

**FATE OF NICKEL IN CONTAMINATED SOIL AND DEVELOPING  
PHYTOREMEDIATION STRATEGY**

**By**

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COIMBATORE - 641 003**

**2015**

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PHYTOREMEDIATION STRATEGY**

*Thesis submitted in part fulfillment of the requirements for the award of the degree of  
**DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL SCIENCES**  
to the Tamil Nadu Agricultural University, Coimbatore.*

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**2015**

## **CERTIFICATE**

This is to certify that the thesis entitled “**FATE OF NICKEL IN CONTAMINATED SOIL AND DEVELOPING PHYTOREMEDIATION STRATEGY**” submitted in part fulfillment of the requirement for the degree of **DOCTOR OF PHILOSOPHY IN ENVIRONMENTAL SCIENCE** to the Tamil Nadu Agricultural University, Coimbatore, is a bonafide record of research work carried out by **V. SATHYA**, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, fellowship or other similar titles of prizes. However part of the thesis work has been published in peer reviewed scientific journals of National/International repute (Copy enclosed).

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## *Acknowledgement*

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*(V. Sathya)*

*Abstract*

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## ABSTRACT

### FATE OF NICKEL IN CONTAMINATED SOIL AND DEVELOPING PHYTOREMEDIATION STRATEGY

By

**V. SATHYA**

Degree : **Doctor of Philosophy (Environmental Sciences)**

Chairman : **Dr. S. MAHIMAIRAJA Ph.D (Massey)**  
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**2015**

Nickel (Ni) is one of the toxic heavy metals and its contamination of soil and water mainly originates from the disposal of industrial wastes. Coimbatore is one of the largest industrial city in Tamil Nadu, where a number of industries including electroplating, textile, steel, foundries and metal based industries etc are located. The effluent and sludge generated from these industries are disposed on agricultural lands and water bodies, resulted in several 'Hot-spots' of heavy metals including nickel (Ni) contamination in and around Coimbatore. In the current study, a series of laboratory incubation and pot experiments were conducted to determine the fate of Ni in soil and based on the results a feasible phytoremediation strategy was developed.

Results of laboratory batch experiments have shown that the adsorption of Ni on soil was described better by the third order, parabolic diffusion and Elovich equations than did the zero-, first-, and second order reactions. A relatively higher correlation coefficient ( $R^2= 0.72$ ) indicated that the Ni sorption was adequately described by the Langmuir isotherm than did Freundlich equation. The adsorption of Ni was found increased significantly with an increase in pH. Addition of bioamendments reduced the rate of adsorption of Ni on soil. Significant reduction in Ni adsorption was found when cadmium (Cd) was present, whereas, the adsorption was found increased in the presence of chromium (Cr) as a co-contaminant. Only about 45 to 65 per cent of adsorbed Ni was found desorbed from the soil.

Data from a series of laboratory and pot experiments showed that the concentration of various species of Ni in contaminated soil follows: Residual-Ni > Organic plus iron oxide bound-Ni > Organic-Ni > Exchangeable-Ni > Water soluble-Ni. Both soil moisture conditions (field capacity and alternate wetting and drying) and addition of bioamendments had significant influence on biotransformation and thus bioavailability of Ni in soil. The water soluble Ni was found higher in soil under field capacity moisture condition than in soil under alternate and wetting drying. In contrast the exchangeable Ni was found higher in soil under alternate wetting and drying. In the presence of bioamendments both the water soluble and exchangeable Ni were found higher in soil under field capacity moisture condition at all days of incubation. Relatively higher concentrations were recorded due to the application of pressmud compost than others. There exists a negative correlation between the soil pH and the water soluble Ni ( $R^2 = -0.796$  to  $-0.93$ ).

The bioamendments, such as farmyard manure, composted poultry manure, pressmud compost and biochar, exerted marked impact on soil pH, soil organic carbon (SOC), water soluble organic carbon (WSOC) and biotransformation of Ni-species soil. The results had shown that the bioamendments greatly influenced various biochemical processes and thus enhanced the bioavailability of Ni (as evident from the increase in water soluble and exchangeable Ni) in the contaminated soil and thus resulted in greater absorption of Ni by the plants. The pressmud compost and poultry manure compost resulted in greater bioavailability of Ni in soil and the effect was also reflected on Ni content and uptake by marigold and alfalfa. A significant positive correlations obtained between Ni uptake by plants and water soluble and exchangeable Ni in soil explains the role of bioavailability in plant uptake of Ni. The Bioconcentration factor (BCF), and enrichment factor (EF) were found lesser than one for both marigold and alfalfa, but the translocation factor (TF) was greater than one in plants grown on soil amended with bioamendments. This suggests that these plants could be considered as potential candidates for phytoremediation of Ni contaminated soil.

The current study has demonstrated that a phytoremediation strategy could be developed by integrating bioamendments and hyperaccumulators (like Marigold and Alfalfa) for remediation of Ni contaminated soil. Further, field experiments are required for confirmatory results.

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## LIST OF ABBREVIATIONS

@	-	At the rate of
CD	-	Critical Difference
CEC	-	Cation Exchange Capacity
cmol (p <sup>+</sup> ) kg <sup>-1</sup>	-	centi mole (proton) per kilo gram
CRD	-	Completely randomized design
dS	-	deci Siemens
EC	-	Electrical conductivity
g	-	Gram
hr	-	hour
ha	-	Hectare
kg	-	Kilogram
L	-	Litre
Mg m <sup>-3</sup>	-	Mega gram per cubic meter
m <sup>3</sup>	-	Cubic metre
mg L <sup>-1</sup>	-	milligram per litre
mg kg <sup>-1</sup>	-	milligram per kilogram
ml	-	Millilitre
NS	-	Non significant
pm	-	Picometer
°C	-	Degree Celcius
%	-	Per cent
SEd	-	Standard Error of deviation
SOC	-	Soil organic carbon
SOM	-	Soil organic matter
WSOC	-	Water soluble organic carbon

# *Introduction*

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## CHAPTER I

### INTRODUCTION

The term 'heavy metal' in general includes elements (both metals and metalloids) with an atomic density greater than  $6 \text{ g/cm}^3$  [with the exception of arsenic (As), boron (B) and selenium (Se)]. This group includes both biologically essential [e.g. cobalt (Co), copper (Cu), chromium (Cr), manganese (Mn) and zinc (Zn)] and non-essential [e.g. cadmium (Cd), lead (Pb) and mercury (Hg)] elements. The essential elements (for plant, animal or human nutrition) are required in low concentrations and hence are known as 'trace elements' or 'micro nutrients'. The non-essential metals are phytotoxic and/or zootoxic and are widely known as 'toxic elements'. Both groups are toxic to plants, animals and/or humans at high concentrations, and are referred to as metal(loids) (Mahimairaja *et al.*, 2005). Heavy metals are most hazardous pollutants because of their non-degradable nature and property to affect all kinds of ecological system (Buccolieri *et al.*, 2005). These heavy metals and metalloids enter into the environment through several natural and anthropogenic sources including industrial wastes. Indiscriminate disposal of industrial wastes results in soil and water pollution in several parts of India.

In Tamil Nadu there are many metal based industries located in various districts like Vellore, Erode, Dindugal and Coimbatore in an unorganized manner. Among them, Coimbatore is the second largest industrial centre in Tamilnadu. The major industries include textile, dyeing, electroplating, motor and pump set, foundry and metal casting industries. According to the present situation, about 500 textiles, 200 electroplating industries, 300 dyeing units and 100 foundries are present in Coimbatore district (Malarkodi *et al.*, 2007). Apart from these industries, unorganized sets of sewers numbering 21,000 (Somasundaram, 2001) are running through various zones and finally discharging into the sewage farm located in Ukkadam, which has been used for irrigating the nearby fields. The contaminants present in the effluent are found to be toxic and hazardous not only to human beings but also to the surroundings (Patil and Hande, 2004).

Nickel (Ni) is an important heavy metal, and pollution by Ni has gained importance due to the greater understanding of its persistence and toxicity in the ecosystems (Alemayehu and Lennartz, 2010). Global input of Ni into the human

environment is approximately 150,000 metric tonnes per year from natural sources and 180,000 metric tonnes per year from anthropogenic sources, including emissions from fossil fuel consumption and the industrial production, use and disposal of nickel compounds and alloys (Hostynek and Maibach, 2002). Nickel may enter and accumulate in agricultural soils through the application of phosphate fertilizers, pesticides and other waste materials from industries like Ni-Cd batteries, Ni electroplating, paints formulation, vegetable fat production, etc (Siddigui *et al.*, 1999; Stefanova, 2000). Human exposure to highly Ni-polluted environments, produce a variety of pathologic effects such as skin allergies, lung fibrosis, cancer and other respiratory tract diseases (Kasprzal *et al.*, 2003). Major toxic effects of Ni include chromosomal damage, alteration of enzyme activity, inhibition of protein and RNA synthesis and decrease in ATP pool (Rajyalaxmi *et al.*, 2004). Fordsmand (1997) reported that the nickel toxicity in plants caused patchy discolorations, premature senescence, yellowing of old leaves growth reduction and thus affecting the photosynthetic functions of higher plants. The normal range of Ni in soils is 2-750 mg kg<sup>-1</sup> and the normal range in plants is 0.02 – 5 mg kg<sup>-1</sup> (Alloway, 1990). The passage of Ni from environment to animals and man involves the transfer of metal through soil-plant link in the terrestrial food chain and hence, the behaviour of Ni in soils and soil-plant system is of considerable environmental significance. In recent years the extent of Ni pollution has been recognized and various chemical and biological remediation techniques have been investigated for its removal (McLaren and Clucas, 2001).

A range of technologies is available for remediation of metals-contaminated soil. General approaches to remediation of metal contamination include isolation, immobilization, toxicity reduction, physical separation and extraction. These general approaches can be used for many types of contaminants but the specific technology selected for treatment of a metals-contaminated site will depend on the form of the contamination and other site-specific characteristics. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, complexation, and changes in valence state. These transformations affect the mobility and bioavailability of metals.

Phytoremediation is an emerging technology in which the plants are employed to absorb and bio-magnify elements from a polluted environment and metabolize them into various biomolecules in their tissues (Preeti *et al.*, 2011). Phytoremediation is a set of processes that uses plants to remove, transfer, stabilize and destroy organic/inorganic contamination in ground water, surface water, and soil (Vishnoi and Srivastava, 2008). Once inside the plant, chemicals can be stored in the roots, stems, or leaves or changed into less harmful chemicals within the plant or changed into gases that are released into the air as the plant transpires. The use of green plants to remove pollutants from the environment or render them harmless is defined as phytoremediation (Cunningham *et al.*, 1993). Phytoextraction, phytostabilization and phytofiltration are the important processes involved in phytoremediation processes which can help reduce metal content of respective environment (Salt *et al.*, 1998). To develop an effective remediation technology, it is imperative to study the fate of heavy metals in soil environment. This includes adsorption and desorption characteristics of metals, biotransformation and subsequent bioavailability of metals in soil environment. In the current study the fate of Ni in contaminated soil was studied and based on that, a phytoremediation strategy was developed. The current study has the following specific objectives.

- Examining the adsorption and desorption characteristics of Ni in soil,
- Studying the bioavailability of Ni in contaminated soil and role of bioamendments on Ni bioavailability,
- Evaluating the potential of certain plants and bioamendments for Ni remediation,
- Developing a phytoremediation strategy for Ni contaminated soil.

# *Review of Literature*

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## CHAPTER II

### REVIEW OF LITERATURE

Metal contaminants are commonly found in soils, sediments, and water. Metal pollutants can be produced through industrial processes such as mining, refining, and electroplating. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation, complexation, and changes in valence state. These transformations affect the mobility and bioavailability of metals. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species. The term 'heavy metal' in general includes elements (both metals and metalloids) with an atomic density greater than  $6 \text{ g/cm}^3$  [with the exception of arsenic (As), boron (B) and selenium (Se)]. This group includes both biologically essential [e.g. cobalt (Co), copper (Cu), chromium (Cr), manganese (Mn) and zinc (Zn)] and non-essential [e.g. cadmium (Cd), lead (Pb) and mercury (Hg)] elements. The essential elements (for plant, animal or human nutrition) are required in low concentrations and hence are known as 'trace elements' or 'micro nutrients' (Mahimairaja *et al.*, 2011).

Excessive metal concentration in soils pose significant hazard to human, animal and plant health, and to the environment in general (Table 2.1). Contamination of soils with toxic metals has often resulted from human activities, especially those related to mining, industrial emissions, disposal of industrial wastes, application of sewage sludge to agricultural soils, manure, fertilizer and pesticide use.

Due to the potential toxicity and high persistence of metals, soils polluted with these elements are an environmental problem that requires an effective and affordable solution. Although a number of techniques have been developed to remove metals from contaminated soils, many sites remain contaminated because economic and environmental costs to clean up those sites with the available technologies are too high. Taking into account such a high demand of economic resources, methods of environmental restoration of metal polluted soils using a plant-based technology have attracted

increasing interest in the last two decades. In this context, phytoremediation has been developed as a cost effective and environment friendly remediation method of contaminated soils.

**Table 2.1. Impact of heavy metals on human health**

<b>Metal</b>	<b>Effects on Humans</b>
Arsenic	Mental disturbance, liver cirrhosis, lung cancer, kidney damage, Ulcers
Cadmium	Diarrhoea, growth retardation, bone deformation, kidney damage, Anemia, hypertension, injury of central nervous system
Chromium	Nephritis, ulcers in gastro- intestinal system diseases in central nervous system, Cancer
Cobalt	Diarrhoea, low blood pressure, lung irritation, bone deformation and paralysis
Copper	Hypertension, coma, sporadic fever, uremia
Lead	Anemia, vomiting, loss of apatite, miscarriage, convulsion, damage of brain, liver and kidney
Mercury	Abdominal pain, head ache, dairrhoea, haemolysis, chest pain
Zinc	Vomiting, renal damage, cramps
Selenium	Damage of liver, kidney and spleen, fever, nervousness, vomiting, low blood pressure, blindness and even death
Nickel	Pneumonitis with adrenal cortical insufficiency, irritant dermatitis, respiratory cancer, pulmonary eosinophilia, asthma, malignant tumor

Source: Adriano, 2001

## **2.1. CHEMISTRY OF NICKEL**

Nickel (Atomic No. 28) belongs to Group VIII of the periodic table, the so called iron-cobalt group of metals, and has an atom. Wt of 58.71, specific gravity of 8.9 and melting point of 1453°C. It is a silvery-white, hard, malleable, ductile, ferromagnetic metal that maintains a high luster and also relatively resistant to corrosion. Although it

forms compounds in several oxidation states, the divalent ion seems to be the most important for both organic and inorganic substances, but the trivalent form may be generated by redox reactions in the cell (Huang, 1993). Nickel is practically insoluble in water but soluble in  $\text{HNO}_3$ , slightly soluble in  $\text{HCl}$  and  $\text{H}_2\text{SO}_4$  and insoluble in  $\text{NH}_4\text{OH}$ .

Nickel has five stable isotopes in nature:  $^{58}\text{Ni}$  (68.27%),  $^{60}\text{Ni}$  (26.10%),  $^{61}\text{Ni}$  (1.13%),  $^{62}\text{Ni}$  (3.59%), and  $^{64}\text{Ni}$  (0.91%). Nickel forms stable complexes with many organic ligands however, complexes with naturally occurring inorganic ligands are formed only to a small degree in the order  $\text{OH}^- > \text{SO}_4^{2-} > \text{Cl}^- > \text{NH}_3$ . Ni occurs in two oxidation states such as 0 and II, although the I and II states can exist under certain conditions but not stable in aqueous solution. Nickel is present as  $\text{Ni}^{2+}$  in common water soluble Ni compounds such as acetate, chloride, sulphate, bromide, fluoride and nitrate salts. Nickel is closely related to Co in both its chemical and biochemical properties. Ni can replace essential metals in metallo-enzymes and can cause disruption of metabolic pathways (Adriano, 2001).

## **2.2. SOURCES AND OCCURRENCE OF NICKEL**

Nickel is widely distributed in nature, forming about 0.008% of the earth's crust. The core of the earth contains 8.5% nickel, deep-sea nodules 1.5%; meteorites have been found to contain 5–50% nickel (IARC, 1990). The natural background levels of nickel in water are relatively low, in open ocean water  $0.228\text{--}0.693 \mu\text{g L}^{-1}$ , in fresh water systems generally less than 2. Agricultural soils contain nickel at levels of  $3\text{--}1000 \text{ mg kg}^{-1}$ ; in 78 forest floor samples from the north-eastern United States of America, concentrations of  $8.5\text{--}15 \text{ mg kg}^{-1}$  were reported (WHO, 2000). The nickel content is enriched in coal and crude oil. Nickel in coals ranges up to  $300 \text{ mg kg}^{-1}$ ; most samples contain less than  $100 \text{ mg kg}^{-1}$  but there is a large variation by region (Swaine, 1980). The nickel content of crude oils is in the range  $<1\text{--}80 \text{ mg kg}^{-1}$  (WHO, 2000).

## **2.3. PRODUCTION AND USES OF NICKEL**

There are two commercial classes of nickel ore, the sulfide ores (pentlandite and pyrrhotite) and the silicate-oxide. Most nickel is produced from the sulfide ores, and the two largest producers, Canada and the Russian Federation, account for 20–25% each of total annual production, which was 784.82 thousand tonnes in 1988 (IARC, 1990).

Intermediate uses of nickel include 42% in steel production and 36% in the production of other alloys. Electroplating in the form of nickel sulfate accounts for about 18%. The most important end uses are transportation 23%, chemical industry 15%, electrical equipment 12%, and construction 10% (WHO, 2000). Nickel in coinage, other manufactured products and household appliances may be important for some health effects (dermatitis). The burning of residual and fuel oils, nickel mining and refining, and municipal waste incineration are the main anthropogenic sources of nickel emissions to the atmosphere (WHO, 2000). These sources account for about 90% of the total global emission, estimated to be  $42.85 \times 10^6$  kg/year.

## **2.4. FATE OF NICKEL IN SOIL**

### **2.4.1. Forms and chemical speciation of Ni in soils**

Nickel in the solid phase of soils occurs in several chemical forms, including occurrence on exchange sites, specific adsorption sites, adsorbed or occluded into the sesquioxides, fixed within the clay lattice, or bound in organic residues and microorganisms (Adriano, 2001). In the aqueous phase (i.e., soil solution), Ni occurs in free ionic forms ( $\text{Ni}^{2+}$ ) and complexed form (i.e., with organic and inorganic ligands).

The most common technique of identifying operationally defined fractions (or forms) of metals in soil is by using sequential extraction with selective chemical reagents. As much as 50% of Ni in soils may be associated with the residual fractions (HF and  $\text{HClO}_4$  soluble) and about 20% in the Fe-Mn oxide fraction, with much of the remainder in the carbonate fraction and only a relatively small fraction in the exchangeable and organic fractions (Hickey and Kittrick, 1984). Application of sewage sludge to soils may result in a shift in the proportion of metal forms in both phases. For example, Ni in aqueous extracts of sludge amended soil has been found to be less than in the sludge itself (Lake *et al.*, 1984). The residual form is considered the most stable of Ni because it requires the most drastic extraction reagent for its removal. For soils heavily enriched with heavy metals, Ni was distributed as follows: residual >> Fe-Mn oxide >> residual=carbonate > organic= exchangeable form. For contaminated soils and river sediments, Ni occurred primarily in the precipitated and 0.5N  $\text{HNO}_3$  extractable forms (Cottienie *et al.*, 1979).

Based on thermodynamic considerations, Ni ferrite ( $\text{NiFe}_2\text{O}_4$ ) is the most probable solid phase that can precipitate in soils (Sadiq and Enfield, 1984). Under acidic and reducing conditions, the sulphides of Ni are likely to control the solubility of Ni in the soil solution, while in acidic soils  $\text{Ni}^{2+}$ ,  $\text{NiSO}_4^0$  and  $\text{NiHPO}_4$  are important species, with the relative proportions depending on the level of  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$ . However, Sposito and Page (1984) explained the importance of the bicarbonates as  $\text{NiHCO}_3^+$  and carbonates as  $\text{NiCO}_3^0$  in Ni speciation, especially in alkaline, calcareous soils. However, the exchangeable form of Ni may constitute a significant portion of the total Ni for certain soils.

#### **2.4.2. Bioavailability of Ni and its importance**

Bioavailability refers to the amount of metal which is available to a living biota. However, the bioavailability of a metal defines the relationship between the concentration of the metal in the terrestrial environment and the level of the metal that actually enters the receptor causing either positive or negative effect on the organism. It is also often referred to as the extent to which a metal can desorb, dissolve, or otherwise dissociate from the environmental medium in which it occurs to become accessible (i.e. bioaccessibility) for absorption (Alexander and Alexander, 2000). Chemical bioavailability is now considered an important consideration in the environment because the availability of chemicals may be mitigated once the chemical comes in contact with the soil and sediment. For this reason, both fertility status of soils as well as risk assessment of contaminated sites requires quantification of chemical bioavailability much like any other parameter in a risk calculation (Hrudey *et al.*, 1996).

Since total Ni in soils is not reliable index of plant bioavailability and mobility of Ni, the extractable fractions is commonly used for this purpose. There are considerable variations in the amount of Ni removed while using various extractants such as DTPA,  $\text{NH}_4\text{OAc-EDTA}$ , DTPA-TEA, HOAc and  $\text{CaCl}_2$ . DTPA extracted 0.5 to 7% of the Ni from the soil (Whitby *et al.*, 1978; El-Rashidi *et al.*, 1997) while  $\text{NH}_4\text{OAc-EDTA}$  removed about 3% of Ni from some polluted soil (Nakos, 1982). DTPA-TEA extracted upto 5.7% of Ni from the sewage sludge treated soil (Valdares *et al.*, 1983) where as 3.8% of Ni extracted by HOAc extractant. The soil treated with sewage sludge yielded higher Ni extractabilities with 0.01M  $\text{CaCl}_2$  and also pH dependent (Sanders *et al.*, 1986).

Just like other trace elements, several extractants to relate plant available Ni to extractable soil Ni has been tested: mild acids, neutral salts and chelating agents. Although some researchers found that a suite of extractants was unsatisfactory in predicting the availability of Ni to plants (Shewry and Peterson, 1976). Some extractants such as DTPA solution could predict plant uptake quite well (Valdares *et al.*, 1983). In addition, the latter investigators found an excellent correlation between DTPA-extractable Ni and the total quantity of Ni added to the soil.

Characterizing the factors affecting bioavailability, leaching and toxicity of metals and metalloids in soil is of paramount importance. Metals and metalloids are significant natural components of all soils where their presence in the mineral fraction comprises a store of potentially-mobile metal species as important components of clays, minerals and iron and manganese oxides that, in turn, have a dramatic influence on soil geochemistry (Gadd, 2008).

Heavy metals and metalloids can be involved in a series of complex chemical and biological interactions. The most important factors which affect their mobility are pH, sorbent nature, presence and concentration of organic and inorganic ligands, including humic and fulvic acids, root exudates and nutrients. Furthermore, redox reactions, both biotic and abiotic, are of great importance in controlling the oxidation state and thus, the mobility and the toxicity of many elements, such as Cr, Se, Co, Pb, As, Ni and Cu. Redox reactions can mobilize or immobilize metals, depending on the particular metal species and microenvironments.

The biogeochemical cycle of heavy metals and metalloids has been greatly accelerated by human activities. Accumulation of metal ions and metalloids in different compartments of the biosphere, and their possible mobilization under environmentally changing conditions induce a perturbation of both the structure and function of the ecosystem and might cause adverse health effects to biota (Fedotov and Mirò, 2008). Heavy metals and metalloids enter an agroecosystem through both natural and anthropogenic processes. Some soils have been found to have a high background of some trace elements, which are toxic to plants and wild life, due to extremely high concentrations of these elements in the parent materials. Anthropogenic processes

include inputs of heavy metals through use of fertilizers, organic manures, and industrial and municipal wastes, irrigation, and wet and/or dry deposits. These processes contribute with variable amounts of heavy metals to the agro ecosystem. Only a small portion of heavy metals and metalloids in soil is bioavailable (Table.2.2).

**Table 2.2. Relative mobility and availability of metals**

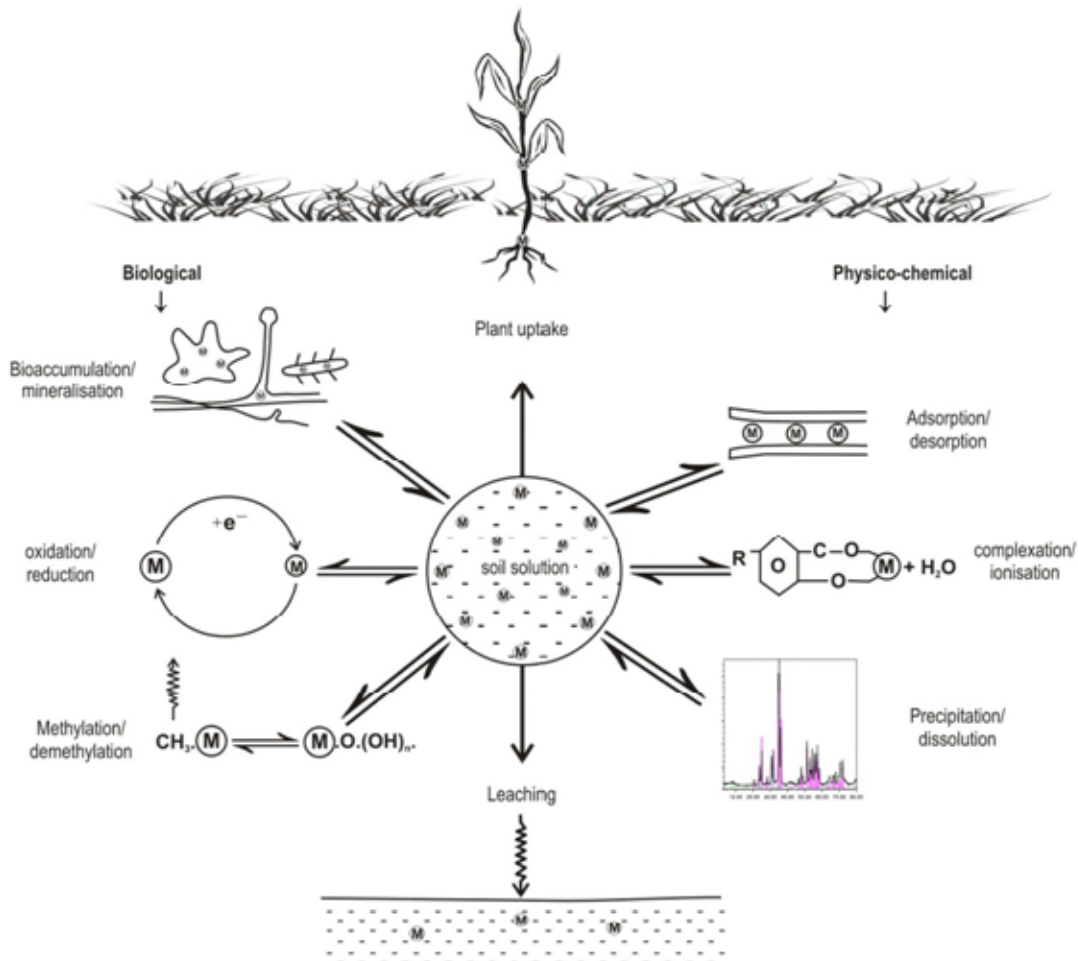
<b>Metal species and association</b>	<b>Mobility</b>
Exchangeable (dissolved) cations	High. Changes in major cationic composition (e.g. estuarine environment) may cause a release due to ion exchange
Metals associated with Fe-Mn oxides	Medium. Changes in redox conditions may cause a release but some metals precipitate if sulfide mineral present is insoluble
Metals associated with organic matter	Medium/High. With time, decomposition/oxidation of organic matter occurs
Metals associated sulfide minerals	Strongly dependent on environmental conditions. Under oxygen-rich conditions, oxidation of sulfide minerals leads to release of metals
Metals fixed in crystalline phase	Low. Only available after weathering or decomposition

(Salomons, 1995)

## **2.5. PROCESSES GOVERNING BIOAVAILABILITY OF NICKEL**

The passage of Ni from environment to animals and man involves the transfer of metal through soil-plant link in the terrestrial food chain and hence, the behaviour of Ni in soils and soil-plant system is of considerable environmental significance. The persistence of Ni in the soil and its reduction in mobility involve the phenomena of sorption, desorption, precipitation, complexation, oxidation/reduction and dissolution (Fig. 2.1) (Ramachandran and D'Souza, 2013). Not all the processes are equally important, but all

of them are affected by soil pH and biological processes. Therefore, it is crucial to understand some major reactions in soils that control the release of Nickel in the soil and the environment in order to overcome problems related to deficiency and contamination of Ni.



**Fig. 2.1. Dynamics of trace elements in soil (Modified from Adriano, 2001)**

### 2.5.1. Sorption process

Charged solute species (ions) are attracted to the charged soil surface by electrostatic attraction and/or through the formation of specific bonds (Mott, 1981). Retention of charged solutes by charged surfaces is broadly grouped into specific and non-specific retention (Bolan *et al.*, 1999). In general terms, nonspecific adsorption is a process in which the charge on the ions balances the charge on the soil particles through

electrostatic attraction; whereas specific adsorption involves chemical bond formation between the ions in the solution and those in the soil surface (Sposito, 1984). Both soil properties and soil solution composition determine the dynamic equilibrium between metals in solution and the soil solid phase. The concentration of metals in soil solution is influenced by the nature of both organic (citrate, oxalate, fulvic, dissolved organic carbon) and inorganic ( $\text{HPO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ ) ligand ions, and soil pH through their influence on metal sorption processes (Harter and Naidu, 1995; Bolan *et al.*, 2003b). Two reasons have been given for the effect of inorganic anions on the sorption of heavy metal cations (Naidu *et al.*, 1994). Firstly, inorganic anions form ion pair complexes with heavy metals, thereby reducing the sorption of heavy metals. Secondly, the specific sorption of ligand anions is likely to increase the negative charge on soil particles, thereby increasing the sorption of heavy metals.

The pH, cation exchange capacity and  $\text{CaCO}_3$  content of the soils are some of the important characteristics which affect the adsorption and subsequent plant uptake of Ni from soil (Bradl, 2004). In general, adsorption capacity of the soils for Ni increased with an increase in pH of the soil, whereas, the reverse was found to be true for the rate of adsorption. Mellis *et al.* (2004) also reported that independent of the treatment, the adsorption of Ni increased with increasing pH of the soils. Adhami *et al.* (2008), who have reported that pH and CEC of soils played major role and were positively correlated with Ni adsorption of soils. Shi *et al.* (2012) have reported that Ni adsorption on soil organic matter (SOM) was dominant in the short term and the slow transfer of adsorbed Ni to Ni-layered double hydroxide (Ni-LDH) phases with longer reaction times. Several researchers have reported about the enhanced sorption of metals with increasing soil pH (Harter, 1983; Msaky and Calvet, 1990; Basta and Tabatai, 1992; Raghupathi and Vasuki, 1993), which might be due to the generation of pH-dependent sites on colloids, reduced competition with H-ions and a change in the hydrolysis state of ions in solution. According to Gomes *et al.* (2001), soils from alfisol and ultisol orders having higher pH values were the ones with highest relative capacity to adsorb metal ions. Soil with high CEC resulted in greater adsorption of Ni and higher  $K_d$  value than the soil having lower CEC. Reddy and Dunn (1986), who reported that the abundance of exchange sites available for Ni on high CEC soils were responsible for the high adsorption rate.

Jalali and Moharrami (2007) also have reported that soil with highest CEC showed the highest sorption capacity of the elements studied. When the pH of the soil decreases, carbonate dissolution increases and ionic exchange is the principal retention mechanism of heavy metals (Plassard *et al.*, 2000; Elzahabi and Yong, 2001).

Ni is retained in certain soils primarily by specific adsorption mechanism at low ( $\leq 10$  ppm) concentrations. Ni sorption by soil can also display a complex (i.e., multiphasic) isotherm (Harter, 1983). Adsorption of metals in soils (pH 4.3 to 5.6) was in the order  $Pb > Cu > Zn > Ni$  (Harter, 1983). Using soil columns, the mobility of metals in soils (pH 3.8 to 7.1) tended to be:  $Cd \leq Ni \leq Zn \gg Cu$  (Tyler and McBride, 1982). In general, differences in sorption or mobility of the metals are affected by differences in physicochemical properties of the soil. Sorption of Ni in soils is largely pH dependent. In examining soils from arable and potential waste disposal sites, Bowman *et al.* (1981) found that sorption of Ni from 0.01N  $CaCl_2$  was extensive for all samples;  $>99\%$  of added Ni sorption was sorbed in some cases. In spite of this almost complete sorption, Ni sorption was not completely irreversible, as some Ni was desorbed by the chelating agent DTPA.

### **2.5.2. Complex formation**

Other chemical interactions that contribute to metal retention by colloid particles include complexation reaction between metals and the inorganic and organic ligand ions. As might be expected, the organic component of soil constituents has a high affinity for heavy metal cations because of the presence of ligands or groups that can form chelates with metals (Harter and Naidu, 1995). With increasing pH, the carboxyl, phenolic, alcoholic and carbonyl functional groups in soil organic matter dissociate, thereby increasing the affinity of ligand ions for metal cations.

The extent of metal-organic complex formation, however, varies with a number of factors including temperature, steric factors and concentration. All these interactions are controlled by solution pH and ionic strength, the nature of the metal species, dominant cation and inorganic and organic ligands present in the soil solution. Since Ni(II) is stable over a wide range of pH and redox conditions, this is the species of Ni expected to occur in most soils. Ni is known to complex readily with a wide variety of inorganic and organic ligands. In the presence of high levels of  $Ca^{2+}$  ions and small

amount of the chelating agent EDTA in the equilibrating solution, Ni sorption by the soils was minimized. In this case, competition between  $\text{Ca}^{2+}$  ions and  $\text{Ni}^{2+}$  ions for adsorption sites prevailed over Ni-Cl complex formation, while the EDTA complexed with Ni, maintaining it in soluble form in the aqueous phase. Ni may bind with variety of synthetic chelates to the virtual exclusion of competing cationic species at typical soil pH (Sommers and Lindsay, 1979). In some instances, inorganic complexation of Ni may be significant in affecting its mobility. Increased Ni mobility or decreased sorption of Ni in the presence of  $\text{Cl}^-$  ions apparently due to  $\text{Cl}^-$  complex formation has been observed (Bowman and O'Connor, 1982; Doner, 1978). Similarly,  $\text{SO}_4^{2-}$  or  $\text{NO}_3^-$  may also have a complexing effect on Ni.

### **2.5.3. Precipitation**

Precipitation appears to be the predominant process in high pH soils and in the presence of anions such as  $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$ ,  $\text{OH}^-$  and  $\text{HPO}_4^{2-}$  and when the concentration of the heavy metal ion is high (Naidu *et al.*, 1996b). Co-precipitation of metals especially in the presence of iron oxyhydroxide has also been reported and often such interactions lead to significant changes in the surface chemical properties of the substrate. Increasingly precipitation of metals using phosphate compounds is becoming a routine technique to immobilize metals such as lead.

Nickel can be precipitated using chemicals like nickel hydrates or sodium hydroxide. Precipitation metals oxide and probably as metal carbonate were two of the mechanisms that contributed to the removal of metals from their solution. Seragadam (2008) reported that the limestone is capable to remove more than 90% of Ni from solution of 2 mg/L. Higher removal was achieved at a final pH of 8.5 with the quantity of 56 g of limestone in 20 ml solution. This implies that lime stone is an important media in the removal of Ni (Benjamin, 1983).

### **2.5.4. Redox reactions**

The reduction/oxidation conditions in a soil can strongly affect the pH and speciation, and hence toxicity of metals in solution, and this is particularly well documented for As, Sb, Cr, Fe and Mn, which are several times more biologically toxic and mobile in their most reduced form (Alloway, 1995; Oorts *et al.*, 2008). However Ni

in soils is mostly present in the divalent form, as the monovalent forms is highly unstable and hence Ni is not significantly reduced under low redox conditions (Reichman, 2002; Whitehead, 2000). Thus redox conditions are considered to play a much smaller role in the solubility and bioavailability Ni.

## 2.6. SOIL PROPERTIES INFLUENCING BIOAVAILABILITY OF NICKEL IN SOILS

Soil plays a significant role in controlling the potential bioavailability of metals in the environment. The primary soil factors influencing the potential bioavailability of contaminants are soil pH, cation and anion exchange capacities (available charged sites on soil surfaces), texture (clay content), soil type and soil organic matter

### 2.6.1. Soil pH

Soil pH is one of the key parameters influencing the sorption of metal(loid)s given that it controls virtually all aspects of biogeochemical processes in soils. These processes include solubility, precipitation, speciation and sorption as well as microbial activity. In most variable charge soils such as the strongly weathered tropical soils and less weathered Andisols, increasing soil pH results in an increase in the number of negatively charged soil sites with a concomitant decrease in the positively charged sites (Naidu *et al.*, 1994, 1996b; Bolan *et al.*, 1999; Fig.2.2).

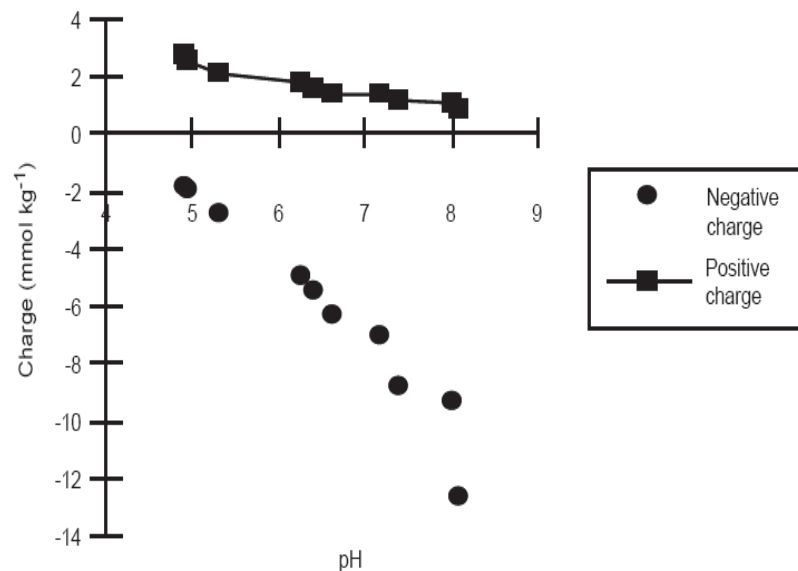


Fig. 2.2. Effect of soil pH on surface charge characteristics of an Oxisol (Adriano, 2001)

In contrast to variable charge soils, the effect of pH on surface charge characteristics in permanent charged soils such as those from temperate Mediterranean region is less marked unless the soil is high in organic matter content. Nevertheless, changing the soil pH directly impacts the sorption and removal from the porewater of metal(loid) (Bohn *et al.*, 1985; Naidu *et al.*, 1996a). In addition to its effect on surface chemical properties, soil pH also controls the speciation of metal(loid)s. The net charge of the metal complexes and their precipitation/dissolution reactions are directly impacted by soil pH. Thus, soil pH plays a major role in controlling Ni bioavailability.

There is a critical pH interval for the solid/solution interfaces, generally smaller than two units, where the metal adsorption percentage may increase sharply from extremely low pH value to 100% at high pH values (Sposito, 1989; Soares *et al.*, 2011). This critical interval, known as the adsorption edge, is related to the hydrolysis constant of the metallic ion. The degree to which the metals are hydrolyzed is probably the main factor that determines the quantity of metal retained at a given pH (James and Healy, 1972; Harter, 1983). Nickel adsorption depended greatly on the pH, regardless of soil or sampling depth, and increased sharply with the increase in pH from 4.0 to 6.0, an interval when 20-90% Ni adsorption occurred (Soares *et al.*, 2011). Many adsorption studies with mineral soils have shown increase in Ni adsorption with increase in pH (Harter, 1983; Elzinga and Sparks, 2001). Harter (1983) and Beukes *et al.* (2000) reported that metal adsorption was pH-dependent and that the intensity of the phenomenon increased drastically above pH 7.0. Practically all the Ni added ( $5 \text{ mg L}^{-1}$ ) disappeared from the soil solution when the pH was over 7.0, regardless of soil or sampling depth (Mellis *et al.*, 2004). Thus, even at the highest pH values reached, the possibility of the presence of  $\text{Ni(OH)}_2^0$  was very small (James and Healy, 1972). Even so, rapid increase in Ni adsorption above pH 8.0 may also be associated to the start of the hydroxide precipitation reactions with the  $\text{Ni}^{2+}$  and  $\text{NiOH}^+$  species (Sen Gupta and Bhattacharyya, 2006). It is expected that  $\text{Ni(H}_2\text{O)}_6^{2+}$  form be found at low pH values and that the complexes with  $\text{OH}^-$  and organic ligands start to predominate as the pH increases (Uren, 1992). Soares *et al.* (2011) suggested that soil pH management would be a promising, efficient and cheap technique as a remediation action in areas contaminated by Ni.

### 2.6.2. Organic matter content

Soil organic matter is a complex polymeric mixture arising from microbial and chemical degradation processes (Khan, 1978). Organic matter in soils is commonly and collectively referred to as humic substances, and this can be further divided into humic acid, fulvic acid, and humin based on solubility (McBride, 1989). Humic and fulvic acids contribute to the majority of cation binding properties in soils (Tipping, 2005) and dissolved organic carbon (DOC) is the main source of fulvic acids which are known to increase the carrying capacity of soil solutions for strongly organic complexing metals such as Cu (Amery *et al.*, 2007; Ashworth and Alloway, 2004), and in some instances Ni (Ashworth and Alloway, 2004; Doig and Liber, 2007). Metal ions can be complexed by the COO<sup>-</sup> and COOH- groups present in both DOC and solid organic matter to form stable complexes (McLaren and Crawford, 1973; Stevenson, 1994). Consequently, the amount of organic matter, in particular DOC increases the opportunity for forming stable organo-metal complexes (McBride, 1994). Organic matter has a high affinity to bind organic compounds as well as some metals in soils thereby, reducing their availability.

Organic matter may affect negatively or positively the metal availability as a result of the formation of metal chelates or complexes. McBride (1989) suggested the following sequence of metal affinity with the organic matter: Cu > Ni > Pb > Co > Ca > Zn > Mn > Mg. As pH increases, only Cu adsorption by organic colloids is inner-sphere (specific adsorption) (Bloom and McBride, 1979), and thus, most cations stay moderately bound by these colloids, when compared to the inorganic forms (Uren, 1992). Although the relevance of organic matter and Fe oxides for the metal adsorption are widely known but studies about the behavior of such elements are scarce (Sauve *et al.*, 2000).

Mellis *et al.* (2004) stated that there is a relevant effect of the organic matter on Ni adsorption due to the difference obtained for Ni adsorption in the original soil samples when compared with the organic matter free soil samples. They obtained results for OM-free soil samples as the average of 21.0, 11.0 and 28.0% lower than those obtained for the original soil samples. For pH above 6.0-6.5, the effect of organic matter on Ni adsorption was not significant, probably because great part of the negative, dependent pH charges would have the H<sup>+</sup> already dissociated. The organic matter

provides sites for cation exchange, but its strong affinity to metals is due to the presence of specific binders or groups that form metal complexes or chelates. Nickel, as well as other heavy metals, form complexes with several soil organic constituents. Nevertheless, these complex formations occur in great part with the fulvic and humic acids, present in the organic matter in large quantities, the insoluble combinations being associated with the humic acid and the soluble ones, with the fulvic acid (Stevenson, 1991). Several researchers have already observed these associations of organic matter with metal chelate formations in many soil types (Mellis *et al.*, 2004).

### **2.6.3. Ion exchange capacities of soils**

pH influences sorption through its effect on surface charge as quantified by cation and anion exchange capacities of soils. The anion exchange capacity (AEC) which is generally associated with oxidic minerals is a measure of available positively charged surface sites which decreases with increasing soil pH (Naidu *et al.*, 1990). The cation exchange capacity (CEC) is a measure of negatively charged sites and is generally associated with layer silicate minerals (such as 2:1 and 1:1 silicates) and organic matter. Both AEC and CEC vary with the clay mineral content and type, organic matter and soil pH.

High clay soils will have a high affinity to sorb cationic species of both organic and inorganic solutes due to high CEC thus making contaminants less bioavailable relative to sandy soils. In addition to soil pH there are a number of other environmental variables that influence ion exchange characteristics of soils. Amongst these is the presence of inorganic and organic ligands that bind specifically to soil colloid surface. Specific sorption of anions onto variable charge components has often been shown to increase the net surface charge (i.e., CEC) of soils and consequently increase the capacity of soils to bind cationic metals and positively charged organic contaminants. This process has been described by a number of researchers as anion-induced cation sorption (Ryden and Syers, 1976; Bolland *et al.*, 1977; Wann and Uehara, 1978; Shuman, 1986).

The Ni<sup>2+</sup> cation is known to form complexes with various organic and inorganic ligands (Bowman *et al.*, 1981). Many studies have considered the effect of cations and anions that constitute the electrolyte support on metal adsorption, principally regarding

to the competitive effect of  $\text{Ca}^{2+}$  for adsorption sites and by the action of  $\text{Cl}^-$  in the formation of chloride complexes with less affinity for the surface (Mattigod *et al.*, 1979; Bowman *et al.*, 1981; Wang *et al.*, 1997; Echeverría *et al.*, 2003). Considering the nature of electrolyte support, the effect of the competition between  $\text{Ca}^{2+}$  and  $\text{Ni}^{2+}$  for the same adsorption sites probably justified the decrease in Ni adsorption with the increase in ionic strength, rather than the probable formation of complexes between Ni and the anion because  $\text{NO}_3^-$  has much less affinity than  $\text{Cl}^-$  for this type of reaction in solution (Criscenty and Sverjensky, 1999).

According to Uren (1992), Ni adsorption in the soils is particularly governed by the formation of outer-sphere type complexes and, because of this, Ni cation species ( $\text{Ni}^{2+}$  e  $\text{NiOH}^+$ ) are the preferential adsorbed forms. Echeverría *et al.* (2003) stated that the decrease in metallic ion adsorption with the increase in the ionic strength are attributed to: (i) the type of metal; (ii) changes in the free ion activity and  $\text{MOH}^+$  species; (iii) the type of surface, surface charge effect and alterations in the electrostatic potential on the adsorption plane and; (iv) the increase in competition for adsorption sites among the cation index and the metallic ions (Boekhold *et al.*, 1993; Naidu *et al.*, 1997; Criscenty and Sverjensky, 1999).

The intensity of the effect of the ionic strength on specific metallic cation adsorption depends greatly on the surface charge signal and on the determined pH (Kosmulski, 1997). For Alfisol, the manifestation of electrostatic adsorption mechanisms did not depend on the pH, due to the higher quantity of permanent charges. In the Oxisols, higher dependence of Ni adsorption started at pH 5.0 in relation to the ionic strength, which implied the manifestation of nonspecific adsorption mechanisms (Soares *et al.*, 2011). Although the essential mechanism for metal adsorption by Fe, Al and Mn (oxy)hydroxides is specific and pH dependent, increasing with the pH, heavy metals can be adsorbed by the electrostatic attraction in the diffuse double layer by ion exchange (Mesquita and Vieira e Silva, 2002). Khan *et al.* (1991) indicated the presence of  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{++}$  as cations and  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  as anions released from applied fertilizers could be regarded to play a dominant role in mobility of Pb and Ni in soil because of blocking of adsorptive site by the above said ions. Sherene (2010)

reported that the major cations  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  at the high concentrations that they are present in the water samples have greater effect in releasing  $\text{Ni}^{2+}$  ions from DOC bound Ni thereby increased mobility.

#### **2.6.4. Soil type**

The nature of soil types varies considerably depending on the geographical location. The different surface charge and chemical properties of the soils, their ability to sorb contaminants varies considerably (Adriano, 2001). Consequently, metal bioavailability varies significantly with soil type due to differences in soil properties. Numerous investigators (Feng *et al.*, 2000) have investigated the effect of soil type on sorption and bioavailability of contaminants by relating  $K_d$  value to either uptake of contaminants by plants or organisms. In these studies they found that soils with large  $K_d$  values have lower metal bioavailability compared to those with low  $K_d$  values. In their studies Feng *et al.* (2000) found that the  $K_d$  increased with ageing of soils but the rate of increase varied with soil type which they attributed to varying soil chemical properties. Naidu and Bolan (2004) recognized that soil consists of a wide range of mineral and organic constituents and that the metal-binding capacity of soils is a function of the partition coefficient ( $K_d$ ) describing the thermodynamics of the interaction between metals and each mineral and organic fraction as shown below:

$$K_d = f(K_{d1}, K_{d2}, \dots, K_{dx})$$

However, the relationship between  $K_d$  and bioavailability (metal uptake) may not be applicable to soils saturated with contaminants given the mass action effect under these conditions may be the major driving force controlling contaminant bioavailability. Research on the mobility and bioavailability of metals in soils varying in organic and mineral contents is still in infancy. Further work is needed on the fate and behaviour of metals in different soil types and in particular how it relates to bioavailability.

