Pathak, K., Prasad, R., Kalamdhad, A.S. (2013). Paper presentation on "Evaluation of Bioavailability and Chemical Speciation of Zn and Cu during Vermicomposting of Water Hyacinth" in **National symposium on environmental issues and challenges in 21th century** held at Bareilly College, Bareilly, U.P. during 3-5 February, 2013.
6. Singh, J., Kalamdhad, A.S. (2013). Paper presentation on "Uptake of bioavailable and leachable fractions of Zn, Cu, Ni, and Cr by natural zeolite during rotary drum composting of water hyacinth", in **International conference on waste management and environment (ICWME)** held at Institute of Biological Sciences, Faculty of Science University of Malaya, Kuala Lumpur Malaysia, during 26-27 August, 2013.

- ***Articles (Submitted revision/Under review)***

1. Singh, J., Kalamdhad, A.S. (2013). Effects of lime on speciation of heavy metals during rotary drum composting of water hyacinth. ***Toxicological & Environmental Chemistry (Submitted revision 1).***
2. Singh, J., Kalamdhad, A.S. (2013). Effect of natural zeolite on speciation of heavy metals during rotary drum composting of water hyacinth (green waste). ***Chemical Speciation & Bioavailability (Submitted revision 1).***
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4. Singh, J., Kalamdhad, A.S. (2013). Uptake of heavy metals by natural zeolite during agitated pile composting of water hyacinth. ***International Journal of Environmental Science (Under review)***.
5. Singh, J., Kalamdhad, A.S. (2013). Effects of natural zeolite on speciation of heavy metals during agitated pile composting of water hyacinth. ***International Journal of Recycling of Organic Waste in Agriculture (Under review)***.