#### **2.6.5. Time dependence**

Contaminants may undergo a time-dependent sequestration in soil that results in a decline in bioavailability. This process known as ageing and it is attributed to the sequestration of contaminants into solid phase (Alexander, 1995; Stewart *et al.*, 2003)

either in mineral nanopores (Pignatello and Xing, 1996; Hatzinger and Alexander, 1997; Nam and Alexander, 1998). Nickel phytoavailability decreases with increasing its residence time in soils (McLaughlin, 2001).

A clear link exists between biodegradation of contaminants, toxicity and bioavailability. The phenomenon of ageing (sequestration) in general results in the apparent decline of bioavailability associated with persistence and increased resistance of the contaminant to extraction with solvents. The reduced bioavailability of contaminant in aged soils is a major constraint in the bioremediation.

## **2.7. PHYTOREMEDIATION OF NICKEL**

The heavy metal toxicity causes serious threats to human and animal health due to their long-term persistence in the environment (Gisbert *et al.*, 2003). Thus the increasing attention on threats caused by heavy metal toxicity necessitated the remediation of polluted soil by using plants and microbes to remove those hazardous metal ions (Winge *et al.*, 1985; Pence *et al.*, 2000). Plants have harvestable stem and leaves aboveground, which is suitable for subsequent post-processing, whereas the microbes possess only a larger specific surface area that activate and remove heavy metals which cannot be harvested (Salt and Wagner, 1993; Salt and Rauser, 1995; Kramer *et al.*, 1996). The technique using hyperaccumulator plants in phytoremediation is a cost-effective technology, to remediate the contaminated soil. The advantage of the technique lies in making the living plants act as a solar-driven pump, which can extract and concentrate certain heavy metals from the environment (Raskin *et al.*, 1997). For phytoremediation of metal-contaminated soils, it is essential to understand the interaction between metal-tolerant plant species and soil chemical properties controlling the bioavailability of metals. Numerous plant species have been identified for the purpose of phytoremediation. Hyperaccumulator plants are attractive candidates as they are able to accumulate potentially phytotoxic elements to concentrations 50-500 times higher than average plants (Lasat, 2002).

The term hyperaccumulator was first applied by Jaffre *et al.* (1976) when they observed the accumulation of Ni in *Sebertia accuminata*. The present idea concerning the concentration of more than 1000 mg/kg of metal in plant tissues was introduced by

Brooks *et al.* (1977), when they examined Ni concentrations in *Homalium* sp. and *Hybanthus* sp. For most elements the threshold uptake concentration is 1000 mg/kg (0.1%) dry mass, except for Zn (10,000 mg/kg), Cd (100 mg/kg). Around 400 plant species from at least 45 plant families hyperaccumulate metals, most of which are Ni hyperaccumulators occurring in ultramafic areas (Reeves, 2006). The ultramafic soil formed due to the weathering of ultramafic rocks which is rich in olivine and pyroxene minerals. The pH of the ultramafic soil ranges between 6 and 8. It also contains higher amount of metals such as nickel, chromium, manganese, cobalt, titanium, iron and other metals. It supports the growth of native plants called as endemic flora. These endemic floras have their own adaptive mechanism to grow in the soil which is having high metal concentration and also they accumulate metals in their biomass. Some of the important Ni hyperaccumulators were given below (Table 2.3).

**Table 2.3. List of important Nickel hyperaccumulators**

<b>Hyperaccumulator plant</b>	<b>Family</b>	<b>References</b>
<i>Sebertia acuminata</i>	Sapotaceae	Jaffre <i>et al.</i> (1976); Perrier (2004)
<i>Allysum pindasilvae</i>	Brassicaceae	Garcia-Leston <i>et al.</i> (2007)
<i>Allysum bertolonii</i>	Brassicaceae	Barzanti <i>et al.</i> (2011)
<i>Allysum serpyllifolium</i>	Brassicaceae	Becerra-Castro <i>et al.</i> (2009)
<i>Phidiasia lindavii</i>	Acanthaceae	Reeves <i>et al.</i> (1999)
<i>Bornmuellera kiyakii</i>	Brassicaceae	Reeves <i>et al.</i> (2009)
<i>Thalapsi goeingense</i>	Brassicaceae	Wenzel <i>et al.</i> (2003)
<i>Berkheya codii</i>	Asteraceae	Robinson <i>et al.</i> (1997); Moradi <i>et al.</i> (2010)

### **2.7.1. Plant metal interaction**

Non-hyperaccumulating plants retain the heavy metals in their root cells and detoxify them by chelating in the cytoplasm or by storing them into vacuoles. Contrastingly, hyperaccumulators release metal chelating compounds (phytochelators/ phytosiderophores) to the rhizosphere, which increase the bioavailability of metals that are tightly bound to the

soil fractions and help to translocate them into plant tissues (Eapen and D'Souza, 2005). Phytochelators are usually low molecular weight organic compounds such as malic, malonic, oxalic acids, acetic acid, succinic acid, sugars, amino acids and phenolics which can change the metal speciation and bioavailability (Cieslinski *et al.*, 1998; Ma *et al.*, 2001; Nascimento *et al.*, 2006). Certain metal chelating compounds (mugenic acid and avenic acids) are released during nutrient metal deficiency and thus increase the bioavailability of metals (Ma and Nomoto, 1996, Pellet *et al.*, 1995, Cakmak *et al.*, 1996) and help to carry them into plant tissues. Metal chelate complexes may also be transported across the plasma-membrane (Romheld, 1991). The rhizosphere provides a complex and dynamic micro-environment where microorganisms form unique communities that have potential for detoxification of hazardous waste compounds. Their interaction can improve metal bioavailability in rhizosphere through the secretion of proton, organic acids, phytochelators (PCs), amino acids, and enzymes (Idris *et al.*, 2004; Yang *et al.*, 2005). Fungal symbiotic associations have the potential to enhance root absorption area and stimulate the acquisition of plant nutrients including metal ions (Khan *et al.*, 2000).

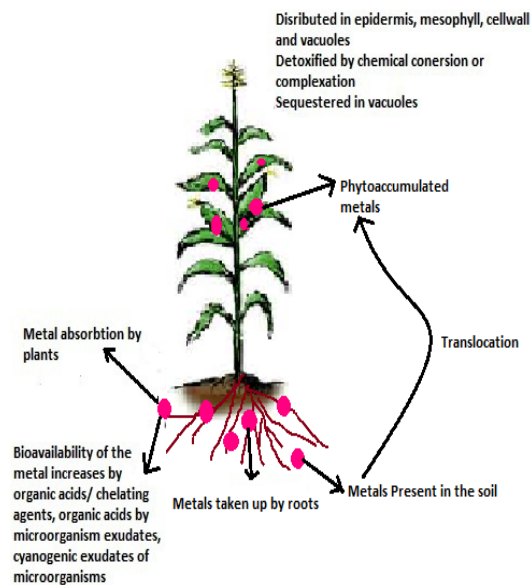
### **2.7.2. Mechanism involved in the phytoremediation of metals**

Technologies for metal phytoremediation include: 1) phytoextraction – the use of plants to remove metals from soils and to transport and concentrate them in above-ground biomass; 2) phytostabilization – the use of plants to minimize metal mobility in contaminated soil through accumulation by roots or precipitation within the rhizosphere; and 3) phytovolatilization – the use of plants to turn volatile chemical species of soil metals (Chaney *et al.*, 1997; Garbisa and Alkorta, 2001; McGrath *et al.*, 2002; Lasat, 2002; Ernst, 2005). Phytoextraction seems to be the most promising technique and has received increasing attention from researchers since it was proposed by Chaney (1983) as a technology for reclaiming metal polluted soils.

Phytoextraction employs metal hyperaccumulating plant species to transport high quantities of metals from soil into the harvestable parts of roots and aboveground shoots (Kumar *et al.*, 1995; Chaney *et al.*, 1997). Ni hyperaccumulation was defined by Brooks *et al.* (1977) as the accumulation of at least 1000 mg kg<sup>-1</sup> Ni in the dry biomass of plants grown on a natural substrate. Subsequently, hyperaccumulators of other heavy

metals were identified. Metal concentrations achieved by naturally occurring hyperaccumulating plant species can be more than 100 times those that occur in non-accumulator plants growing in the same substrates. The extraordinary ability of hyperaccumulator plants to tolerate and accumulate heavy metals has stimulated research into possible uses of phytoextraction (Chaney *et al.*, 1995; Salt *et al.*, 1995).

Phytoextraction is the most feasible method and widely utilized to extract metals from soils. Hyperaccumulators absorb metals through roots and translocate them to shoots. Then, the metals can be harvested from the plant biomass by incineration techniques. Phytoextraction involves various steps such as solubilization of metal from the soil matrix, root uptake and transport to shoots, and finally translocation and sequestration of metals in the plant biomass (Fig. 2.3).



**Fig.2.3. Mechanism of phytoextraction**

### 2.7.2.1. Solubilization of metal from the soil matrix

Out of the total metal concentration in soil matrix, metals are present in three forms: available form, potentially available form, and unavailable form. In available form, metals are available to organisms at any time. In potentially available form, metal is available once the available fraction has been removed. Metals in unavailable form

have extremely low solubility and are chemically bound to an organic or silicate matrix (Robinson *et al.*, 1999, 2003). Plants use various methods to desorb metals from the soil matrix.

Acidification of the rhizosphere and exudation of carboxylate are considered potential targets for enhancing metal accumulation. Secretion of H<sup>+</sup> by roots could acidify the rhizosphere and increase the metal dissolution. The proton extrusion of the roots is operated by plasma membrane H<sup>+</sup> ATPase and H<sup>+</sup> pumps (Ghosh and Singh, 2005).

Plant roots secrete various ligands such as organic acids (malonic and oxalic acids), metal chelating compounds (phytosiderophores), and enzymes (reductase). These act as chelating agents and enhance metal desorption from soil thus increasing the bioavailability of metals in the soil solution and a greater accumulation in plants (Ma *et al.*, 2001; Callahan *et al.*, 2006). Rhizosphere is populated by large concentrations of microorganisms which mainly consist of bacteria and mycorrhizal fungi. These root-colonizing bacteria and mycorrhizae have been shown to catalyze redox transformations leading to increase in soil metal bioavailability (Idris *et al.*, 2004; Chen *et al.*, 2005).

Low molecular-weight organic acids are probably the most important exudates in natural phytoextraction systems. They influence the acquisition of metals by either forming complexes with metal ions or decreasing the pH around the roots and altering soil characteristics. Despite the fact that metals uptake may be increased due to decreasing pH (Brown *et al.*, 1994), it is clear that the complexing capacity of organic acids, rather than their capacity to decrease pH, is the main factor related to mobilization of metals in soil and their accumulation in plants (Bernal *et al.*, 1994; McGrath *et al.*, 1997; Gupta *et al.*, 2000). Indirect effects of root exudates on microbial activity, rhizosphere physical properties and root growth dynamics may also influence ion solubility and uptake (Marschner, 1995; Walker *et al.*, 2003).

Salt *et al.* (2000) were unable to identify any high-affinity Ni-chelator compound in the rhizosphere of the Ni hyperaccumulator *Thlaspi goesingense*. In contrast, they found that Ni-chelators histidine and citrate accumulated in the root exudates of the non- hyperaccumulator *Thlaspi arvense* exposed to Ni. Such findings led the authors to suggest that the release of these exudates by *T. arvense* may be a strategy to reduce

Ni uptake and toxicity, but exudate releasing is not involved in the hyperaccumulation of Ni by *T.goelsingense*. Persans *et al.* (1999) also established that Ni hyperaccumulation in *T. goesingense* is not determined by the overproduction of histidine in response to Ni. Since at non-toxic Ni concentrations, both plant species translocate Ni to shoots at equivalent rates (Kramer *et al.*, 1997), the existence of a more efficient translocation mechanism in *T. goesingense* does not seem to explain the capability of this species in accumulating Ni. Kramer *et al.* (2000) provided evidence that free histidine may be also involved in shuttling Ni across the cytoplasm into the vacuole in *T.goelsingense*, which could be responsible for Ni tolerance and accumulation. Kramer *et al.* (1996) have already reported a 36-fold increase in the concentration of free histidine in the xylem exudates of the Ni hyperaccumulator *Alyssum lesbiacum* after exposure to Ni, suggesting that histidine could be involved in the transport and storage of Ni in such species. Kerkeb & Krämer (2003) recently provided further evidence that histidine enhances the release of Ni from roots into the xylem, not only in *A. lesbiacum* but also in the non-hyperaccumulator *B. juncea*.

#### **2.7.2.2. Root absorption and transport to shoot**

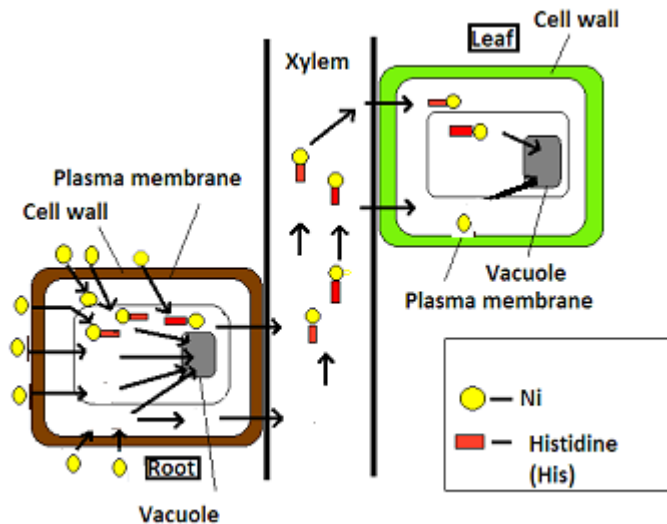
Soluble metals can enter into the root symplast by crossing the plasma-membrane of the root endodermal cells or they can enter the root apoplast through the space between cells. While it is possible for solutes to travel up through the plant by apoplastic flow, the more efficient method of moving up the plant is through the vasculature of the plant, called xylem. To enter the xylem, solutes must cross the casparian strip, a waxy coating, which is impermeable to solutes, unless they pass through the cells of the endodermis. Therefore, to enter the xylem, metals must cross a membrane, probably through the action of membrane pump or channel. This type of transport of metals which takes place in xylem after they cross the casparian strip is called symplast transport. It is more regulated due to the selectively permeable plasma-membrane of the cells that control access to the symplast by specific or generic metal ion carriers or channels (Gaymard, 1998; Hall, 2002). Once loaded into the xylem, the flow of xylem sap will transport the metals to the shoots. Several classes of proteins have been implicated in heavy metal transport in plants. These include the heavy metal or CPx-type ATPase, the natural resistance- associated macrophage protein (Nramp) family of proteins, the cation

diffusion facilitator (CDF) family proteins, zinc–iron permease (ZIP) family proteins, etc. Xylem loading is operating through cation–proton antiport, cation-ATPases or ion channel (Williams *et al.*, 2000; Yang *et al.*, 2005).

### **2.7.2.3. Distribution, detoxification, and sequestration of metal ion**

At any point along the pathway, metal could be converted to a less toxic form through chemical conversion or by complexation with organic acid such as malate, citrate, and nicotianamine (Mari *et al.*, 2006; Gendre *et al.*, 2007). Various oxidation states of heavy metals have very different uptake, transport, and detoxification characteristics in plants. Once the metals are translocated to shoot cells, they are stored in cellular locations such as trichome (apoplast tissue), epidermis, mesophyll, cell wall, etc., where the metal will not damage the vital cellular processes (Shah and Nongkynrih, 2007). Membrane transport systems play a vital role in the translocation process (Revathi and Venugopal., 2013). Hyperaccumulators rapidly and efficiently translocate them into the leaf vacuoles via xylem (Rascio and Navari-Izzo., 2011). The final step for accumulation of most of the metals is the sequestration of the metal away from any cellular processes it might disrupt. Metal binding proteins such as metallothioneins (MTs) and phytochelatins (PCs) in plants play important role in sequestration and also enhance metal tolerance and accumulation. Metal such as Ag (I), Cu (II), and Ni (II) are sequestered by bonding with organic sulphur (R-SH) on the cysteine residues of these peptides. MTs and PCs complex the metal ions to inactivate and, transport them into vacuoles for long-term sequestration (Eapen and D'Souza, 2005).

The main role in heavy metal accumulation is played by free amino acid such as histidine (His) and nicotianamine, which forms stable complexes with the bivalent cations. Free histidine (His) is considered as the most important ligand in the hyperaccumulation of Ni (Callahan *et al.*, 2006). Presence of high concentration of His in the roots of *Thlaspi* species which is Ni hyperaccumulators, suggest the same mode of operation of amino acid in other hyperaccumulators (Assuncao *et al.*, 2003). Because of the presence of carrier for the transport of Ni in plants, heavy metal get absorbed from the soil easily, crosses the cell wall and plasma membrane of the root and through xylem gets accumulated in the leaf vacuole (Fig 2.4).

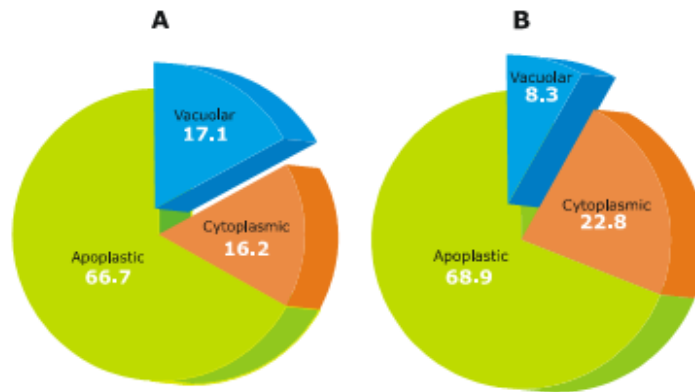


Source: Kumar and Maiti, 2013

**Fig. 2.4. Schematic representation of translocation of Ni from root to leaf**

The root to shoot translocation in hyperaccumulator plants relies on enhanced xylem loading by constitutive overexpression of genes coding for transport system common to non-hyperaccumulators (Rascio and Navari-Izzo., 2011). Moreover, Heavy Metal Accumulation (HMAs) plays a vital role in metal homeostasis and tolerance (Axelsen and Palmgren, 1988). The MATE (Multidrug And Toxin Efflux) family of small organic molecule transporter seems to be another transport protein that is active in translocation of heavy metals in hyperaccumulation plants (Rascio and Navari-Izzo., 2011).

Metal transport from the cytosol to the vacuole is considered an important mechanism of both metal tolerance and accumulation in plants. For this reason, much work has been dedicated to investigating subcellular localization of metals in hyperaccumulators (Vázquez *et al.*, 1992; Kupper *et al.*, 1999; Hirschi *et al.*, 2000; Kramer *et al.*, 2000; Sarret *et al.*, 2002). Kramer *et al.* (2000) isolated vacuoles from Ni-tolerant *T. goesingense* and Ni-sensitive *T. arvense* aiming directly to address the role of vacuolar Ni storage in Ni tolerance. They found that *T. goesingense* accumulated two-fold more Ni in the vacuole than *T. arvense* (Fig.2.5). Since protoplast and apoplast Ni contents were similar in both species, vacuolar compartmentalization in *T. goesingense* seems to play a major role in Ni-accumulation and tolerance.

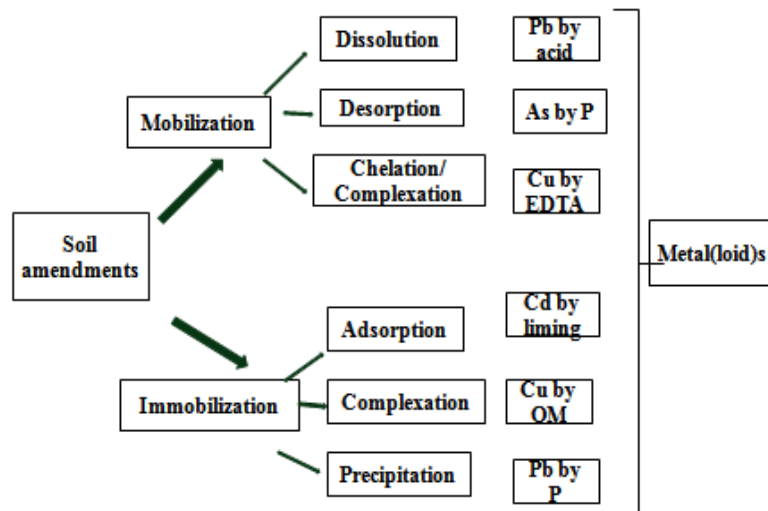


**Fig. 2.5. Subcellular localization of Nickel in leaves of *T. goesingense* (A) and *T. arvensis* (B) as a percentage of total leaf Nickel**

(Adapted from Kramer *et al.*, 2000)

## 2.8.SOIL AMENDMENTS FOR NICKEL (IM)MOBILIZATION

A number of amendments are used either to mobilize or immobilize heavy metals in soils. The basic principle involved in the mobilization technique is to release the metals into soil solution, which is subsequently removed using higher plants. In contrast, in the case of the immobilization technique the metal concerned is removed from soil solution either through adsorption, complexation and precipitation reactions, thereby rendering the metals unavailable for plant uptake and leaching to groundwater (Fig. 2.6).



**Fig. 2.6 Role of soil amendments in the im(mobilization) and bioavailability of metals in soils**

### **2.8.1. Chelating and complexing agents**

When a metal ion combines with an electron donor, the resulting substance is called a complex or coordination compound. If the substance which combines with the metal contains two or more donor groups so that one or more rings are formed, the resulting structure is called a metal chelate, and the donor is called chelating agent. The term 'chelate' is derived from the Greek word 'chela' which means 'claw' and it is so named because these species can coordinate at several or all positions around a central metal ion by literally wrapping themselves around the metal ion. Chelating agents which have high affinity for metal ions can be used to enhance the solubilization of metals in soils through the formation of soluble metal chelates. A number of synthetic chelating agents are available including EDTA, EDHA, DTPA and EHPG.

### **2.8.2. Liming materials**

Although liming is primarily aimed at ameliorating soil acidity, it is increasingly being accepted as an important management tool in reducing the toxicity of heavy metals in soils (Bolan *et al.*, 2003a, 2003b). A range of liming materials is available, which vary in their ability to neutralize the acidity. These include calcite ( $\text{CaCO}_3$ ), burnt lime ( $\text{CaO}$ ), slaked lime ( $\text{Ca(OH)}_2$ ), dolomite ( $\text{CaMg(CO}_3)_2$ ) and slag ( $\text{CaSiO}_3$ ). The acid neutralizing value of liming materials is expressed in terms of calcium carbonate equivalent (CCE), defined as the acid neutralizing capacity of a liming material expressed as a weight percentage of pure  $\text{CaCO}_3$ .

Liming, as part of the normal cultural practices, has often been shown to reduce the concentration of Cd, Pb and other metals in edible parts of crops. Similarly, liming serpentine soils containing toxic levels of Ni has shown to alleviate the phytotoxic effects of Ni. In these cases, the effect of liming materials in decreasing metal uptake by plants has been attributed to both decreased mobility in soils (through adsorption/precipitation) and to the competition between  $\text{Ca}^{2+}$  and metals ions on the root surface.

### **2.8.3. Organic composts**

The major sources of organic composts include biosolid and animal manures (Bolan *et al.*, 2004). Traditionally biosolid is viewed as one of the major sources of

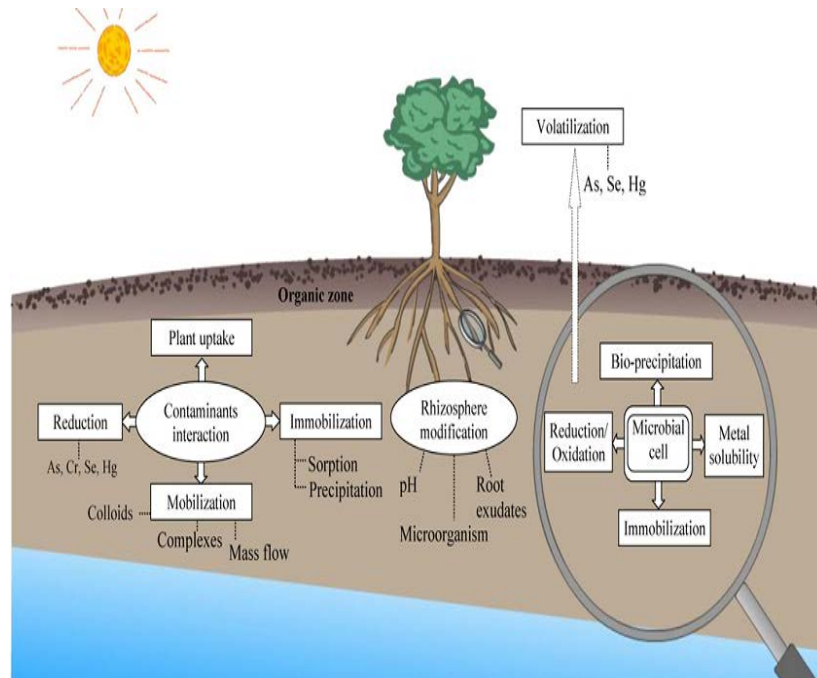
metal accumulation in soils. Advances in the treatment of sewage water and isolation of industrial wastewater in the sewage treatment plants have resulted in a steady decline in the metal content of biosolid. Furthermore, stabilization using alkaline materials has resulted in the immobilization of metals in biosolid. Most manure products contain low levels of heavy metals (except Cu and Zn in swine manure and As in poultry manure) (Bolan *et al.*, 2004). Furthermore, recent advances in the treatment of manure byproducts have resulted in reduced bioavailability of metals. Similarly, treatment of poultry manure with alum [ $\text{Al}_2(\text{SO}_4)_3$ ] decreased the concentration of water-soluble Zn, Cu and Cd. Hence unlike sewage sludge application, where land application is limited based on allowable trace element loadings, regulations governing livestock and poultry manure byproducts are generally based on total N and P loading. Manure byproducts that are low in metal content can be used to immobilize metal contaminants in soils.

Although a number of studies have examined the role of biosolid as a source of metal contamination in soil, only limited work has been reported on the beneficial effect of organic amendments as a sink for the immobilization of metals in soils. Recent studies have shown that alkaline-stabilized biosolid that are low in total and/or bioavailable metal content (known as ‘exception quality’ biosolid or ‘designer sludge’) can be used as an effective sink for reducing the bioavailability of metals in contaminated soils and sediments. Immobilization of metals by such amendments is achieved through adsorption, complexation and redox reactions.

Addition of organic amendments has often been shown to increase the CEC of soils, thereby resulting in increased metal adsorption (Bolan *et al.*, 2003c). The presence of phosphates, aluminium compounds and other inorganic minerals in some organic amendments, such as typical municipal sewage sludge, is also believed to be responsible for the retention of metals, thereby inducing the ‘plateau effect’ in metal uptake by crops and preventing the increased metal availability suggested in the ‘time bomb’ hypothesis.

Metals form both soluble and insoluble complexes with organic constituents in soils, a process which apparently depends on the nature of the organic matter (Bolan *et al.*, 2003d). As might be expected, the organic component of soil constituents has a high affinity for metal cations because of the presence of ligands or functional groups that can

form chelates with metals. With increasing pH, the carboxyl, phenolic, alcoholic and carbonyl functional groups in soil organic matter dissociate, thereby increasing the affinity of ligand ions for metal cations.



**Fig. 2.7 The role of organic amendments in regulating various bioremediation processes**

Arnesen and Singh (1999) found that the application of peat increased the amount of DTPA-extractable Ni. They suggested that the lowering of pH in the peat-amended to soil decreased the sorption of Ni in the soil and this effect became more significant after degradation of organic matter. Karaca (2004) reported that the amount of DTPA extractable Ni has been increased after the addition of mushroom compost due to the higher organic matter content. Tobacco dust application also increased the DTPA extractable Ni (Karaca, 2004).

## 2.9. ACCUMULATION OF NICKEL IN PLANTS

Since Ni is ubiquitous in the environment, it is a normal constituent of plant tissues. Nickel content of field-grown crops and natural vegetation ranges from 0.05 to 5.0 ppm in the dry matter (Vanselow, 1966). In normal plants, the following ranges of values have been reported (Kabata-Pendias and Pendias, 1992): grasses, 0.10 to 1.7 ppm

DW; clovers, 1.2 to 2.7 ppm; vegetables, 0.20 to 3.7 ppm; cereals, average of 0.50 ppm. Generally, the level of Ni in most plant species that may produce phytotoxicity ranges from 10 to 100 ppm (Adriano, 2001).

Nickel is usually absorbed in the ionic form  $\text{Ni}^{2+}$  from the soil or culture medium. Nickel is apparently more easily absorbed by plants when supplied in the ionic form than when chelated (Mishra and Kar, 1974). A possible explanation is that the charge on the chelated molecule inhibits its absorption by the roots, i.e., a molecule with no charge or a slight negative charge can be taken up, while a complexed molecule with strong negative charge cannot. High Ni content of the soil, particularly when the exchangeable form is also high, facilitates the absorption of this element by plants when other conditions are also favourable (Adriano, 2001).

A nickel hyperaccumulator grown under natural conditions should contain 1000 mg  $\text{kg}^{-1}$  dry matter or more Ni in its aboveground tissues (Baker & Brooks, 1989; Reeves *et al.*, 2001, Reeves *et al.*, 2009). The nickel content of hyperaccumulators may range between 4200 and 20,400 mg  $\text{kg}^{-1}$  (Li *et al.*, 2003). Nickel-accumulating species comprise about three-quarters of all hyperaccumulator plants (Baker *et al.*, 2000; Reeves & Baker, 2000; Ghaderian *et al.*, 2007). The genus *Alyssum* L. (Brassicaceae) has the highest number of such members, with 48 taxa accumulating up to 3% Ni in their foliage (Brooks *et al.*, 1977; Reeves & Baker, 2000). The number of discovered Brassicaceae members, some of which are Ni hyperaccumulators, has increased day by day (Ozhatay *et al.*, 2009; Mutlu, 2010; Ozhatay *et al.*, 2011). Hyperaccumulators do not usually compete with normal flora (Baker *et al.*, 2000). Soleimani *et al.* (2009) reported that tall fescue grass accumulated more Ni than the Bermuda grass. Not likewise other metal the Ni has accumulated more in shoots than the roots. Motesharezadeh and Savaghebi-Firoozabadi (2011) studied the effect of different inoculants on the efficiency of Ni phytoextraction by sunflower, amaranthus and alfalfa. They have obtained the highest accumulation with the *Bacillus safensis* inoculants in amaranthus. Bosiacki and Wojciechowska (2012) studied the phytoextraction capacity of different ornamental plants such as marigold, sunflower and amaranthus. They have assessed the Ni accumulation in different plant parts like inflorescences, leaves and stem. Among the three plants, marigold and amaranthus accumulated higher amount of Ni than the

sunflower. Several researchers used wide variety of plant species to study the accumulation capacity and pattern for Ni in the selected plant species. The list of plants used so far in the remediation of Ni is given in the table 2.4.

**Table 2.4. Plants used for the Nickel remediation and their accumulating capacity**

<b>Plant</b>	<b>Family</b>	<b>Amount of Ni accumulated (mg kg<sup>-1</sup>)</b>	<b>References</b>
<i>Alyssum caricum</i>	Brassicaceae	7576	Altinozlu <i>et al.</i> , 2012
<i>Alyssum peltariodes</i>	Brassicaceae	4411	
<i>Isatis pinnatiloba</i>	Brassicaceae	275	
<i>Aegopordon berardioides</i>	Asteraceae	20.0	Ghaderian and Baker., 2007.
<i>Cleome heratensis</i>	Capparaceae	21.0	
<i>A. serpyllifolium</i>	Brassicaceae	38105	Freitas <i>et al.</i> , 2004.
<i>L. spartea (L.)</i>	Scrophulariaceae	492.0	
<i>A. serpyllifolium</i>	Brassicaceae	670-31200	Lazaro <i>et al.</i> , 2006
<i>Cistus ladanifer</i>	Cistaceae	3-50	
<i>Plantago subulata subsp. Radicata</i>	Plantaginaceae	46.4-267	
<i>Silene armeria</i>	Caryophyllaceae	2540	Lombini <i>et al.</i> , 1998.
<i>Cerastium arvense</i>		2685	
<i>Minuartia laricifolia</i>		2629	
<i>Dianthus sylvestris</i>		2501	
<i>Biscutella laevigata</i>		2399	
<i>Alyssum bertolonii</i>	Brassicaceae	2594	Hsiao <i>et al.</i> , 2007.
<i>Brassica juncea</i>	Brassicaceae	18	

Plant	Family	Amount of Ni accumulated (mg kg <sup>-1</sup> )	References
<i>Cynanchum schlechtendalii</i>	Asclepiadaceae	235	Reeves <i>et al.</i> , 2007
<i>Macroptilium gracile</i>	Fabaceae/Papilionaceae	114	
<i>Hyptis suaveolens</i>	Lamiaceae	175	
<i>Oxalis frutescens</i>	Oxalidaceae	106	
<i>Paspalum pectinatum</i>	Poaceae	170	
<i>Diodia teres</i>	Rubiaceae	246	
<i>Buchnera pusilla</i>	Scrophulariaceae	185	
<i>Russelia sarmentosa</i>	Scrophulariaceae	130	

## 2.10. NICKEL TOXICITY IN PLANTS

Nickel is metabolically important and essential minor element for the development of the plants but increase in concentration results in toxicity (Fargasova, 2008). In general, when Ni concentrations in vegetative tissue of plants exceed 50 ppm DW, plants may suffer from excess Ni and manifest toxicity symptoms (Adriano, 2001). Different plants species have different resistivity against nickel. While some plants are introduced as Ni hyperaccumulators other are very sensitive and introduced as non-accumulators (Freeman *et al.*, 2004). In the cytoplasm, high levels of free nickel generally avoid removal of the metal ions to the vacuoles and the formation of complexes with organic acids (Ernst *et al.*, 1990). High concentration of nickel inevitably binds organic macromolecules and denatures them. Furthermore, nickel can replace iron, zinc and magnesium due to the chemical affinity with those elements, interfering with their metabolism (Woolhouse, 1983). Ni is transported to underground plant parts by the oxygen atoms either as metal complexes of organic acids or as hydrated cations (Salt *et al.*, 2002). High Ni concentrations retard shoot and root growth, affect branching development, deform various plant parts, produce abnormal flower

shape, decrease biomass production, induce leaf spotting, disturb mitotic root tips, and produce Fe deficiency that leads to chlorosis and foliar necrosis. Additionally, excess Ni also affects nutrient absorption by roots, impairs plant metabolism, inhibits photosynthesis and transpiration, and causes ultrastructural modifications (Ahmad and Ashraf, 2011).

## **2.11. CONCLUSION**

Phytoextraction has emerged as a novel approach to clean up metal-polluted soils. Important advances have been made in the last few years in understanding the processes involved in phytoextraction of metals from contaminated soils, especially on the processes that contribute to uptake and hyperaccumulation of metals. But better understanding of the interactions taking place in the rhizosphere will be important to the ultimate success of phytoextraction as a technique to clean up soils. Advances in this research field are expected to produce a substantial impact on phytoextraction potential. The identification of specific exudates associated with solubilization of metals in soils could enable alternative ways of manipulating the plant rhizosphere to enhance metal bioavailability. For chemically-assisted phytoextraction, the dynamics of metal chelates in the rhizosphere need to be examined, either to overcome the risks associated with low degradability of synthetic chelators, or to optimize the use of more biodegradable compounds. A complete understanding of plant metal tolerance will be essential to develop strategies to genetically enhance the metal accumulation ability of plants. Many genes are involved in metal accumulation, translocation and sequestration in the plant biomass. Since most of the known hyperaccumulator species are slow-growing and have small biomass, expressing their metal-accumulating genes in fast-growing, high biomass plants, is a promising approach for developing plants that can be used in phytoremediation technology. Environment friendly and biodegradable chelators should be developed. Agronomic techniques to successfully grow plants for phytoremediation purposes such as fertilization, timing for chelators application and practices to minimize spread of metals through the food chain, are also awaiting more detailed study.

## *Materials and Methods*

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## CHAPTER III

### MATERIALS AND METHODS

Due to continuous irrigation of sewage water in agricultural field, application of sewage sludge to the crops, indiscriminate disposal of electroplating industry effluent and some fertilizer usage lead to the Ni contamination in soil. From the contaminated soil, Ni has taken up by the plants and enters into human food chain. It causes various health effects including cancer. So there is an urgent need to remediate the contaminated soil. A series of laboratory, pot and field experiments were conducted and based on the results, a phytoremediation technology was developed by integrating crops and bioamendments. The materials used and methods adopted in various experiments are presented in this chapter.

#### 3.1. DESCRIPTION OF THE STUDY AREA

Coimbatore is located at 11.0161<sup>0</sup> N, 77.971<sup>0</sup> E and 426.72 meter above sea level and has an area of about 7,469 sq.km in the north western Tamil Nadu, very close to Western Ghats. The population of Coimbatore city is 4,224,107. In 1865, Coimbatore was established as the capital of the newly formed Coimbatore district and in 1866 it was accorded the municipality status. In 1981, Coimbatore was constituted as corporation. Coimbatore is situated in the extreme west of Tamil Nadu, near the state of Kerala. It is surrounded by mountains on the west, with reserve forests and the Nilgris biosphere reserve on the Northern side. The eastern side of the district, including the city is predominantly dry. The entire western and northern part of the district borders the Western Ghats and Nilgris biosphere as well as Anaimalai and Munnar ranges. The city of Coimbatore has nine lakes (Wetlands like Singanallur Lake, Kuruchi Lake, Valankulam Lake, Krishnapathi Lake and Seevagasinthamani Lake are some of them. Large number of textile mills in this region made it as “The Manchester of South India”. Some of the major industries are Lakshmi machine works, Premier Instruments & Control, Premier Evolvics, ELGI equipments, Shanthy gears and Root industries. There are so many metal based industries located in Coimbatore in an unorganized manner and is the second largest industries in Tamil Nadu. The major industries include textile, dyeing, electroplating, motor and pumpset, foundry and metal casting industries. 4500 textiles, 300 dyeing, 1200 electroplating, 100 foundries, 413 medium scale industries, 1653 small scale industries and cottage industries and 275 units of transport equipments and automobile

components, 4155 units of machinery and machine spare units, 625 printing units, 1145 paper and paper products units, 1265 rubber, plastic and petrol based units are present in Coimbatore. The city has six major industries arterial roads and three national highways are NH-47 (Cochin-Salem), NH-67 (Mysore-Nagapattinam), NH-209 (Bangalore-Dindigul). A new highway was completed in 1998 has helped to reduce truck traffic in the city. The number of town buses in city is around 800 in 228 different routes.

### **3.2. EVALUATION OF METHOD OF MEASUREMENT OF NICKEL – LABORATORY INCUBATION EXPERIMENT**

The recovery percentage of Ni in soil was assessed through a laboratory closed incubation experiment. Bulk soil samples were collected from a farmer's field located in Kamatchipuram, Coimbatore. Hundred grams of air-dried soil (< 2 mm) was weighed in plastic containers. The Ni was added at the rate of 0, 20, 40, 80, 100  $\mu\text{g g}^{-1}$  in the form of  $\text{NiCl}_2$  and thoroughly mixed with the soil. The plastic containers were covered with polyethylene bags containing small pin-sized holes to permit aeration. Three replicates of each treatment were prepared, randomly placed and incubated in the laboratory at  $25 \pm 2^\circ\text{C}$  for 3 days. At the end of 3<sup>rd</sup> day the incubating mixtures were thoroughly mixed and allowed to dry for 3 days. After that, the samples were digested with aquaregia and analysed for Ni recovery percentage. The recovery percentage of Ni was obtained as 86 percent for 20  $\text{mg kg}^{-1}$  of Ni added soil, 88.4 percent for 40  $\text{mg kg}^{-1}$  added soil, 115 percent for 60  $\text{mg kg}^{-1}$  of Ni added soil, 92.7 percent for 80  $\text{mg kg}^{-1}$  of Ni added soil and 110.1 percent for 100  $\text{mg kg}^{-1}$  added soil. The overall recovery percentage of Ni was 98.6 percent.

**Table 3.1. Recovery of Nickel from soil digested with aquaregia**

<b>Treatments</b>	<b>Percentage recovery</b>
T <sub>1</sub> - Control (Soil alone)	-
T <sub>2</sub> - Soil + 20 ppm of Ni	86.00
T <sub>3</sub> - Soil + 40 ppm of Ni	88.40
T <sub>4</sub> - Soil + 60 ppm of Ni	115.8
T <sub>5</sub> - Soil + 80 ppm of Ni	92.70
T <sub>6</sub> - Soil + 100 ppm of Ni	110.1
<b>Overall recovery percentage</b>	<b>98.6</b>

Metals are found naturally throughout the earth, in rocks, soils and sediments trapped in stable form. Toxic anthropogenic inputs result in contamination of the soil. The chemical behavior of these contaminants are controlled by soil composition, soil properties such as pH and a number of processes, such as metal cation release from contamination source, cation exchange and specific adsorption onto surfaces of minerals, etc. (Guveni and Akinci, 2011). The impact of metals depends on the total metal concentrations in soil, speciation, mobility, and bioavailability (Roundhill *et al.*, 2009). Several methods have been used by different researchers in the digestion of soil samples for the determination of metals. Such methods have been through the use of fluxes or inorganic acids such as HCl, H<sub>3</sub>PO<sub>4</sub>, HNO<sub>3</sub>, HClO<sub>4</sub>, HF, H<sub>2</sub>SO<sub>4</sub> or their combination. These acids (extractants) exhibit various peculiar properties which enables each acid to carry out specific functions during extraction (Alam and Tokunaga, 2006; Kislik, 2002). The choice of extractants depends on the aim of the study, type of contaminants, properties of the extractant, experimental conditions, need to readily release extracted metal and need for minimum interference from contaminants (Kaasalainen and Yli-Halla, 2003). Improper selection of extractants could cause effects such as partial dissolution of soil sample resulting in decreased metal content levels in soil samples (Hlavay *et al.*, 2004; Twyman, 2005). Extractants may be acidic or basic depending on their mode of action which depends on interacting mechanisms, such as the metal ion-extractant affinity, metal ion concentration, extraction temperature and acidity of the medium. The extent ions of extractant show affinity for metals depends on the concentration of the metal in soil and inter-metal interference in heterogeneous contaminated soils during extraction. The specificity can be improved by combining a series of acids during extraction (Szakova *et al.*, 2010). Mixtures of HCl, HNO<sub>3</sub>, HClO<sub>4</sub> and HF dissolve most metals in soils and this is reported to analyses with great accuracy multi-elements (Twyman, 2005). The use of aquaregia in the dissolution of sulfides, phosphates, and many metals and alloys including Ni, Au, Pt and Pd has been reported (Tam and Yao, 1999).

According to Wilson *et al.* (2005) aquaregia produced the most accurate, efficient and reproducible results. Berrow and Stein (1983) reported 80 per cent recovery of the total Cr, Cu, Pb and Mn from sewage sludges and sludge-treated soil, when using aquaregia as an extractant. However, Abreu *et al.* (1996) and Kaasalainen and Yli-Halla (2003)

reported more than 90 per cent of Fe, Mn, Zn, Cu, Pb and Cd from the aquaregia digestion of soil. The percentage of extractability in this study corroborates the results of previous studies. The extractants had no specific pattern for the extractability observed. The variations in the extractability observed could be due to the type, size and the geometry of the extractant ions and also due to inter-metal interference in heterogeneous contaminated soils during extraction. Similar results were obtained by Roundhill *et al.* (2009); Nogales *et al.* (1995); Kislik, (2002); Guveni and Akinci, (2011). Other reasons for variation in extractability of Ni could be as a result of the mineralogy of soils, soil properties, continual deposition of heavy metal on soil surface from various sources through atmospheric transportation, sampling, sample preparation methods and soil texture which could vary on a micro scale of about 1 to 100 mm altering metal solubility and extractability concentrations (Hlavay *et al.*, 2004; Szakova *et al.*, 2010; Twyman, 2005; Kislik, 2002).

### **3.3. ADSORPTION AND DESORPTION OF NICKEL - LABORATORY BATCH EXPERIMENT**

A series of laboratory batch experiments were conducted to study the adsorption of Ni on soil. The soil used for the experiment was collected from a farmer's field located in Kamatchipuram village, Coimbatore district.

#### **3.3.1. Batch Experiment to study the adsorption of Ni in soil**

Firstly, the adsorption at a range of equilibrium period (0.5, 1, 2, 5, 8, 12, 24, 36, and 48 hrs) was examined at a single input concentration of 100 mg of Ni L<sup>-1</sup>. It was found that sorption equilibrium was achieved within 12 hrs. The adsorption isotherm for Ni was obtained by batch studies at concentrations ranged between 0 and 100 mg L<sup>-1</sup> using NiCl<sub>2</sub>. The soil (1g) was mixed with Ni solutions in polypropylene tube (50 ml) at a soil: solution ratio of 1:25 by shaking on an end-over-end shaker for 12 hrs at 20±2<sup>0</sup>C. At the end of equilibrium time, the samples were centrifuged at 8000 rpm for 10 minutes and filtered through Whatman No. 40 filter paper. The extracts were then analysed for Ni content using AAS (VARION AA200). Differences in initial and the equilibrium solution concentrations were computed to determine the amount of Ni sorbed per unit mass of soil. The sorption data were fitted to Freundlich isotherm (Eq 1) and Langmuir isotherm (Eq 2):

$$(x/m) = K_f C_e^n \longrightarrow \text{(Eq 1)}$$

Where, (x/m) is amount adsorbed (mg kg<sup>-1</sup>), K<sub>f</sub> is Freundlich distribution coefficient, C<sub>e</sub> is equilibrium solution concentration (mg/L), and 'n' is equilibrium constant (dimensionless).

$$Y = (MbC) / (1+bC) \longrightarrow \text{(Eq 2)}$$

Where Y = amount adsorbed (mg kg<sup>-1</sup>), C = equilibrium solution concentration (mg L<sup>-1</sup>), and b is the Langmuir constant, related adsorption energy.

To describe the adsorption kinetics, the zero, first, second and third order reactions, the parabolic diffusion and the Elovich equations were tested for goodness of fit by least square regression analysis. The equation that best described the adsorption of Ni by the soil was determined by computing standard error of estimate (SE) using the following equation:

$$SE = [\sum(q_t - q_t^*)^2 / (n-2)]^{(1/2)}$$

Where q<sub>t</sub> and q<sub>t</sub><sup>\*</sup> are the measured and calculated sorbed Ni in soil at time t and n is the number of time points for measurements. The standard error of estimate is considered to be the best measure of the agreement of the calculated values with the observed values (Steel and Torric, 1960).

**Table 3.2. Various kinetic models used**

Model	Equation	Parameters
Zero-order reaction	$q_t = q_0 + k_0 t$	k <sub>0</sub> , zero-order rate constant (mg Ni kg <sup>-1</sup> )
First-order reaction	$\ln q_t = \ln q_0 + k_1 t$	k <sub>1</sub> , first-order rate constant (1/s)
Second-order reaction	$1/q_t = 1/q_0 - k_2 t$	k <sub>2</sub> , second-order rate constant [(mg Ni kg <sup>-1</sup> ) <sup>-1</sup> ]
Third-order reaction	$1/q_t^2 = 1/q_0^2 - k_3 t$	k <sub>3</sub> , third-order rate constant [(mg Ni kg <sup>-1</sup> ) <sup>-2</sup> S <sup>-2</sup> ]
Parabolic diffusion	$Q = \alpha + k_d \sqrt{t}$	k <sub>d</sub> , diffusion rate constant [(mg Ni kg <sup>-1</sup> ) <sup>0.5</sup> ]
Elovich-type equation	$q_t = q_0 + (1+\beta) \ln(\alpha\beta) + (1/\beta) \ln t$	α, initial Ni sorption rate (mg Ni kg <sup>-1</sup> h <sup>-1</sup> ), and β, Ni desorption constant [(mg Ni kg <sup>-1</sup> ) <sup>-1</sup> ]

### **3.3.2. Factors affecting adsorption of Nickel on soil**

The adsorption of Ni on soil was governed by various factors such as pH, organic matter content and competitive ions present in soil solution. Therefore, experiment was also conducted to study the factors affecting Ni adsorption on soil.

#### ***3.3.2.1. Effect of pH on adsorption of Ni***

The effect of pH on Ni adsorption was assessed between pH of 5 and 9. Firstly, the soil was mixed with the Ni solutions of single input concentration ( $100 \text{ mg kg}^{-1}$ ) in polypropylene tube at a soil: solution ratio of 1:25. Then the pH of the solution concentration was adjusted to a desirable pH range such as 5, 6, 7, 8 and 9 using 0.1M HCl or 0.1M NaOH. Then the samples were shaken on an end-over-end shaker for 12 hrs at  $20 \pm 2^{\circ}\text{C}$ . At the end of equilibrium time, the samples were centrifuged at 8000 rpm for 10 minutes and filtered through Whatman No. 40 filter paper. The extracts were analysed for Ni content using AAS (Varion, 200). Differences in initial and the equilibrium solution concentrations were computed to determine the amount of Ni sorbed per unit mass of soil.

#### ***3.3.2.2. Effect of organic matter on adsorption of Ni***

The effect of organic matter on Ni adsorption was examined by using various bioamendments such as farmyard manure, composted poultry manure, pressmud compost and prosopis biochar at the level of  $0.5 \text{ g g}^{-1}$  of soil based on a preliminary experiments conducted. The bioamendments were collected, processed, sieved through 2mm sieve except biochar and analysed for important characteristics (Table 3). Firstly, the soil was equilibrated with 0.5g of each bioamendment in polypropylene tube and kept for three days. Then the soil was exposed to single input concentration of Ni  $100 \text{ mg kg}^{-1}$ ). The soil: solution ratio was maintained at 1:25. Then the samples were shaken on an end-over-end shaker for 12 hrs at  $20 \pm 2^{\circ}\text{C}$ . At the end of equilibrium time, the samples were centrifuged, filtered and analysed for Ni content as discussed above.

#### ***3.3.2.3. Effect of competitive ions/ co-contaminants on adsorption of Ni***

The effect of competitive ions or co-contaminants usually present in the contaminated soils on Ni adsorption was examined. The major heavy metals present

along with the Ni in the environment are chromium (Cr) and cadmium (Cd). Therefore these two metals were taken for the present study. Firstly, the soil was equilibrated with the Cr/Cd solutions at a single input concentration ( $100 \text{ mg kg}^{-1}$ ) in the polypropylene tube for three days. Then the soil was exposed to different concentrations of Ni ranged from 0 to  $100 \text{ mg kg}^{-1}$ . The soil: solution ratio was maintained at 1:25. Then the samples were shaken on an end-over-end shaker for 12 hrs at  $20 \pm 2^\circ\text{C}$ . At the end of equilibrium time, the samples were centrifuged, filtered and analysed for Ni content. Differences in initial and the equilibrium solution concentrations were computed to determine the amount of Ni sorbed per unit mass of soil

### **3.3.3. Desorption of Nickel from soil**

Batch experiments were also conducted to examine the desorption of Ni from soil using different extractants such as 0.005M DTPA and 0.05M  $\text{Ca}(\text{NO}_3)_2$ . These two extractants are found effective in desorbing Ni content from soil. Firstly, the soil was equilibrated with Ni solutions ( $100 \text{ mg kg}^{-1}$ ) in polypropylene tube at a soil: solution ratio of 1:25 for three days and shaken on an end-over-end shaker for 12 hrs at  $20 \pm 2^\circ\text{C}$ . The samples were centrifuged at 8000 rpm for 10 minutes, filtered through Whatman No. 40 filter paper and discarded. Then, 25 ml of extractant (0.005M DTPA or 0.05M  $\text{Ca}(\text{NO}_3)_2$ ) was added to the Ni adsorbed soil. The desorption at a range of equilibrium period (0.5, 1, 2, 5, 8, 12, 24, 36, and 48 hrs) was examined. It was found that maximum desorption equilibrium was achieved within 30 minutes for 0.005M DTPA and 2 hours for 0.05M  $\text{Ca}(\text{NO}_3)_2$ . Likewise, the desorption of Ni has been calculated by adding different concentrations of Ni between 0 and  $100 \text{ mg kg}^{-1}$  to the soil and the extractants (0.005M DTPA or 0.05M  $\text{Ca}(\text{NO}_3)_2$ ) were added to the Ni adsorbed soil to find out the maximum desorption rate of Ni from soil.

## **3.4. BIOAVAILABILITY AND BIOTRANSFORMATION OF NICKEL IN SOIL – LABORATORY INCUBATION EXPERIMENT**

The factors governing the bioavailability of Ni in soil were examined through a laboratory closed incubation experiment. Bulk soil samples were collected from a farmer's field located in Kamachipuram, Coimbatore. Hundred grams of air-dried soil (< 2 mm) was weighed in plastic containers. The Ni was added as per the treatment

details given below. The Ni was added in the form of NiCl<sub>2</sub> and thoroughly mixed with the soil. Required quantity of distilled water was added to achieve a final moisture content equivalent to field capacity (0.37 g g<sup>-1</sup>). The plastic containers were covered with polyethylene bags containing small pin-sized holes to permit aeration. Three replicates of each treatment were prepared, randomly placed and incubated in the laboratory at 25±2°C for 30 days (Fig. 3.1). The above treatments were maintained under two moisture conditions such as field capacity moisture and alternate wetting & drying. Based on the weight loss distilled water was added once in three days to the containers maintained under field capacity moisture condition and once in a week to the containers maintained under alternate wetting & drying condition for achieving an uniform moisture content throughout the incubation experiment. At the end of 0, 15 and 30 days the incubating mixtures were thoroughly mixed and samples (≈25 g) were removed from all the treatments and analysed for pH, total Ni content and species of Ni.

#### **Treatment details**

T <sub>1</sub>	:	Control (Soil alone)
T <sub>2</sub>	:	Soil + 20 ppm of Ni
T <sub>3</sub>	:	Soil + 40 ppm of Ni
T <sub>4</sub>	:	Soil + 60 ppm of Ni
T <sub>5</sub>	:	Soil + 80 ppm of Ni
T <sub>6</sub>	:	Soil + 100 ppm of Ni

#### **Factors**

M<sub>1</sub> - Field capacity moisture

M<sub>2</sub> - Alternate wetting and drying

### **3.5. EFFECT OF BIOAMENDMENTS ON BIOTRANSFORMATION AND BIOAVAILABILITY OF NICKEL IN CONTAMINATED SOIL – LABORATORY INCUBATION EXPERIMENT**

The effect of different bioamendments on the fractions and bioavailability of Ni in soil was studied by conducting a laboratory closed incubation experiment. Bulk soil samples were collected from Ni contaminated area nearby Nanjundapuram, Coimbatore



**Fig. 3.1 Laboratory incubation experiment**



**Fig. 3.2 Laboratory incubation experiment with organic amendments**

where large number of industries including electroplating industries exist. After air drying for three days, the soil was passed through 2mm sieve to achieve a homogenized sample. Different bioamendments such as farmyard manure, composted poultry manure, pressmud compost, prosopis biochar were collected.

Hundred grams of air-dried soil (< 2 mm) was weighed in plastic containers and required amount of bioamendments were added as per the following treatments. Distilled water was added to achieve two different moisture conditions namely field capacity moisture and alternate wetting & drying and thoroughly mixed. The plastic containers were covered with polyethylene bags containing small pin-sized holes to permit aeration. Three replicates of each treatment were prepared, randomly placed and incubated in the laboratory at  $25\pm 2^{\circ}\text{C}$  for 60 days. The above incubation experiment was maintained under two different moisture conditions throughout the experiment (Fig. 3.2). At the end of 0, 15, 30, 45 and 60 days the samples were thoroughly mixed and about 10 grams of samples were taken and analysed for the pH, soil organic carbon, water soluble organic carbon and different fractions of Ni. The experiment was carried out using by completely randomized design and the results were statistically scrutinized using AGRES software.

### **Treatment details**

- T<sub>1</sub> : Control - Soil\* (No amendments)
- T<sub>2</sub> : Soil\* + FYM (12.5t ha<sup>-1</sup>)
- T<sub>3</sub> : Soil\*+ Poultry manure (5t ha<sup>-1</sup>)
- T<sub>4</sub> : Soil\*+ Pressmud (5t ha<sup>-1</sup>)
- T<sub>5</sub> : Soil\*+ Biochar (5t ha<sup>-1</sup>)

### **Factors**

M<sub>1</sub> - Field capacity moisture

M<sub>2</sub> - Alternate wetting and drying

## **3.6. PHYTOEXTRACTION OF NICKEL FROM THE CONTAMINATED SOIL – POT EXPERIMENT**

Two pot experiments were conducted to examine the effect of bioamendments on Ni bioavailability in soil with the plants such as marigold and alfalfa (Fig. 3.3 & 3.4).



**Fig. 3.3 Pot experiment with Marigold**



**Fig. 3.4 Pot experiment with Alfalfa**

The soil used in this study was collected from the field located at Nanjudapuram checkpost, Coimbatore which is contaminated by Ni containing electroplating industry effluent and sewage water. Syntax pots with a capacity of 2kg were used. Two kg of soil was weighed and transferred into each pot. The nickel content of the soil is 300 mg kg<sup>-1</sup>. The bioamendments such as FYM, poultry manure, pressmud and biochar were added in as per the treatment schedule and uniformly mixed. The rate of application of bioamendments was based on TNAU recommendation (Crop production guide, 2005). The pots were incubated at field capacity (0.37g g<sup>-1</sup>). The Marigold seedlings were transplanted and alfalfa seeds were sown after three days incubation of bioamendment addition. At fortnight intervals, soil samples were removed from each pot and analysed for pH, total Ni content and speciation of Ni

Periodically known amount of water was added uniformly to all pots to compensate moisture loss. The plants were grown up to active vegetation stage and harvested to examine the Ni accumulation in the plants. Soil and plant samples were collected at the end of the experiment for various analyses. The experiment was carried out using by completely randomized design and the results were statistically scrutinized using AGRES software.

### **Treatment details**

T <sub>1</sub>	:	Control - Soil* (No amendments)
T <sub>2</sub>	:	Soil* + FYM (12.5t ha <sup>-1</sup> )
T <sub>3</sub>	:	Soil*+ Poultry manure (5t ha <sup>-1</sup> )
T <sub>4</sub>	:	Soil*+ Pressmud (5t ha <sup>-1</sup> )
T <sub>5</sub>	:	Soil*+ Biochar (5t ha <sup>-1</sup> )

## **3.7. ANALYTICAL METHODS**

### **3.7.1. Analysis of Soil**

#### **3.7.1.1. Physical properties**

The bulk density and particle density of the sample were determined by cylindrical method. The surface core soil samples (undisturbed) were collected as per the standardized procedure by Gupta and Dakshinamoorthi (1981).

### ***3.7.1.2. Chemical properties***

#### ***pH and Electrical Conductivity***

The pH of soil was measured in water (1:2.5) after half an hour equilibration with a glass electrode pH meter. The Electrical Conductivity (EC) of the supernatant suspension was measured using a conductivity bridge (Jackson, 1973).

#### ***Cation Exchange Capacity***

The CEC of soil was determined by the method described by Schollenberger and Dreibelbis (1930). Ten gram of sample was added with 50 ml of neutral  $\text{CH}_3\text{COONH}_4$  and kept overnight. The sample was then transferred to the filter paper (Whatman No.3) and leached with  $\text{CH}_3\text{COONH}_4$  for 6-8 times. After that, a pinch of  $\text{NH}_4\text{Cl}$  was added and leached with 60 %  $\text{C}_2\text{H}_5\text{OH}$  until the filtrate runs free of chloride. The sample along with the filter paper was transferred to the distillation flask and added 50 ml of distilled water and 10ml of 40 % NaOH. The  $\text{NH}_3$  evolved during distillation was collected in 25ml of 0.1N  $\text{H}_2\text{SO}_4$  added with 2 drops of methyl red indicator. The excess acid was titrated against 0.1N KOH and CEC was calculated.

#### ***Organic carbon***

Organic carbon content of the soil was estimated by the wet digestion method (Walkley and Black, 1934). A 0.5 g of soil (<1 mm) was taken in a 500 ml conical flask and added 10 ml of 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  and 20 ml of conc.  $\text{H}_2\text{SO}_4$ . The contents were allowed to stand for 30 min. Then distilled water (200 ml),  $\text{H}_3\text{PO}_4$  (10 ml) and diphenylamine (1 ml) indicator were added. This was titrated against 0.5 N  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4) \cdot 6\text{H}_2\text{O}$  towards the end point of a bright green colour.

#### ***Water soluble organic carbon***

The water soluble organic C was determined as per the procedure outlined by McGill *et al.*, (1986). Water soluble organic C was extracted from field-moist samples within 24 hrs of sampling by shaking 5 g of soil and 10 ml of distilled water for 60 min followed by centrifugation at 10,000 rpm for 30 min. Supernatant solution was filtered and 5 ml of aliquot was taken in a conical flask and treated with 5 ml of 0.07N  $\text{K}_2\text{Cr}_2\text{O}_7$ , 10ml of concentrated  $\text{H}_2\text{SO}_4$  and 5 ml of orthophosphoric acid. The sample was mixed

carefully and digested at 150° C for 30 min using a digestion block. After cooling, one ml of diphenylamine indicator was added and titrated against 0.35N Fe (NH<sub>4</sub>)<sub>2</sub> (SO<sub>4</sub>). 6H<sub>2</sub>O in 0.4M H<sub>2</sub>SO<sub>4</sub>. The end point was the appearance of dark green colour.

#### ***KMnO<sub>4</sub>-N***

The KMnO<sub>4</sub>- N content of the soil was determined by the alkaline permanganate method (Subbiah and Asija, 1956). A 5 g of soil (2 mm) was taken in a distillation flask and 25 ml of each 0.32 % KMnO<sub>4</sub>-N and 2.5 % NaOH was added to the soil. Twenty ml of 2 % boric acid with a drop of double indicator was taken in a beaker and kept near the delivery end. The distillation was carried out and the liberated NH<sub>3</sub> was collected and titrated against 0.02 N sulphuric acid. From the titer value the KMnO<sub>4</sub>-N was calculated.

#### ***NaHCO<sub>3</sub> - P***

NaHCO<sub>3</sub> - P was determined by a colorimetric method (Olsen *et al.*, 1954). A 5 g of soil (2 mm) was taken in a polycarbonate shaking bottle. Fifty ml of 0.5 M NaHCO<sub>3</sub> and a pinch of activated carbon were added to the soil, and shaken for 30 min. The extract was filtered using Whatman No. 40 filter paper. A 5 ml of filtrate was pipetted out into a 25 ml volumetric flask and 4 ml of reagent B was added and made up to 25 ml. After 30 min. the absorbance value of the colour developed in the sample was read at 660 nm in a colorimeter and the available phosphorus was calculated from the standard curve.

#### ***NH<sub>4</sub>OAc - K and exchangeable Na***

The available K and Na as extracted in neutral normal NH<sub>4</sub>OAc was determined. A 5 g of the soil was taken in a 100 ml shaking bottle and 25 ml of 1 N NH<sub>4</sub>OAc was added, shaken for 5 min. and then filtered. The NH<sub>4</sub>OAc - K and Na in the extract were determined using a flame photometer (Jackson, 1973).

#### ***Total Nickel***

One gram of sample was weighed in a acid washed 100 ml conical flask and added 15 ml of aqua-regia (HCl : HNO<sub>3</sub> @ 3:1). The sample was digested in a hot plate at 110° C for about 2 h. After obtaining a white slurry, the flask was cooled, added 5 ml of distilled water and boiled for few minutes. The volume of the content was made to 50 ml and kept overnight. Then the content was filtered through Whatman No. 40 filter paper

and the Ni concentration was measured using an Atomic Absorption Spectrophotometer (AAS) with air- acetylene flame (VARIAN, AA200). A wave length of 232 nm was used with a spectral slit width of 0.2 nm (USEPA, 1979).

### ***Speciation of Nickel***

A sequential extraction procedure was followed to quantify the relative proportion of different species of Ni. It is helpful in determining the bioavailability of Ni as species determine the toxicity of heavy metals. Sequential extraction is a series of chemical extractions performed on same sample. The extractants used in the first few steps of a sequential extraction tend to selectively target specific components of adsorbent structure whereas extractants used in the last step are less specific and more vigorous and destructive (Beckett, 1989).

A method described by Noble and Hughes (1991) was employed to determine the species of retained Ni in the soil as outlined below.

***Step 1 (Water soluble fraction):*** One gram of air-dried soil sample was weighed in a 50 ml polypropylene centrifuge tube and added 25 ml of double distilled water. It was shaken in an end-over-end shaker for 2 hrs at  $25\pm 2^\circ$  C. Then centrifuged the tubes at 8000 rpm for 10 minutes and filtered through Whatman No.40 filter paper. The soluble Ni in the water extract was determined using an Atomic Absorption Spectrophotometer with air- acetylene flame of new VARIAN, AA240 (USEPA, 1979).

***Step 2 (Exchangeable fraction):*** To the residue from step 1, 25 ml of 0.5 M  $\text{KNO}_3$  was added and shaken it for 16 hrs. The centrifugation, filtration and measurement were followed as in step 1.

***Step 3 (Organic fraction):*** Added 25 ml of 0.5 M NaOH to the soil remaining after the exchangeable fractions (step 2) and shaken it for 16 hrs. The centrifugation, filtration and measurement were followed as in step 1.

***Step 4 (Organic plus iron-oxide bound fraction):*** The residue from step 3 was shaken with 0.05 M  $\text{Na}_2\text{EDTA}$  for 6 hrs and followed centrifugation, filtration and measurement as in step 1.

***Step 5 (Residual fraction):*** The soil residue from step 4 was transferred to a 150 ml conical flask using a jet of water and dried in an oven. Then added 10 ml concentrated

HNO<sub>3</sub> and digested the contents at 110° C. After digestion, the contents were diluted and filtered before taking measurements.

The tube plus contents were weighed before and after extraction to calculate the volume of entrapped solution and transfer of heavy metal between extractants. The amounts of Ni extracted by each extractant were computed by using the following equation.

$$\text{Ni extracted } (\mu\text{g g}^{-1}) = C \times (E+M) - (C' \times M) / \text{weight of soil}$$

Where,

C - Concentration of heavy metal in the extraction solution

M – Mass (g) of the entrained solution carried over from previous extraction

C' - Concentration of the heavy metal in the extraction solution of proceeding step of the sequence

E – Mass (g) of the extractant

### **3.7.2. Characterization of bioamendments for their physico-chemical properties**

Partially decomposed poultry manure was collected from the Poultry Unit of the Department of Animal Husbandry, TNAU, Coimbatore. Bulk sample of FYM was collected from the Department of Farm management. The required quantity of pressmud compost was collected from Bannari amman sugars Pvt. Ltd, Sathiyamangalam. Biochar has been procured from the private agency, Coimbatore. The above bioamendments were analysed for the some important parameters.

#### **3.7.2.1. pH and Electrical Conductivity**

The pH of manure was measured in water (1:10) after half an hour equilibration with a glass electrode pH meter. The electrical conductivity of the supernatant suspension was measured using a conductivity bridge (Falcon *et al.*, 1987).

#### **3.7.2.2. Organic Carbon**

Organic carbon content of the manure was estimated by the wet digestion method (Walkley and Black, 1934). A 0.5g of air dried manure (<1mm) was taken in a 500ml conical flask and added 10 ml of 1 N K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 20 ml of conc. H<sub>2</sub>SO<sub>4</sub>. The contents

were allowed to stand for 30 min. Then, distilled water (200 ml) orthophosphoric acid (10ml) and diphenylamine (1ml) indicator were added. This was titrated against 0.5 N Fe.  $(\text{NH}_4)_2(\text{SO}_4)_2$  towards the end point of a bright green colour.

#### **3.7.2.3. Total nitrogen**

The bioamendment was digested with diacid ( $\text{H}_2\text{SO}_4$  and  $\text{HClO}_4$  at 5:2) and volume was made up with distilled water. The extractant was analysed by Kjeldahl distillation unit (Biswas *et al.*, 1977; Humphries, 1956).

#### **3.7.2.4. Total Phosphorus**

The samples were digested using tri acid ( $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$  and  $\text{HClO}_4$  at 9:2:1) and analysed total phosphorus by Vanadomolybdate colorimetric (ELICO-SL159) method (Jackson, 1973).

#### **3.7.2.5. Total Potassium**

The triacid extract was analysed for total K by Flame photometer (Jackson, 1973).

#### **3.7.2.6. Total Calcium and Magnesium**

The triacid extract was used for Ca and Mg measurement by Versenate titration method (Jackson, 1973).

### **3.7.3. Plant sample collection and analysis**

Plant samples (marigold and alfalfa) were collected from the pot culture at harvest stage. The plant samples were cleaned with water and separated into roots and above ground biomass. The samples were kept in paper covers and shade dried and later oven dried ( $70^\circ\text{C}$ ). After recording the dry weight, each sample was ground in a Wiley mill and sub-samples were obtained for laboratory analysis.

#### **3.7.3.1. Total Ni in plant parts**

The plant materials (root, stem, leaves and flowers) were weighed (0.5-1g) and digested with 10 ml of concentrated  $\text{HNO}_3$  and Ni was determined as described in soil analysis.

### ***3.7.3.2. Total Ni in plants***

The plant samples were harvested from Ni incubated soils and oven dried. The whole powdered plant samples were analysed for Ni content and Ni was determined as described in soil analysis.

### **3.7.4. Statistical analysis**

The data on various characters studied during the course of investigation were statistically analysed as suggested by Gomez and Gomez (1984) to find out the influence of various treatment on the soil properties and crop response. The critical difference was worked out at five per cent probability level. Treatment differences that were not significant were denoted as 'NS'.

## *Results*

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## CHAPTER IV

### EXPERIMENTAL RESULTS

Nickel contamination is widespread nowadays due to variety of industrial activities such as electroplating, production of Ni-Cd batteries, steel alloy etc. Many 'hot-spots' of Ni contamination are found in and around Coimbatore city where large number of dye and textiles, foundries and electroplating industries are located. To develop a viable remediation technology, an understanding of the fate of Ni in soil is needed. Therefore, in the current study a series of laboratory and pot experiments were conducted to examine the adsorption & desorption, bioavailability and plant uptake of Ni etc. The results obtained from these experiments are presented in this chapter.

#### 4.1. CHARACTERIZATION OF EXPERIMENTAL SOIL

Bulk soil sample was collected from a farmer's field in Kamatchipuram, near Coimbatore, air dried (at  $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$ ), sieved (2mm) and used. The soil was analysed for important characteristics and presented in the Table 4.1. The soil belongs to Pelamedu series and *Typic Haplusterts* in USDA classification. The soil was neutral in pH (6.41) and non saline ( $\text{EC} = 0.27 \text{ dSm}^{-1}$ ). The soil organic carbon (SOC) was 0.75 per cent with low CEC (8.29). With regard to nutrient status, the soil was low in N ( $168 \text{ kg ha}^{-1}$ ), medium in P ( $12 \text{ kg ha}^{-1}$ ) and high in K ( $412 \text{ kg ha}^{-1}$ ) levels. The total Ni content of the soil was  $72.6 \text{ mg kg}^{-1}$ .

#### 4.2. EXPERIMENT I - ADSORPTION AND DESORPTION OF NICKEL

It is well known that adsorption is the foremost process that controls the bioavailability and mobility of Ni in soil and thus its environmental impact. Therefore, laboratory batch experiments were conducted to examine the adsorption and desorption of Ni on the experimental soil.

##### 4.2.1. Adsorption of Nickel

The adsorption of Ni in soil as a function of equilibrium period is important, since Ni should be retained by soil particles for a reasonably sufficient period of time for any change to occur. Therefore, the equilibrium period was determined. The result showed that

**Table 4.1. Initial characteristics of experimental soil (Experiment I &II)**

<b>S. No.</b>	<b>Parameters</b>	<b>Values</b>
1.	Soil series	Peelamedu series
2.	USDA classification	<i>Typic Haplusterts</i>
3.	Bulk density ( $\text{Mg m}^{-3}$ )	1.31
4.	pH (1:2.5 water)	6.41
5.	EC ( $\text{dS m}^{-1}$ )	0.27
6.	Soil organic carbon (%)	0.75
7.	Cation exchange capacity ( $\text{cmol (p}^+) \text{ kg}^{-1}$ )	8.29
8.	$\text{KMnO}_4\text{-N}$ ( $\text{kg ha}^{-1}$ )	168
9.	$\text{NaHCO}_3\text{-P}$ ( $\text{kg ha}^{-1}$ )	12.0
10.	$\text{NH}_4\text{OAc- K}$ ( $\text{kg ha}^{-1}$ )	412
11.	Exchangeable Na ( $\text{mg kg}^{-1}$ )	208
12.	Exchangeable Ca ( $\text{mg kg}^{-1}$ )	1800
13.	Exchangeable Mg ( $\text{mg kg}^{-1}$ )	612
14.	Total Nickel ( $\text{mg kg}^{-1}$ )	72.6

with a single input concentration ( $100 \text{ mg L}^{-1}$ ) the adsorption of Ni progressively increased with time and attained a maximum at 12 hrs and continued up to 48 hrs (Table 4.2). It was clearly showed that Ni adsorption attained steady state equilibrium at 12 hrs. However, more than 80 percent of added Ni was found adsorbed on soil particles within 30 minutes. Therefore, in the subsequent experiments, an equilibrium period of 12 hrs was used.

#### ***4.2.1.1. Adsorption as a function of Nickel concentration***

The effect of different initial concentration (0, 20, 40, 60, 80 and  $100 \text{ mg L}^{-1}$ ) of Ni on adsorption was examined. The result showed that the Ni adsorption was greatly dependent on initial concentration. The equilibrium concentration ranged from 0.136 to  $0.382 \text{ mg L}^{-1}$  (Table 4.3). The amount of Ni adsorbed on soil ranged from 6.6 to  $90.45 \text{ mg kg}^{-1}$ . With the increase in the initial concentration, the adsorption was found increased progressively and the maximum adsorption (90.45 percent) was observed at the highest equilibrium concentration of  $0.382 \text{ mg L}^{-1}$ .

#### ***4.2.1.2. Factors affecting adsorption of Nickel on soil***

The adsorption of Ni on soil is affected by various factors such as pH, organic matter content and competitive ions present in soil along with the Ni. Therefore, a batch experiment was conducted to study the effect of pH, organic matter content and co-contaminants (Viz., Cd and Cr) on Ni adsorption. The results are presented in Tables 4.4-4.7

##### ***4.2.1.2.1. Effect of soil pH***

The effect of different pH (5 to 9) on the adsorption of Ni are presented in Table 4.4. The pH plays an important role on Ni dynamics and thus could affect adsorption of Ni. The study of pH influence is important for establishing the optimum sorption of Ni. For Ni adsorption the initial pH (adjusted) ranged from 5.0 to 9.0. The equilibrium concentration ranged from 0.21 to  $0.54 \text{ mg L}^{-1}$ . The amount of Ni adsorbed at different pH ranged from 86.46 to  $94.84 \text{ mg kg}^{-1}$ . With an increase in pH, the adsorption was found increased significantly. The highest amount of Ni ( $94.84 \text{ mg kg}^{-1}$ ) adsorbed on soil was observed at pH 9.

##### ***4.2.1.2.2. Effect of soil organic matter content***

The effects of different bioamendments on the adsorption of Ni is presented in Table 4.5. The SOM plays a major role on Ni adsorption on soil. To study the influence of

**Table 4.2. Adsorption of Nickel on soil as a function of equilibrium period**

<b>Hours</b>	<b>Equilibrium concentration (mg L<sup>-1</sup>)</b>	<b>Amount adsorbed (mg kg<sup>-1</sup>)</b>
0	0	Bdl
0.5	0.648	83.80
1	0.642	83.95
2	0.585	85.38
5	0.557	86.08
8	0.521	86.98
12	0.416	89.60
24	0.413	89.67
36	0.412	89.70
48	0.413	89.68

**Table 4.3. Adsorption of Nickel on soil as a function of equilibrium concentration**

<b>Initial concentration (mg L<sup>-1</sup>)</b>	<b>Equilibrium concentration (mg L<sup>-1</sup>)</b>	<b>Amount adsorbed (mg kg<sup>-1</sup>)</b>
<b>0</b>	0	Bdl
<b>10</b>	0.136	6.60
<b>20</b>	0.147	16.33
<b>40</b>	0.17	35.75
<b>60</b>	0.181	55.48
<b>80</b>	0.262	73.45
<b>100</b>	0.382	90.45

**Table 4.4. Effect of soil pH on adsorption of Nickel**

<b>pH level</b>	<b>Equilibrium concentration (<math>\mu\text{g ml}^{-1}</math>)</b>	<b>Amount adsorbed (<math>\text{mg kg}^{-1}</math>)</b>
5	0.54	86.46
6	0.37	90.73
7	0.27	93.28
8	0.26	93.50
9	0.21	94.84

**Table 4.5. Effect of bioamendments on adsorption of Nickel**

<b>Bioamendments</b>	<b>Equilibrium concentration (<math>\mu\text{g ml}^{-1}</math>)</b>	<b>Amount of Ni adsorbed (<math>\text{mg kg}^{-1}</math>)</b>
Control	0.27	93.18
FYM	0.41	89.85
Poultry manure	0.44	88.91
Pressmud	0.98	75.43
Biochar	0.43	89.27

**Table 4.6. Effect of co-contaminant (Cd) on adsorption of Nickel**

<b>Amt of Ni added (<math>\text{mg kg}^{-1}</math>)</b>	<b>Equilibrium concentration (<math>\mu\text{g ml}^{-1}</math>)</b>	<b>Amount of Ni adsorbed (<math>\text{mg kg}^{-1}</math>)</b>
0	0.25	Bdl
10	0.79	Bdl
20	1.49	Bdl
40	2.60	Bdl
60	3.42	Bdl
80	4.49	Bdl
100	5.24	Bdl

organic matter content of soil on Ni adsorption, different bioamendments such as farmyard manure, composted poultry manure, pressmud compost and prosopis biochar were used at the rate of  $0.5\text{g g}^{-1}$  of soil along with the single input concentration of Ni ( $100\text{ mg kg}^{-1}$ ). The equilibrium concentration ranged from  $0.27$  to  $0.98\text{ mg L}^{-1}$ . The amount of Ni adsorbed due to the application of bioamendments, ranged from  $75.43$  to  $93.18\text{ mg kg}^{-1}$ . In general with the application of bioamendment, the Ni adsorption was found decreased significantly. While the highest amount of Ni adsorbed ( $93.18\text{ mg kg}^{-1}$ ) on soil was observed with the control soil and the lowest adsorption ( $75.43\text{ mg kg}^{-1}$ ) was recorded with the application of pressmud compost of followed by composted poultry manure ( $88.91\text{ mg kg}^{-1}$ ).

#### 4.2.1.2.3. Effect of co-contaminants

To study the influence of co-contaminants on Ni adsorption, the single input concentration of Cd and Cr ( $100\text{ mg kg}^{-1}$ ) was used along with various concentration of Ni ranged from  $0$  to  $100\text{ mg kg}^{-1}$ . The presence of different co-contaminants or competitive ions was found to significantly affect the adsorption of Ni on soil (Table 4.6 & 4.7). When Cd is used as a co-contaminant, the equilibrium concentration ranged from  $0.25$  to  $5.24\text{ mg L}^{-1}$ . The Ni adsorption was found decreased significantly in the presence of Cd. When Cr was present, the equilibrium concentration ranged from  $0.14$  to  $0.54\text{ mg L}^{-1}$ . The amount of Ni adsorbed in the presence of Cr, ranged from  $6.41$  to  $86.57\text{ mg kg}^{-1}$ , and the Ni adsorption was found increased significantly when Cr was present as a co-contaminant.

#### **4.2.2. Desorption of Nickel from soil**

Like adsorption, desorption of Ni also gained much importance to predict the mobility of Ni in soil and its environmental impact. At first a batch experiment was conducted for adsorption of Ni in soil using various concentration of Ni ranged from  $0$  to  $100\text{ mg kg}^{-1}$  of soil. The amount of adsorbed Ni has been calculated. Then, various extractants such as  $0.005\text{M DTPA}$  and  $0.05\text{M Ca(NO}_3)_2$  have been used to determine the desorption of Ni from soil.

The equilibrium period for desorption with  $0.005\text{M DTPA}$  was determined as  $30$  minutes with maximum desorption of Ni ( $56.76\text{ }\mu\text{g}$ ) from the soil. The amount of Ni adsorbed in the soil ranged between  $7.09$  and  $93.32\text{ mg kg}^{-1}$  of soil. Then, desorption of Ni when using  $0.005\text{M DTPA}$  ranged from  $7.08$  to  $60.42\text{ }\mu\text{g}$  of Ni in the  $25\text{ml}$  of solution (Table 4.8).

**Table 4.7. Effect of co-contaminant (Cr) on adsorption of Nickel**

<b>Amount of Ni added (mg kg<sup>-1</sup>)</b>	<b>Equilibrium concentration (µg ml<sup>-1</sup>)</b>	<b>Amount of Ni adsorbed (mg kg<sup>-1</sup>)</b>
0	0.14	Bdl
10	0.14	6.41
20	0.18	15.56
40	0.19	35.31
60	0.25	53.80
80	0.37	70.75
100	0.54	86.57

**Table 4.8. Desorption of Nickel with 0.005M DTPA**

<b>Amount of Ni added (mg kg<sup>-1</sup>)</b>	<b>Amount of Ni adsorbed (mg kg<sup>-1</sup>)</b>	<b>Amount of Ni desorbed in the solution (µg/25ml)</b>	<b>Percentage of Desorption</b>
0	0.0	0.0	-
10	7.09	7.08	99.85
20	16.76	16.16	96.42
40	36.26	30.73	84.75
60	56.67	43.19	76.21
80	74.51	56.14	75.34
100	93.32	60.42	64.74

The equilibrium period for desorption with 0.05M  $\text{Ca}(\text{NO}_3)_2$  was determined as 2 hours with maximum desorption of Ni (21.28  $\mu\text{g}$ ) from the soil. The amount of Ni adsorbed in the soil ranged between 6.41 and 88.66  $\text{mg kg}^{-1}$  of soil. When using 0.05M  $\text{Ca}(\text{NO}_3)_2$ , the amount of Ni desorbed from the soil ranged from 0.62 to 40.27  $\mu\text{g}$  to the 25ml of solution (Table 4.9).

### **4.3. EXPERIMENT II - BIOTRANSFORMATION AND BIOAVAILABILITY OF NICKEL IN SOIL**

A laboratory closed incubation experiment was conducted to examine the bioavailability and factors governing bioavailability of Ni in soil. The Ni was added to the soil at the rate of 0, 20, 40, 80, 100 $\mu\text{g g}^{-1}$  as  $\text{NiCl}_2$  and thoroughly mixed. The experiment was maintained under two moisture conditions such as field capacity moisture (0.37  $\text{g g}^{-1}$ ) and alternate wetting & drying. At the end of 15 and 30 days, the soil was thoroughly mixed and sub samples ( $\approx 25$  g) were removed from all the treatments and analysed for soil pH, SOC and species of Ni.

#### **4.3.1. Changes in soil pH during incubation**

The pH of the soil ranged from 7.40 to 8.37 during the 30 days of incubation. The two moisture conditions had significant influence on the soil pH (Table 4.10). When comparing the two different moisture conditions the soil under field capacity moisture recorded relatively lower pH than the soil under alternate wetting and drying condition. The pH of the soil was found increased slightly at 15<sup>th</sup> day but decreased at 30<sup>th</sup> day of the incubation in the soil under field capacity moisture conditions. Under alternate wetting and drying condition, the soil pH was decreased gradually from 0<sup>th</sup> day to 30<sup>th</sup> day of incubation. The Ni concentration in soil had marked effect on soil pH. It was found maximum in the control soil ( $T_1$ ) and minimum in the soil ( $T_6$ ) containing 100  $\text{mg kg}^{-1}$  of Ni in soil during both initial and final stages of the incubation.

#### **4.3.2. Changes in soil organic carbon during incubation**

The changes observed in SOC during the incubation period are presented in the Table 4.11. The soil organic carbon (SOC) was ranged from 0.23 to 0.52 percent during 30 days of incubation. Marked difference in SOC was observed due to the moisture

**Table 4.9. Desorption of Nickel with 0.05M Ca(NO<sub>3</sub>)<sub>2</sub>**

Amount of Ni added (mg kg <sup>-1</sup> )	Amount of Ni adsorbed (mg kg <sup>-1</sup> )	Amount of Ni desorbed in the solution (µg/25ml)	Percentage of Desorption
0	0.0	0.0	-
10	6.14	0.62	0.10
20	15.53	3.32	21.38
40	34.52	11.98	34.70
60	53.23	23.43	44.02
80	71.97	34.12	47.40
100	88.66	40.27	45.42

**Table 4.10. Variations in pH during incubation of soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub> - Control (Soil alone)	8.37	8.30	8.34	<b>8.42</b>	8.20	8.32	<b>8.26</b>
T <sub>2</sub> - Soil + 20 ppm of Ni	8.17	8.14	8.16	<b>8.18</b>	7.94	7.96	<b>7.95</b>
T <sub>3</sub> - Soil + 40 ppm of Ni	8.01	7.92	7.95	<b>7.94</b>	7.71	7.86	<b>7.79</b>
T <sub>4</sub> - Soil + 60 ppm of Ni	7.87	7.79	7.86	<b>7.83</b>	7.67	7.61	<b>7.64</b>
T <sub>5</sub> - Soil + 80 ppm of Ni	7.73	7.75	7.80	<b>7.78</b>	7.60	7.52	<b>7.56</b>
T <sub>6</sub> - Soil + 100 ppm of Ni	7.63	7.69	7.73	<b>7.71</b>	7.42	7.40	<b>7.41</b>
<b>Mean</b>	<b>7.96</b>	<b>7.93</b>	<b>8.02</b>	<b>7.97</b>	<b>7.76</b>	<b>7.78</b>	<b>7.77</b>
<b>T M TXM</b>	<b>SEd</b>		<b>CD (0.05)</b>		<b>SEd</b>		<b>CD (0.05)</b>
	0.011		0.023**		0.007		0.014**
	0.007		0.014**		0.004		0.008**
0.016		0.033**		0.010		0.020**	

**Table 4.11. Variations in soil organic carbon (%) during incubation of soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	30 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub> - Control (Soil alone)	0.52	0.40	0.49	<b>0.45</b>
T <sub>2</sub> - Soil + 20 ppm of Ni	0.49	0.39	0.43	<b>0.41</b>
T <sub>3</sub> - Soil + 40 ppm of Ni	0.46	0.39	0.38	<b>0.39</b>
T <sub>4</sub> - Soil + 60 ppm of Ni	0.41	0.38	0.35	<b>0.37</b>
T <sub>5</sub> - Soil + 80 ppm of Ni	0.39	0.37	0.32	<b>0.35</b>
T <sub>6</sub> - Soil + 100 ppm of Ni	0.35	0.32	0.23	<b>0.28</b>
<b>Mean</b>	<b>0.44</b>	<b>0.38</b>	<b>0.37</b>	<b>0.37</b>
<b>T M TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>
		0.03		0.06**
		NS		NS
		NS		NS

conditions. At all days, SOC was found relatively higher in soil under field capacity moisture than in soil under alternate wetting and drying. The SOC was found decreased during incubation under both moisture conditions. The treatments had significant effect on SOC. Immediately after mixing different concentration of Ni, it was found maximum (0.52 percent) in the control soil ( $T_1$ ) and minimum (0.34 per cent) in the soil containing  $100 \text{ mg kg}^{-1}$  of Ni ( $T_6$ ). After 30 days of incubation, the maximum SOC (0.40 per cent) was recorded in the control soil ( $T_1$ ) and minimum (0.35 per cent) in the soil that had  $100 \text{ mg Ni kg}^{-1}$  ( $T_6$ ) under field capacity moisture. Under alternate wetting and drying condition, final SOC was found maximum (0.49 per cent) in the soil ( $T_1$ ) and minimum (0.23 per cent) in the soil that had the Ni of  $100 \text{ mg kg}^{-1}$  ( $T_6$ ).

### **4.3.3. Biotransformation of Nickel**

#### ***4.3.3.1. Water soluble Nickel***

The changes in the water soluble fraction of Ni observed during incubation are presented in the Table 4.12. The concentration of water soluble Ni ranged from 4.30 to  $11.33 \text{ mg kg}^{-1}$ . The moisture conditions had a significant effect on water soluble fraction of Ni. Initially, the water soluble Ni was found higher in soil under alternate wetting and drying. But at final stage it was significantly higher in soil under field capacity moisture condition. The water soluble Ni showed a gradual increase over a period of time from 0<sup>th</sup> day to 15<sup>th</sup> day of incubation. After that, it showed a slight decrease at 30<sup>th</sup> day under both moisture conditions. Initially, the water soluble Ni was found higher ( $6.19 \text{ mg kg}^{-1}$ ) in soil that contained  $100 \text{ mg Ni kg}^{-1}$  ( $T_6$ ) and lower ( $5.10 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ). At final stage, the highest values of water soluble Ni were recorded in the soil contained  $100 \text{ mg kg}^{-1}$  of Ni ( $T_6$ ) under field capacity moisture ( $5.45 \text{ mg kg}^{-1}$ ) and alternate wetting and drying ( $5.03 \text{ mg kg}^{-1}$ ). The lowest water soluble Ni were recorded in the control soil ( $T_1$ ) under field capacity moisture ( $4.58 \text{ mg kg}^{-1}$ ) and alternate wetting and drying ( $4.30 \text{ mg kg}^{-1}$ ).

#### ***4.3.3.2. Exchangeable Nickel***

The soil moisture had significant effect on exchangeable Ni (Table 4.13). The exchangeable Ni in soil ranged between 7.43 and  $36.81 \text{ mg kg}^{-1}$ . Initially, exchangeable fraction was found higher in soil under field capacity moisture condition than in the soil under alternate wetting and drying. At the end of 30 days of incubation, the exchangeable fraction was

**Table 4.12. Variations in water soluble fractions of Nickel ( $\text{mg kg}^{-1}$ ) during incubation of soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub> - Control (Soil alone)	5.10	5.28	4.98	<b>5.13</b>	4.58	4.30	<b>4.44</b>
T <sub>2</sub> - Soil + 20 ppm of Ni	5.37	5.30	5.05	<b>5.18</b>	5.03	4.43	<b>4.73</b>
T <sub>3</sub> - Soil + 40 ppm of Ni	5.67	5.38	5.43	<b>5.41</b>	5.13	4.50	<b>4.82</b>
T <sub>4</sub> - Soil + 60 ppm of Ni	5.72	5.65	6.28	<b>5.97</b>	5.18	4.68	<b>4.93</b>
T <sub>5</sub> - Soil + 80 ppm of Ni	5.93	6.55	7.05	<b>6.80</b>	5.25	4.95	<b>5.10</b>
T <sub>6</sub> - Soil + 100 ppm of Ni	6.19	7.20	11.33	<b>9.27</b>	5.45	5.03	<b>5.24</b>
<b>Mean</b>	<b>5.66</b>	<b>5.89</b>	<b>6.69</b>	<b>6.29</b>	<b>5.10</b>	<b>4.65</b>	<b>4.88</b>
		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
<b>T</b>		0.09		0.20**	0.08		0.16**
<b>M</b>		0.06		0.12**	0.04		0.09**
<b>TXM</b>		0.14		0.29**	0.11		NS

**Table 4.13. Variations in exchangeable fractions of Nickel ( $\text{mg kg}^{-1}$ ) during incubation of soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub> - Control (Soil alone)	11.11	7.65	7.43	<b>7.54</b>	31.26	30.56	<b>30.91</b>
T <sub>2</sub> - Soil + 20 ppm of Ni	11.31	7.97	7.54	<b>7.76</b>	31.82	35.17	<b>33.50</b>
T <sub>3</sub> - Soil + 40 ppm of Ni	11.43	8.14	7.78	<b>7.96</b>	32.06	35.31	<b>33.69</b>
T <sub>4</sub> - Soil + 60 ppm of Ni	11.44	8.51	7.93	<b>8.22</b>	32.86	35.53	<b>34.20</b>
T <sub>5</sub> - Soil + 80 ppm of Ni	11.69	8.91	7.99	<b>8.45</b>	33.31	35.74	<b>34.53</b>
T <sub>6</sub> - Soil + 100 ppm of Ni	11.91	8.94	8.30	<b>8.62</b>	34.38	36.81	<b>35.60</b>
<b>Mean</b>	<b>11.48</b>	<b>8.35</b>	<b>7.83</b>	<b>8.09</b>	<b>32.62</b>	<b>34.85</b>	<b>33.73</b>
		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
<b>T</b>		0.29		NS	0.41		NS
<b>M</b>		0.17		0.35**	0.23		0.48**
<b>TXM</b>		0.41		NS	0.57		NS

found relatively higher in soil under alternate wetting and drying condition. Irrespective of moisture conditions, the exchangeable Ni in soil was found decreased gradually up to 15<sup>th</sup> day of incubation and thereafter it was increased rapidly at 30<sup>th</sup> day of incubation.

Further the results showed that the highest exchangeable Ni was recorded in the soil (T<sub>6</sub>) with 100 mg kg<sup>-1</sup> (11.91 mg kg<sup>-1</sup>) and lowest (11.11 mg kg<sup>-1</sup>) in the control (T<sub>1</sub>) at initial stage. Under both moisture condition, the highest values were registered in the soil (T<sub>6</sub>) with 100 mg kg<sup>-1</sup> (34.38 mg kg<sup>-1</sup> in field capacity moisture; 36.81 mg kg<sup>-1</sup> in alternate wetting and drying) and lowest values were recorded in the control (31.26 mg kg<sup>-1</sup> in field capacity moisture; 30.56 mg kg<sup>-1</sup> in alternate wetting and drying) at final stage of the incubation.

#### ***4.3.3.3. Organic Nickel***

The organic fraction of Ni varied significantly between the two moisture conditions and the concentration ranged from 4.76 and 57.58 mg kg<sup>-1</sup> (Table 4.14). The organic Ni was found higher in the soils under alternate wetting and drying than under field capacity moisture. Irrespective of moisture, the concentration of organic Ni was decreased gradually from 0<sup>th</sup> day to 15<sup>th</sup> day, and thereafter showed a rapid increase at 30<sup>th</sup> day of incubation.

There was a marked changes in the concentration of organic Ni due to different concentration of Ni. Initially, the organic fraction of Ni is high in the soil (T<sub>6</sub>) added with 100 mg kg<sup>-1</sup> (15.35 mg kg<sup>-1</sup>) and low in control soil (6.82 mg kg<sup>-1</sup>). The organic fraction of Ni was found increased over a period of time. Under both moisture conditions, the highest values were (50.52 mg kg<sup>-1</sup> in field capacity moisture; 57.58 mg kg<sup>-1</sup> in alternate wetting and drying) recorded in the soil (T<sub>6</sub>) contained 100 mg kg<sup>-1</sup> and lowest values were found (15.06 mg kg<sup>-1</sup> in field capacity moisture; 17.73 mg kg<sup>-1</sup> in alternate wetting and drying) in the control soil (T<sub>1</sub>) after 30 days of incubation.

#### ***4.3.3.4. Organic plus iron oxide bound Nickel***

The organic plus iron oxide bound fraction of Ni was markedly influenced both moisture conditions and the concentration of Ni added to the soil. The concentration of organic plus iron oxide bound Ni ranged between 4.69 and 158.35 mg kg<sup>-1</sup> (Table 4.15). It was found higher in soil under alternate wetting and drying condition than under field capacity moisture conditions. The concentration of organic plus iron oxide bound Ni was found increased during 30 days of incubation under both moisture conditions.

**Table 4.14. Variations in organic fractions of Nickel ( $\text{mg kg}^{-1}$ ) during incubation of soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub> - Control (Soil alone)	6.82	4.76	5.84	<b>5.30</b>	15.06	17.73	<b>16.40</b>
T <sub>2</sub> - Soil + 20 ppm of Ni	10.23	7.63	8.56	<b>8.10</b>	29.06	29.38	<b>29.22</b>
T <sub>3</sub> - Soil + 40 ppm of Ni	11.37	10.05	11.75	<b>10.90</b>	33.28	34.75	<b>34.02</b>
T <sub>4</sub> - Soil + 60 ppm of Ni	13.01	12.61	14.88	<b>13.75</b>	36.50	39.21	<b>37.86</b>
T <sub>5</sub> - Soil + 80 ppm of Ni	14.82	14.42	14.98	<b>14.70</b>	46.99	48.79	<b>47.89</b>
T <sub>6</sub> - Soil + 100 ppm of Ni	15.35	15.81	15.38	<b>15.60</b>	50.52	57.58	<b>54.05</b>
<b>Mean</b>	<b>11.93</b>	<b>10.88</b>	<b>11.90</b>	<b>11.39</b>	<b>35.24</b>	<b>37.91</b>	<b>36.57</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		2.50		5.15**	2.68		5.53**
		1.44		NS	1.55		NS
		3.53		NS	3.79		NS

**Table 4.15. Variations in Organic plus iron oxide fractions of Nickel ( $\text{mg kg}^{-1}$ ) during incubation of soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub> - Control (Soil alone)	6.83	4.69	5.77	<b>5.23</b>	10.44	11.28	<b>10.86</b>
T <sub>2</sub> - Soil + 20 ppm of Ni	17.54	19.58	23.42	<b>21.50</b>	56.31	48.73	<b>52.52</b>
T <sub>3</sub> - Soil + 40 ppm of Ni	35.80	32.46	40.32	<b>36.39</b>	81.69	90.58	<b>86.14</b>
T <sub>4</sub> - Soil + 60 ppm of Ni	49.19	43.46	49.56	<b>46.51</b>	115.57	116.74	<b>116.16</b>
T <sub>5</sub> - Soil + 80 ppm of Ni	57.77	58.95	60.60	<b>59.78</b>	141.37	138.82	<b>140.10</b>
T <sub>6</sub> - Soil + 100 ppm of Ni	65.91	75.94	65.44	<b>70.69</b>	148.53	158.35	<b>153.44</b>
<b>Mean</b>	<b>38.84</b>	<b>39.18</b>	<b>40.85</b>	<b>40.02</b>	<b>92.32</b>	<b>94.08</b>	<b>93.20</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		7.00		14.45**	10.76		22.21**
		4.04		NS	6.21		NS
		9.90		NS	15.22		NS

Remarkable changes were observed in the concentration of organic plus iron oxide bound Ni due to the different concentration of Ni in soil under two moisture conditions. Initially, the concentration of organic plus iron oxide bound Ni was found higher ( $65.91 \text{ mg kg}^{-1}$ ) in the soil added with  $100 \text{ mg Ni kg}^{-1}$  ( $T_6$ ) and lower ( $6.83 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ). Finally, it was found higher in the treatment added with  $100 \text{ mg Ni kg}^{-1}$  ( $T_6$ ) under field capacity moisture ( $148.53 \text{ mg kg}^{-1}$ ) and alternate wetting & drying ( $158.35 \text{ mg kg}^{-1}$ ) and lower ( $10.44 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ) under field capacity moisture and alternate wetting & drying ( $11.28 \text{ mg kg}^{-1}$ ).

#### ***4.3.3.5. Residual Nickel***

The concentration of residual Ni in soil ranged from  $38.25$  to  $157.48 \text{ mg kg}^{-1}$  (Table 4.16). There was marked change due to the two different moisture conditions. Initially, the highest concentration of residual Ni was registered in the soil under alternate wetting and drying condition than under field capacity moisture. However at the end of incubation, it was found higher in soil under field capacity moisture condition. Under both moisture conditions, the concentration of residual Ni was found increased gradually from 0<sup>th</sup> day to 30<sup>th</sup> day of incubation.

Different concentration of Ni added to the soil showed significant changes in the concentration of residual Ni. Initially, the highest value of residual Ni was observed in the soil added with  $100 \text{ mg kg}^{-1}$  of Ni ( $T_6$ ) and the lowest value ( $38.25 \text{ mg kg}^{-1}$ ) in control soil ( $T_1$ ). After 30 days of incubation, the highest concentrations of residual Ni were recorded in the treatment added with  $100 \text{ mg kg}^{-1}$  of Ni in soil ( $T_6$ ) under field capacity moisture ( $150.96 \text{ mg kg}^{-1}$ ) and alternate wetting & drying ( $157.48 \text{ mg kg}^{-1}$ ). The lowest concentrations were recorded in the control soil ( $T_1$ ) under field capacity moisture ( $105.73 \text{ mg kg}^{-1}$ ) and alternate wetting & drying ( $100.03 \text{ mg kg}^{-1}$ ) was recorded in the control soil ( $T_1$ ).

#### **4.4. EXPERIMENT III - EFFECT OF BIOAMENDMENTS ON BIOTRANSFORMATION AND BIOAVAILABILITY OF NICKEL IN CONTAMINATED SOIL**

The soil organic matter (SOM) plays a major role in determining the bioavailability of Ni in soil because it significantly alters the soil chemical and biological characters. The important characteristics of soil that altered by the SOM are soil pH, SOC, CEC etc.

**Table 4.16. Variations in residual fractions of Nickel ( $\text{mg kg}^{-1}$ ) during incubation of soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub> - Control (Soil alone)	38.25	56.10	45.98	<b>51.04</b>	105.73	100.03	<b>102.88</b>
T <sub>2</sub> - Soil + 20 ppm of Ni	44.42	60.45	63.65	<b>62.05</b>	118.65	105.43	<b>112.04</b>
T <sub>3</sub> - Soil + 40 ppm of Ni	46.65	64.73	69.99	<b>67.36</b>	125.52	111.85	<b>118.69</b>
T <sub>4</sub> - Soil + 60 ppm of Ni	48.16	68.38	77.00	<b>72.69</b>	130.26	117.25	<b>123.76</b>
T <sub>5</sub> - Soil + 80 ppm of Ni	49.88	74.46	77.61	<b>76.04</b>	135.92	124.31	<b>130.12</b>
T <sub>6</sub> - Soil + 100 ppm of Ni	50.94	86.33	78.25	<b>82.29</b>	150.96	157.48	<b>154.22</b>
<b>Mean</b>	<b>46.38</b>	<b>68.41</b>	<b>68.75</b>	<b>68.58</b>	<b>127.84</b>	<b>119.39</b>	<b>123.62</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		3.32		6.85**	6.35		NS
		1.92		NS	3.67		NS
		4.70		NS	9.00		NS

To study the effect of SOM on bioavailability of Ni, a laboratory closed incubation experiment was conducted using different bioamendments under two different moisture conditions. Bulk soil samples were collected from Ni contaminated area nearby Nanjundapuram and used for this experiment.

The experimental soil was a clay soil, belongs to Kilayur series and *Typic Haplusterts* in USDA classification. The soil was alkaline in pH (8.49) with an EC of 1.11 dSm<sup>-1</sup>. The SOC content of the soil was 0.45 per cent with CEC of 19.2 (cmol (p<sup>+</sup>) kg<sup>-1</sup>). With regard to nutrient status, the soil was low in N (216 kg ha<sup>-1</sup>), medium in P (18 kg ha<sup>-1</sup>) but high in K (321 kg ha<sup>-1</sup>) levels. The total Ni content of the soil was 300 mg kg<sup>-1</sup> (Table 4.17). The bioamendments such as farmyard, composted poultry manure, pressmud compost and prosopis biochar were also analyzed for important characteristics and presented in the Table 4.18.

#### **4.4.1. Soil pH**

The pH of Ni contaminated soil amended with different bioamendments varied significantly during incubation and ranged from 6.99 to 7.50 (Table 4.19). Consistently, the soils under field capacity moisture had higher pHs than the soils under alternate wetting and drying. The soil pH was found decreased gradually from 0<sup>th</sup> day to 60<sup>th</sup> day of the experiment under both moisture conditions. The bioamendments had marked influence on the soil pH. The pH was found maximum in the control soil (T<sub>1</sub>) and minimum in the soil amended with pressmud compost (T<sub>4</sub>) during all stages of the incubation.

#### **4.4.2. Soil organic carbon**

The changes observed in the soil organic carbon (SOC) during incubation are presented in Table 4.20. The SOC ranged from 0.528 to 0.261 percent during 60 days of incubation. There was no significant change in SOC due to two different moisture conditions. The SOC was constant in soil under both the moisture conditions. The SOC was found decreased during incubation. The bioamendments had significant effect on SOC. Initially, it was found maximum in the soil amended with farmyard manure (T<sub>2</sub>) and minimum in control soil (T<sub>1</sub>). However at the end of incubation, the maximum SOC was recorded in the soil amended with composted poultry manure (T<sub>3</sub>) and minimum in the control soil (T<sub>1</sub>) under both moisture conditions.

**Table 4.17. Initial characteristics of experimental soil (Experiment III & IV)**

S. No	Parameters	Values
1.	Soil series	Kilaiyur series
2.	USDA classification	<i>Typic Haplusterts</i>
3.	Bulk density (Mg m <sup>-3</sup> )	1.44
4.	pH (1:2.5 water)	8.49
5.	EC (dS m <sup>-1</sup> )	1.11
6.	Organic carbon (%)	0.45
7.	Cation exchange capacity (cmol (p <sup>+</sup> ) kg <sup>-1</sup> )	19.2
8.	KMnO <sub>4</sub> -N (kg ha <sup>-1</sup> )	216
9.	NaHCO <sub>3</sub> -P (kg ha <sup>-1</sup> )	18.0
10.	NH <sub>4</sub> OAc- K (kg ha <sup>-1</sup> )	321
11.	Exchangeable Na (mg kg <sup>-1</sup> )	295
12.	Exchangeable Ca (mg kg <sup>-1</sup> )	3154
13.	Exchangeable Mg (mg kg <sup>-1</sup> )	2457
14.	Total Nickel (mg kg <sup>-1</sup> )	300

**Table 4.18. Some important characteristics of bioamendments**

Parameters	Farmyard manure	Composted poultry manure	Pressmud compost	Prosopis biochar
pH (1:5 H <sub>2</sub> O)	8.25	6.88	7.85	7.64
EC (dSm <sup>-1</sup> )	0.69	1.44	1.57	1.82
Organic Carbon (%)	16.54	21.07	14.80	3.62
Total N (%)	0.57	1.71	1.13	0.50
Total P (%)	0.26	0.66	0.73	0.14
Total K (%)	0.21	0.67	1.95	0.39
Total Na (%)	0.060	0.287	0.160	0.073

**Table 4.19. Effect of bioamendments on soil pH during incubation of Nickel contaminated soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day			45 <sup>th</sup> day			60 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	7.50	7.46	7.40	<b>7.43</b>	7.40	7.37	<b>7.39</b>	7.37	7.35	7.36	7.36	7.33	<b>7.35</b>
T <sub>2</sub>	7.45	7.26	7.22	<b>7.24</b>	7.25	7.19	<b>7.22</b>	7.22	7.18	7.20	7.20	7.15	<b>7.18</b>
T <sub>3</sub>	7.39	7.21	7.15	<b>7.18</b>	7.21	7.10	<b>7.16</b>	7.19	7.08	7.14	7.17	7.04	<b>7.11</b>
T <sub>4</sub>	7.15	7.12	7.06	<b>7.09</b>	7.09	7.02	<b>7.06</b>	7.04	7.00	7.02	6.99	7.04	<b>7.02</b>
T <sub>5</sub>	7.47	7.41	7.24	<b>7.33</b>	7.39	7.21	<b>7.30</b>	7.33	7.19	7.26	7.27	7.16	<b>7.22</b>
<b>Mean</b>	<b>7.39</b>	<b>7.29</b>	<b>7.21</b>	<b>7.25</b>	<b>7.27</b>	<b>7.18</b>	<b>7.22</b>	<b>7.23</b>	<b>7.16</b>	<b>7.20</b>	<b>7.20</b>	<b>7.14</b>	<b>7.17</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		0.017		0.035**	0.031		0.064**	0.029		0.061**	0.051		0.106**
		0.011		0.022**	0.019		0.041**	0.019		0.039**	0.032		NS
		0.024		NS	0.043		NS	0.042		NS	0.072		NS
<p>T<sub>1</sub>.Control - Soil* (No amendments); T<sub>2</sub>. Soil* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil*+ Composted poultry manure (5t ha<sup>-1</sup>)  T<sub>4</sub>. Soil*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil*+ Prosopis biochar (5t ha<sup>-1</sup>)</p>													

**Table 4.20. Effect of bioamendments on soil organic carbon (%) during incubation of Nickel contaminated soil under two different moisture conditions**

Treatments	Initial	Final		
		M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	0.472	0.261	0.348	<b>0.305</b>
T <sub>2</sub>	0.528	0.319	0.406	<b>0.363</b>
T <sub>3</sub>	0.490	0.377	0.464	<b>0.421</b>
T <sub>4</sub>	0.497	0.348	0.377	<b>0.363</b>
T <sub>5</sub>	0.471	0.338	0.406	<b>0.372</b>
<b>Mean</b>	<b>0.491</b>	<b>0.329</b>	<b>0.400</b>	<b>0.364</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>
		0.02		0.03**
		0.01		0.02**
		0.02		NS
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )				

#### **4.4.3. Water soluble organic carbon**

Bioamendment addition showed marked influence on water soluble carbon (WSOC) of soil (Table 4.21). The concentration of WSOC ranged from 0.014 to 0.036 percent during the 60 days of incubation. Marked difference in WSOC was observed between the two moisture conditions. It was found higher in the soil under field capacity moisture than the soil under alternate wetting and drying condition. In general, irrespective of treatments WSOC was found increased during the 60 days of incubation under both moisture conditions. The bioamendments had a significant effect on WSOC. Initially, it was found maximum (0.029 per cent) in the soil amended with composted poultry manure (T<sub>3</sub>) and minimum (0.018 per cent) in control soil (T<sub>1</sub>). Under field capacity moisture, WSOC was significantly higher (0.036 per cent) in the soil amended with composted poultry manure (T<sub>3</sub>) and lower (0.016 per cent) in the control soil (T<sub>1</sub>) after 60 days of incubation. Under alternate wetting and drying, it was found relatively higher (0.033 per cent) in prosopis biochar amended soil (T<sub>5</sub>) and lower (0.014 per cent) in the control soil (T<sub>1</sub>) at final stage of incubation.

#### **4.4.4. Biotransformation and bioavailability of Ni in contaminated soil**

##### ***4.4.4.1. Water soluble Nickel***

Both the moisture conditions and bioamendments had significant effect on the concentration of water soluble Ni (Table 4.22). The concentration of water soluble Ni ranged from 0.35 to 13.02 mg kg<sup>-1</sup>. At 60<sup>th</sup> day of incubation, the concentration of water soluble Ni was found significantly higher in soil under field capacity moisture condition. But during the incubation, it was found high in soil under alternate wetting and drying. Under field capacity moisture, the concentration of water soluble Ni showed a gradual decrease over a period of time up to 45<sup>th</sup> day of incubation. After that, it was found slightly increased at 60<sup>th</sup> day of incubation. Initially, the concentration of water soluble Ni was found relatively higher (13.02 mg kg<sup>-1</sup>) in the soil amended with farmyard manure (T<sub>2</sub>) and low (11.53 mg kg<sup>-1</sup>) in the soil amended with prosopis biochar (T<sub>5</sub>). At the end of incubation the highest value (2.54 mg kg<sup>-1</sup>) was recorded in the soil amended with pressmud (T<sub>4</sub>) and lowest in the control soil (0.88 mg kg<sup>-1</sup>).

**Table 4.21. Effect of bioamendments on water soluble organic carbon (%) during incubation of Nickel contaminated soil under two different moisture conditions**

Treatments	Initial	Final		
		M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	0.018	0.016	0.014	<b>0.015</b>
T <sub>2</sub>	0.024	0.028	0.027	<b>0.028</b>
T <sub>3</sub>	0.029	0.036	0.032	<b>0.034</b>
T <sub>4</sub>	0.028	0.030	0.029	<b>0.030</b>
T <sub>5</sub>	0.027	0.028	0.033	<b>0.031</b>
<b>Mean</b>	<b>0.025</b>	<b>0.028</b>	<b>0.027</b>	<b>0.027</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>
		0.0003		0.0007**
		0.0002		0.0004**
		0.0005		0.0010**
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )				

**Table 4.22. Effect of bioamendments on water soluble fractions of Nickel (mg kg<sup>-1</sup>) during incubation of Ni contaminated soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day			45 <sup>th</sup> day			60 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	12.17	9.24	8.29	<b>8.77</b>	1.70	2.15	<b>1.93</b>	0.35	0.43	<b>0.39</b>	0.88	0.93	<b>0.91</b>
T <sub>2</sub>	13.02	9.65	12.36	<b>11.01</b>	1.99	2.21	<b>2.10</b>	0.67	0.70	<b>0.69</b>	1.24	1.43	<b>1.34</b>
T <sub>3</sub>	12.68	9.21	14.26	<b>11.74</b>	2.43	2.70	<b>2.57</b>	1.41	1.54	<b>1.48</b>	2.18	1.70	<b>1.94</b>
T <sub>4</sub>	12.32	8.81	18.76	<b>13.79</b>	3.23	3.20	<b>3.22</b>	1.67	1.66	<b>1.67</b>	2.54	1.97	<b>2.26</b>
T <sub>5</sub>	11.53	8.70	10.77	<b>9.74</b>	2.23	2.34	<b>2.29</b>	1.13	1.33	<b>1.23</b>	1.56	1.45	<b>1.51</b>
<b>Mean</b>	<b>12.34</b>	<b>9.12</b>	<b>12.89</b>	<b>11.01</b>	<b>2.32</b>	<b>2.52</b>	<b>2.42</b>	<b>1.05</b>	<b>1.13</b>	<b>1.09</b>	<b>1.68</b>	<b>1.50</b>	<b>1.59</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>	<b>CD (0.05)</b>		<b>SEd</b>	<b>CD (0.05)</b>		<b>SEd</b>	<b>CD (0.05)</b>		<b>SEd</b>	<b>CD (0.05)</b>	
		0.41	0.84**		0.19	0.40**		0.03	0.07**		0.13	0.27**	
		0.26	0.53**		0.12	NS		0.02	0.04**		0.08	NS	
		0.57	1.19**		0.27	NS		0.05	NS		0.18	NS	
<p>T<sub>1</sub>.Control - Soil* (No amendments); T<sub>2</sub>. Soil* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil*+ Composted poultry manure (5t ha<sup>-1</sup>)  T<sub>4</sub>. Soil*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil*+ Prosopis biochar (5t ha<sup>-1</sup>)</p>													

The concentration of water soluble Ni in soils under alternate wetting and drying condition showed a different trend. At first, the concentration was found increased from 0<sup>th</sup> day to 15<sup>th</sup> day in the treatments with composted poultry manure and pressmud compost. Thereafter, it gradually decreased up to 45<sup>th</sup> day and then showed an increasing trend at 60<sup>th</sup> day of incubation. The highest value for the water soluble Ni was recorded in the soil amended with pressmud compost (T<sub>4</sub>) of 1.97 mg kg<sup>-1</sup> and the lowest value was recorded in the control (T<sub>1</sub>) of 0.93 mg kg<sup>-1</sup> at 60<sup>th</sup> day of incubation.

#### ***4.4.4.2. Exchangeable Nickel***

During the incubation, concentration of exchangeable Ni ranged between 7.39 and 24.10 mg kg<sup>-1</sup>. In most of the stages of incubation, the concentration of exchangeable Ni was found higher in soil under field capacity moisture condition than in soil under alternate wetting and drying. Irrespective of moisture conditions, the concentration of exchangeable Ni was found decreased gradually from 0<sup>th</sup> day to 30<sup>th</sup> day of incubation and then it was found increased rapidly upto 60<sup>th</sup> day of incubation (Table 4.23).

The data showed that the addition of bioamendments had significant impact on exchangeable Ni. Initially, the highest concentration of exchangeable Ni was recorded in the soil (T<sub>3</sub>) amended with composted poultry manure (24.10 mg kg<sup>-1</sup>) and the lowest (17.74 mg kg<sup>-1</sup>) in the control soil (T<sub>1</sub>). Under field capacity moisture, the highest value was registered in the soil (T<sub>4</sub>) amended with pressmud compost (13.97 mg kg<sup>-1</sup>) and the lowest value was recorded in the control soil (12.18 mg kg<sup>-1</sup>). Under alternate wetting and drying condition, the highest concentration (12.31 mg kg<sup>-1</sup>) was observed in the pressmud compost amended soil (T<sub>4</sub>) and lowest (11.25 mg kg<sup>-1</sup>) in control soil (T<sub>1</sub>) after 60 days of incubation.

#### ***4.4.4.3. Organic Nickel***

There was a significant difference among the moisture conditions in influencing the organic Ni content of soil. The concentration of organic Ni ranged between 14.19 and 86.78 mg kg<sup>-1</sup>. The concentration of organic Ni was found higher in the soil under alternate wetting and drying than the soil under field capacity moisture condition. The concentration of organic Ni was found gradually increased from 0<sup>th</sup> day to 30<sup>th</sup> day in all treatments. After that, it showed a slight decrease at 45<sup>th</sup> day but increased significantly at 60<sup>th</sup> day of incubation under both moisture conditions (Table 4.24).

**Table 4.23. Effect of bioamendments on exchangeable fractions of Nickel ( $\text{mg kg}^{-1}$ ) during incubation of Ni contaminated soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day			45 <sup>th</sup> day			60 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	17.74	7.49	7.39	<b>7.44</b>	8.24	7.39	<b>7.82</b>	12.21	12.25	<b>12.23</b>	12.18	11.25	<b>11.72</b>
T <sub>2</sub>	21.05	9.16	8.12	<b>8.64</b>	9.24	8.96	<b>9.10</b>	12.72	13.04	<b>12.88</b>	12.53	11.75	<b>12.14</b>
T <sub>3</sub>	24.10	12.23	10.66	<b>11.45</b>	10.79	9.62	<b>10.21</b>	12.51	13.05	<b>12.78</b>	13.34	11.94	<b>12.64</b>
T <sub>4</sub>	20.05	11.38	10.03	<b>10.71</b>	10.45	10.01	<b>10.23</b>	12.69	13.27	<b>12.98</b>	13.97	12.31	<b>13.14</b>
T <sub>5</sub>	21.90	9.97	9.93	<b>9.95</b>	9.15	9.00	<b>9.08</b>	12.48	12.65	<b>12.57</b>	12.15	11.99	<b>12.07</b>
<b>Mean</b>	<b>20.97</b>	<b>10.05</b>	<b>9.23</b>	<b>9.64</b>	<b>9.57</b>	<b>9.00</b>	<b>9.29</b>	<b>12.52</b>	<b>12.85</b>	<b>12.69</b>	<b>12.83</b>	<b>11.85</b>	<b>12.34</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		0.28		0.58**	0.31		0.65**	0.12		0.26**	0.23		0.48**
		0.18		0.37**	0.20		0.41**	0.08		0.16**	0.15		0.30**
		0.39		NS	0.44		NS	0.17		NS	0.33		NS
<p>T<sub>1</sub>.Control - Soil* (No amendments); T<sub>2</sub>. Soil* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil*+ Composted poultry manure (5t ha<sup>-1</sup>)  T<sub>4</sub>. Soil*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil*+ Prosopis biochar (5t ha<sup>-1</sup>)</p>													

**Table 4.24. Effect of bioamendments on organic fractions of Ni (mg kg<sup>-1</sup>) during incubation of Nickel contaminated soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day			45 <sup>th</sup> day			60 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	15.87	22.80	34.77	<b>28.79</b>	31.27	30.31	<b>30.79</b>	19.55	21.33	<b>20.44</b>	25.58	26.28	<b>25.93</b>
T <sub>2</sub>	18.23	30.22	43.17	<b>36.70</b>	30.87	29.40	<b>30.14</b>	23.93	23.45	<b>23.69</b>	30.17	36.70	<b>33.44</b>
T <sub>3</sub>	14.19	29.16	38.65	<b>33.91</b>	35.73	35.49	<b>35.61</b>	25.92	25.55	<b>25.74</b>	56.90	64.86	<b>60.88</b>
T <sub>4</sub>	16.29	28.40	41.17	<b>34.79</b>	35.24	38.19	<b>36.72</b>	28.70	31.87	<b>30.29</b>	81.51	86.78	<b>84.15</b>
T <sub>5</sub>	15.35	28.85	35.64	<b>32.25</b>	31.46	34.14	<b>32.80</b>	25.43	23.96	<b>24.70</b>	52.37	57.71	<b>55.04</b>
<b>Mean</b>	<b>15.98</b>	<b>27.89</b>	<b>38.68</b>	<b>33.28</b>	<b>32.91</b>	<b>33.51</b>	<b>33.21</b>	<b>24.71</b>	<b>25.23</b>	<b>24.97</b>	<b>49.31</b>	<b>54.47</b>	<b>51.89</b>
<b>T M TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		0.54		1.13**	0.46		0.96**	0.90		1.87**	1.86		3.88**
		0.34		0.71**	0.29		NS	0.57		NS	1.18		2.45**
		0.76		1.60**	0.65		1.36**	1.27		NS	2.63		NS
<p>T<sub>1</sub>.Control - Soil* (No amendments); T<sub>2</sub>. Soil* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil*+ Composted poultry manure (5t ha<sup>-1</sup>)  T<sub>4</sub>. Soil*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil*+ Prosopis biochar (5t ha<sup>-1</sup>)</p>													

There was a marked changes in the concentration of organic Ni due to the application of bioamendments. Initially, the concentration of organic Ni was found higher in the soil (T<sub>2</sub>) with farmyard manure (18.23 mg kg<sup>-1</sup>) and lower in control soil (15.87mg kg<sup>-1</sup>). Under field capacity moisture, the concentration of organic Ni increased over a period of time and finally the highest value of 81.51 mg kg<sup>-1</sup> in the soil with pressmud compost (T<sub>4</sub>) and the lowest value of 25.58 mg kg<sup>-1</sup> in the control soil (T<sub>1</sub>) at 60<sup>th</sup> day. Under alternate wetting and drying, the highest concentration of organic Ni (86.78 mg kg<sup>-1</sup>) was obtained in the pressmud compost amended soil (T<sub>4</sub>) and lowest value (26.28 mg kg<sup>-1</sup>) in the control soil (T<sub>1</sub>) at final stage of incubation.

#### ***4.4.4.4. Organic plus iron oxide bound Nickel***

The concentration of organic plus iron oxide bound Ni ranged from 43.45 from 131.4 mg kg<sup>-1</sup> during the incubation. Consistently, it was found significantly higher in soil under alternate wetting and drying condition than in soil under field capacity moisture. The concentration of organic plus iron oxide Ni was found decreased and increased and did not follow any trend during 60 days of incubation (Table 4.25).

Different bioamendments had significant impact on the biotransformation of Ni in soil and thus influenced the concentration of organic plus iron oxide bound Ni. Initially, the organic plus iron oxide bound fraction of Ni was found higher (112.11 mg kg<sup>-1</sup>) in soil applied with pressmud (T<sub>4</sub>) and lower (97.79 mg kg<sup>-1</sup>) in the control soil. Finally it was found higher (97.56 mg kg<sup>-1</sup>) in the soil applied with composted poultry manure (T<sub>3</sub>) and lower (70.64 mg kg<sup>-1</sup>) in the control soil under field capacity moisture. In contrast, under alternate wetting and drying moisture, it was found higher (94.95 mg kg<sup>-1</sup>) in the soil amended with composted poultry manure (T<sub>3</sub>) and lower (63.45 mg kg<sup>-1</sup>) in the soil amended with the prosopis biochar (T<sub>5</sub>) after 60 days of incubation.

#### ***4.4.4.5. Residual Nickel***

There was marked changes in the concentration of residual Ni due to the two moisture conditions. The concentration of residual Ni ranged from 106.58 to 206.33 mg kg<sup>-1</sup> in the soil during incubation. The highest concentration of residual Ni was observed in the soil under field capacity moisture than the soil under alternate wetting and drying condition.

**Table 4.25. Effect of bioamendments on organic plus iron oxide fractions of Nickel (mg kg<sup>-1</sup>) during incubation of Ni contaminated soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day			45 <sup>th</sup> day			60 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	97.79	70.03	58.21	<b>64.12</b>	83.14	101.1	<b>92.12</b>	43.45	47.57	<b>45.51</b>	70.64	67.89	<b>69.27</b>
T <sub>2</sub>	110.26	80.98	70.21	<b>75.60</b>	91.06	110.3	<b>100.68</b>	56.09	59.23	<b>57.66</b>	89.12	80.69	<b>84.91</b>
T <sub>3</sub>	110.68	100.07	95.92	<b>98.00</b>	99.43	112.1	<b>105.77</b>	69.10	71.32	<b>70.21</b>	97.56	94.95	<b>96.26</b>
T <sub>4</sub>	112.11	88.12	81.80	<b>84.96</b>	104.25	131.4	<b>117.83</b>	68.29	75.42	<b>71.86</b>	90.25	85.33	<b>87.79</b>
T <sub>5</sub>	109.28	88.84	87.31	<b>88.08</b>	93.81	109.8	<b>101.81</b>	57.95	58.74	<b>58.35</b>	68.52	63.45	<b>65.99</b>
<b>Mean</b>	<b>108.02</b>	<b>85.61</b>	<b>78.69</b>	<b>82.15</b>	<b>94.34</b>	<b>112.94</b>	<b>103.64</b>	<b>58.98</b>	<b>62.46</b>	<b>60.72</b>	<b>83.22</b>	<b>78.46</b>	<b>80.84</b>
<b>T M TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		2.16		4.50**	2.25		4.70**	2.60		5.42**	5.50		11.46**
		1.36		2.90**	1.43		2.97**	1.64		NS	3.47		NS
		3.05		NS	3.19		NS	3.68		NS	7.77		NS
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ) T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )													

Irrespective of the moisture conditions, the concentration of residual Ni was found increased gradually from 0<sup>th</sup> day to 45<sup>th</sup> day and then found decreased at 60<sup>th</sup> day of incubation (Table 4.26).

The bioamendments addition brought about significant changes in the concentration of residual Ni. Initially, the highest value (132.67 mg kg<sup>-1</sup>) of residual Ni was obtained in the soil with pressmud compost application (T<sub>4</sub>) and the lowest value (116.83 mg kg<sup>-1</sup>) in control soil. At final stage of the incubation, the highest concentration of residual Ni (161.01 mg kg<sup>-1</sup>) was recorded in the prosopis biochar amended soil (T<sub>5</sub>) and the lowest (114.62 mg kg<sup>-1</sup>) in the soil amended with pressmud compost (T<sub>4</sub>) under field capacity moisture. But under alternate wetting and drying, the highest concentration of residual Ni (158.17 mg kg<sup>-1</sup>) was recorded in the prosopis biochar amended soil (T<sub>5</sub>) and lowest value (106.58 mg kg<sup>-1</sup>) recorded in the soil with pressmud compost (T<sub>4</sub>) after 60 days of incubation.

#### **4.5. EXPERIMENT IV – PHYTOEXTRACTION OF NICKEL FROM THE CONTAMINATED SOIL**

Two pot experiments were conducted to examine the effect of bioamendments on bioavailability and subsequent phytoextraction of Ni from soil by plants such as marigold and alfalfa. The soil used in this study was collected from the field located at Nanjudapuram check post, Coimbatore which is contaminated by Ni due to the effluent from electroplating industry and sewage water. The bioamendments such as farmyard manure, composted poultry manure, pressmud compost and prosopis biochar were added to the soil as per the treatment. The soil used in the pot experiment was clay soil and belongs to Kilayur series. The soil is classified taxonomically as *Typic Haplusterts* according to USDA classification. Some important physico - chemical characteristics of the soil were presented in Table 4.17.

##### **4.5.1. Pot experiment with Marigold**

###### **4.5.1.1. Soil pH**

Similar to the results of incubation experiments, the soil pH was changed significantly due to the application of bioamendments. The pH varied between 7.73 and 8.57 in soil with marigold (Table 4.27). The soil pH was found decreased gradually from 0<sup>th</sup> day to 60<sup>th</sup> day. The bioamendments had marked influence on soil pH.

**Table 4.26. Effect of bioamendments on residual fractions of Ni (mg kg<sup>-1</sup>) during incubation of Nickel contaminated soil under two different moisture conditions**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day			30 <sup>th</sup> day			45 <sup>th</sup> day			60 <sup>th</sup> day		
		M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean	M <sub>1</sub>	M <sub>2</sub>	Mean
T <sub>1</sub>	116.83	135.37	131.90	<b>133.64</b>	137.19	113.11	<b>125.15</b>	186.86	166.31	<b>176.59</b>	139.68	142.46	<b>141.07</b>
T <sub>2</sub>	121.05	160.47	152.38	<b>156.43</b>	155.36	137.64	<b>146.50</b>	197.76	193.82	<b>195.79</b>	150.28	151.24	<b>150.76</b>
T <sub>3</sub>	118.66	152.06	123.29	<b>137.68</b>	155.05	125.42	<b>140.24</b>	192.13	170.83	<b>181.48</b>	140.78	113.11	<b>126.95</b>
T <sub>4</sub>	132.67	162.12	136.13	<b>149.13</b>	141.75	107.28	<b>124.52</b>	186.90	166.19	<b>176.55</b>	114.62	106.58	<b>110.60</b>
T <sub>5</sub>	121.88	167.35	149.62	<b>158.49</b>	166.73	138.23	<b>152.48</b>	206.33	199.31	<b>202.82</b>	161.01	158.17	<b>159.59</b>
<b>Mean</b>	<b>122.22</b>	<b>155.47</b>	<b>138.66</b>	<b>147.07</b>	<b>151.22</b>	<b>124.34</b>	<b>137.78</b>	<b>194.00</b>	<b>179.29</b>	<b>186.64</b>	<b>141.27</b>	<b>134.31</b>	<b>137.79</b>
<b>T</b> <b>M</b> <b>TXM</b>		<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>	<b>SEd</b>		<b>CD (0.05)</b>
		0.71		1.49**	2.61		5.44**	1.43		2.99**	3.35		6.98**
		0.45		0.94**	1.65		3.44**	0.91		1.89**	2.12		4.42**
		1.01		2.10**	3.69		NS	2.02		4.22**	4.73		9.87**
<p>T<sub>1</sub>.Control - Soil* (No amendments); T<sub>2</sub>. Soil* + Farmacyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil*+ Composted poultry manure (5t ha<sup>-1</sup>)  T<sub>4</sub>. Soil*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil*+ Prosopis biochar (5t ha<sup>-1</sup>)</p>													

**Table 4.27. Effect of bioamendments on pH of soil with Marigold**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	8.40	8.27	8.23	8.18	8.17
T <sub>2</sub>	8.57	8.04	8.01	7.98	7.93
T <sub>3</sub>	8.51	8.18	8.14	8.07	8.03
T <sub>4</sub>	8.50	7.89	7.89	7.82	7.73
T <sub>5</sub>	8.53	8.12	8.04	7.94	7.86
<b>Mean</b>	<b>8.50</b>	<b>8.10</b>	<b>8.06</b>	<b>8.00</b>	<b>7.94</b>
<b>SEd</b>	0.07	0.07	0.02	0.13	0.08
<b>CD (0.05)</b>	NS	0.15**	0.05**	NS	0.17**
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )					

**Table 4.28. Effect of bioamendments on soil organic carbon (%) in pot experiment with Marigold**

Treatments	Initial	Final
T <sub>1</sub>	0.446	0.297
T <sub>2</sub>	0.525	0.347
T <sub>3</sub>	0.485	0.317
T <sub>4</sub>	0.455	0.356
T <sub>5</sub>	0.446	0.327
<b>Mean</b>	<b>0.471</b>	<b>0.329</b>
<b>SEd</b>	0.02	0.03
<b>CD (0.05)</b>	0.04**	NS
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )		

Initially, the soil pH was found maximum (8.57) in the treatment that had farmyard manure (T<sub>2</sub>) and minimum in the control soil (T<sub>1</sub>). Thereafter, a sudden decrease in pH at 15<sup>th</sup> day in soil amended with bioamendments. After that a gradual decrease was observed throughout the period of experiment. At final stage, a significantly higher pH (8.17) was observed in the control soil (T<sub>1</sub>) and lower pH (7.73) was obtained in the soil amended with pressmud compost (T<sub>4</sub>).

#### ***4.5.1.2. Soil organic carbon***

The SOC ranged from 0.297 to 0.525 percent during 60 days of growth of marigold (Table 4.28). The bioamendments have shown a marked influence on SOC. Initially, the SOC was found maximum (0.525 percent) in the soil amended with farmyard manure (T<sub>2</sub>) and minimum (0.446 percent) in the control soil (T<sub>1</sub>) followed by the soil amended with prosopis biochar (T<sub>5</sub>). However at later stage the maximum SOC was (0.356 percent) recorded in the soil amended with pressmud compost (T<sub>4</sub>) and minimum (0.297 percent) in the control soil (T<sub>1</sub>).

#### ***4.5.1.3. Water soluble organic carbon of soil***

The addition of bioamendment had significant effect on WSOC of soil (Table 4.29). The WSOC ranged from 0.012 to 0.039 percent in soil during pot experiment with marigold and in general WSOC was found increased with advancement of time. It was found maximum (0.030 per cent) in the soil amended with prosopis biochar (T<sub>5</sub>) and minimum (0.017 per cent) in control (T<sub>1</sub>) initially. At later stage, the maximum (0.039 per cent) value was recorded in the soil added with pressmud compost (T<sub>4</sub>) and minimum (0.012 per cent) in the control (T<sub>1</sub>).

#### ***4.5.1.4. Biotransformation and bioavailability of Nickel in soil***

##### ***4.5.1.4.1. Water soluble Nickel***

Remarkable changes were observed in the concentration of water soluble Ni due to the application of bioamendments. The concentration of water soluble Ni varied between 0.63 and 6.73 mg kg<sup>-1</sup>. The water soluble Ni was found slightly increased from 0<sup>th</sup> day to 15<sup>th</sup> day and thereafter, it was found decreased gradually upto 60<sup>th</sup> day of pot experiment (Table 4.30).

**Table 4.29. Effect of bioamendments on water soluble organic carbon (%) in soil with Marigold**

Treatments	Initial	Final
T <sub>1</sub>	0.017	0.012
T <sub>2</sub>	0.027	0.029
T <sub>3</sub>	0.029	0.035
T <sub>4</sub>	0.029	0.039
T <sub>5</sub>	0.030	0.037
<b>Mean</b>	<b>0.026</b>	<b>0.030</b>
<b>SEd</b>	0.0005	0.0006
<b>CD (0.05)</b>	0.0011**	0.0012**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Table 4.30. Effect of bioamendments on water soluble fraction of Nickel (mg kg<sup>-1</sup>) in soil with Marigold**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	4.43	4.84	1.82	0.63	0.66
T <sub>2</sub>	5.09	5.42	2.38	0.94	0.91
T <sub>3</sub>	5.31	6.08	3.48	2.16	1.91
T <sub>4</sub>	5.25	6.73	3.87	2.49	1.99
T <sub>5</sub>	4.92	5.21	2.77	1.12	1.38
<b>Mean</b>	<b>5.00</b>	<b>5.66</b>	<b>2.86</b>	<b>1.47</b>	<b>1.37</b>
<b>SEd</b>	0.47	0.22	0.32	0.06	0.09
<b>CD (0.05)</b>	NS	0.50**	0.72**	0.14**	0.20**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

Initially, the concentration of water soluble Ni was found significantly higher ( $5.31 \text{ mg kg}^{-1}$ ) in the soil amended with composted poultry manure ( $T_3$ ) and lower ( $4.43 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ). At final stage (60<sup>th</sup> day) the highest value ( $1.99 \text{ mg kg}^{-1}$ ) was recorded in the soil amended with pressmud compost ( $T_4$ ) and the lowest in the control ( $0.66 \text{ mg kg}^{-1}$ ).

#### 4.5.1.4.2. Exchangeable Nickel

The exchangeable Ni showed a marked changes as the concentrations ranged between  $6.97$  and  $15.18 \text{ mg kg}^{-1}$ . The concentration was found decreased gradually from 0<sup>th</sup> day to 30<sup>th</sup> day, and thereafter it was increased rapidly up to 60<sup>th</sup> day (Table 4.31). Initially the highest concentration of exchangeable Ni ( $8.27 \text{ mg kg}^{-1}$ ) was recorded in the soil amended with composted poultry manure ( $T_3$ ) and the lowest ( $7.94 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ). Whereas, at the final stage the highest value was registered in the treatment ( $T_4$ ) with pressmud compost ( $15.18 \text{ mg kg}^{-1}$ ) and the lowest value was recorded in the control ( $12.61 \text{ mg kg}^{-1}$ ).

#### 4.5.1.4.3. Organic Nickel

The organic Ni in soil ranged from  $15.52$  to  $103.13 \text{ mg kg}^{-1}$ . During the experimental period, the concentration of organic Ni was found gradually increased from 0<sup>th</sup> day to 30<sup>th</sup> day. After that, it showed a slight decrease at 45<sup>th</sup> day and a sudden increase at 60<sup>th</sup> day (Table 4.32). There was marked changes in the concentration of organic Ni due to the application of bioamendments. Initially, the concentration of organic Ni was found high in the treatment ( $T_5$ ) with prosopis biochar ( $20.46 \text{ mg kg}^{-1}$ ) and low in the control soil ( $15.52 \text{ mg kg}^{-1}$ ). The concentration of organic Ni was found increased over a period of time and finally the highest value of  $103.13 \text{ mg kg}^{-1}$  was recorded in the treatment that had pressmud compost ( $T_4$ ). The lowest value of  $26.50 \text{ mg kg}^{-1}$  was observed in the control soil ( $T_1$ ).

#### 4.5.1.4.4. Organic plus iron oxide bound Nickel

The organic plus iron oxide fraction of Ni was influenced significantly by the addition of different bioamendments. The concentration of organic plus iron oxide bound Ni ranged from  $50.66$  to  $128.61 \text{ mg kg}^{-1}$ , and it was decreased up to 30<sup>th</sup> day and thereafter after that increased slightly during 45<sup>th</sup> and 60<sup>th</sup> day of experiment with marigold (Table 4.33).

**Table 4.31. Effect of bioamendments on exchangeable fractions of Nickel (mg kg<sup>-1</sup>) in soil with Marigold**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	7.94	6.97	10.66	12.32	12.61
T <sub>2</sub>	8.10	7.22	11.10	12.42	13.07
T <sub>3</sub>	8.27	7.39	10.91	14.46	14.52
T <sub>4</sub>	7.86	7.73	10.90	15.17	15.18
T <sub>5</sub>	7.24	7.57	10.41	13.20	12.94
<b>Mean</b>	<b>7.88</b>	<b>7.38</b>	<b>10.80</b>	<b>13.51</b>	<b>13.66</b>
<b>SEd</b>	0.67	0.14	0.40	0.35	0.51
<b>CD (0.05)</b>	NS	0.32**	NS	0.78**	1.15**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Table 4.32. Effect of bioamendments on organic fractions of Nickel (mg kg<sup>-1</sup>) in soil with Marigold**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	15.52	16.98	22.67	18.59	26.50
T <sub>2</sub>	18.28	18.90	24.20	21.85	39.33
T <sub>3</sub>	17.43	19.07	28.32	22.65	77.49
T <sub>4</sub>	16.74	18.25	31.04	24.80	103.13
T <sub>5</sub>	20.46	18.70	25.90	21.26	49.34
<b>Mean</b>	<b>17.69</b>	<b>18.38</b>	<b>26.43</b>	<b>21.83</b>	<b>59.16</b>
<b>SEd</b>	1.98	0.66	0.69	2.32	4.59
<b>CD (0.05)</b>	NS	NS	1.54**	NS	10.23**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Table 4.33. Effect of bioamendments on organic plus iron oxide bound fractions of Nickel ( $\text{mg kg}^{-1}$ ) in soil with Marigold**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	97.92	65.86	50.66	64.95	72.83
T <sub>2</sub>	106.01	69.68	61.67	69.37	84.59
T <sub>3</sub>	119.36	77.84	79.27	80.66	96.74
T <sub>4</sub>	128.61	78.35	74.43	108.36	58.43
T <sub>5</sub>	111.76	72.69	67.21	74.45	82.64
<b>Mean</b>	<b>112.73</b>	<b>72.88</b>	<b>66.65</b>	<b>79.56</b>	<b>79.05</b>
<b>SEd</b>	11.83	2.14	1.90	3.84	10.16
<b>CD (0.05)</b>	NS	4.76**	4.24**	8.55**	22.63**
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )					

**Table 4.34. Effect of bioamendments on residual fractions of Nickel ( $\text{mg kg}^{-1}$ ) in soil with Marigold**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	114.12	175.84	185.54	170.82	142.29
T <sub>2</sub>	118.37	150.50	152.96	145.95	113.16
T <sub>3</sub>	117.18	123.72	107.77	110.11	35.41
T <sub>4</sub>	113.72	125.38	112.86	77.85	49.45
T <sub>5</sub>	121.95	159.76	157.94	148.67	115.48
<b>Mean</b>	<b>117.07</b>	<b>147.04</b>	<b>143.41</b>	<b>130.68</b>	<b>91.16</b>
<b>SEd</b>	5.96	2.39	2.67	2.89	4.33
<b>CD (0.05)</b>	NS	5.33**	5.95**	6.43**	9.65**
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )					

There was significant changes in organic plus iron oxide bound Ni in soil due to various treatments. During initial stage of the experiment, the organic plus iron oxide bound Ni was found high (128.61 mg kg<sup>-1</sup>) in soil amended with pressmud compost (T<sub>4</sub>) and low (97.92 mg kg<sup>-1</sup>) in the control soil. Finally it was high (96.74 mg kg<sup>-1</sup>) in the treatment that had composted poultry manure (T<sub>3</sub>) and low (58.43 mg kg<sup>-1</sup>) in the soil amended with pressmud compost (T<sub>4</sub>).

#### 4.5.1.4.5. Residual Nickel

The residual fraction of Ni in soil ranged from 35.41 to 185.54 mg kg<sup>-1</sup>. The concentration of residual Ni increased gradually from 0<sup>th</sup> day to 30<sup>th</sup> day and then decreased at 60<sup>th</sup> day in soil with marigold (Table 4.34).

Initially, the highest value (121.95 mg kg<sup>-1</sup>) of residual Ni was obtained in the treatment that had prosopis biochar (T<sub>5</sub>) and the lowest value (113.72 mg kg<sup>-1</sup>) was with the treatment that had pressmud compost. However, during the final stage of the experiment, the highest concentration of residual Ni (142.29 mg kg<sup>-1</sup>) was recorded in the control soil (T<sub>1</sub>) and the lowest residual Ni (35.41mg kg<sup>-1</sup>) in the composted poultry manure amended soil (T<sub>3</sub>).

#### **4.5.1.5. Nickel content and uptake by Marigold**

The Ni content in above ground biomass and below ground biomass of marigold is depicted in Table 4.35. The application of different bioamendments had significant influence on the Ni content of marigold. In the above ground biomass, the Ni content varied between 3.32 µg g<sup>-1</sup> and 103 µg g<sup>-1</sup>. While, the lowest Ni content was found in plants grown on control soil (T<sub>1</sub>) and the highest Ni content (103 µg g<sup>-1</sup>) was observed in plants that grown on soil amended with pressmud compost @ 5t ha<sup>-1</sup> (T<sub>4</sub>). All the bioamendments had significant influence on Ni content.

The application of different bioamendments had significant influence on the Ni content of marigold root biomass. The Ni content of root biomass showed differential pattern varying Ni content ranged from 48.9 to 101.6 µg g<sup>-1</sup>. The plants grown on soil amended with poultry manure was found to have lowest Ni content in root biomass whereas the highest Ni content in root biomass was observed in plants grown on soil amended with FYM @ 12.5t ha<sup>-1</sup>.

**Table 4.35. Nickel accumulation in Marigold**

Treatments	Ni content in above ground biomass ( $\mu\text{g g}^{-1}$ )	Ni content in root biomass ( $\mu\text{g g}^{-1}$ )	Biomass of Marigold (g)	Ni uptake ( $\mu\text{g/plant}$ )
T <sub>1</sub>	3.31	84.1	7.17	635
T <sub>2</sub>	5.71	101.6	8.25	883
T <sub>3</sub>	79.1	48.9	11.26	1441
T <sub>4</sub>	103	54.9	12.37	1948
T <sub>5</sub>	24.9	87.6	9.21	1037
<b>Mean</b>	<b>43.1</b>	<b>75.4</b>	<b>9.65</b>	<b>1189</b>
<b>SEd</b>	7.18	9.16	0.59	110.15
<b>CD (0.05)</b>	16.00**	20.40**	1.33**	245.41**
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )				

**Table 4.36. Effect of bioamendments on pH of soil with Alfalfa**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	8.49	8.26	8.21	8.16	8.13
T <sub>2</sub>	8.55	8.05	7.97	7.90	7.83
T <sub>3</sub>	8.44	7.91	7.85	7.80	7.74
T <sub>4</sub>	8.53	7.97	7.91	7.88	7.84
T <sub>5</sub>	8.55	8.04	7.98	7.95	7.92
<b>Mean</b>	<b>8.51</b>	<b>8.05</b>	<b>7.98</b>	<b>7.94</b>	<b>7.89</b>
<b>SEd</b>	0.05	0.04	0.03	0.04	0.14
<b>CD (0.05)</b>	NS	0.10**	0.06**	0.08**	NS
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )					

The application of different bioamendments had significant influence on the biomass of marigold. The biomass of marigold ranged from 7.17 g to 12.37 g. The plants grown on soil amended with pressmud compost (T<sub>4</sub>) was found to have highest biomass (12.37 g) followed by composted poultry manure (T<sub>3</sub>) whereas the lowest biomass (7.17 g) was recorded in plants grown on control soil (T<sub>1</sub>).

The application of different bioamendments had significant influence on the uptake of Ni by marigold. The Ni uptake by marigold varied between 635 µg plant<sup>-1</sup> and 1948 µg plant<sup>-1</sup>. The plants grown on control soil recorded the lowest uptake whereas the plants grown on the soil amended with pressmud compost recorded the highest uptake of Ni.

#### **4.5.2. Pot experiment with Alfalfa**

##### ***4.5.2.1. Soil pH***

Similar to the results of incubation experiments, the soil pH was changed significantly due to the application of bioamendments. The pH varied between 7.74 and 8.55 in soil with alfalfa (Table 4.36). The soil pH was found decreased gradually from 0<sup>th</sup> day to 60<sup>th</sup> day. The bioamendments had marked influence on soil pH.

Initially, the soil pH was found maximum (8.55) in the treatment that had farmyard manure (T<sub>2</sub>) followed by prosopis biochar and minimum (8.44) in the composted poultry manure amended soil (T<sub>3</sub>). Thereafter, a sudden decrease in pH at 15<sup>th</sup> day in soil amended with bioamendments. After that a gradual decrease was observed throughout the period of experiment. At final stage, a significantly higher pH (8.13) was observed in the control soil (T<sub>1</sub>) and lower pH (7.74) was obtained in the soil amended with composted poultry manure (T<sub>3</sub>).

##### ***4.5.2.2. Soil organic carbon***

The SOC ranged from 0.317 to 0.541 percent during 60 days of growth of alfalfa (Table 4.37). The bioamendments have shown a marked influence on SOC. Initially, the SOC was found maximum (0.541 percent) in the soil amended with farmyard manure (T<sub>2</sub>) and minimum (0.436 percent) in the control soil (T<sub>1</sub>) followed by the soil amended with prosopis biochar (T<sub>5</sub>). However at later stage the maximum SOC was (0.386 percent) recorded in the soil amended with pressmud compost (T<sub>4</sub>) and minimum (0.317 percent) in the control soil (T<sub>1</sub>).

**Table 4.37. Effect of bioamendments on soil organic carbon (%) in pot experiment with Alfalfa**

<b>Treatments</b>	<b>Initial</b>	<b>Final</b>
T <sub>1</sub>	0.436	0.317
T <sub>2</sub>	0.541	0.376
T <sub>3</sub>	0.460	0.347
T <sub>4</sub>	0.462	0.386
T <sub>5</sub>	0.439	0.337
<b>Mean</b>	<b>0.468</b>	<b>0.353</b>
<b>SEd</b>	0.01	0.02
<b>CD (0.05)</b>	0.03**	0.04**
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )		

**Table 4.38. Effect of bioamendments on water soluble organic carbon (%) in soil with Alfalfa**

<b>Treatments</b>	<b>Initial</b>	<b>Final</b>
T <sub>1</sub>	0.014	0.017
T <sub>2</sub>	0.025	0.032
T <sub>3</sub>	0.028	0.029
T <sub>4</sub>	0.029	0.035
T <sub>5</sub>	0.028	0.036
<b>Mean</b>	<b>0.025</b>	<b>0.030</b>
<b>SEd</b>	0.0007	0.0006
<b>CD (0.05)</b>	0.0015**	0.0014**
T <sub>1</sub> .Control - Soil* (No amendments); T <sub>2</sub> . Soil* + Farmyard manure (12.5t ha <sup>-1</sup> ); T <sub>3</sub> . Soil*+ Composted poultry manure (5t ha <sup>-1</sup> ); T <sub>4</sub> . Soil*+ Pressmud compost (5t ha <sup>-1</sup> ); T <sub>5</sub> . Soil*+ Prosopis biochar (5t ha <sup>-1</sup> )		

#### ***4.5.2.3. Water soluble organic carbon of soil***

The addition of bioamendment had significant effect on WSOC of soil (Table 4.38). The WSOC ranged from 0.014 to 0.036 percent in soil during pot experiment with alfalfa and in general WSOC was found increased with advancement of time. It was found maximum (0.029 per cent) in the soil amended with pressmud compost (T<sub>4</sub>) and minimum (0.014 per cent) in control (T<sub>1</sub>) initially. At later stage, the maximum (0.036 per cent) value was recorded in the soil added with prosopis biochar (T<sub>5</sub>) and minimum (0.017 per cent) in the control (T<sub>1</sub>).

#### ***4.5.2.4. Bioavailability and biotransformation of Nickel in soil***

##### ***4.5.2.4.1. Water soluble Nickel***

Remarkable changes were observed in the concentration of water soluble Ni due to the application of bioamendments. The concentration of water soluble Ni varied between 0.75 and 6.78 mg kg<sup>-1</sup>. The concentration of water soluble Ni was found decreased gradually from 0<sup>th</sup> day to 60<sup>th</sup> day (Table 4.39).

Initially, the concentration of water soluble Ni was found significantly higher (6.78 mg kg<sup>-1</sup>) in the soil amended with composted poultry manure (T<sub>3</sub>) and lower (6.08 mg kg<sup>-1</sup>) in the soil amended with prosopis biochar (T<sub>5</sub>). At final stage (60<sup>th</sup> day) the highest value ((2.38 mg kg<sup>-1</sup>) was recorded in the soil amended with pressmud compost (T<sub>4</sub>) and the lowest in the control (0.75 mg kg<sup>-1</sup>).

##### ***4.5.2.4.2. Exchangeable Nickel***

The exchangeable Ni showed a marked changes as the concentrations ranged between 6.84 and 15.97 mg kg<sup>-1</sup>. The concentration was found decreased gradually from 0<sup>th</sup> day to 15<sup>th</sup> day, and thereafter it was increased rapidly up to 60<sup>th</sup> day (Table 4.40). Initially the highest concentration of exchangeable Ni (10.46 mg kg<sup>-1</sup>) was recorded in the soil amended with composted poultry manure (T<sub>3</sub>) and the lowest (9.06 mg kg<sup>-1</sup>) in the control soil (T<sub>1</sub>). Whereas, at the final stage the highest value was registered in the treatment (T<sub>4</sub>) with pressmud compost (15.97 mg kg<sup>-1</sup>) and the lowest value was recorded in the control (12.86 mg kg<sup>-1</sup>).

**Table 4.39. Effect of bioamendments on water soluble fraction of Nickel (mg kg<sup>-1</sup>) in soil with Alfalfa**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	6.16	4.16	1.78	0.77	0.75
T <sub>2</sub>	6.41	4.90	2.37	1.46	0.90
T <sub>3</sub>	6.78	5.39	2.76	2.38	2.13
T <sub>4</sub>	6.44	5.87	3.28	2.51	2.38
T <sub>5</sub>	6.08	4.93	2.11	1.24	1.61
<b>Mean</b>	<b>6.37</b>	<b>5.05</b>	<b>2.46</b>	<b>1.67</b>	<b>1.55</b>
<b>SEd</b>	1.23	0.10	0.10	0.30	0.10
<b>CD (0.05)</b>	2.75**	0.20**	0.23**	0.67**	0.22**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Table 4.40. Effect of bioamendments on exchangeable fractions of Nickel (mg kg<sup>-1</sup>) in soil with Alfalfa**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	9.06	6.84	8.95	12.60	12.86
T <sub>2</sub>	9.51	7.62	10.46	12.54	14.58
T <sub>3</sub>	10.46	7.40	11.81	12.51	14.97
T <sub>4</sub>	10.05	7.61	12.37	13.62	15.97
T <sub>5</sub>	9.44	7.05	11.98	13.32	12.73
<b>Mean</b>	<b>9.70</b>	<b>7.30</b>	<b>11.11</b>	<b>12.92</b>	<b>14.22</b>
<b>SEd</b>	1.94	0.13	0.65	0.37	0.43
<b>CD (0.05)</b>	NS	0.30**	1.46**	0.82**	0.95**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

#### 4.5.2.4.3. Organic Nickel

The organic Ni in soil ranged from 16.41 and 100.57 mg kg<sup>-1</sup>. During the experimental period, the concentration of organic Ni was found gradually increased from 0<sup>th</sup> day to 30<sup>th</sup> day. After that, it showed a slight decrease at 45<sup>th</sup> day and a sudden increase at 60<sup>th</sup> day (Table 4.41). There was marked changes in the concentration of organic Ni due to the application of bioamendments. Initially, the concentration of organic Ni was found high in the treatment (T<sub>5</sub>) with composted poultry manure (21.08 mg kg<sup>-1</sup>) and low in the control soil (16.41mg kg<sup>-1</sup>). The concentration of organic Ni was found increased over a period of time and finally the highest value of 100.57 mg kg<sup>-1</sup> was recorded in the treatment that had pressmud compost (T<sub>4</sub>). The lowest value of 33.59 mg kg<sup>-1</sup> was observed in the control soil (T<sub>1</sub>).

#### 4.5.2.4.4. Organic plus iron oxide bound Nickel

The organic plus iron oxide fraction of Ni was influenced significantly by the addition of different bioamendments. The concentration of organic plus iron oxide bound Ni ranged from 62.77 to 106.11 mg kg<sup>-1</sup>, and it was decreased up to 15<sup>th</sup> day and thereafter after that increased slightly during 45<sup>th</sup> and 60<sup>th</sup> day of experiment with alfalfa (Table 4.42).

There was significant changes in organic plus iron oxide bound Ni in soil due to various treatments. During initial stage of the experiment, the organic plus iron oxide bound Ni was found high (102.06 mg kg<sup>-1</sup>) in soil amended with composted poultry manure (T<sub>3</sub>) and low (95.78 mg kg<sup>-1</sup>) in the soil amended with prosopis biochar. Finally it was high (95.75 mg kg<sup>-1</sup>) in the treatment that had composted poultry manure (T<sub>3</sub>) and low (70.53 mg kg<sup>-1</sup>) in the control soil.

#### 4.5.2.4.5. Residual Nickel

The residual fraction of Ni in soil ranged from 8.43 to 199.24 mg kg<sup>-1</sup>. The concentration of residual Ni increased gradually from 0<sup>th</sup> day to 15<sup>th</sup> day and then decreased at 60<sup>th</sup> day in soil with alfalfa (Table 4.43). Initially, the highest value (128.80 mg kg<sup>-1</sup>) of residual Ni was obtained in the treatment that had composted poultry manure (T<sub>3</sub>) and the lowest value (105.17 mg kg<sup>-1</sup>) in the control soil. However, during the

**Table 4.41. Effect of bioamendments on organic fraction of Nickel ( $\text{mg kg}^{-1}$ ) in soil with Alfalfa**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	16.41	17.33	24.02	23.05	33.59
T <sub>2</sub>	19.17	18.54	28.03	24.25	40.61
T <sub>3</sub>	21.08	20.08	28.63	29.77	89.93
T <sub>4</sub>	18.60	20.49	36.16	30.00	100.57
T <sub>5</sub>	17.27	18.97	26.81	23.74	61.61
<b>Mean</b>	<b>18.51</b>	<b>19.08</b>	<b>28.73</b>	<b>26.16</b>	<b>65.26</b>
<b>SEd</b>	2.14	0.57	0.81	2.02	2.68
<b>CD (0.05)</b>	NS	1.26**	1.81**	4.51**	5.96**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Table 4.42. Effect of bioamendments on organic plus iron oxide bound fractions of Nickel ( $\text{mg kg}^{-1}$ ) in soil with Alfalfa**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	96.77	62.77	44.18	65.27	70.53
T <sub>2</sub>	100.28	68.10	85.20	62.27	84.44
T <sub>3</sub>	102.06	74.86	83.78	82.88	95.75
T <sub>4</sub>	99.07	78.04	89.18	106.11	87.73
T <sub>5</sub>	95.78	69.55	83.82	74.73	77.77
<b>Mean</b>	<b>98.79</b>	<b>70.66</b>	<b>77.23</b>	<b>78.25</b>	<b>83.24</b>
<b>SEd</b>	12.89	1.39	2.72	5.41	6.98
<b>CD (0.05)</b>	NS	3.09**	6.06**	12.06**	15.56**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Table 4.43. Effect of bioamendments on residual fractions of Nickel (mg kg<sup>-1</sup>) in soil with Alfalfa**

Treatments	0 <sup>th</sup> day	15 <sup>th</sup> day	30 <sup>th</sup> day	45 <sup>th</sup> day	60 <sup>th</sup> day
T <sub>1</sub>	105.17	187.29	199.24	175.33	128.66
T <sub>2</sub>	121.23	156.25	129.66	149.11	99.36
T <sub>3</sub>	128.80	133.94	115.73	102.93	17.92
T <sub>4</sub>	124.33	129.88	93.61	80.71	8.43
T <sub>5</sub>	127.66	166.89	138.61	151.25	94.73
<b>Mean</b>	<b>121.44</b>	<b>154.85</b>	<b>135.37</b>	<b>131.87</b>	<b>69.82</b>
<b>SEd</b>	10.09	1.22	4.13	3.88	1.85
<b>CD (0.05)</b>	NS	2.72**	9.21**	8.64**	4.12**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Table 4.44. Nickel accumulation in Alfalfa**

Treatments	Ni content in above ground biomass (µg g <sup>-1</sup> )	Ni content in root biomass (µg g <sup>-1</sup> )	Biomass of Alfalfa (g)	Ni uptake (µg/plant)
T <sub>1</sub>	8.23	125.3	1.13	150
T <sub>2</sub>	43.3	109.5	1.54	234
T <sub>3</sub>	114.3	63.6	1.85	328
T <sub>4</sub>	147.0	51.7	2.00	393
T <sub>5</sub>	14.90	131.7	1.90	275
<b>Mean</b>	<b>65.6</b>	<b>96.4</b>	<b>1.68</b>	<b>276</b>
<b>SEd</b>	3.57	6.52	0.064	13.28
<b>CD (0.05)</b>	7.95**	14.53**	0.144**	29.59**

T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

final stage of the experiment, the highest concentration of residual Ni ( $128.66 \text{ mg kg}^{-1}$ ) was recorded in the control soil ( $T_1$ ) and the lowest residual Ni ( $8.43 \text{ mg kg}^{-1}$ ) in the pressmud compost amended soil ( $T_4$ ).

#### ***4.5.2.5. Nickel content and uptake by Alfalfa***

The Ni content in above ground biomass and below ground biomass of alfalfa is depicted in Table 4.44. The application of different bioamendments had significant influence on the Ni content of alfalfa. In the above ground biomass, the Ni content varied between  $8.23 \text{ } \mu\text{g g}^{-1}$  and  $147 \text{ } \mu\text{g g}^{-1}$ . While, the lowest Ni content was found in plants grown on control soil ( $T_1$ ) and the highest Ni content ( $147 \text{ } \mu\text{g g}^{-1}$ ) was observed in plants that grown on soil amended with pressmud compost @  $5 \text{ t ha}^{-1}$  ( $T_4$ ). All the bioamendments had significant influence on Ni content.

The application of different bioamendments had significant influence on the Ni content of alfalfa root biomass. The Ni content of root biomass showed differential pattern varying Ni content ranged from  $51.67$  to  $131.67 \text{ } \mu\text{g g}^{-1}$ . The plants grown on soil amended with poultry manure was found to have lowest Ni content in root biomass whereas the highest Ni content in root biomass was observed in plants grown on soil amended with prosopis biochar @  $5 \text{ t ha}^{-1}$ .

The application of different bioamendments had significant influence on the biomass of alfalfa. The biomass of alfalfa ranged from  $1.13 \text{ g}$  to  $2.0 \text{ g}$ . The plant grown on soil amended with pressmud compost ( $T_4$ ) was found to have highest biomass ( $2.0 \text{ g}$ ) followed by prosopis biochar ( $T_5$ ) whereas the lowest biomass ( $1.13 \text{ g}$ ) was recorded in plants grown on control soil ( $T_1$ ).

The application of different bioamendments had significant influence on the uptake of Ni by alfalfa. The Ni uptake by alfalfa varied between  $150 \text{ } \mu\text{g plant}^{-1}$  and  $393 \text{ } \mu\text{g plant}^{-1}$ . The plants grown on control soil recorded the lowest uptake whereas the plants grown on the soil amended with pressmud compost recorded the highest uptake of Ni.

## *Discussion*

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## **CHAPTER V**

### **DISCUSSION**

Nickel (Ni) is a heavy metal that generally exists in the environment at very low levels (0.5-25 mg kg<sup>-1</sup>). It is considered as a micronutrient for some crops, but may become toxic to plants, animals and humans if normal levels are exceeded. High input of Ni to soil may arise from land disposal of industrial wastes and from sewage sludge. Once added to the soil, Ni accumulates and enters into the food chain. Thus, it produces number of health effects including cancer in animal and humans. Therefore, the study of Ni behavior, fate in the environment and bioavailability has gained importance to predict the toxicity effects of Ni. It also helps to develop remediation strategy for Ni contaminated soil. In the current study, adsorption/desorption, biotransformation and bioavailability of Ni in soil and subsequent phytoextraction by plants were studied by conducting a series of laboratory and pot experiments. Based on the results, a phytoremediation strategy has been suggested for Ni-contaminated soil. The results obtained from these experiments are discussed in this chapter.

#### **5.1. ADSORPTION AND DESORPTION OF NICKEL**

Characterizing the factors affecting bioavailability, leaching and toxicity of Ni in soil is of paramount importance. The mobility and availability of the metals are controlled by many chemical and biochemical processes such as sorption-desorption, precipitation-dissolution, complexation-dissociation, and oxidation-reduction. Although all these reactions can occur simultaneously, adsorption is the single most important mechanism which determines the metal availability and solubility in the soil and thus environmental impacts (Sparks, 1999).

Adsorption refers to the attachment or accumulation of a material at the solution-surface interface (Sparks, 2003). Charged solute species (ions) are attracted to the charged soil surface by electrostatic attraction and/or through the formation of specific bonds (Mott, 1981). Retention of charged solutes by charged surfaces is broadly grouped into specific and non-specific retention (Bolan *et al.*, 1999). In general terms, nonspecific adsorption is a process in which the charge on the ions balances the charge on the soil particles through electrostatic attraction; whereas specific adsorption involves chemical bond

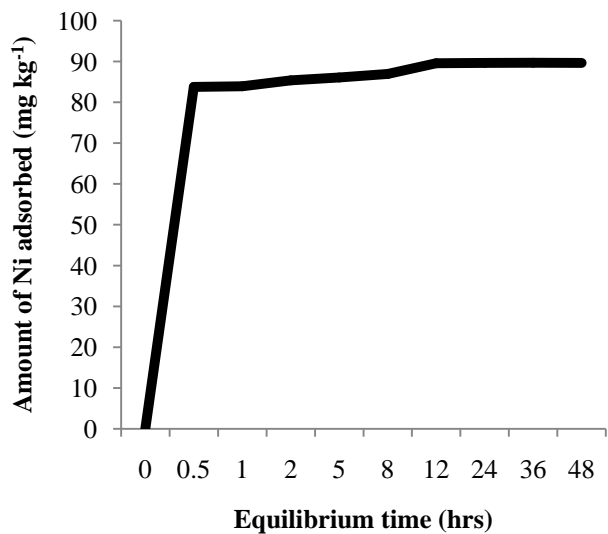
formation between the ions in the solution and those in the soil surface (Sposito, 1984). Ni is retained soil primarily by specific sorption mechanism at low concentrations (Adriano, 2001). Both soil properties and soil solution composition determine the dynamic equilibrium between metals in solution and the soil solid phase. Number of soil chemical characteristics such as pH, CEC, organic matter content and co-contaminants or competitive ions present in the soil significantly influence the adsorption of Ni in soil. Therefore, the adsorption and desorption character of Ni were studied by conducting a series of laboratory batch experiments. The impact of pH, soil organic matter (SOM) and co-contaminants present in soil were also studied.

The data showed that the adsorption of Ni on soil continued up to 48 hrs, although more than 89 per cent occurred within 12 hrs (Fig. 5.1). The adsorption was found rapid initially (>80% within 30 minutes) and continued afterwards at a slow rate up to 48 hrs. An initial rapid process followed by a slower and finally a steady-state condition generally characterized the Ni sorption patterns. The zero-, first-, second- and third order reactions, the parabolic diffusion and the Elovich equation were tested for suitability to describe Ni sorption pattern in soil.

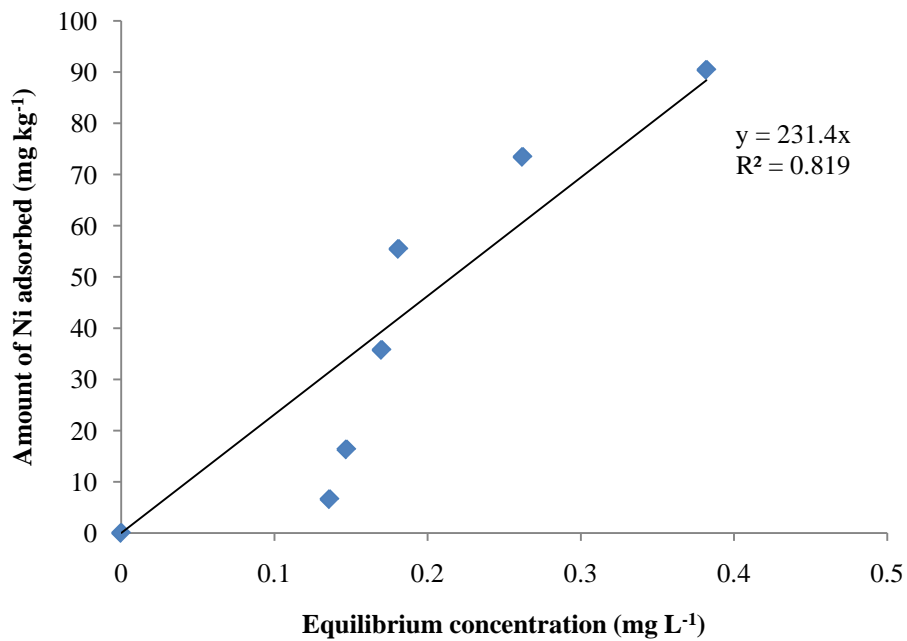
The amount of Ni sorbed by the soil particles was poorly described by the zero-, first-, and second order reactions. The third order, parabolic diffusion and Elovich equations described Ni sorption much better than did the zero-, first-, and second order reactions (Table 5.1). The relatively highest  $R^2$  over the whole time range indicated that the Ni sorption in this soil was better described by third order reaction kinetics ( $R^2=0.998$ ) followed by Elovich equation ( $R^2=0.922$ ) and parabolic diffusion ( $R^2=0.829$ ). Similar, observation was reported by Mahimairaja and Bolan (2009) for arsenic adsorption.

### **5.1.1. Adsorption isotherms**

The sorption data for Ni were fitted in both Langmuir and Freundlich equations. A relatively higher correlation coefficient ( $R^2= 0.72$ ) indicated that the Ni sorption was adequately described by the Langmuir isotherm than did Freundlich equation (Table 5.2). The adsorption isotherm was depicted in Fig. 5.2. The adsorption and retention of Ni by soil determine its persistence, reactions, movement, transformation and ecological effects



**Fig. 5.1 Determination of equilibrium period**



**Fig. 5.2 Nickel adsorption isotherm**

**Table 5.1. Coefficients of determination ( $R^2$ ) and standard error of estimate (SE) of the various kinetics equations used for Ni**

<b>Equations</b>	<b><math>R^2</math></b>	<b>SE</b>
Zero-order reaction	0.658	1.58
First-order reaction	0.655	93.86
Second-order reaction	0.652	98.90
Third-order reaction	0.998	98.91
Parabolic diffusion	0.829	21.71
Elovich -type	0.922	34.21

**Table 5.2. Langmuir and Freundlich equations describing the adsorption of Ni in soil**

<b>Equation</b>	<b>Isotherm</b>	<b><math>R^2</math> value</b>
Langmuir	$Y = 1566.88C / 1 + 191.2C$	0.72
Freundlich	$x/m = 191.2C_e^{8.195}$	0.39

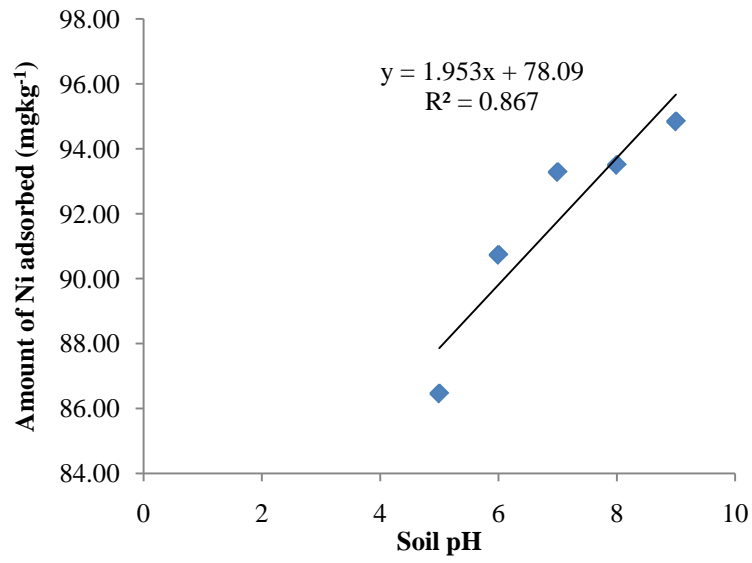
(Mahimairaja and Bolan, 2009). As in case of other metals and metalloids, one of the most commonly reported and the first reaction to occur in soil is adsorption on to soil particles. Adsorption of Ni has been found to correlate with soil properties such as soil particle size, organic matter, type and nature of constituent minerals, pH, redox potential and competing ions (Bolan *et al.*, 1999). According to Adhami *et al.* (2008) an adsorption adequately explained by Langmuir adsorption isotherm of Ni was correlated with CEC of the soil. Further pH, CEC, clay and organic matter content were the main factors which regulate retention of Ni in soils.

### **5.1.2. Effect of pH**

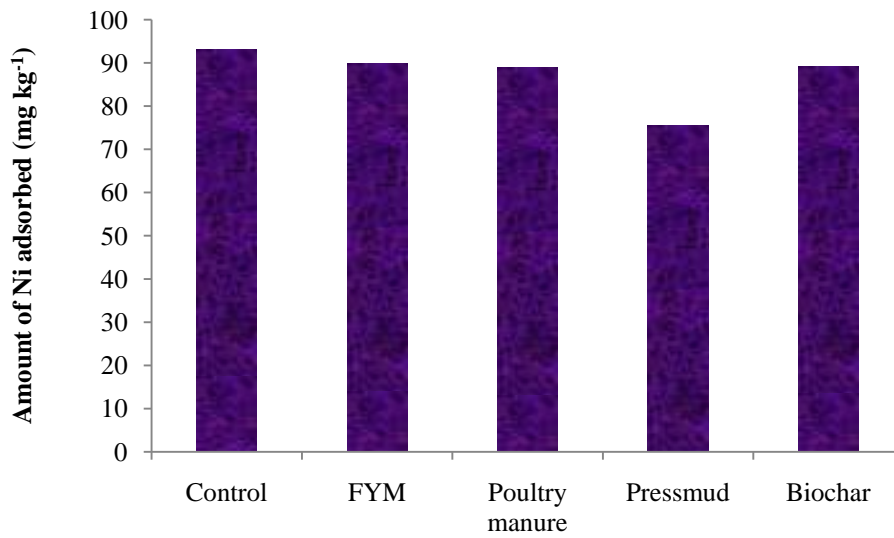
The pH of the soil is the single most determinant that affects several biochemical processes in soil. It has been found that the pHs of soil play a dominant role in adsorption process. The amount of Ni adsorbed at different pH ranged from 86.46 to 94.84 mg kg<sup>-1</sup>. With an increase in pH, the Ni adsorption was found increased significantly (Fig.5.3). The highest amount of Ni adsorbed on soil was observed when the pH was the highest (9.0). The adsorption rate of Ni was found increased with increasing pH which could be due to the presence of hydroxyl ions in the high pH soil. The enhancement in Ni adsorption at high pH is attributed to an increase in negative surface charge density, precipitation of hydroxide and formation of hydroxyl species that led to an increased concentration of NiOH<sup>+</sup> species in soil (Adriano, 2001; Sen Gupta and Bhattacharyya, 2006). The formation of multi-nuclear metal hydroxyl species in solution is the precursor to formation of metal-hydroxy precipitates from a solution (Park *et al.*, 2011). At high pH level, the presence of carbonates may also lead to an increase in the retention of Ni as carbonate salt in soil (Ramachandran and D'Souza, 2013).

### **5.1.3. Effect of bioamendments**

The effect of bioamendments on Ni adsorption on soil was examined. Different bioamendments such as farmyard manure, composted poultry manure, pressmud compost and prosopis biochar at the rate of 0.5g g<sup>-1</sup> were added to soil along a the single input concentration of Ni (100 mg kg<sup>-1</sup>). The highest amount (93.18 mg kg<sup>-1</sup>) of Ni adsorbed on soil was observed in soil with no addition of bioamendments (control treatment). The addition of bioamendments was found to reduce the Ni adsorption on soil (Fig. 5.4).



**Fig. 5.3 Effect of pH on Nickel adsorption**



**Fig. 5.4 Effect of bioamendments on Nickel adsorption**

The lowest adsorption ( $75.43 \text{ mg kg}^{-1}$ ) of Ni was recorded with the application of pressmud compost followed by composted poultry manure ( $88.91 \text{ mg kg}^{-1}$ ). The bioamendments significantly reduced the adsorption of Ni on soil which may be attributed to the formation of organic complexes with metals or the release of organic acids from bioamendments that might have reduced the negative charges present in soil by protonation reaction. The organic matter provides sites for cation exchange, but its strong affinity to metals is due to the presence of specific binders or groups that form metal complexes or chelates. Nickel, as well as other heavy metals, forms complexes with several soil organic constituents (Mellis *et al.*, 2004). An important aspect of metal-organic matter interactions is the formation of multi-dentate complexes, thereby increasing the stability of the bond (Park *et al.*, 2011) Nevertheless, these complex formations occur in great part with the fulvic and humic acids, present in the organic matter in large quantities, the insoluble combinations being associated with the humic acid and the soluble ones, with the fulvic acid (Stevenson, 1994). In many soil types, the metal and organic matter forms metal chelate. It greatly enhances the mobility of heavy metal and reduces the adsorption (Bradl, 2004). The difference in adsorption of Ni might also be due to the differential characteristics of bioamendments used. The difference in organic matter content, pH and CEC of bioamendments might have resulted in significant variation in the Ni adsorption on soil. The pHs also played an important role in the adsorption of Ni in the presence of bioamendments. According to Mellis *et al.* (2004) for pH above 6.5, the effect of organic matter on Ni adsorption was not significant, probably because greater part of the negative, dependent pH changes would have the  $\text{H}^+$  already dissociated.

#### **5.1.4. Effect of co-contaminants or competitive ions**

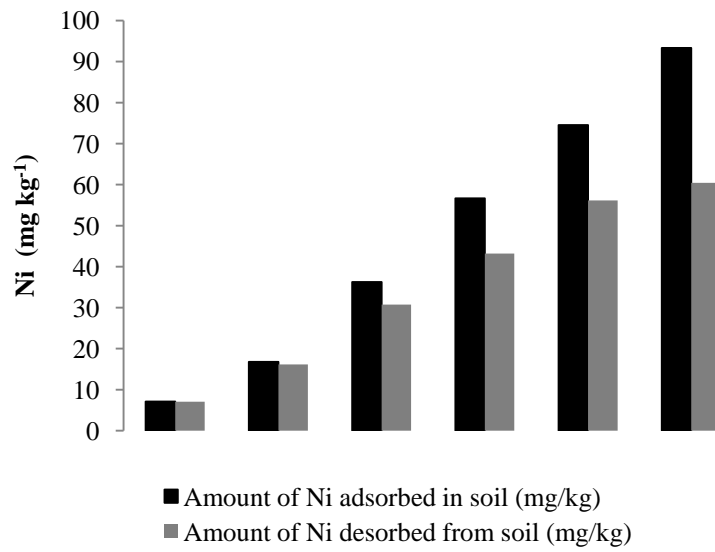
The presence of different co-contaminants or competitive ions significantly affects the adsorption of Ni on soil. To study the influence of co-contaminants on Ni adsorption, a single input concentration of Cd and Cr (each at  $100 \text{ mg kg}^{-1}$ ) was used along with various concentration of Ni that ranged from 0 to  $100 \text{ mg kg}^{-1}$ . The Ni adsorption was found decreased significantly due to the presence of Cd even at its low concentration. The decrease in adsorption capacity of Ni under competitive condition may be attributed to the occupation of some sorption sites by Cd as the ionic size ( $97 \text{ pm}$ )

is relatively greater than Ni (69 pm). In contrast, the Ni adsorption was found increased significantly in the presence of Cr as a co-contaminant which may be attributed to the greater ionic size of Ni than Cr (52 pm). It has been reported that the sorption capacities of each metal in binary system and multi metal systems were lower than that of single metal system (Chotpantararat *et al.*, 2011).

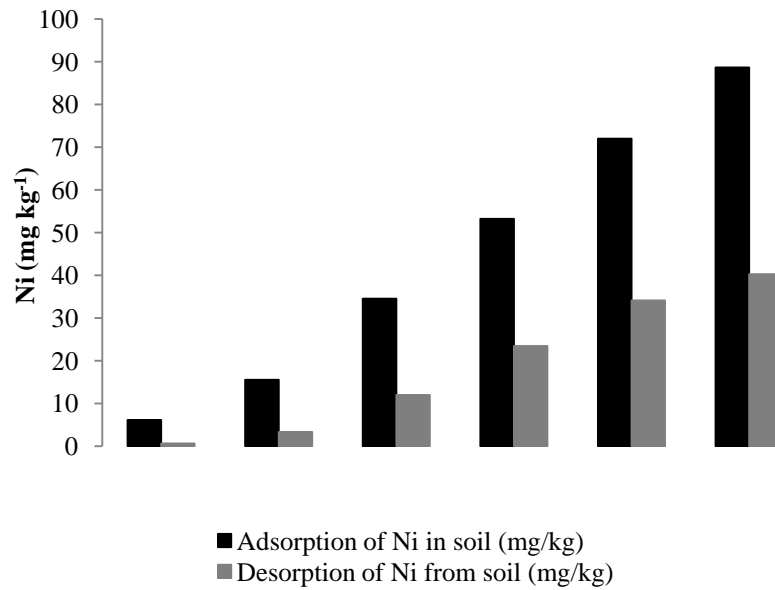
#### **5.1.5. Desorption of Nickel from soil**

Like adsorption, desorption of Ni is also gained much importance to predict the mobility of Ni in soil and subsequent contamination of ground water. At first, a batch experiment was conducted for adsorption of Ni in soil using various concentration of Ni that ranged from 0 to 100 mg Ni kg<sup>-1</sup> of soil and subsequently the soil was extracted with 0.005M DTPA or 0.05M Ca(NO<sub>3</sub>)<sub>2</sub> to quantify desorption of Ni from the soil.

The amount of Ni adsorbed in the soil ranged between 7.09 and 93.32 mg kg<sup>-1</sup>. Then, the soil was extracted by 0.005M DTPA where is the desorption of Ni from soil ranged from 7.08 to 60.4 mg of Ni (Fig. 5.5). Only about 65 per cent of adsorbed Ni was found desorbed from the soil while using 0.005M DTPA as an extractant. In case of 0.05M Ca(NO<sub>3</sub>)<sub>2</sub> extraction, the amount of Ni adsorbed in the soil ranged between 6.14 and 88.66 mg kg<sup>-1</sup> and only 45 per cent of adsorbed Ni was found desorbed from the soil using 0.05M Ca(NO<sub>3</sub>)<sub>2</sub> (Fig. 5.6). When two extractants were compared, 0.005M DTPA extracted relatively more amount of Ni than 0.05M Ca(NO<sub>3</sub>)<sub>2</sub>. This may be due to the complete or partial reversible sorption of Ni while using suitable extractants. Adriano (2001) reported that the Ni sorption was not completely irreversible as some Ni was desorbed from soil by the chelating agent DTPA. The DTPA extractant was found to predict the plant uptake quite well (Valdares *et al.*, 1983). In addition several investigations found an excellent correlation between DTPA extractable Ni and plant uptake (Adriano, 2001). Moreover, desorption of Ni is mainly depends on the soil properties to a greater extent. Hence, retention mobility and availability of applied Ni vary in different soils (Barman *et al.*, 2013).



**Fig. 5.5 Desorption of Nickel by 0.005M DTPA**



**Fig. 5.6 Desorption of Nickel by 0.05M Ca(NO<sub>3</sub>)<sub>2</sub>**

## **5.2. BIOTRANSFORMATION AND BIOAVAILABILITY OF NICKEL IN SOIL**

The bioavailability of a metal in the soil environment has often been defined as the fraction of the total metal in the interstitial porewater (i.e., soil solution) and soil particles that is available to the receptor organism. Considerable controversy exists in the literature relating to 'what constitutes the bioavailable fraction' including the definition itself and the methods used for its measurements. A more generic definition of bioavailability is the potential for living organisms to take up chemicals from food (i.e., oral) or from the abiotic environment (i.e., external) to the extent that the chemicals may become involved in the metabolism of the organism. More specifically, it refers to the biologically available chemical fraction (or pool) that can be taken up by an organism and can react with its metabolic machinery (Campbell, 1995); or it refers to the fraction of the total chemical that can interact with a biological target (Vangronsveld and Cunningham, 1998). In order to be bioavailable, the metals have to come in contact with the organism (i.e., physical accessibility). Moreover, metals need to be in a particular form (i.e., chemical accessibility) to be able to enter a plant root. In essence, for a metal to be bioavailable, it will have to be mobile and transportable and be in an accessible form to the plant. Based on the bioavailability of metal, suitable remediation strategies can be developed.

Number of soil properties have been affected the bioavailability of metal including soil pH, organic matter content, ion exchange capacities, soil type and residence time etc. Although several factors are affecting the bioavailability of metals, soil pH is the single determinant which interrelate all other soil properties and thus it affects the mobility of metals. In order to study the biotransformation and bioavailability of Ni in soil, a laboratory incubation experiment was conducted by adding different concentrations of Ni in soil under two different moisture conditions such as field capacity moisture and alternate wetting & drying conditions.

### **5.2.1. Soil pH**

From the results, it is evident that the soil moisture and the concentration of Ni are the prime factors which affect the soil pH significantly. The soil pH was found relatively higher in the soil under alternate wetting & drying than the field capacity moisture

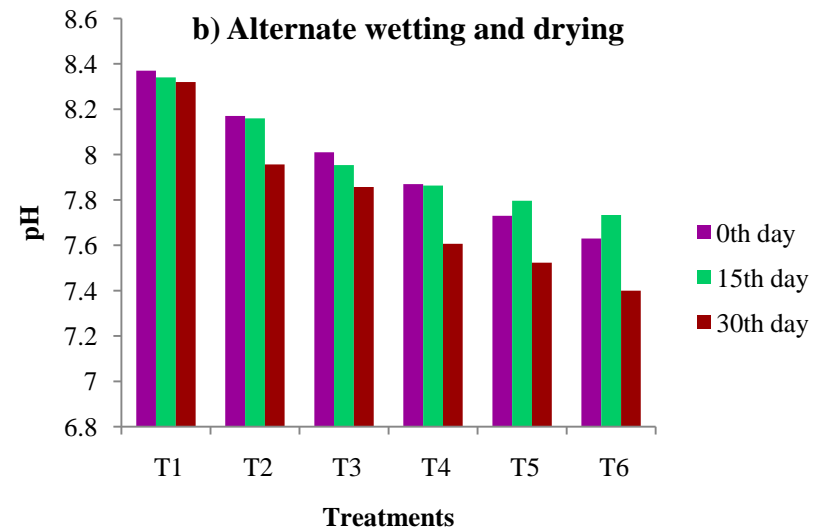
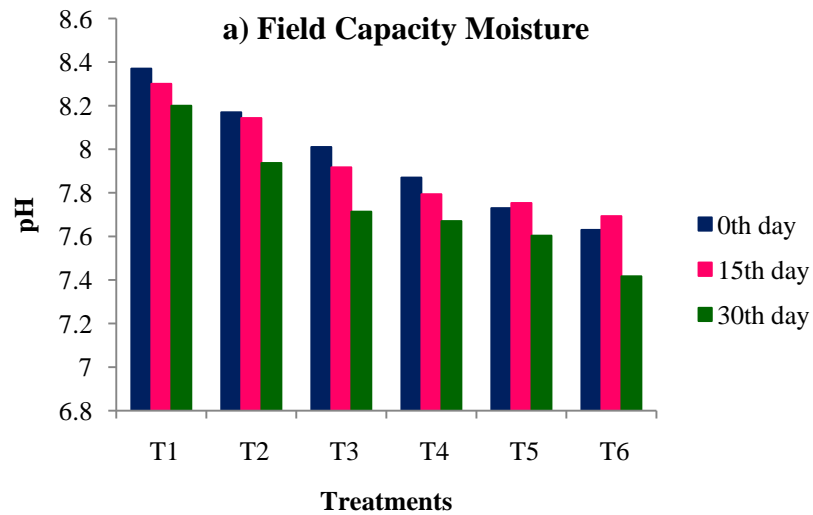
condition. The pH was found increased at 15<sup>th</sup> day and then decreased at the 30<sup>th</sup> day of incubation in the soil under field capacity moisture. But under alternate wetting and drying, the soil pH was decreased at a slow steady rate from 0<sup>th</sup> day to 30<sup>th</sup> day of incubation. During 30 days of incubation, the pH was maximum in control and minimum in the soil that had the highest concentration of Ni under both moisture conditions (Fig 5.7). The pH of soil was found decreased over a period of 30 days which might be due to the production of organic acid during the decomposition of SOM. The amount of Ni retained on soil is mainly affected by the soil pH. Soil pH controls all aspects metal and biogeochemical processes of soil including solubility, precipitation, speciation and sorption as well as microbial activity. The solubility and bioavailability of Ni is inversely related to soil pH (Adriano, 2001). For metals, the net charge of the metal complexes and their precipitation/dissolution reactions are directly impacted by soil pH. Thus, soil pH plays a major role in controlling Ni bioavailability (Naidu *et al.*, 1996b).

### **5.2.2. Soil organic carbon**

Soil organic matter (SOM) strongly reacts with metals. However it undergoes transformations and affects the retention or solubilization of metals. Significant changes were observed in the SOC during the incubation (Fig 5.8). The moisture condition markedly influenced the SOC. Soil under field capacity moisture recorded relatively higher SOC than the soil under alternate wetting and drying at all stages of incubation. The SOC was found decreased over a time period under both moisture conditions. The SOC was found relatively higher in control soil and lower in soil that had greater amount of Ni. The reduction in the SOC during the incubation could be attributed to the release of carbon through CO<sub>2</sub> and CH<sub>4</sub> emissions due to microbial activity of soil, that might have reduced the SOC content. Similar results were observed by Walker *et al.* (2003) and Karaca, 2004.

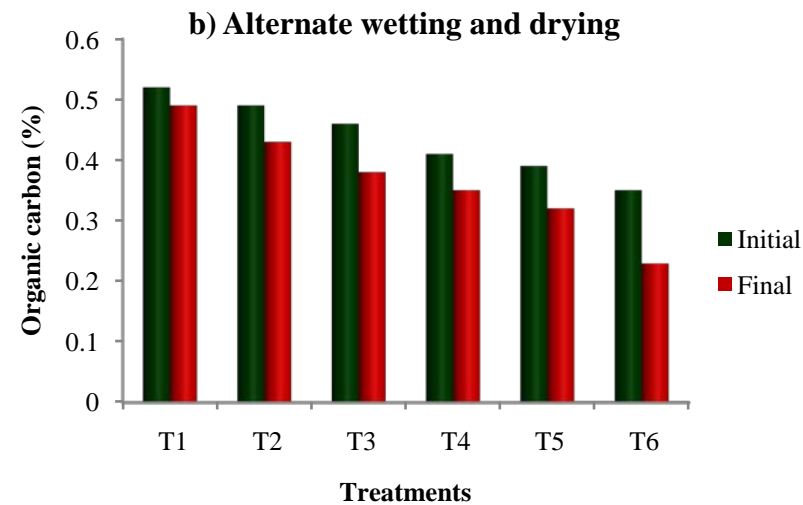
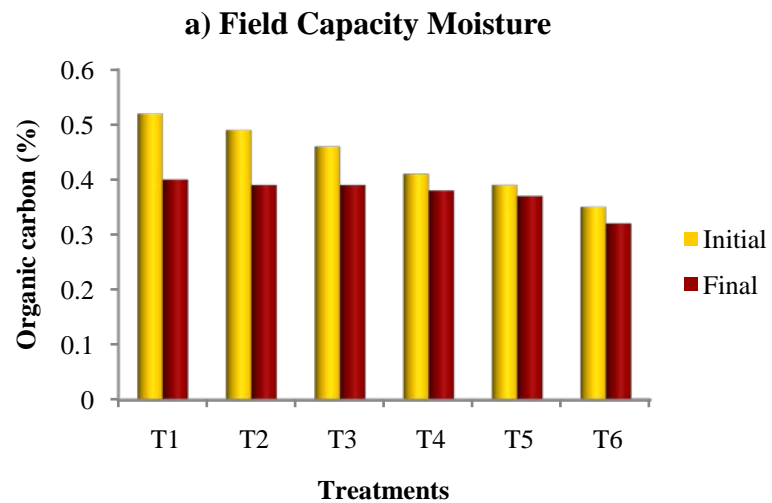
### **5.2.3. Biotransformation of Nickel**

Nickel in the solid phase of soil occurs in several chemical forms such as exchangeable, adsorbed, organically bound, carbonate and residual forms. In the aqueous phase it occurs in ionic and complexed forms (Adriano, 2001). In this study, the species of Ni occurs in the order of Residual > Organic plus iron oxide bound > Organic >



T<sub>1</sub>.Control (Soil alone); T<sub>2</sub>. Soil + 20 ppm of Ni; T<sub>3</sub>. Soil + 40 ppm of Ni; T<sub>4</sub>. Soil+ 60 ppm of Ni; T<sub>5</sub>. Soil+ 80 ppm of Ni; T<sub>6</sub>. Soil+ 100 ppm of Ni

**Fig. 5.7 Changes in soil pH during incubation of soil under two different moisture conditions**



T<sub>1</sub>.Control (Soil alone); T<sub>2</sub>. Soil + 20 ppm of Ni; T<sub>3</sub>. Soil + 40 ppm of Ni; T<sub>4</sub>. Soil+ 60 ppm of Ni; T<sub>5</sub>. Soil+ 80 ppm of Ni; T<sub>6</sub>. Soil+ 100 ppm of Ni

**Fig. 5.8 Changes in soil organic carbon during incubation of soil under two different moisture conditions**

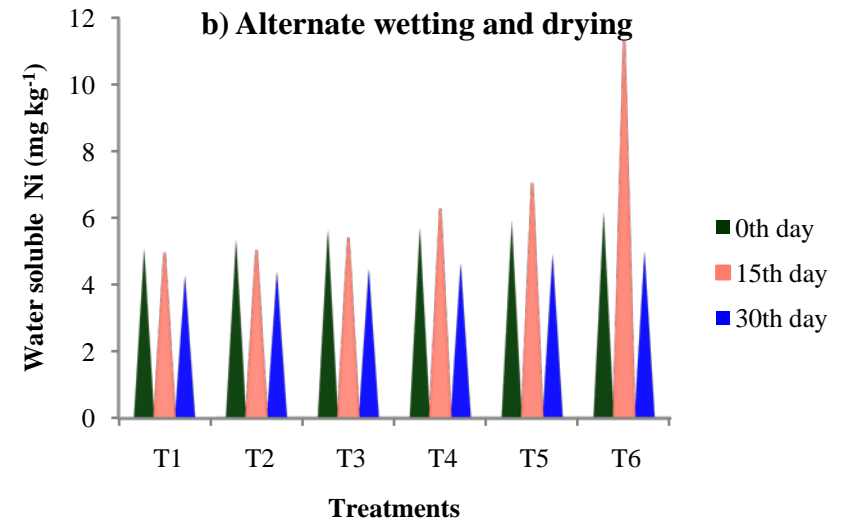
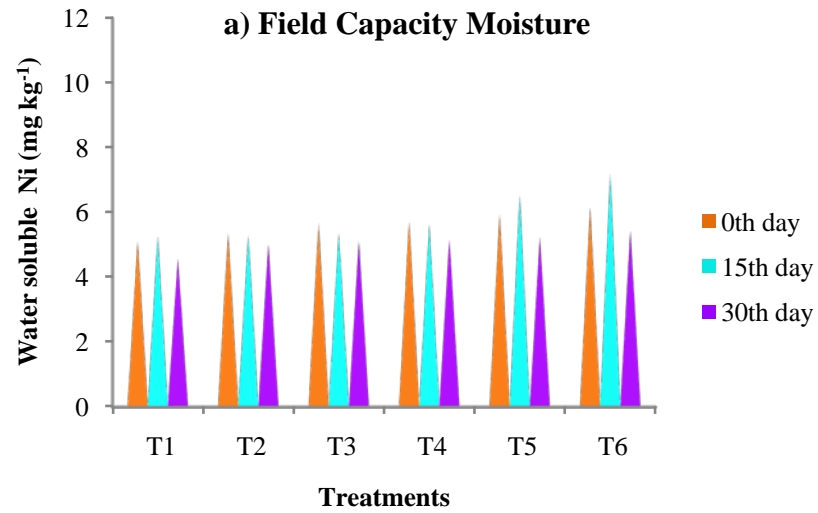
Exchangeable > Water soluble fraction. Hickey and Kittrick (1984) also reported that the 50 per cent of Ni in soils is associated with the residual fraction, and about 20 per cent in the Fe-Mn oxide fraction, with much of the remainder in the carbonate portion and only a relatively small fraction in the exchangeable and organic fractions.

#### **5.2.3.1. Water soluble Nickel**

The water soluble Ni is certainly the most biologically active fraction contributing significantly towards its bioavailability. It has high potential of entering into food chain, surface water and ground water (Leita and De Nobili, 1991; He *et al.*, 1992). The changes occurred in the water soluble Ni during incubation were observed periodically (Fig 5.9). It was differed significantly with the moisture conditions. During the initial stage, it was found higher in soil under alternate wetting and drying. However, at the final stage, it was found higher in soil under field capacity moisture condition. The water soluble Ni showed a gradual increase from 0<sup>th</sup> day to 15<sup>th</sup> day of incubation. After that, it showed a slight decrease at 30<sup>th</sup> day. The water soluble fraction of Ni was found high in the soil with 100 mg Ni kg<sup>-1</sup> (T<sub>6</sub>) and low in the control soil (T<sub>1</sub>) irrespective of stages of incubation and moisture conditions. The increase in water soluble Ni could be attributed to the biotransformation of other forms of Ni mediated by a variety of microorganisms in soil. Alternate wetting and drying favours microbial activity that resulted in greater transformation of Ni from other fractions into water soluble fraction. The changes in the water soluble fraction of Ni also could be attributed to the large effects of equilibrium pH. Reddy *et al.* (1995) suggested that the availability and mobility of metal ions in soil solution increased with decreased soil pH.

#### **5.2.3.2. Exchangeable Nickel**

Heavy metals are associated with different soil components in different ways and these associations determine their mobility and availability (Kabata-Pendias and Pendias, 1992; Kabala and Singh, 2001). Water soluble and exchangeable forms are considered readily mobile and available to plants, thus considered as the bioavailable form of metals. The exchangeable Ni was markedly affected by the moisture conditions. During initial stage of incubation, concentration of exchangeable Ni was found relatively higher in soil under field capacity moisture condition than in soil under alternate wetting



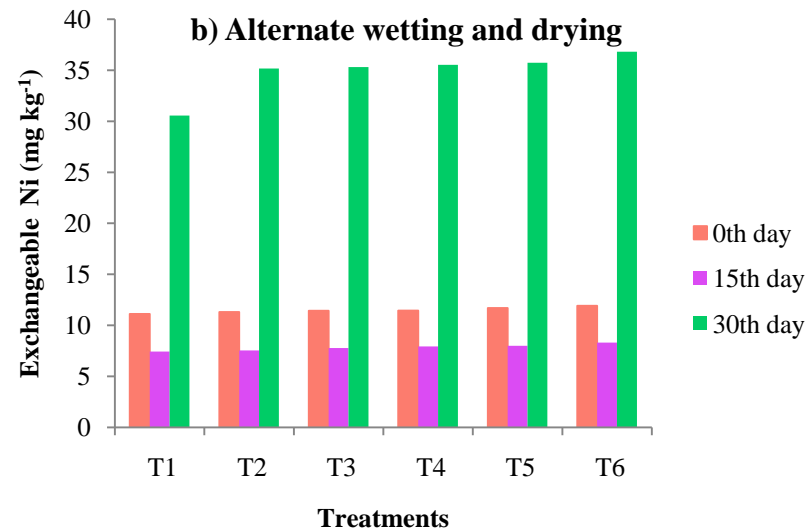
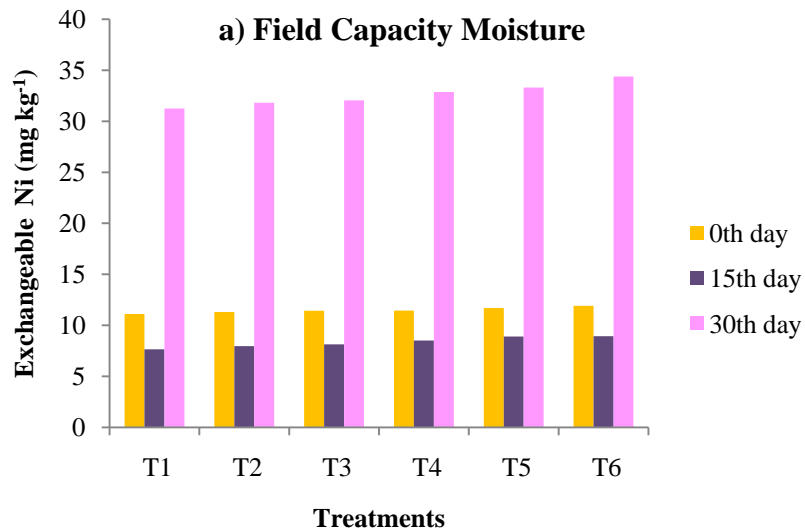
T<sub>1</sub>.Control (Soil alone); T<sub>2</sub>.Soil + 20 ppm of Ni; T<sub>3</sub>. Soil + 40 ppm of Ni; T<sub>4</sub>. Soil+ 60 ppm of Ni; T<sub>5</sub>. Soil+ 80 ppm of Ni; T<sub>6</sub>. Soil+ 100 ppm of Ni

**Fig. 5.9 Changes in Water soluble Nickel during incubation of soil under two different moisture conditions**

and drying. After 30 days of incubation, the concentration of exchangeable Ni was found higher in alternate wetting and drying condition. Irrespective of moisture conditions, the exchangeable fraction of Ni in soil was found gradually decreased up to 15<sup>th</sup> day and then found increased rapidly (Fig. 5.10). Under both moisture conditions, the highest concentration of exchangeable Ni was observed in the treatment (T<sub>6</sub>) added with 100 mg Ni kg<sup>-1</sup> and the lowest in the control soil (T<sub>1</sub>) at all the stages of incubation. The changes occurred in the concentration of exchangeable Ni might be correlated well with the changes in the soil pH during the incubation period. Tewari *et al.* (2010) reported that the increase in exchangeable fraction of metal was due to a higher acidity condition. The addition of Ni as a soluble salt also might be a reason for increased concentration of exchangeable Ni (Cunningham *et al.*, 1975).

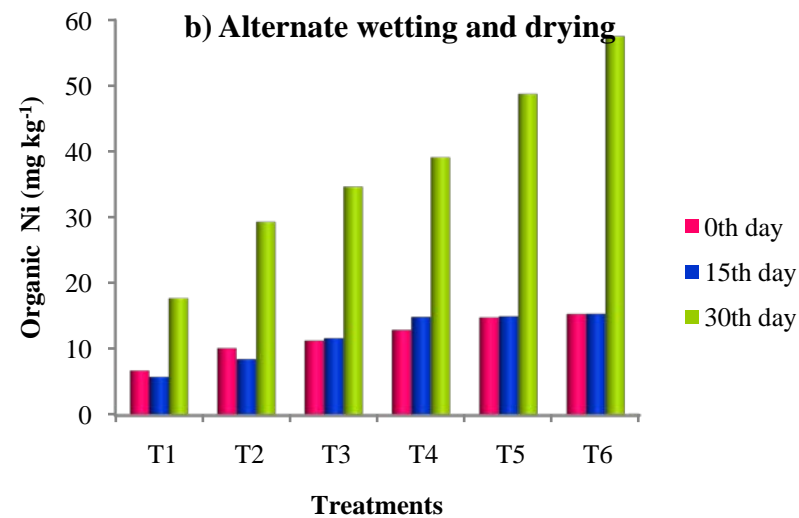
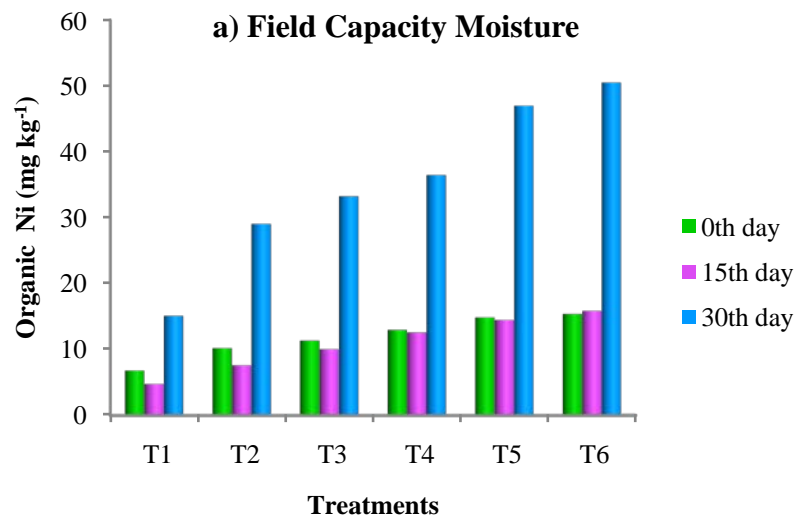
#### **5.2.3.3. Organic Nickel**

Trace metals in the soluble, exchangeable and organically bound forms are believed to be highly available for plant uptake (Petruzzelli, 1989; He *et al.*, 1992). The concentration of organic Ni in soil varied significantly between the two moisture conditions. The organic Ni was found relatively higher in the soil under alternate wetting and drying conditions than the soil under field capacity moisture condition. Irrespective of moisture condition, the concentration of organic Ni was found decreased gradually at 15<sup>th</sup> day. After that, it showed a rapid increase at the end of incubation (Fig. 5.11). The concentration of organic Ni was found high in the soil treated with the highest level of Ni (100 mg kg<sup>-1</sup>) and the lowest in control soil at all stages of incubation. The organic fraction consists of metals bound to various forms of organic matter. The changes occurred in the concentration of organic Ni could be attributed to the microbial conversion of soluble and exchangeable Ni into organic-Ni and subsequent formation of organic-Ni complex. Salomons (1995) reported that the mobility of organic fraction metals ranged from medium to high depending upon the time, decomposition or oxidation of organic matter. Obrador *et al.* (1998) also reported that there was an increase in potentially bioavailable fraction of all cations initially, which then remained more or less constant or decreased over the rest of the period.



T<sub>1</sub>.Control (Soil alone); T<sub>2</sub>. Soil + 20 ppm of Ni; T<sub>3</sub>. Soil + 40 ppm of Ni; T<sub>4</sub>. Soil+ 60 ppm of Ni; T<sub>5</sub>. Soil+ 80 ppm of Ni; T<sub>6</sub>. Soil+ 100 ppm of Ni

**Fig. 5.10 Changes in Exchangeable Nickel during incubation of soil under two different moisture conditions**



T<sub>1</sub>.Control (Soil alone); T<sub>2</sub>. Soil + 20 ppm of Ni; T<sub>3</sub>. Soil + 40 ppm of Ni; T<sub>4</sub>. Soil+ 60 ppm of Ni; T<sub>5</sub>. Soil+ 80 ppm of Ni; T<sub>6</sub>. Soil+ 100 ppm of Ni

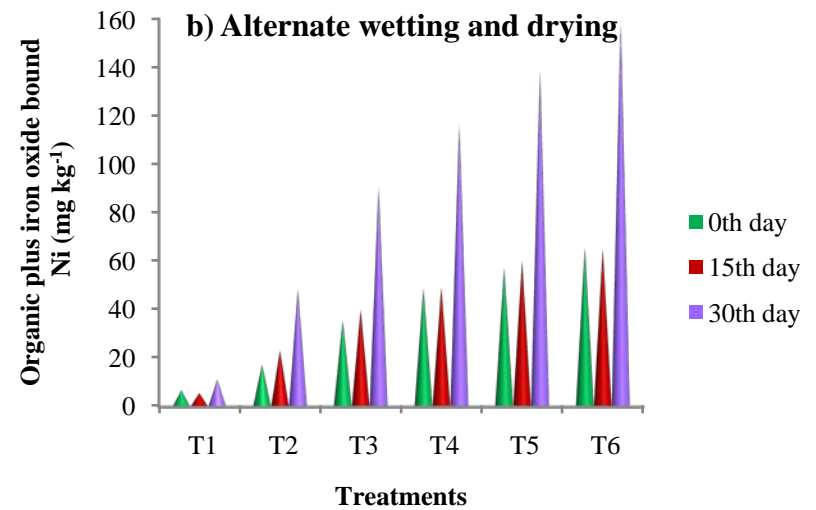
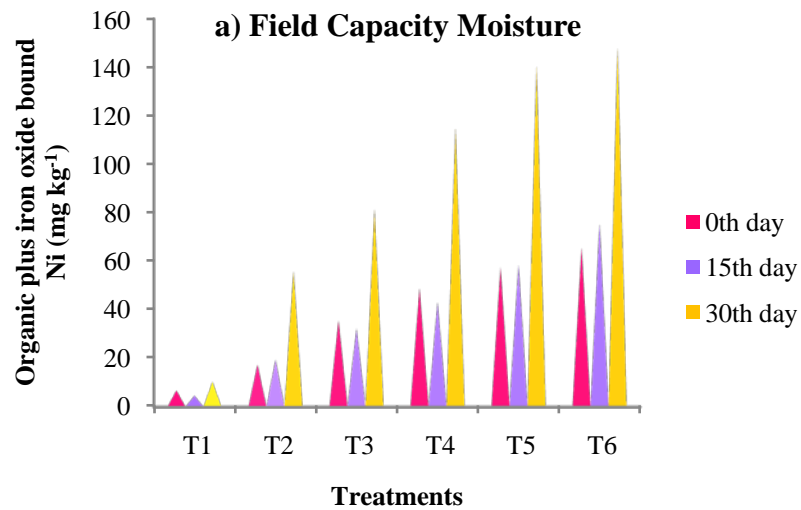
**Fig. 5.11 Changes in Organic Nickel during incubation of soil under two different moisture conditions**

#### **5.2.3.4. Organic plus iron oxide bound Nickel**

The organic plus iron oxide bound fraction consists of metals adsorbed to organic matter or iron oxide particles. The concentration of organic plus iron oxide bound Ni was markedly influenced by the two different moisture conditions (Fig. 5.12). The concentration was relatively higher in soil under alternate wetting and drying than in soil under field capacity moisture. In both moisture conditions, the concentration of organic plus iron oxide bound Ni was found increased over a period of time. Irrespective of moisture conditions, the organic plus iron oxide bound Ni was high in the soil with 100 mg kg<sup>-1</sup> of Ni and low in the control soil at all stages of incubation. The changes in the concentration of organic plus iron oxide bound Ni might be due to the variations in the redox conditions which might have favoured microbial conversion of Ni species. The higher amount of soluble and exchangeable Ni available in the soil due to Ni addition might have favoured greater amount of Ni bound on organic matter and iron oxide surfaces. The mobility of this fraction is medium and the organic complexed form is relatively stable throughout the period (Tu-Cong and Tu-C, 1996).

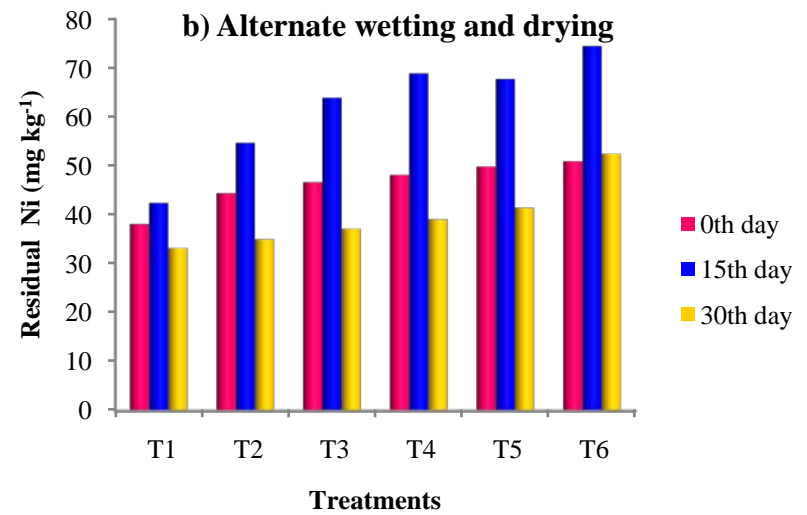
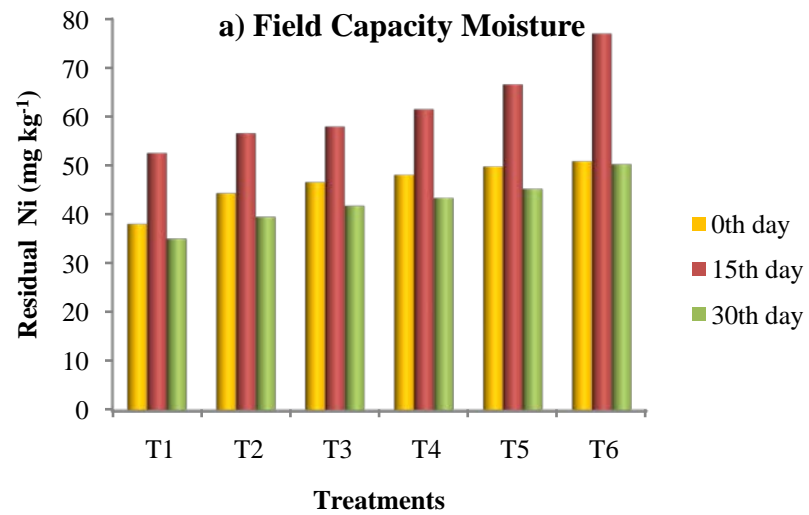
#### **5.2.3.5. Residual Nickel**

The most abundant pool of Ni is the residual fraction which accounts about 60-89 per cent in soil (Tewari *et al.*, 2010). Initially the highest concentration of residual Ni was registered in the soil under alternate wetting and drying condition than in the soil under field capacity moisture. However at the end of incubation, it was found high in soil under field capacity moisture condition. Under both moisture conditions, the concentration of residual Ni was increased gradually from initial to final stage of incubation (Fig 5.13). At all stages of incubation, the highest concentration of residual Ni was recorded in the treatment with 100 mg Ni kg<sup>-1</sup> in soil and the lowest value in the control soil (T<sub>1</sub>). The increase in the concentration of residual Ni over a period of time could be attributed to the transformation of other forms/species of Ni into insoluble form due to the formation of Ni precipitates (Tewari *et al.*, 2010). The increase in the residual fraction of Ni could also be due to the irreversible adsorption of Ni onto silicates (Lindsay and Norvell, 1969).



T<sub>1</sub>.Control (Soil alone); T<sub>2</sub>. Soil + 20 ppm of Ni; T<sub>3</sub>. Soil + 40 ppm of Ni; T<sub>4</sub>. Soil+ 60 ppm of Ni; T<sub>5</sub>. Soil+ 80 ppm of Ni; T<sub>6</sub>. Soil+ 100 ppm of Ni

**Fig. 5.12 Changes in Organic plus iron oxide bound Nickel during incubation of soil under two different moisture conditions**



T<sub>1</sub>.Control (Soil alone); T<sub>2</sub>. Soil + 20 ppm of Ni; T<sub>3</sub>. Soil + 40 ppm of Ni; T<sub>4</sub>. Soil+ 60 ppm of Ni; T<sub>5</sub>. Soil+ 80 ppm of Ni; T<sub>6</sub>. Soil+ 100 ppm of Ni

**Fig. 5.13 Changes in Residual Nickel during incubation of soil under two different moisture conditions**

Soil moisture conditions and metal source strongly influence metal transformation in soil and control the overall availability of metals in the soil. Field capacity moisture increased the exchangeable fraction of Ni in soil and thus increased the bioavailability of Ni. All other fractions of Ni have increased under alternate wetting and drying conditions.

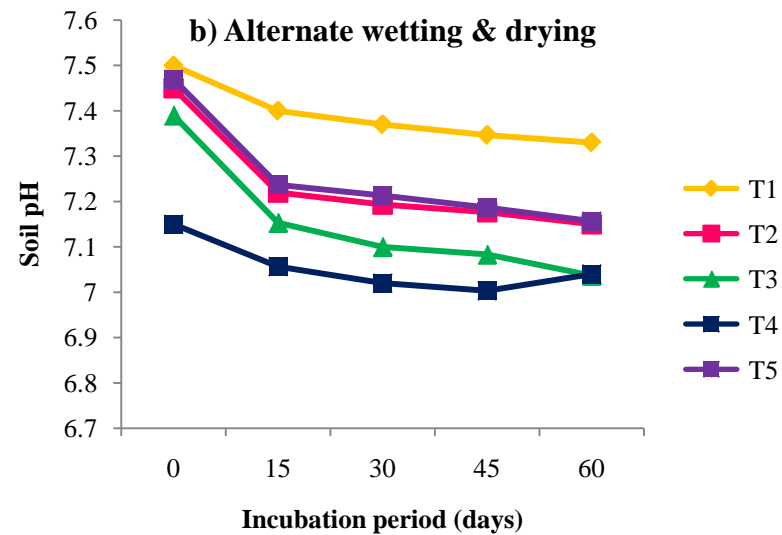
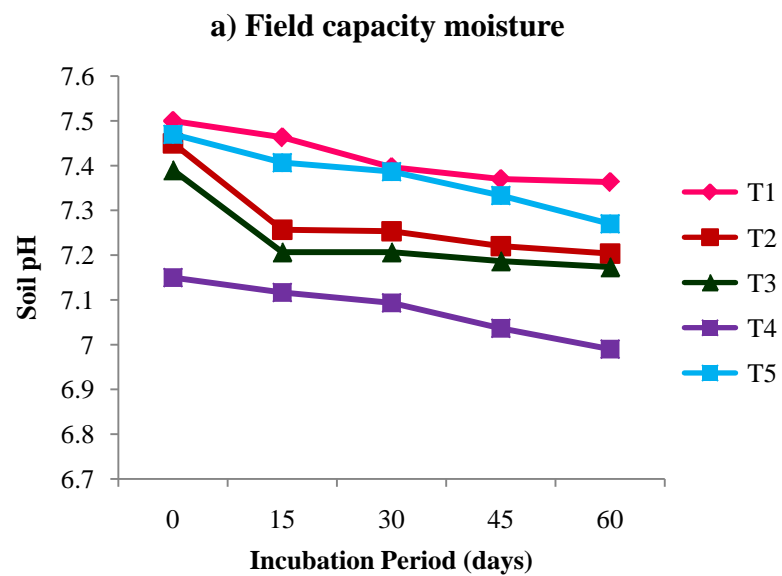
### **5.3. EFFECT OF BIOAMENDMENTS ON BIOTRANSFORMATION AND BIOAVAILABILITY OF NICKEL IN CONTAMINATED SOIL**

The bioamendments such as farmyard manure, composted poultry manure, pressmud compost and prosopis biochar have significant effect on soil pH, SOC, WSOC and different fractions of Ni in soil. At the same time, moisture condition also determines the variability in the soil pH, SOC, WSOC and different fractions of Ni in soil.

#### **5.3.1. Soil pH**

The parameter with the most influence on soil solution ion content is pH. As soil pH relates to metal bioavailability, it generally stands that as the pH of a system is decreased more metals are released to the soil solution because of proton competition for exchange sites of clay minerals or organic matter, or through proton promoted dissolution of soil minerals. The release of metals can be detrimental to plant and microorganism viability. Conversely, as the pH increases, the soil retains more base saturating cations which may lead to nutrient deficiency. Increase in pH can also induce metal complexation or precipitation which can also account for nutrient or metal sequestration.

Metals interaction with organic matter depends on soil pH that determines mobilization or immobilization of metals in soils. When two different moisture conditions were compared, the field capacity moisture recorded relatively higher pH than the alternate wetting & drying condition. The pH of soil was decreased gradually from initial to final stage of the incubation under both moisture conditions (Fig.5.14). The bioamendments has markedly influenced the pH of soil. The pH was maximum in the control soil ( $T_1$ ) and minimum in the soil amended with pressmud compost at all stages of incubation. The decrease in soil pH was due to the organic acids produced during the decomposition of soil organic matter and added bioamendments. Similar results were reported by Mahimairaja *et al.* (1999) and Prapagar *et al.* (2012). Low soil pH usually



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

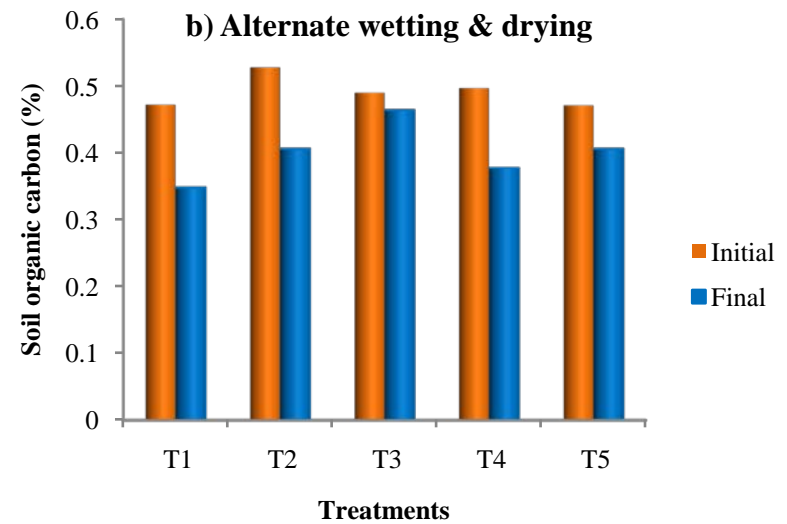
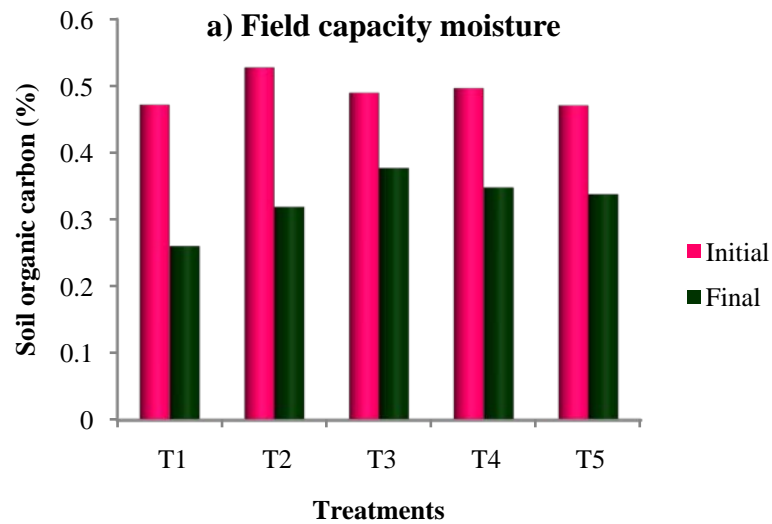
**Fig. 5.14 Effect of bioamendments on soil pH during incubation of Nickel contaminated soil under two different moisture conditions**

causes protonation of organic matter and other exchange sites which could decrease metal sorption from soil solution (Clemente *et al.*, 2005).

### **5.3.2. Soil organic carbon**

Soil organic matter has a marked influence on the ability of soil to retain metals. The SOM or humus includes the total organic matter content of soils and is composed of plant, microbial and animal residues and exudates at various stages of decomposition (Sparks 2003). The SOM is typically fractionated into three components – fulvic acid, humic acid, and humin based on differences in their solubility in acid or alkali solutions. As described by Sposito (1989) humic and fulvic acids exhibit four characteristics that strongly influence their chemical reactivity in soils such as polyfunctionality, macromolecular charge, hydrophilicity and structural lability. With respect to polyfunctionality, the surface functional groups that exhibit the most influence on metal retention are the carboxyl (COOH) and phenolic OH (aromatic ring-OH) groups. The charge of the surface functional group is dependent on the pH of the soil solution and thus the charge on organic matter is considered variable or pH dependent. The complexation of metals with organic matter has traditionally been studied by determining conditional metal stability constants and complexation capacities (Xia *et al.*, 1997).

The SOM plays an important role in accumulation and transportation of Ni and other metals through formation of chelates of various stabilities (Stevenson and Cole, 1999). Stability of organic matter complexes with divalent metal ions like Ni(II) varies in the decreasing order of Cu > Ni > Pb > Co > Ca > Zn > Mn > Mg (McBride, 1989). During initial stage of incubation experiment, the SOC was higher in the soil under field capacity moisture than in the soil under alternate wetting and drying condition. However, at final stage no significant difference existed between the two moisture conditions. The SOC was found decreased over a period of time under both moisture conditions (Fig. 5.15). Initially, the SOC was found maximum in the soil amended with farmyard manure (T<sub>2</sub>) and minimum in the control soil (T<sub>1</sub>). At the end of 60 days, under field capacity moisture the content of SOC was recorded high in the soil amended with pressmud (T<sub>4</sub>) and minimum in the control soil (T<sub>1</sub>). Under alternate wetting and drying, it was found maximum in poultry manure amended soil (T<sub>3</sub>) and minimum in the control soil (T<sub>1</sub>)



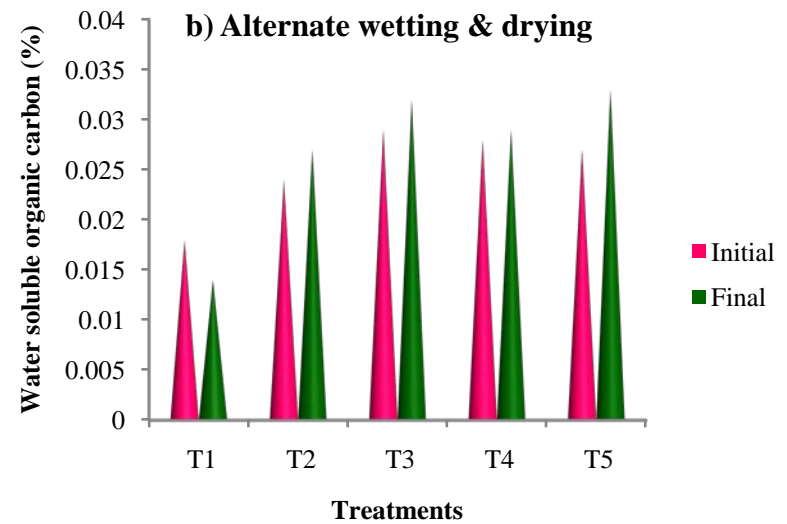
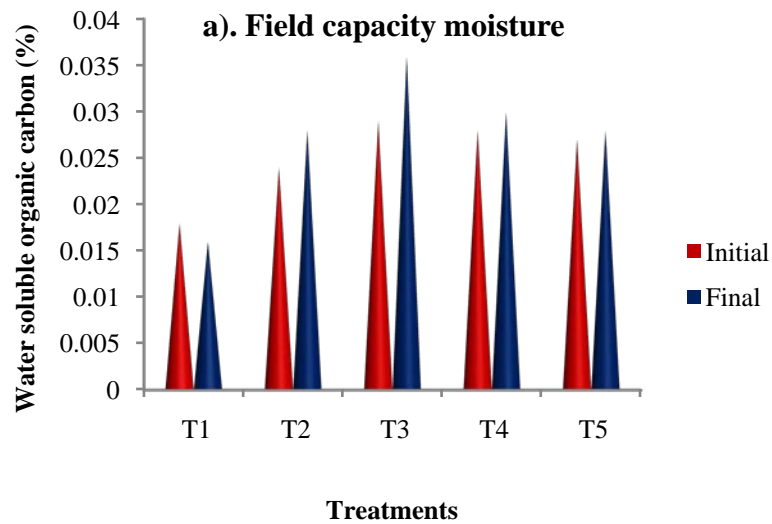
T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig. 5.15 Effect of bioamendments on soil organic carbon during incubation of Nickel contaminated soil under two different moisture conditions**

The SOC content was decreased during the incubation period which could be attributed to the mineralization of soluble organic compounds over a period of time (decomposition) and also due to the microbial activity of the soil which results in the emission of CO<sub>2</sub> and CH<sub>4</sub> and thus carbon loss (Walker *et al.*, 2003; Karaca, 2004). However decrease in SOC content varied with amendments which could be attributed to varying stability of organic matter content of the bioamendments (Walker *et al.*, 2003; Karaca, 2004). The interaction of organic matter with the metals mainly depends on soil pH which determines mobilization or immobilization of metals in soils. Nickel chelate forms weak complexes with the monocarboxylates and stronger chelates, with the polycarboxylates. It was concluded that the Ni(II) complexes with soil fulvic acid were inner sphere and complexation occurred with one or more carboxylate groups. In general, the maximum amount of metal that can be complexed by SOM is roughly equal to the number of carboxylic functional groups (Stevenson, 1994).

### **5.3.3. Water soluble organic carbon of soil**

The water soluble organic carbon (WSOC) of soil is important as it determines the metal dynamics in soil besides pH and redox potential. The addition of bioamendment has showed marked influence on water soluble organic carbon (Fig 5.16). It was found relatively higher in the soil under field capacity moisture than in the soil under alternate wetting and drying throughout the experimental period. The WSOC was found increased over a period of 60 days under both moisture conditions. Initially, the soil amended with composted poultry manure (T<sub>3</sub>) had significantly higher WSOC and control had lower WSOC. Under field capacity moisture, the maximum WSOC was recorded in the treatment that had composted poultry manure (T<sub>3</sub>) and minimum in the control (T<sub>1</sub>) after 60 days of incubation. The poultry manure was reported to contain higher amount of soluble carbon (Mahimairaja *et al.*, 1999). Under alternate wetting and drying condition, it was found maximum in prosopis biochar amended soil (T<sub>5</sub>) and minimum in the control soil at final stage. Different biochemical processes occurred during SOM transformation like hydrolysis, oxidation and/or depolymerization. These processes result in the release of soluble organic matter, an increase in metal ions mobility and bioavailability (Martinez *et al.*, 2003).



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig. 5.16 Effect of bioamendments on soil water soluble organic carbon during incubation of Nickel contaminated soil under two different moisture conditions**

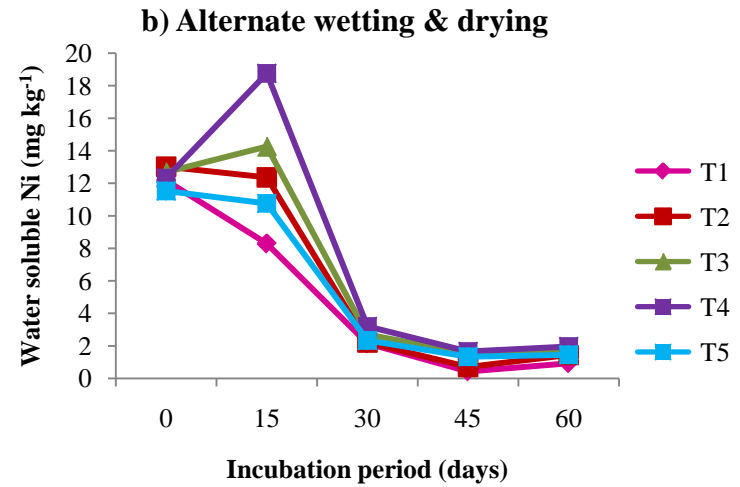
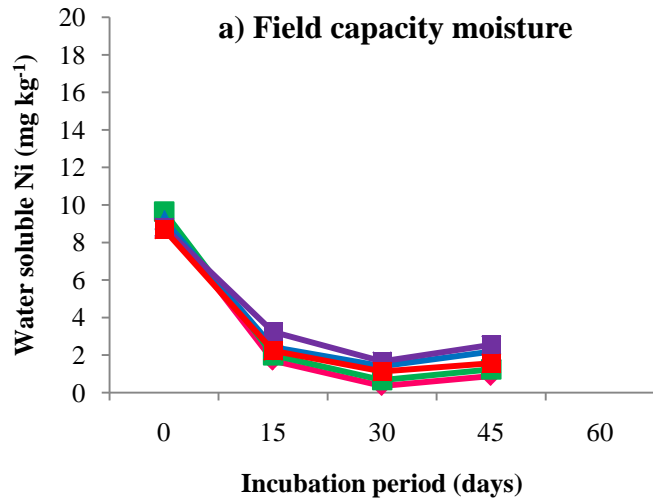
### **5.3.4. Biotransformation and bioavailability of Nickel in contaminated soil**

The species of metals in environmental samples is a vital factor in assessing its potential environmental impacts. Fractionation of heavy metals in soils is needed to predict elemental mobility in soil and bioavailability to plants. The bioamendments alter the speciation of Ni through changes in soil chemical properties (pH, CEC, nutrient content) and by metal chelating ability of organic matter. In the current study, the effect of certain bioamendments on biotransformation and bioavailability of Ni was examined. A sequential extraction procedure was adopted to determine various fractions of Ni in soil.

#### ***5.3.4.1. Water soluble Nickel***

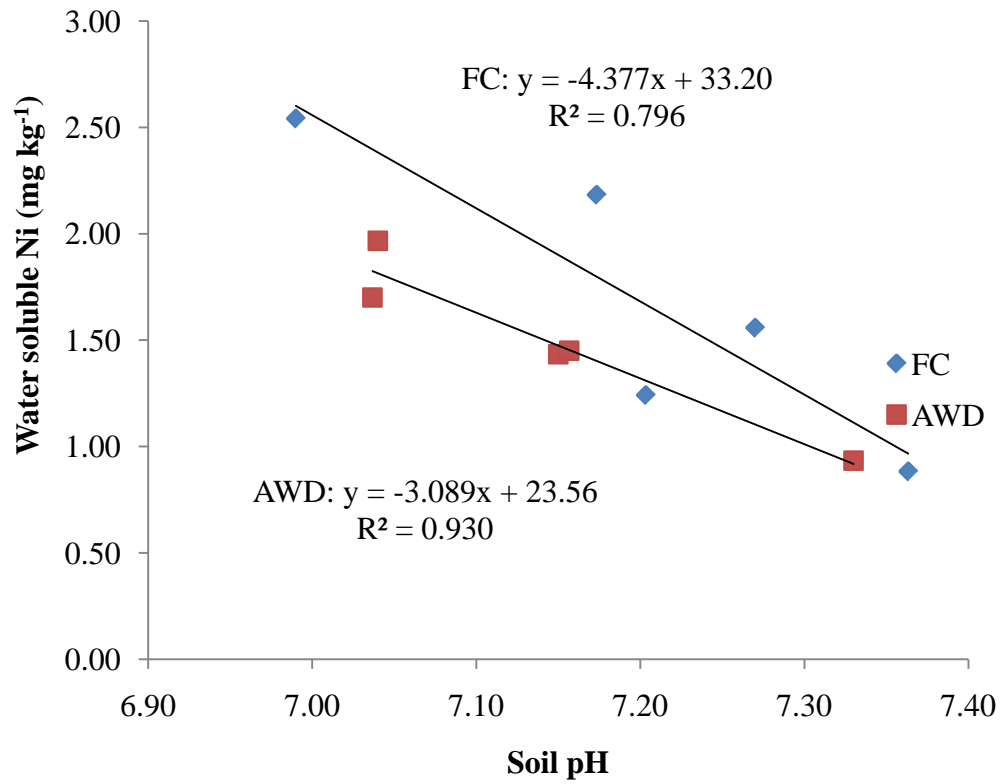
The water soluble fraction was found high in soil under field capacity moisture condition both at initial and final stage. However, during the incubation it was high in soil under alternate wetting and drying. Under field capacity moisture, the water soluble Ni showed a gradual decrease over a period of time upto 45<sup>th</sup> day of incubation. After that, it showed a slight increase at 60<sup>th</sup> day (Fig. 5.17). Initially, the concentration of water soluble Ni was high (13.02 mg kg<sup>-1</sup>) in the soil amended with farmyard manure (T<sub>2</sub>) and low (11.53 mg kg<sup>-1</sup>) in the soil amended with biochar (T<sub>5</sub>). At the end of incubation (60<sup>th</sup> day) the highest value (2.44 mg kg<sup>-1</sup>) was recorded in the soil amended with pressmud compost (T<sub>4</sub>) and the lowest (0.88 mg kg<sup>-1</sup>) (T<sub>1</sub>) was found in the control soil.

The water soluble Ni in soils maintained under alternate wetting and drying condition showed a different trend. At first, the concentration was found increased from 0<sup>th</sup> day to 15<sup>th</sup> day. Thereafter, gradually decreased up to 45<sup>th</sup> day and then, showed an increasing trend at 60<sup>th</sup> day of incubation. Finally, the highest concentration of water soluble Ni (1.97 mg kg<sup>-1</sup>) was recorded in the soil treated with pressmud compost (T<sub>4</sub>) and the lowest concentration (0.93 mg kg<sup>-1</sup>) in the control soil (T<sub>1</sub>) at 60<sup>th</sup> day of incubation. Among the soil characteristics, the pH was found to have significant effect on water soluble fraction of Ni in soil under both field capacity moisture and alternate wetting & drying, as there was a negative correlation exists between soil pH and water soluble fraction of Ni (Fig. 5.18. R<sup>2</sup> for FC= 0.796, AWD =0.93). The metal speciation can be changed with pH of the soil, which in turn affects the adsorption of metals on soil particles. It has reported that the Ni adsorption was more at high pH due to hydrolysis of



T<sub>1</sub>. Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig. 5.17 Effect of bioamendments on Water soluble Nickel during incubation of Nickel contaminated soil under two different moisture conditions**



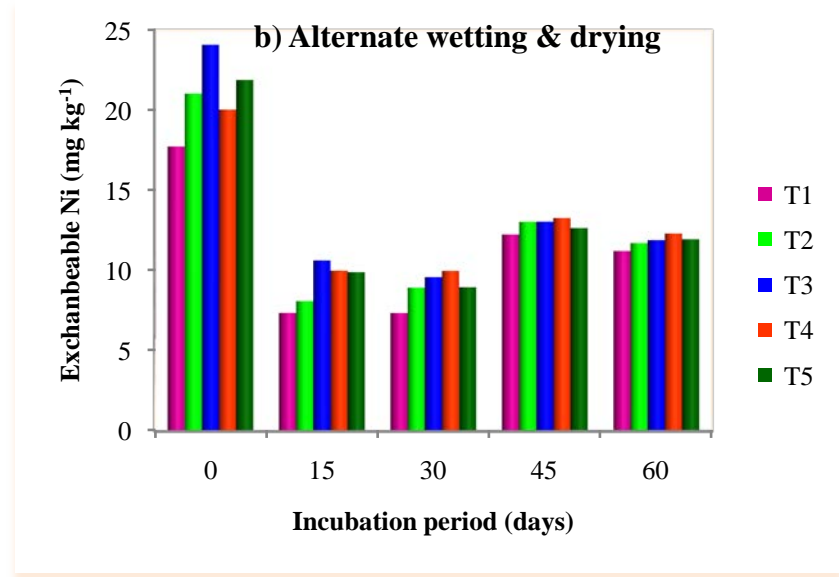
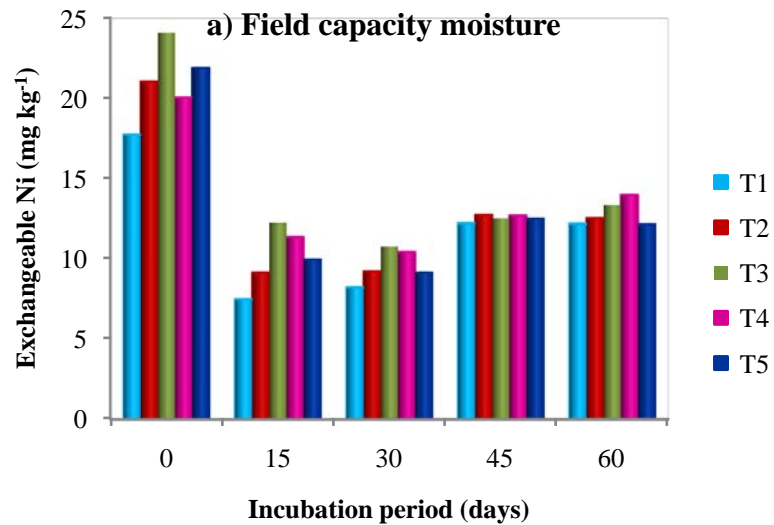
**Fig.5.18 Relationship between soil pH and water soluble fraction of Nickel at final stage**

Ni ions (McBride, 1989). The soluble concentration of Ni could be predicted on the basis of pH, total metal concentration and organic matter content (Yin *et al.*, 2002). The decrease in the concentration of water soluble during incubation could be due to the formation of insoluble complexes of Ni with humic acid (Silveria *et al.*, 2003)

#### **5.3.4.2. Exchangeable Nickel**

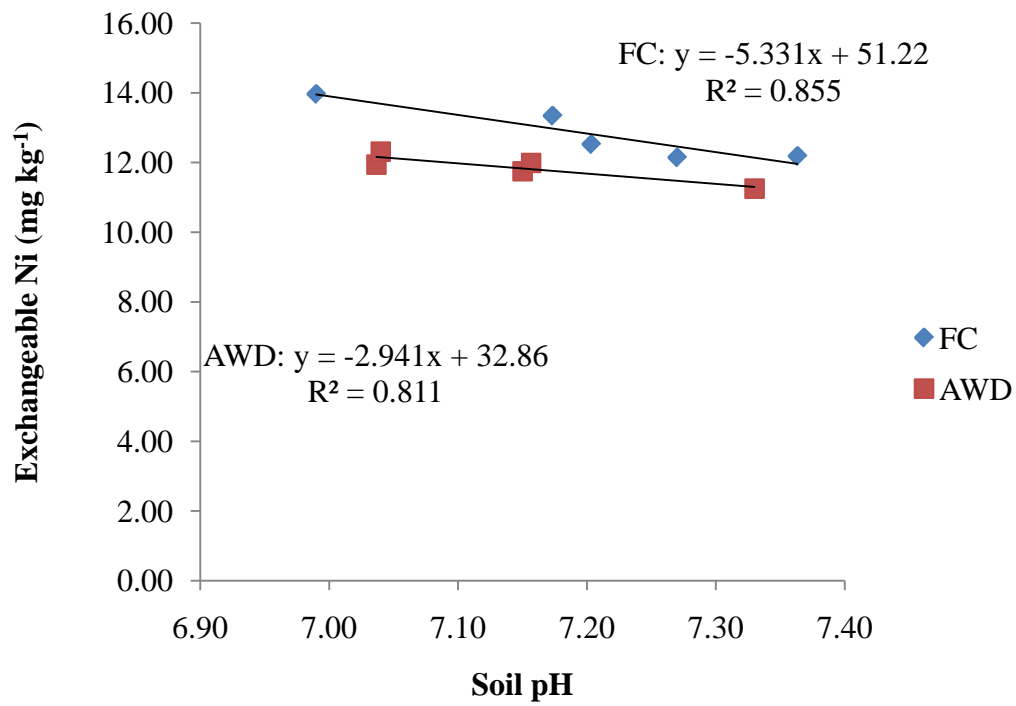
At most of the stages of incubation, the exchangeable Ni was found higher in soil under field capacity moisture condition than in soil under alternate wetting and drying (Fig. 5.19). Irrespective of moisture conditions, the exchangeable Ni was found gradually decreased upto 30<sup>th</sup> day of incubation and then it was found increased rapidly upto 60<sup>th</sup> day. Such effect was more pronounced in soil under alternate wetting and drying condition.

Immediately after mixing with bioamendments, the exchangeable Ni was found higher (24.10 mg kg<sup>-1</sup>) in soil amended with poultry manure (T<sub>3</sub>), whereas it was found lower (15.71 mg kg<sup>-1</sup>) in the soil amended with no bioamendments (T<sub>1</sub>). However, at the end of incubation, the soil amended with pressmud compost (T<sub>4</sub>) was recorded the highest concentration (13.97 mg kg<sup>-1</sup>) of exchangeable Ni and the control soil (T<sub>1</sub>) recorded the lowest concentration (12.18 mg kg<sup>-1</sup>) under field capacity moisture. It was found high in the pressmud compost amended soil (T<sub>4</sub>) and low in the control soil (T<sub>1</sub>) at 60<sup>th</sup> day under alternate wetting and drying condition. The reduction in exchangeable Ni was due to its adsorption on soil particles and/or due to its transformation to other species of Ni. Transformation of metals in soil mainly depends on soil type, moisture content and time of incubation. The total soluble fraction is a reliable indicator of bioavailability of metals. The increase in exchangeable fraction of Ni could be attributed to the formation of soluble complexes of fulvic acid of organic matter. Bioamendments would affect the redox equilibrium responsible for the adsorption of cationic metals. It also can mobilize the adsorbed metals towards the labile form (Hernandez- Sariano and Jimenez- Lopez, 2012). Similar to water soluble fraction of Ni, a negative correlation was observed between soil pHs and exchangeable Ni (Fig. 5.20; R<sup>2</sup> value for FC= 0.855, AWD =0.811). When the pH of the soil decreases, the adsorption of metal decreases and thereby the available fraction increased (Ramachandran and D'Souza, 2013).



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig. 5.19 Effect of bioamendments on Exchangeable Nickel during incubation of Nickel contaminated soil under two different moisture conditions**

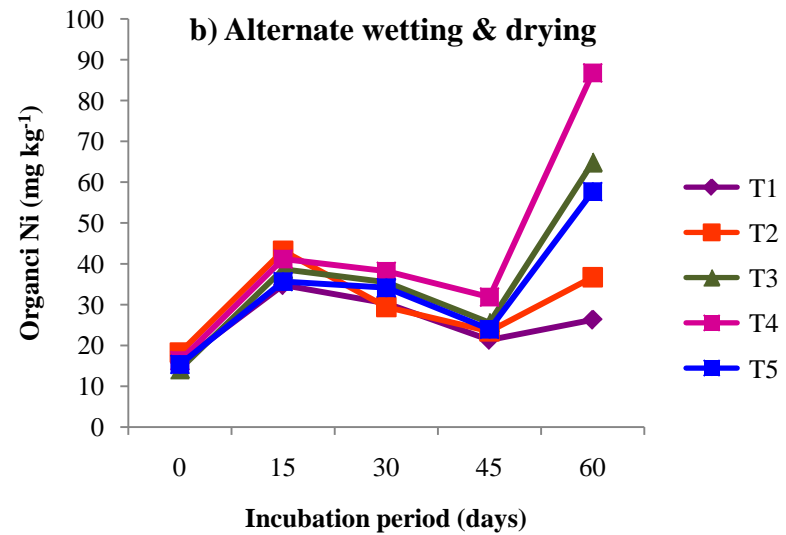
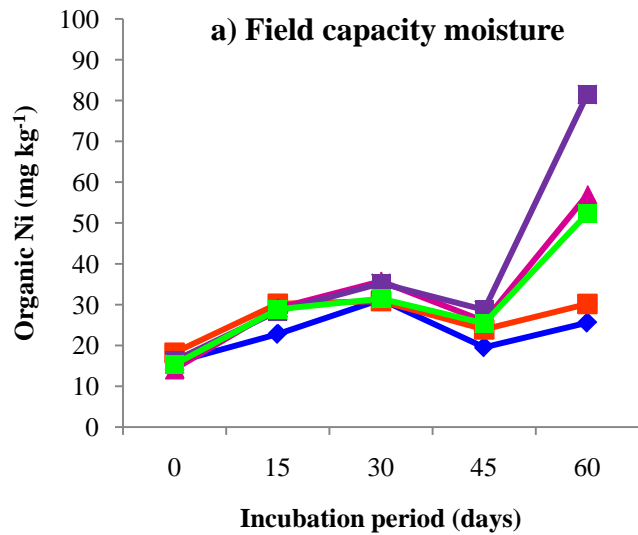


**Fig.5.20 Relationship between soil pH and Exchangeable Nickel at final stage**

#### 5.3.4.3. Organic Nickel

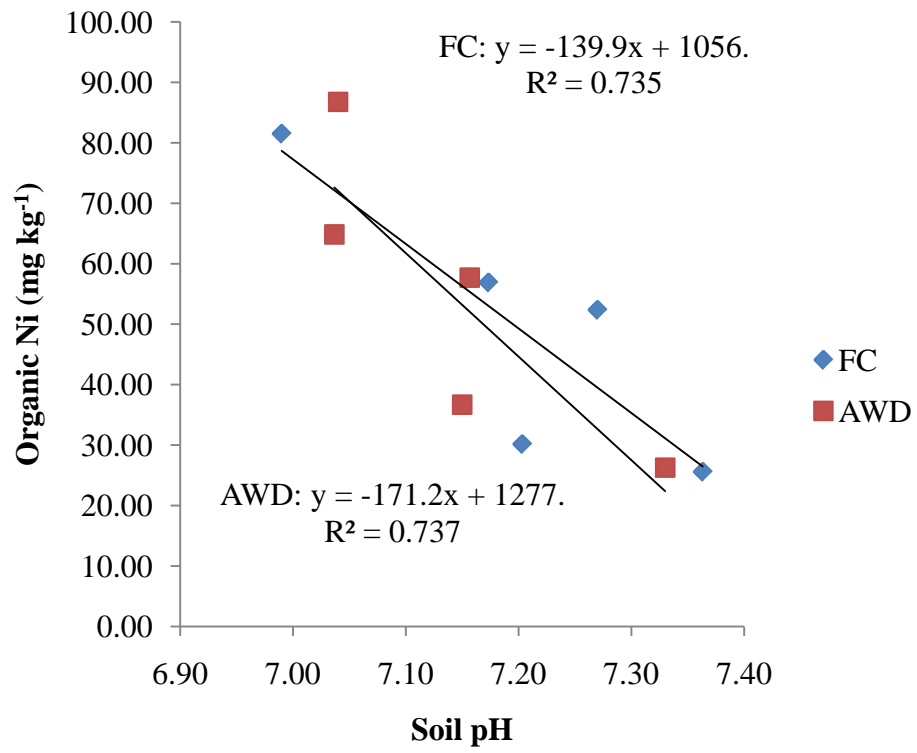
The organic Ni was extracted by 0.5M NaOH. There was a significant difference in the concentration of organic Ni due to moisture conditions. The organic fraction was found higher in soil under alternate wetting and drying conditions than under field capacity moisture condition. Irrespective of moisture conditions, the concentration of organic Ni was found gradually increased from 0<sup>th</sup> day to 30<sup>th</sup> day. Afterwards, it showed a slight decrease at 45<sup>th</sup> day and a sudden increase at 60<sup>th</sup> day of incubation (Fig. 5.21).

There was a marked change in the organic Ni content due to the application of bioamendments. Initially, the concentration of organic Ni was found high (18.23 mg kg<sup>-1</sup>) in the soil treated with farmyard manure (T<sub>2</sub>) and low in the control soil (15.87 mg kg<sup>-1</sup>). The organic Ni was found increased over a period of time and finally the highest concentration (81.51 mg kg<sup>-1</sup>) was recorded in the soil with pressmud compost (T<sub>4</sub>) and lowest value of 25.58 mg kg<sup>-1</sup> in the control soil (T<sub>1</sub>) under field capacity moisture. In the case of alternate wetting and drying condition, the highest organic Ni (86.78 mg kg<sup>-1</sup>) was obtained in the soil amended with pressmud compost (T<sub>4</sub>) and the lowest value (26.28 mg kg<sup>-1</sup>) in the control soil (T<sub>1</sub>) after 60 days of incubation. The changes occurred in the organic Ni might also be due to the alteration of chemical properties of soil and by the chelating ability of organic matter present in the applied bioamendments. Decrease in availability of metals with time is generally attributed to different physico-chemical reactions between metals and soils, like complexation, adsorption and precipitation (McLaughlin, 2001). Whenever the organic fraction decreased, the exchangeable Ni and water soluble Ni were found increased due to the biotransformation mediated by the microbial activities. A negative correlation was obtained between soil pH and organic Ni (Fig. 5.22; R<sup>2</sup> value for FC= 0.735, AWD =0.737). At low pH, relatively large amount of Ni might have solubilized and available for microbial conversion into organic form whereas at high pH relatively large amount of Ni might have been adsorbed on soil particles resulted in lower amount of Ni available for microbial conversion into organic Ni. The results of adsorption study also have shown greater adsorption of Ni at high pHs.



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig. 5.21 Effect of bioamendments on Organic Nickel during incubation of Nickel contaminated soil under two different moisture conditions**



**Fig.5.22 Relationship between soil pH and Organic Nickel at final stage**

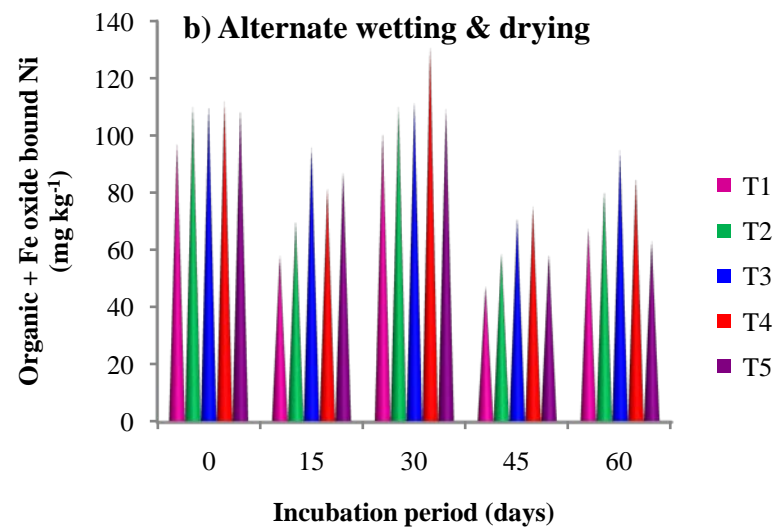
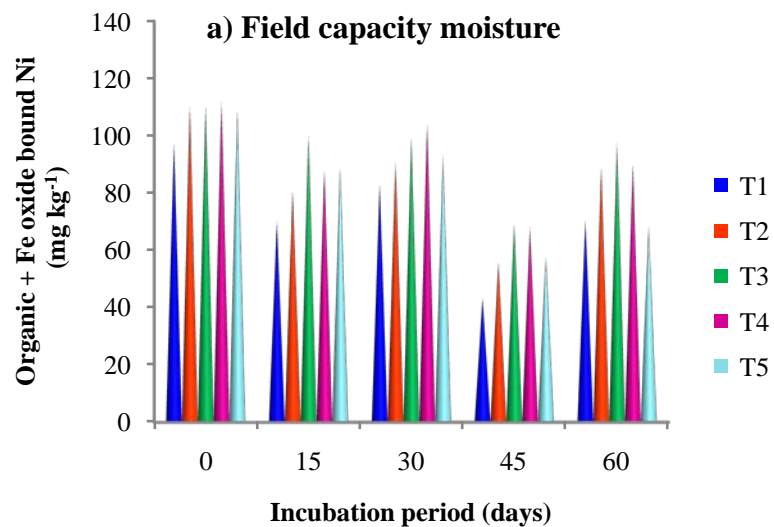
#### **5.3.4.4. Organic plus iron oxide bound fraction of Ni**

The concentration of organic plus iron oxide bound Ni was found higher in the soil under alternate wetting and drying condition than in the soil under field capacity moisture. In both moisture conditions, the concentration of organic plus iron oxide bound Ni was found decreased and increased alternatively over a period of time (Fig. 5.23). The application of different bioamendments resulted in significant changes in organic plus iron oxide fraction of Ni. Initially, the organic plus iron oxide bound fraction of Ni was found higher ( $112.11 \text{ mg kg}^{-1}$ ) in pressmud compost amended soil ( $T_4$ ) and lower ( $97.79 \text{ mg kg}^{-1}$ ) in control soil ( $T_1$ ). Under field capacity moisture condition, it was found higher ( $97.56 \text{ mg kg}^{-1}$ ) in the soil with poultry manure ( $T_3$ ) and lower ( $70.64 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ) at final stage. But under alternate wetting and drying condition, the highest concentration ( $94.95 \text{ mg kg}^{-1}$ ) was recorded in the soil amended with poultry manure ( $T_3$ ) and the lowest concentration ( $63.45 \text{ mg kg}^{-1}$ ) in the treatment amended with the biochar ( $T_5$ ). The variation in organic plus iron oxide bound fraction of Ni could be attributed to the complexation or chelation of Ni with the organic matter (Almas *et al.*, 1999). The characteristics and nature of organic carbon present in various bioamendments significantly affected the biotransformation of Ni in soil and resulted in differential concentration. The metal could precipitate as inorganic compounds during mineralization of bioamendments (Walker *et al.*, 2003).

#### **5.3.4 .5. Residual fraction of Ni**

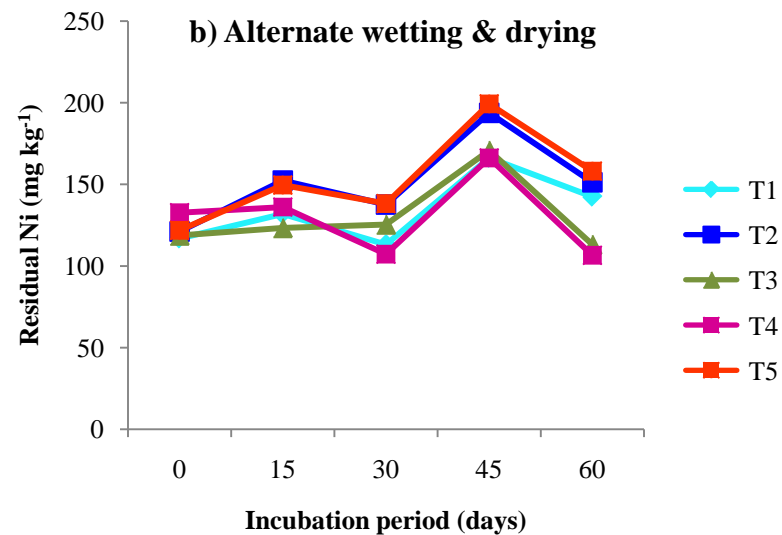
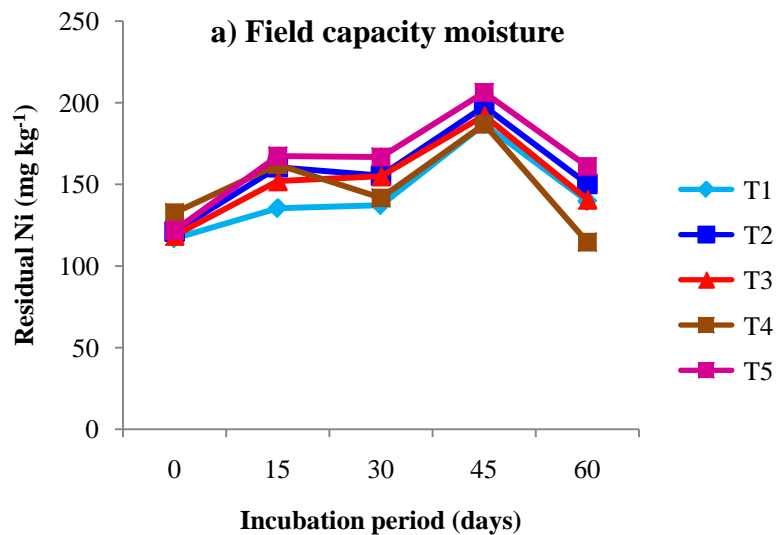
The concentration of residual Ni was significantly higher in the soil under field capacity moisture than in the soil under alternate wetting and drying condition. Under both moisture conditions, the residual Ni was found increased gradually from 0<sup>th</sup> day to 45<sup>th</sup> day and thereafter it was found decreased at 60<sup>th</sup> day of incubation (Fig. 5.24).

The bioamendments addition determines important changes in the residual fraction of Ni. Initially, the highest residual Ni ( $132.67 \text{ mg kg}^{-1}$ ) was observed in the treatment that had pressmud compost ( $T_4$ ) and the lowest ( $116.83 \text{ mg kg}^{-1}$ ) in the control soil ( $T_1$ ). But after 60 days of incubation, the concentration of residual Ni ( $161.01 \text{ mg kg}^{-1}$ ) was highest in the soil amended with biochar ( $T_5$ ) and the lowest ( $114.62 \text{ mg kg}^{-1}$ ) in the soil amended with pressmud compost ( $T_4$ ) under field capacity moisture. But under



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig. 5.23 Effect of bioamendments on Organic plus iron oxide bound Nickel during incubation of Nickel contaminated soil under two different moisture conditions**



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>); T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>)  
 T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>); T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig. 5.24 Effect of bioamendments on Residual Nickel during incubation of Nickel contaminated soil under two different moisture conditions**

alternate wetting and drying condition, the highest concentration of residual Ni ( $158.17 \text{ mg kg}^{-1}$ ) was recorded in the biochar amended soil ( $T_5$ ) and lowest ( $106.58 \text{ mg kg}^{-1}$ ) in the soil with pressmud compost ( $T_4$ ) after 60 days of incubation. Biochar has been reported to adsorb larger amount of heavy metals and thus might have retained greater amount of Ni (Zhang *et al.*, 2013). The persistence of Ni in soil may be due to the immobile nature. The significant increase in the residual fraction Ni can be attributed to the sorption of Ni. It is the dominating speciation process and thus, the largest fraction of Ni in soil is associated with the solid phase of that metal. It also exhibits low mobility in the soil (Sherene, 2010).

#### **5.4. PHYTOEXTRACTION OF NICKEL FROM THE CONTAMINATED SOIL**

Phytoremediation is the major mechanism through which metal is absorbed by plants and accumulated in their biomass. Then the plants may be harvested and metals recovered by burning/ incinerating the plant biomass. The major criteria for selecting plant species for phytoremediation are hyperaccumulating, fast growing species with high biomass. Three important processes take place during the extraction of metals from soil such as solubilization of metals in soil matrix, root uptake of metals and translocation of metals in the shoot parts of plants. Usually Ni is stable in the soil and cannot readily be taken up by the plants. Bioavailability of Ni is the prime factor that decides the phytoextraction process. Bioavailable concentration of a metal may be defined as fraction of metal in soils that can be readily absorbed by plants and interferes in the biochemical processes. Plant absorption of Ni and other metals is influenced by soil pH, CEC, SOM, lime and concentration of micro- and macro-nutrients in soils (Seregin and Kozhevnikova, 2006). The SOM is the most important as it could influence other soil physical and chemical properties which ultimately determine phytoavailability of Ni and other metals. Organic matter plays an important role in accumulation and transportation of Ni and other metals through formation of chelates of various stabilities. Humic acid, fulvic acid and humin are the most stable compounds resulting from bio-chemical decomposition of OM (Stevenson and Cole, 1999). The SOM plays different roles in controlling uptake of Ni and other metals by plants depending upon whether its fraction in soil is soluble (fulvic acid) or insoluble (humic acid). The SOM may (a) inhibit metal sorption through formation of complexes with metals or competition with metals at the

surface for sorption sites, (b) enhance metal sorption as ligands form stable complexes with metal ions which has strong affinity for adsorption onto soil surface and /or (c) may have no or little effect on sorption of metals due to weak complexation (Huang and Lin, 1981; McBride, 1994; Harter and Naidu, 1995). To increase the bioavailable fraction of Ni, manipulating soil properties such as pH, organic matter content, CEC etc is necessary. Therefore, in the current study bioamendments were used to examine their effect on Ni bioavailability in soil. From the results of laboratory incubation study, it is evident that the bioamendments influenced the soil biochemical properties and increased the bioavailability of Ni. Therefore, the effect of bioamendments on bioavailability of Ni in soil and subsequent uptake (removal) by Marigold and Alfalfa plants were examined by conducting two pot experiments.

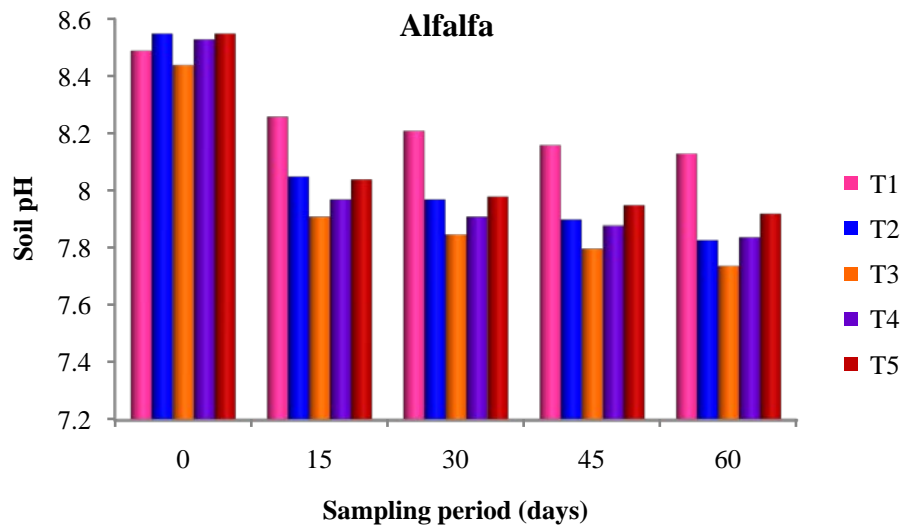
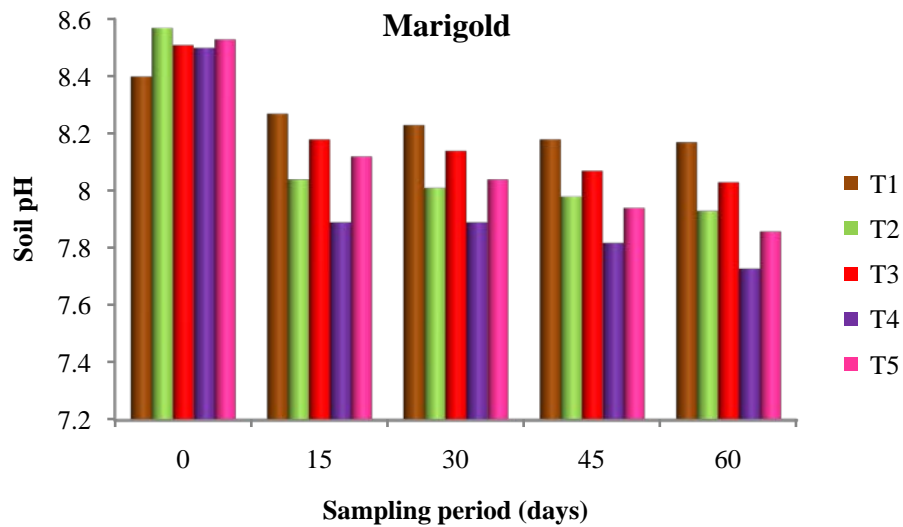
#### **5.4.1. Pot experiment with Marigold and Alfalfa**

##### **5.4.1.1. Soil pH**

Similar to the results of incubation experiments, the pHs of soil with marigold and alfalfa was found decreased gradually from 0<sup>th</sup> day to 60<sup>th</sup> day (Fig. 5.25). The bioamendments had markedly influenced the pH of soil. Initially the soil pH ranged from 8.4 to 8.57 after with various bioamendments. At the end of 60 days, the maximum pH was observed in the control (T<sub>1</sub>) and minimum in the pressmud compost applied soil (T<sub>4</sub>) in both crops. Decrease in soil pH could be attributed to the organic acid produced during decomposition of bioamendments in soil (Mahimairaja *et al.*, 1999).

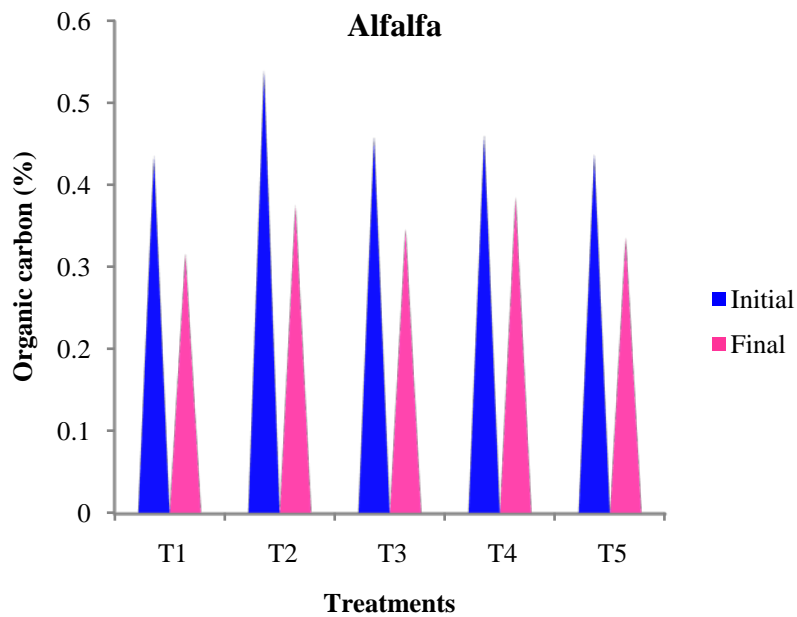
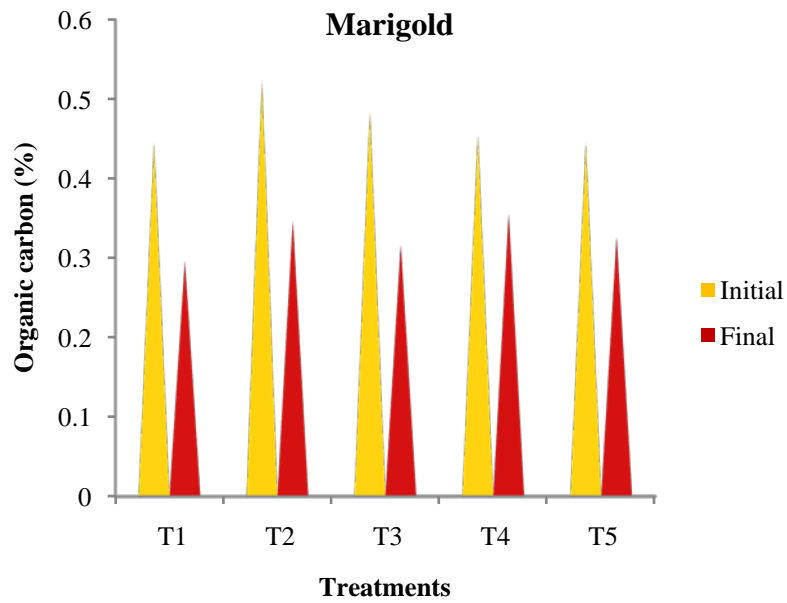
##### **5.4.1.2. Soil organic carbon**

In both pot experiments (Marigold and Alfalfa), the SOC was found decreased over a time period (Fig. 5.26). Initially, it was found maximum in the soil amended with farmyard manure (T<sub>2</sub>) and minimum in control (T<sub>1</sub>). During later stage the maximum SOC was recorded in the soil treated with pressmud compost (T<sub>4</sub>) and minimum in the control soil (T<sub>1</sub>). The bioamendments enhanced the microbial activity of soil and it acted as a source of carbon for microorganisms. Subsequently the carbon is released as CO<sub>2</sub> and/or CH<sub>4</sub> from soil might be the reason for the reduction in SOC. Initially, the SOC content was found maximum in soil treated with farmyard manure but it did not increase the organic matter to a greater extent. It could be due to presence of soluble organic



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.25 Effect of bioamendments on pH of soil with Marigold and Alfalfa**



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.26 Effect of bioamendments on soil organic carbon in pot experiment with Marigold and Alfalfa**

compounds that mineralized rapidly and thus contributed less SOC at the end of the experiment (Sabir *et al.*, 2008). During final stage, SOC was found significantly high due to pressmud compost application suggests that the organic matter contributed by pressmud compost was relatively stable when compared to other amendments (Karaca, 2004).

#### ***5.4.1.3. Water soluble organic carbon of soil***

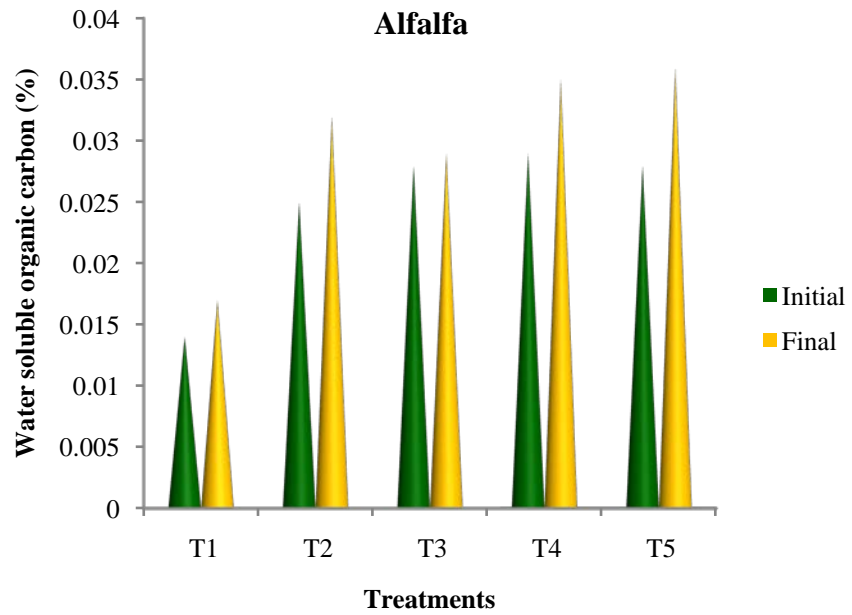
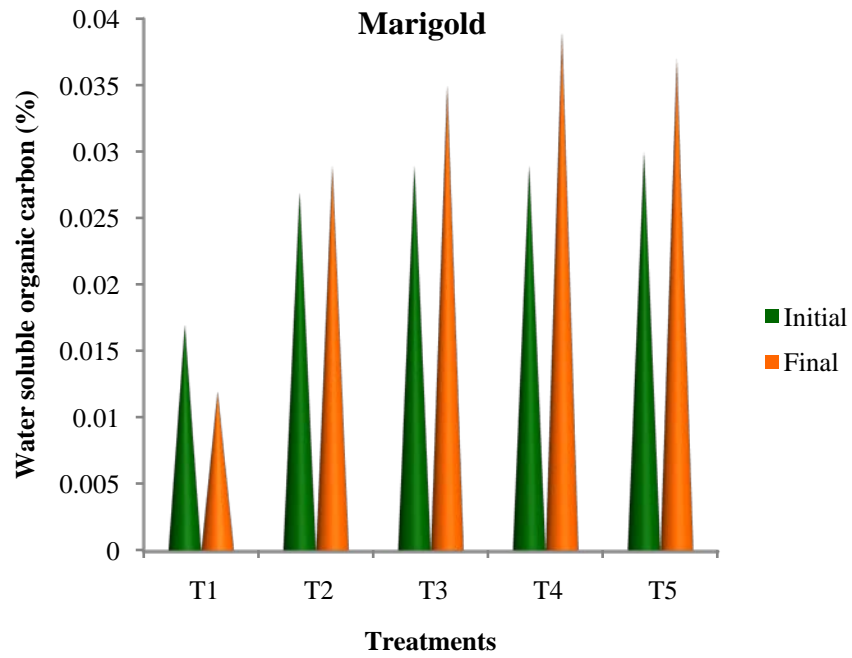
The WSOC was found increased soil under marigold as well as alfalfa pot culture plants (Fig. 5.27). With marigold, the maximum WSOC was observed in the soil amended with prosopis biochar (T<sub>5</sub>) and minimum in the control soil initially but at the end, the highest concentration of WSOC was registered in soil amended with pressmud compost (T<sub>4</sub>) and the lowest in the control (T<sub>1</sub>). Likewise with alfalfa, the maximum WSOC was found in the soil amended with pressmud compost (T<sub>4</sub>) and minimum in control (T<sub>1</sub>) initially. During later stage the highest concentration was recorded due to the addition of prosopis biochar (T<sub>5</sub>) and the lowest in the control (T<sub>1</sub>). The higher amount of WSOC was found in the soil with pressmud compost and prosopis biochar might be due to the addition of soluble carbon through bioamendments or enhanced carbon mineralization by the microbial activity favoured by these bioamendments. Thus Ni might have formed soluble complexes easily and increased the bioavailability of Ni in soil (Silveria *et al.*, 2003; Chirenje *et al.*, 2002).

#### ***5.4.1.4. Biotransformation and bioavailability of Nickel in soil***

The effect of bioamendments on the biotransformation of Ni differed significantly in soils under marigold and alfalfa

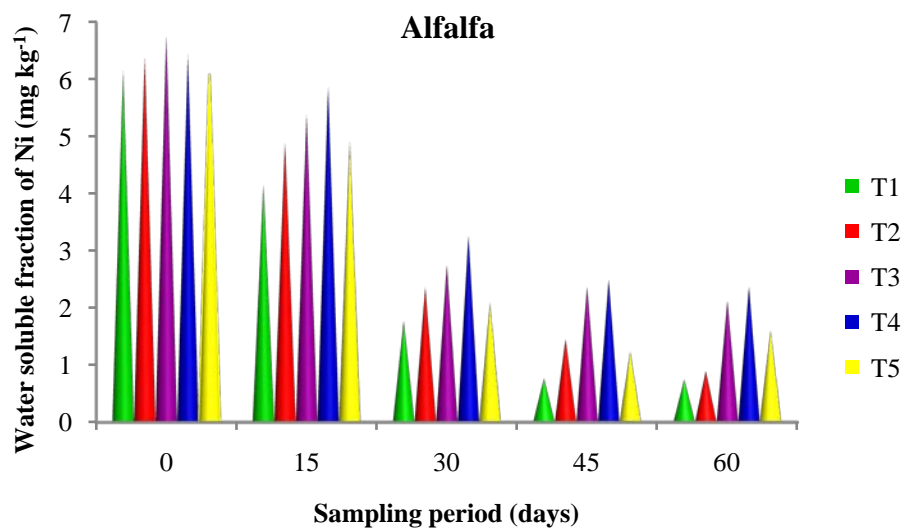
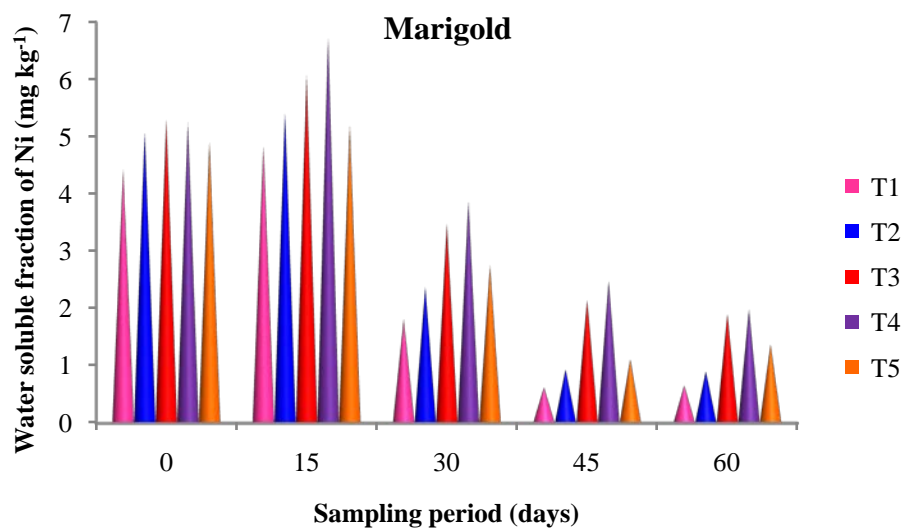
##### ***5.4.1.4.1. Water soluble Nickel***

With marigold, the water soluble Ni in soil showed a slight increase initially, afterwards it was found gradually decreased up to final stage of pot experiment (Fig. 5.28). The water soluble Ni was significantly found higher in the soil amended with pressmud compost (T<sub>4</sub>) and lower in the control soil at all stages (T<sub>1</sub>). Whereas in soils with alfalfa, the water soluble Ni was found gradually decreased from initial to final stage of pot experiment. Initially, the water soluble Ni was recorded high in the soil amended with composted poultry manure (T<sub>3</sub>) and low in the soil amended with prosopis biochar (T<sub>5</sub>).



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>.Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.27 Effect of bioamendments on water soluble organic carbon in soil with Marigold and Alfalfa**



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.28 Effect of bioamendments on Water soluble Nickel in soil with Marigold and Alfalfa**

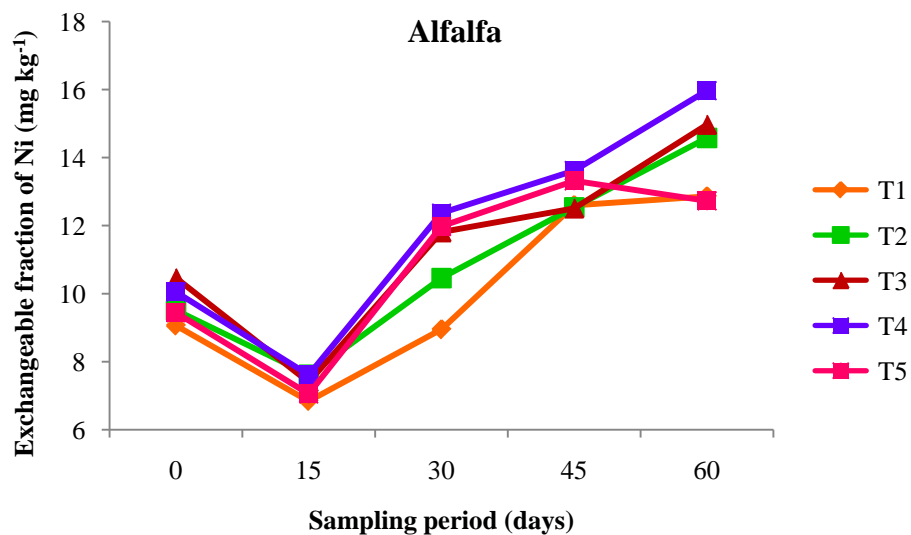
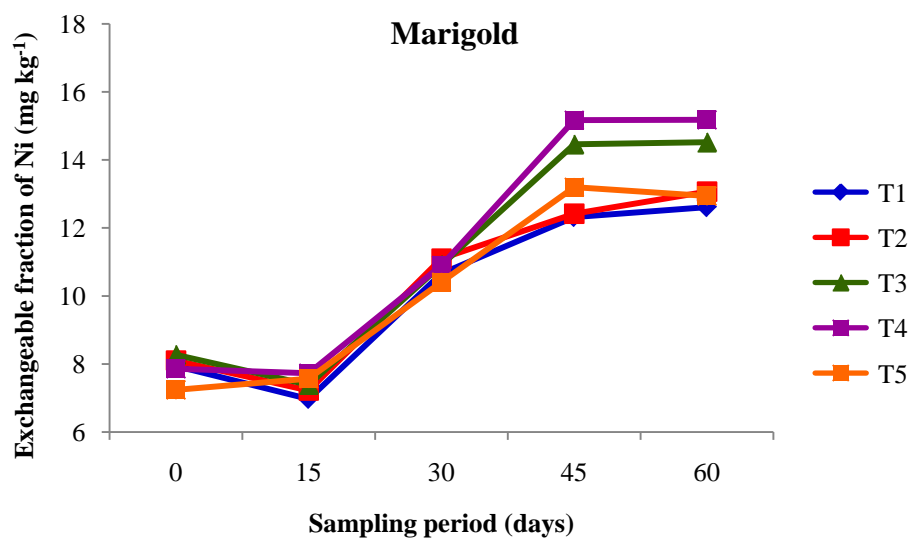
However, at the end of 60 days the highest value was recorded in the soil with pressmud compost application (T<sub>4</sub>) and the lowest in the control. The increase in water soluble Ni might be due to the solubilization or microbial conversion of Ni in to soluble form favoured by the reduction in soil pH. Thus the soluble fraction of Ni was increased and extracted by plants throughout the period. The Ni solubilization enhanced was also due to the increase in humic and fulvic substances of bioamendments (Gungor and Bekbolet, 2010).

#### 5.4.1.4.2. Exchangeable Nickel

The exchangeable Ni in soil was found gradually decreased during initial stages and then increased rapidly in soil with both marigold and alfalfa plants (Fig. 5.29). Initially, the highest concentration of exchangeable Ni was recorded in the soil amended with composted poultry manure (T<sub>3</sub>) and the lowest in the control (T<sub>1</sub>). At the end of 60 days of experiment the highest value was registered in the soil amended with pressmud compost (T<sub>4</sub>) and the lowest value in the control soil (T<sub>1</sub>). Application of pressmud compost was found to enhance the exchangeable Ni in soil with both the crops. The enhancement of exchangeable Ni might be due to the mobilization of Ni by dissolved organic carbon present in the pressmud compost. Bioamendments could affect the bioavailability of Ni through different mechanisms. For example, Ni-organic matter association could occur both in soil solution and soil surface (Silveria *et al.*, 2003). Both the nature and stability of soil-applied organic materials could influence metal solid-solution phase partitioning and thus alter the bioavailability of Ni. Different biochemical transformation of organic matter leads to the release of DOC and thus it increases mobility and bioavailability of metals (Martinez *et al.*, 2003).

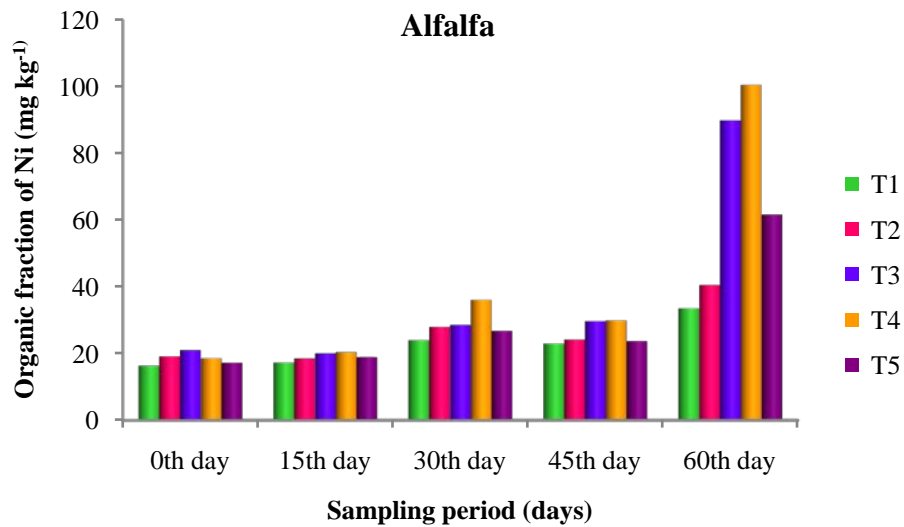
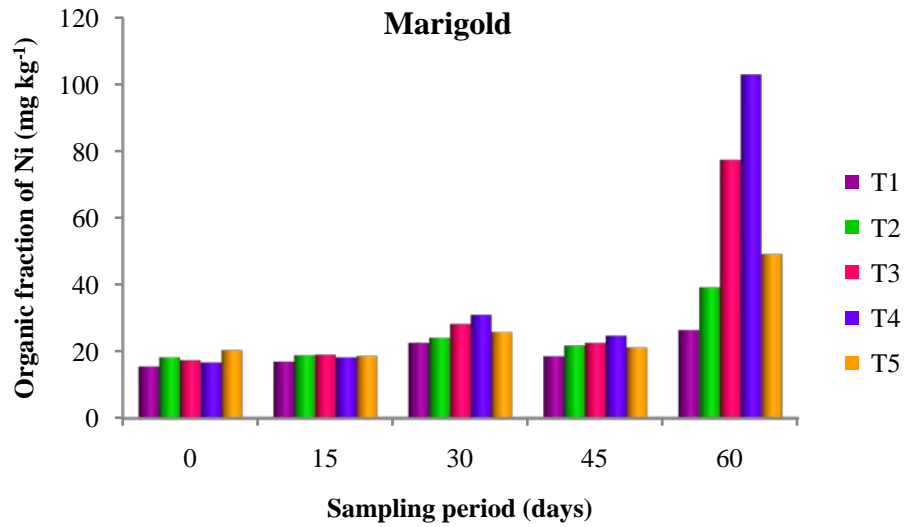
#### 5.4.1.4.3. Organic Nickel

In both marigold and alfalfa, the concentration of organic Ni was gradually increased upto 30<sup>th</sup> day slightly decreased at 45<sup>th</sup> day and rapidly increased at 60<sup>th</sup> day of experimentation (Fig. 5.30). During early stage of marigold, the concentration of organic Ni was found high in the treatment (T<sub>5</sub>) that had prosopis biochar and low in the control soil. However at the end of 60 days, the highest value was recorded in the soil amended with pressmud compost (T<sub>4</sub>) and the lowest value in the control (T<sub>1</sub>). In alfalfa grown soil, during early stage, the organic Ni was high in the treatment (T<sub>3</sub>) that supplied



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>.Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.29 Effect of bioamendments on Exchangeable Nickel in soil with Marigold and Alfalfa**



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.30 Effect of bioamendments on Organic Nickel in soil with Marigold and Alfalfa**

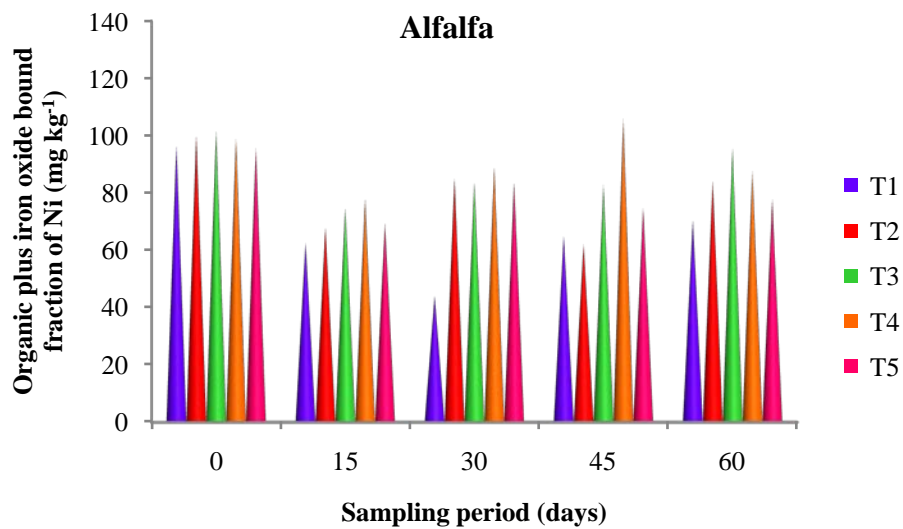
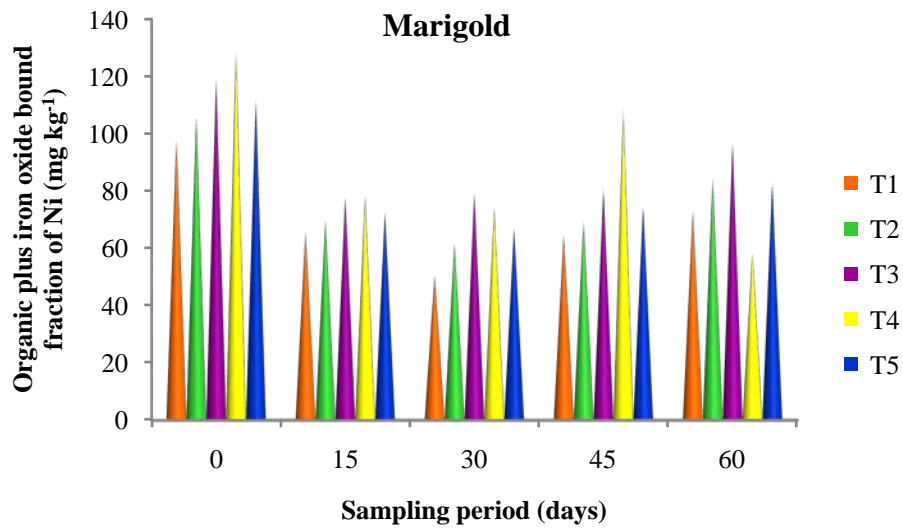
composted poultry manure and low in control soil. Finally the highest value was recorded in the treatment that had pressmud compost (T<sub>4</sub>) and the lowest value in the control treatment (T<sub>1</sub>). The increase of organic Ni is attributed to the complex formation with organic matter. Organic matter could chelate metals by coordinating with them directly and through inner-sphere complex formation with acid functional groups (Mcbride, 1989). Organic matter could re-distribute Ni from available forms to fractions associated with organic matter in soil as well as other soil components (Karaca, 2004).

#### 5.4.1.4.4. Organic plus iron oxide bound Nickel

The organic plus iron oxide bound Ni was found decreased initially but increased slightly towards the end of experiment with both marigold and alfalfa (Fig. 5.31). During initial stages of marigold, the concentration of organic plus iron oxide bound Ni was high in soil amended with pressmud compost (T<sub>4</sub>) and low in control soil. Finally it was recorded high in the treatment applied with composted poultry manure (T<sub>3</sub>) and low in the control soil. In contrast, the concentration of organic plus iron oxide bound Ni in soil with alfalfa was high due to composted poultry manure application (T<sub>3</sub>) and low in control soil during both initial and final stages. The increase in the concentration of organic plus iron oxide bound Ni might be due to the increased complexation of Ni with humic acid of organic matter which is insoluble in nature. Nickel may form soluble complexes with fulvic acid while humic acid forms insoluble complexes (Chirenje *et al.*, 2002).

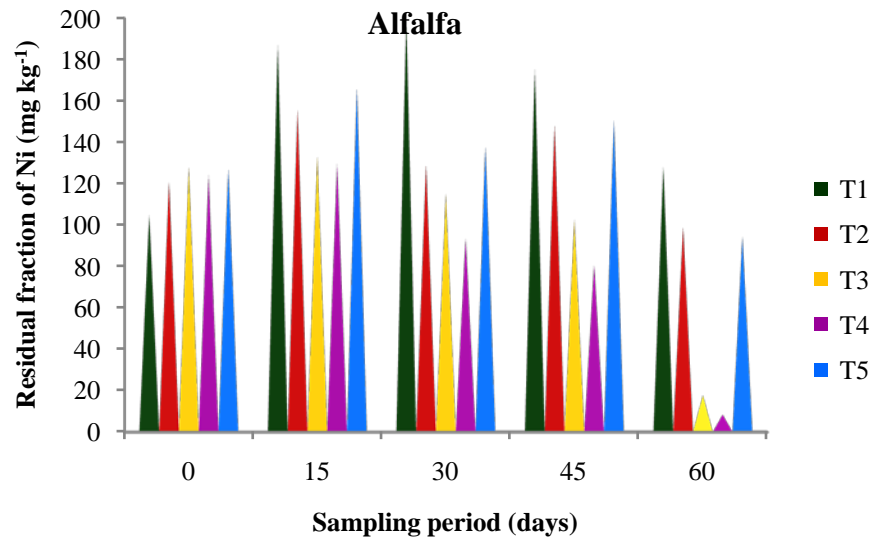
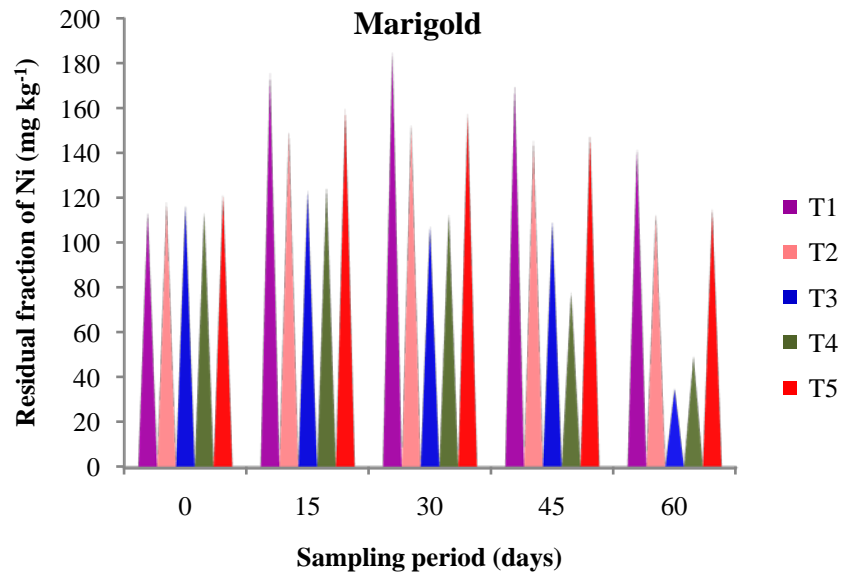
#### 5.4.1.4.5. Residual Nickel

In both marigold and alfalfa grown soil, the concentration of residual Ni was increased gradually during initial stages and then decreased drastically towards end of experiment (Fig. 5.32). Initially, the highest concentration of residual Ni was obtained in the soil amended with prosopis biochar (T<sub>5</sub>) and the lowest concentration in the soil amended with pressmud compost (T<sub>4</sub>). However, during final stage of the experiment, relatively higher concentration of residual Ni was recorded in the control soil (T<sub>1</sub>) and relatively lower concentrations was observed in soil treated with composted poultry manure amended soil (T<sub>3</sub>) in marigold experiment. In alfalfa experiment, initially the highest value of residual Ni was obtained due to the application of prosopis biochar (T<sub>5</sub>) and the lowest value in control soil (T<sub>1</sub>). During final stage of the experiment, the



T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.31 Effect of bioamendments on Organic plus iron oxide bound Nickel in soil with Marigold and Alfalfa**



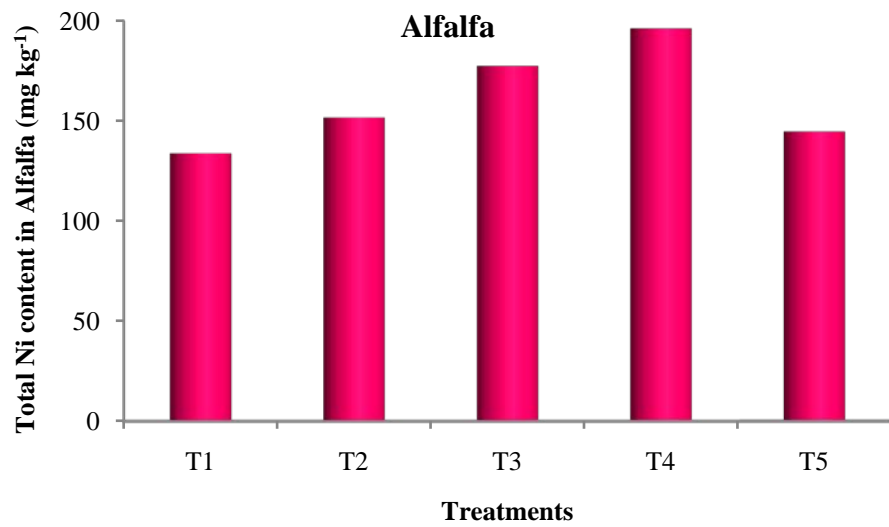
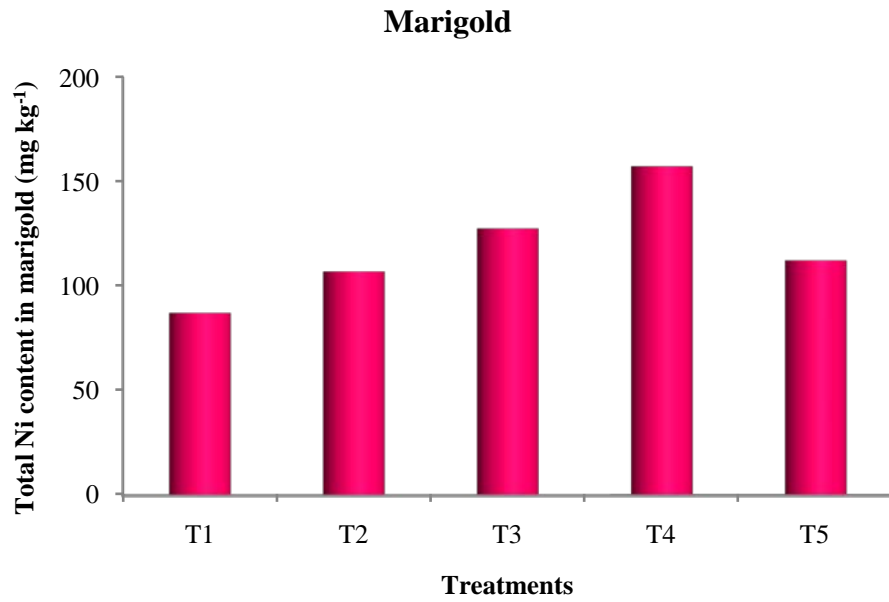
T<sub>1</sub>. Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.32 Effect of bioamendments on Residual Nickel in soil with Marigold and Alfalfa**

residual Ni was recorded higher in the control soil (T<sub>1</sub>) and lower in the pressmud compost amended soil (T<sub>3</sub>). The increase in the concentration of residual Ni might be due to various processes such as adsorption, precipitation or complexation reactions of Ni. The soil amended with prosopis biochar showed increased amount of residual Ni which could be attributed to strong complexation of Ni with its humified organic matter contents that has strong affinity for adsorption on to soil surface (Narwal and Singh, 1998; Misra and Chaturvardi, 2007). Prosopis biochar effectively immobilized Ni due to its high cation exchange capacity also.

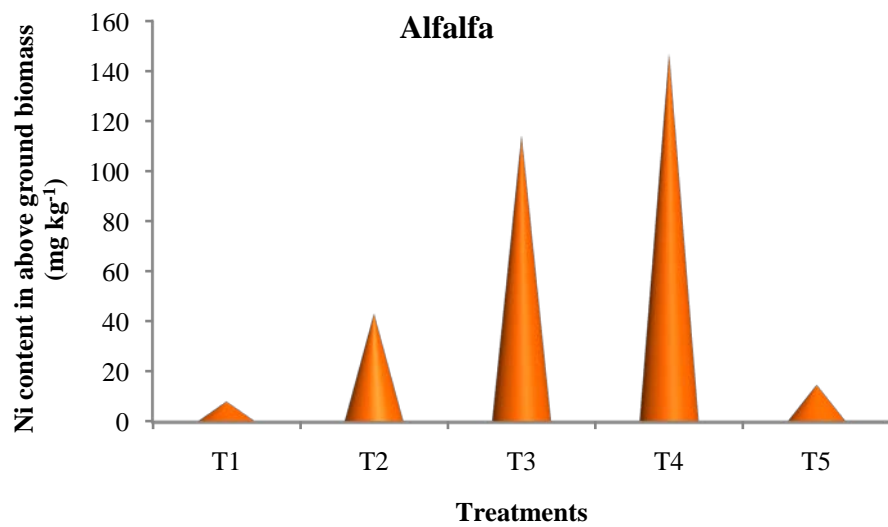
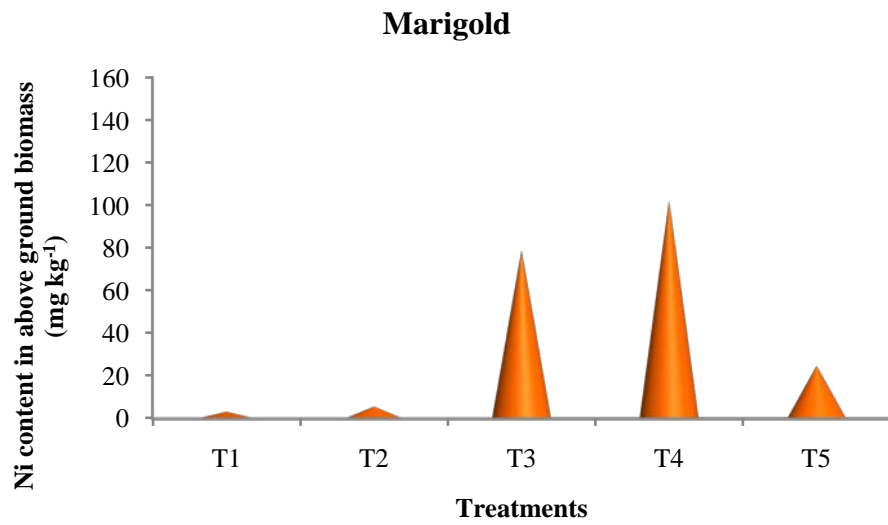
#### **5.4.1.5. Nickel content and uptake by crops**

Plants can accumulate Ni usually less than 0.1 per cent of total dry weight (Lee *et al.*, 1987). Nickel is very mobile and can be accumulated in vegetative and reproductive parts (Soon *et al.*, 1980). The results from both marigold and alfalfa experiment showed that the pressmud application resulted in the highest Ni content in plants. Plants grown on control soil had the lowest content of Ni (Fig. 5.33). Relatively higher concentration of Ni was observed in root biomass than in above ground biomass. In general the plants grown on the control soil had greater amount of Ni in root biomass than in the above ground biomass. In the above ground biomass the highest Ni content was found in the plants grown on soil amended with pressmud compost (T<sub>4</sub>) followed by composted poultry manure (T<sub>3</sub>). The lowest Ni in above ground biomass was observed in the control (T<sub>1</sub>) (Fig. 5.34). In contrast the root biomass of marigold, had significantly higher Ni content due to the application of farmyard manure (T<sub>2</sub>) followed by prosopis biochar (T<sub>5</sub>) and the lowest Ni content in the root biomass was found in the plants grown on soil amended with composted poultry manure (T<sub>3</sub>). In alfalfa, the Ni content of root biomass was relatively higher when the plants grown on soil with prosopis biochar (T<sub>5</sub>) followed by control soil (T<sub>1</sub>). However, the alfalfa had lower Ni content when they grown on soil amended pressmud compost (T<sub>4</sub>) (Fig. 5.35). The uptake of Ni by marigold and alfalfa had shown similar effect of bioamendments (Fig.5.36). A positive correlation was obtained between Ni uptake by marigold and bioavailable fractions of Ni in soil (Fig. 5.37 & 5.38; R<sup>2</sup> for Water soluble Ni=0.887; R<sup>2</sup> for Exchangeable Ni=0.941). Similarly for alfalfa also positive correlation was obtained (Fig. 5.39 & 5.40; R<sup>2</sup> for Water soluble Ni=0.893; R<sup>2</sup> for Exchangeable Ni=0.684). All the bioamendments



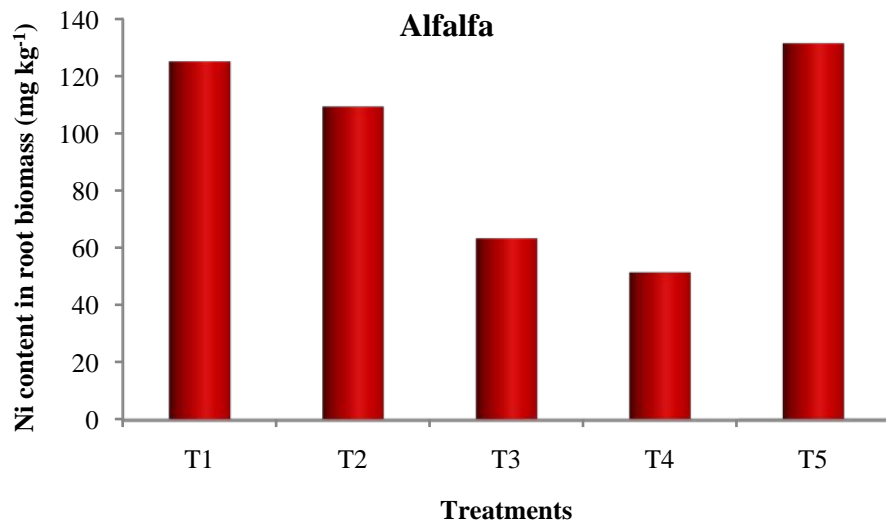
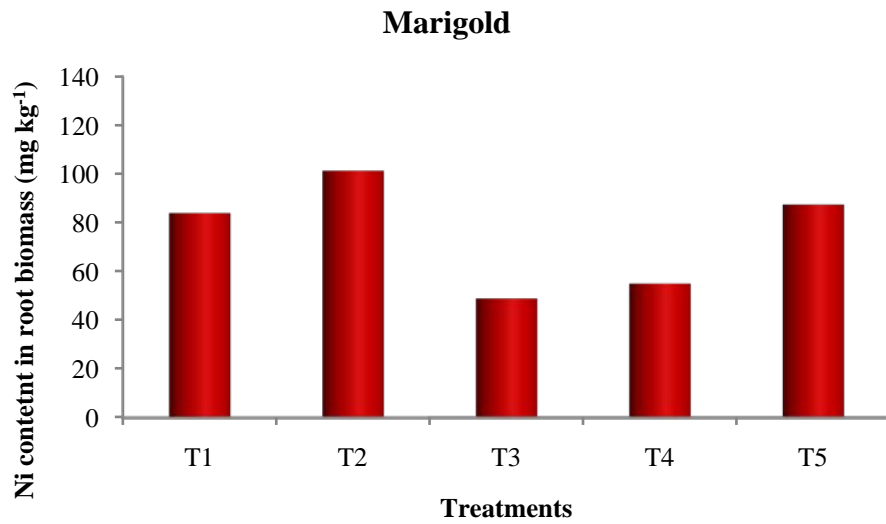
T<sub>1</sub>. Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.33 Effect of bioamendments on Nickel content of Marigold and Alfalfa**



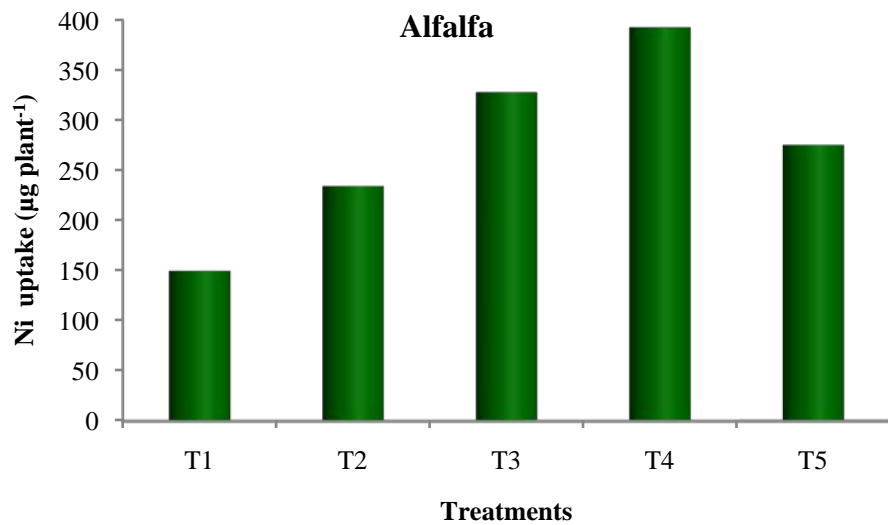
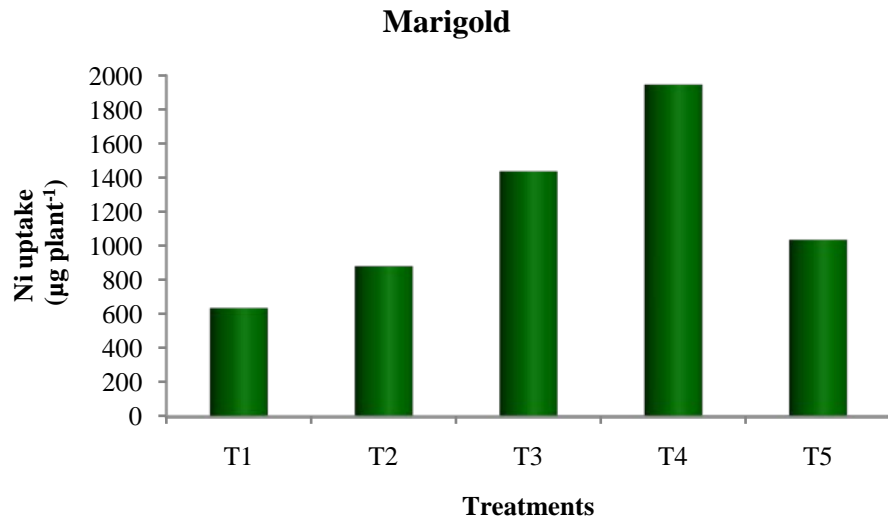
T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>.Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.34 Effect of bioamendments on Nickel content in above ground biomass of Marigold and Alfalfa**



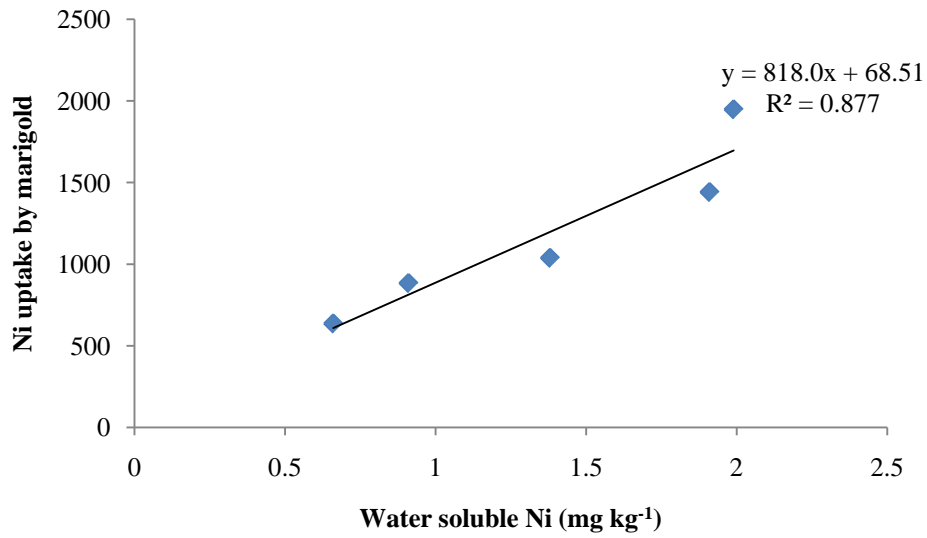
T<sub>1</sub>. Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

**Fig 5.35 Effect of bioamendments on Nickel content in root biomass of Marigold and Alfalfa**

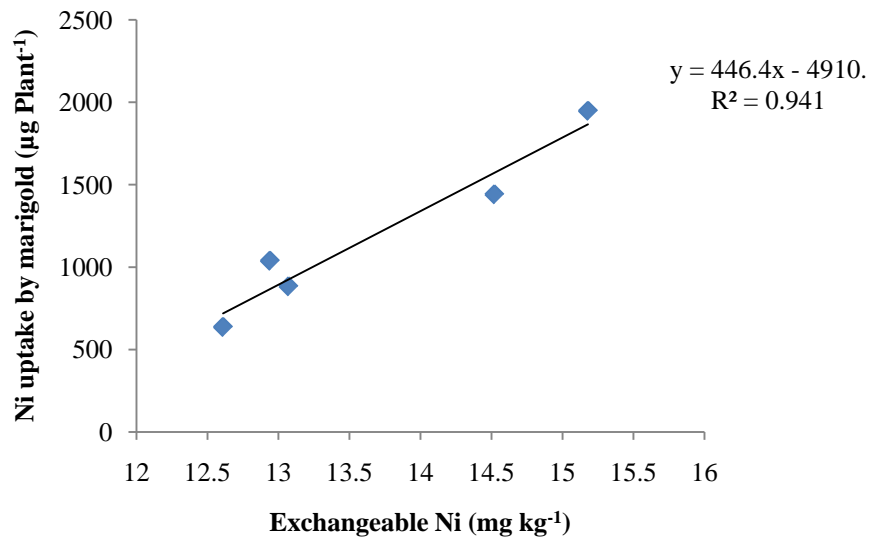


T<sub>1</sub>.Control - Soil\* (No amendments); T<sub>2</sub>. Soil\* + Farmyard manure (12.5t ha<sup>-1</sup>);  
 T<sub>3</sub>. Soil\*+ Composted poultry manure (5t ha<sup>-1</sup>); T<sub>4</sub>. Soil\*+ Pressmud compost (5t ha<sup>-1</sup>);  
 T<sub>5</sub>. Soil\*+ Prosopis biochar (5t ha<sup>-1</sup>)

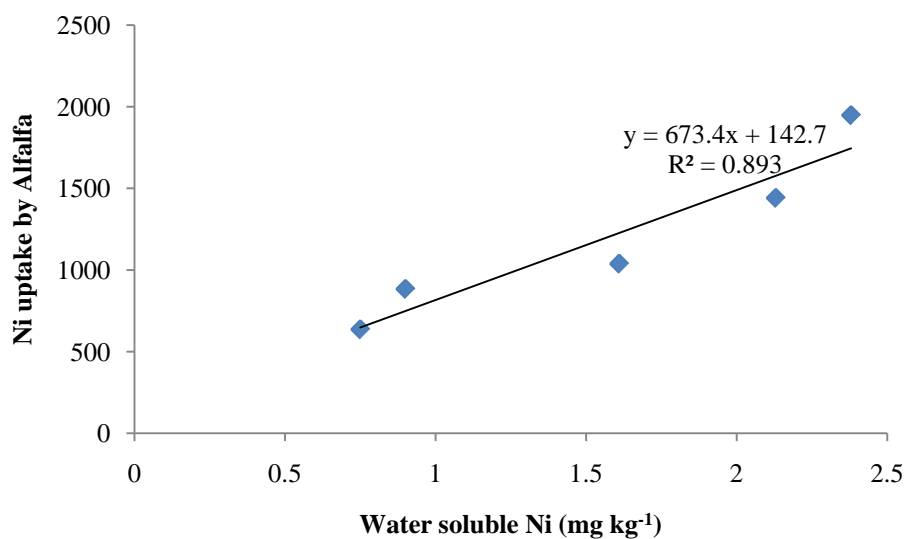
**Fig 5.36 Effect of bioamendments on Nickel uptake by Marigold and Alfalfa**



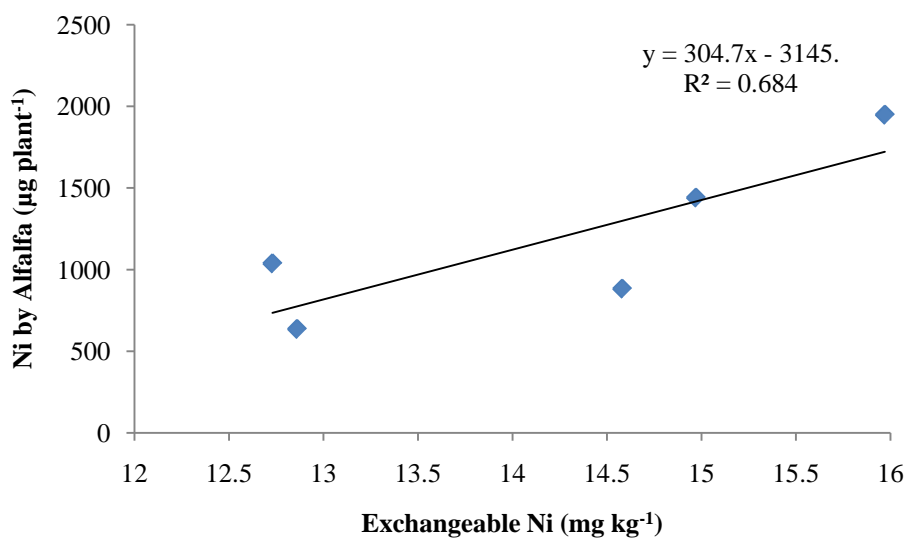
**Fig. 5.37 Relationship between Water soluble Nickel in soil and Nickel uptake by marigold**



**Fig. 5.38 Relationship between Exchangeable Nickel in soil and Nickel uptake by marigold**



**Fig. 5.39 Relationship between Water soluble Nickel in soil and Nickel uptake by alfalfa**



**Fig. 5.40 Relationship between Exchangeable Nickel in soil and Nickel uptake by alfalfa**

significantly increased the biomass of marigold and alfalfa. The higher biomass production due to the application of bioamendments could be due to addition of plant nutrients particularly nitrogen released during decomposition of bioamendments (Clemente *et al.*, 2007) and improvement in the soil physical properties. The plants grown on soil amended with pressmud compost had shown relatively higher biomass. This could be due to its higher nutrients and organic matter contents, which on mineralization can supply additional nutrients, improve buffering capacity and enhance nutrient cycling and thus can improve plant growth (Stewart *et al.*, 2000; Clemente *et al.*, 2007). While prosopis biochar was found to reduce the Ni concentration in marigold and alfalfa, pressmud compost and composted poultry manure were found to increase Ni content and uptake by plants. This could be due to the immobilization of Ni with prosopis biochar in the soil compared to other amendments as evidenced from less exchangeable fractions of Ni in soil amended with prosopis biochar.

#### **5.4.1.6. Bioconcentration factor and Translocation factor**

The mobility of Ni from the polluted soil into the roots of marigold and alfalfa and the ability to translocate the Ni from roots to above ground parts were evaluated by computing the Bioconcentration factor (BCF), the translocation factor (TF) and enrichment factor (EF) as follows (Lorestani *et al.*, 2011);

$$\text{BCF} = \text{Ni in roots (mg kg}^{-1}\text{)} / \text{Ni in soil (mg kg}^{-1}\text{)}$$

$$\text{TF} = \text{Ni in stover/stalks (mg kg}^{-1}\text{)} / \text{Ni in roots (mg kg}^{-1}\text{)}$$

$$\text{EF} = \text{Ni in stover/stalk (mg kg}^{-1}\text{)} / \text{Ni in soil (mg kg}^{-1}\text{)}$$

The ability of marigold and alfalfa to tolerate and accumulate Ni is useful for phytoextraction and thus for phytoremediation. Plants with both BCF and TF greater than one have the potential to be used in phytoextraction. Besides, plants with BCF greater than one and TF less than one have the potential for Phytostabilization (Yoon *et al.*, 2006). The lesser values of BCF may suggest the restriction in soil-root transfer at this Ni concentration in the soil (Gafoori *et al.*, 2011). The hyper accumulator plant should have EF greater than 1, or TF >1. The results obtained from the pot experiments are presented in Table 5.3 and showed that the BCF and EF were less than one for both marigold and

**Table 5.3 Bioconcentration factor (BCF), Translocation co-efficient factor (TF) and Enrichment factor (EF) for marigold and alfalfa crop**

Treatments	Marigold			Alfalfa		
	BCF	TF	EF	BCF	TF	EF
T <sub>1</sub>	0.280	0.042	0.011	0.418	0.101	0.027
T <sub>2</sub>	0.339	0.056	0.019	0.365	0.427	0.144
T <sub>3</sub>	0.163	1.921	0.264	0.212	2.626	0.381
T <sub>4</sub>	0.183	1.866	0.342	0.172	2.674	0.490
T <sub>5</sub>	0.292	0.285	0.083	0.439	0.170	0.050
<b>Mean</b>	<b>0.2514</b>	<b>0.834</b>	<b>0.1438</b>	<b>0.3212</b>	<b>1.1996</b>	<b>0.2184</b>

alfalfa but the TF was greater than one in plants grown on soil amended with bioamendments. Therefore, they may be considered as Ni hyperaccumulator. Heavy metal tolerance with high TF and low BCF value was suggested for phytoaccumulator for contaminated soil (Yoon *et al.*, 2006). However, these plants showed greater potential in tolerating and accumulating higher concentration Ni and therefore could be integrated along with bioamendments for phytoextraction and thus phytoremediation of Ni contaminated soil.

## *Summary and Conclusion*

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## CHAPTER VI

### SUMMARY AND CONCLUSIONS

Coimbatore is one of the largest industrial city in Tamil Nadu, where a number of industries including electroplating, textile, steel, foundries and metal based industries etc are located. The effluent and sludge generated from these industries are disposed on agricultural lands and water bodies, resulted in several 'Hot-spots' of heavy metals including nickel (Ni) contamination in and around Coimbatore. In the current study, a series of laboratory incubation and pot experiments were conducted to determine the fate of Ni in soil and based on the results a feasible phytoremediation strategy was developed. The results and salient findings of the study are summarized and conclusions drawn.

#### 6.1 ADSORPTION AND DESORPTION OF NICKEL

The mobility and bioavailability of Ni is governed by many chemical and biochemical processes such as sorption-desorption, precipitation-dissolution, complexation-dissociation, oxidation-reduction and biotransformation. Amongst all these reactions, adsorption is the single most important mechanism which determines fate and bioavailability of Ni in soil and thus its environmental impacts. Therefore, a series of laboratory batch experiments were conducted to examine the adsorption and desorption characters of Ni. The impact of pH, soil organic matter (SOM) and co-contaminants present in soil was also studied. The following are the salient findings.

- ❖ Adsorption of Ni on soil continued up to 48 hrs, although more than 89 per cent occurred within 12 hrs. The adsorption was found rapid initially (>80% within 30 minutes) and continued afterwards at a slow rate.
- ❖ The third order, parabolic diffusion and Elovich equations described Ni sorption much better than did the zero-, first-, and second order reactions. The relatively higher  $R^2$  over the whole time range indicated that the Ni sorption in this soil was better described by third order reaction kinetics ( $R^2=0.998$ ) followed by Elovich equation ( $R^2=0.922$ ) and parabolic diffusion ( $R^2=0.829$ ).

- ❖ A relatively higher correlation coefficient ( $R^2 = 0.72$ ) indicated that the Ni sorption was adequately described by the Langmuir isotherm than did Freundlich equation.
- ❖ The adsorption of Ni was found increased significantly with an increase in pH and the highest amount of Ni adsorbed was observed when the pH was the highest (9.0).
- ❖ Addition of bioamendments reduced the rate of adsorption of Ni on soil, mainly due to the formation of organic complexes and/or the release of organic acids from bioamendments that reduces the negative charges present in soil by protonation reaction.
- ❖ Presence of co-contaminants or competitive ions significantly affected the adsorption of Ni on soil. Significant reduction in Ni adsorption was found when Cd was present even at its low concentration, due to greater ionic size of Cd (97 pm) than Ni (69 pm). In contrast, the adsorption was found increased in the presence of Cr. as a co-contaminant due to greater ionic size of Ni than Cr (52 pm).
- ❖ Only about 45 to 65 per cent of adsorbed Ni was found desorbed from the soil. The 0.005M DTPA was found to extract relatively large amounts of desorbed Ni than did 0.05M  $\text{Ca}(\text{NO}_3)_2$ .

## **6.2 BIOTRANSFORMATION AND BIOAVAILABILITY OF NICKEL IN SOIL**

Bioavailability of Ni in contaminated soils plays an important role as it determines plant uptake, biotoxicity and leaching and subsequent contamination of groundwater. Therefore, the biotransformation and bioavailability of Ni in soil were examined by conducting a laboratory closed incubation experiment. The results are as follows:

- ❖ The soil pH was found relatively higher in the soil under alternate wetting and drying than in the soil under field capacity moisture condition. The soil pH was found increased at 15<sup>th</sup> day and then decreased at the 30<sup>th</sup> day of incubation in the soil under field capacity moisture. But under alternate wetting and drying, the soil pH was decreased at a slow steady rate from 0<sup>th</sup> day to 30<sup>th</sup> day of incubation.

- ❖ Significant changes were observed in SOC during the incubation. The moisture condition markedly influenced the SOC. The soil under field capacity moisture recorded relatively higher SOC than the soil under alternate wetting and drying. The SOC was found decreased during incubation irrespective of moisture conditions.
- ❖ The concentration of various species of Ni in soil follows: Residual-Ni > Organic plus iron oxide bound-Ni > Organic-Ni > Exchangeable-Ni > Water soluble-Ni.
- ❖ Soil moisture conditions had significant influence on biotransformation of Ni in soil.
- ❖ Water soluble and exchangeable forms are considered as the bioavailable form of Ni. The water soluble Ni was found higher in soil under field capacity moisture condition than in soil under alternate and wetting drying. In contrast the exchangeable Ni was found higher in soil under alternate wetting and drying.
- ❖ The water soluble Ni showed a gradual increase from 0<sup>th</sup> day to 15<sup>th</sup> day of incubation. After that, it showed a slight decrease at 30<sup>th</sup> day. Whereas, the exchangeable Ni showed just opposite trend, a notable increase was observed after 15 days.
- ❖ The organic Ni was found relatively higher in soil under alternate wetting and drying conditions than in soil under field capacity moisture condition. Irrespective of moisture condition, the concentration of organic Ni was found decreased gradually up to 15<sup>th</sup> day and increased rapidly towards the end of incubation.
- ❖ Alternate wetting and drying resulted in significantly higher concentrations of organic plus iron oxide bound Ni and residual Ni at all days of incubation. Both the concentrations were found increased during the incubation.

### **6.3 EFFECT OF BIOAMENDMENTS ON BIOTRANSFORMATION AND BIOAVAILABILITY OF NICKEL IN CONTAMINATED SOIL**

Bioamendments such as farmyard manure, composted poultry manure, pressmud compost and biochar had significant effect on soil pH, SOC, WSOC and different species of Ni in soil besides soil moisture condition.

#### **6.3.1. Soil pH and soil organic carbon**

- ❖ The field capacity moisture resulted in relatively greater pHs in soil than the alternate wetting and drying condition. Irrespective of moisture conditions, the pH of soil was found reduced gradually during the incubation. All bioamendments markedly reduced the soil pH, and the effect was more pronounced due to pressmud compost.
- ❖ Though not consistent, the SOC was not significantly affected due to the moisture conditions and found decreased during the incubation. The bioamendments had significant effect on SOC. At the end of 60 days, the SOC was recorded high in the soil amended with pressmud, followed by composted poultry manure.
- ❖ The addition of bioamendment showed marked influence on soil water soluble carbon (WSOC). It was found relatively higher in the soil under field capacity moisture throughout the experimental period. Irrespective of moisture conditions, the WSOC was found increased during 60 days of incubation. The soil amended with composted poultry manure had significantly higher WSOC under field capacity moisture, whereas, the soil amended with biochar had higher content of WSOC under alternate wetting and drying condition.

#### **6.3.2. Biotransformation and bioavailability of Nickel in contaminated soil**

The effect of certain bioamendments on biotransformation and bioavailability of Ni was examined. A sequential fractionation procedure was adopted to determine various species of Ni in soil. The following are the results.

- ❖ Contradicting the earlier results, both the water soluble and exchangeable Ni were found higher in soil under field capacity moisture condition at all days of incubation. The concentrations of both species were found decreased at 45<sup>th</sup> day

and thereafter increased at 60<sup>th</sup> days. Relatively higher concentrations were recorded due to the application of pressmud compost than others. There exists a negative correlation between the soil pH and the water soluble Ni ( $R^2 = -0.796$  to  $-0.93$ ).

- ❖ The alternate wetting and drying resulted in significantly higher concentrations of organic Ni, and organic plus iron oxide bound Ni were found in soil than the field capacity moisture condition. Irrespective of moisture conditions, the concentration of organic Ni was found increased at 30<sup>th</sup> day, slightly decreased at 45<sup>th</sup> day and rapidly increased at 60<sup>th</sup> day of incubation.
- ❖ The application of poultry manure to soil resulted in significantly higher concentration of organic plus iron oxide bound Ni under both moisture conditions. In contrast, the application of biochar resulted in relatively lesser concentration, particularly under alternate wetting and drying condition.
- ❖ The concentration of residual Ni was significantly higher in the soil under field capacity moisture than in the soil under alternate wetting and drying condition. Under both moisture conditions, the residual Ni was found increased gradually from 0<sup>th</sup> day to 45<sup>th</sup> day and thereafter was found decreased at 60<sup>th</sup> day of incubation. The application of biochar resulted in higher concentration of residual Ni in soil under both the moisture conditions, due to greater adsorption and retention of Ni.

#### **6.4 PHYTOEXTRACTION OF NICKEL FROM THE CONTAMINATED SOIL**

Phytoremediation is the major mechanism through which metal is absorbed by plants and accumulated in their biomass. It is evident from the laboratory experiments that the bioamendments greatly influences various biochemical processes and thus enhance the bioavailability of Ni (increase in water soluble and exchangeable Ni) in the contaminated soil. It should favour greater absorption (removal) of Ni by plants. Therefore, two pot experiments were conducted to examine the bioavailability and subsequent uptake of Ni by marigold and alfalfa. The following are the salient findings.

#### **6.4.1. Soil pH and soil organic carbon**

- ❖ Similar to the results of incubation experiments, the pH and SOC content in soil with marigold and alfalfa were found decreased during the experimental period. The bioamendments had marked influence on pH and SOC. The soil amended with pressmud recorded greater amount of SOC.
- ❖ The WSOC was found increased in soil under marigold and alfalfa plants. The soils amended with pressmud (in marigold) and biochar (alfalfa) had relatively higher amount of WSOC.

#### **6.4.2. Biotransformation and bioavailability of Nickel in soil**

- ❖ Consistent with the incubation experiments, the results of pot experiments have also shown the potential of bioamendments in enhancing the bioavailability of Ni in soil.
- ❖ The water soluble Ni was found increased in soil due to the application of bioamendments initially, but was found decreased at the end of pot experiment. The concentration was found significantly higher in the soil amended with pressmud compost. Whereas, in soil with alfalfa, the water soluble Ni was found gradually decreased from initial to final stage and the highest value was recorded in the soil with pressmud compost application.
- ❖ The exchangeable Ni in soil was found increased rapidly in soil with both marigold and alfalfa plants. The soil amended with pressmud compost had relatively higher amount of exchangeable-Ni than other amendments.
- ❖ In both marigold and alfalfa, the concentration of organic Ni was gradually increased at 30<sup>th</sup> days, slightly decreased at 45<sup>th</sup> day and rapidly increased at 60<sup>th</sup> day of experimentation. The highest value was recorded in the soil amended with pressmud compost.
- ❖ The organic plus iron oxide bound Ni was found decreased initially but increased slightly towards the end of experiment with both marigold and alfalfa plants. The treatment with composted poultry manure had shown relatively higher

concentration in soil with marigold. In contrast, the concentration of organic plus iron oxide bound Ni in soil with alfalfa was high due to composted poultry manure application.

- ❖ In both marigold and alfalfa grown soil, the concentration of residual Ni was increased gradually during initial stages and then decreased notably towards end of experiment. Relatively lower concentration was observed in soil treated with composted poultry manure in marigold experiment. In alfalfa experiment, the residual Ni was recorded higher in the control soil and lower in the pressmud compost amended soil.

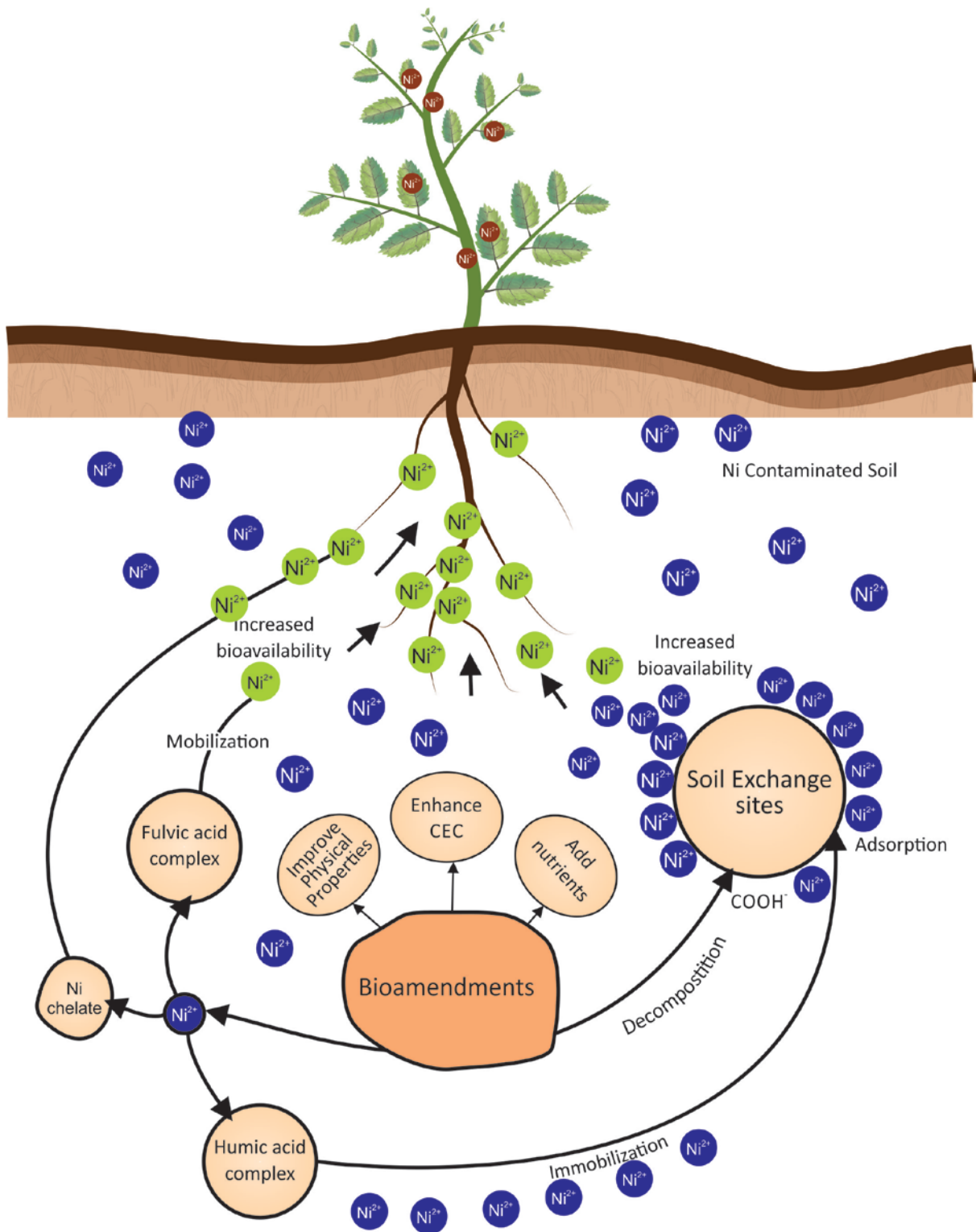
#### **6.4.3. Nickel content and uptake by crops**

- ❖ The results from both marigold and alfalfa experiment showed that the bioamendment application enhanced the Ni content in plants.
- ❖ Significantly higher Ni content was observed in the root biomass than in the above ground biomass. In general the plants grown on the control soil had greater amount of Ni in root biomass than in the above ground biomass.
- ❖ In the above ground biomass the highest Ni content was found in the plants grown on soil amended with pressmud compost, followed by composted poultry manure.
- ❖ In contrast, the root biomass of marigold had significantly higher Ni content due to the application of farmyard manure followed by biochar. The lowest Ni content in the root biomass was found in the plants grown on soil amended with composted poultry manure. In alfalfa, the Ni content of root biomass was relatively higher when the plants were grown on soil with biochar.
- ❖ The alfalfa plants had lesser Ni content when they grown on soil amended pressmud compost. The uptake of Ni by marigold and alfalfa had shown similar effect of bioamendments. All the bioamendments significantly increased the biomass of marigold and alfalfa. The plants grown on soil amended with pressmud compost produced greater biomass.

- ❖ While biochar application resulted in Ni concentration in marigold and alfalfa, pressmud compost and composted poultry manure application resulted in greater Ni content and uptake by plants.
- ❖ A significant positive correlations were obtained between Ni uptake by plants and water soluble and exchangeable Ni in soil with marigold and alfalfa. This explains the role of bioavailability in plant uptake of Ni.
- ❖ The mobility of Ni from the polluted soil into the roots of marigold and alfalfa and the ability to translocate the Ni from roots to above ground parts were evaluated by computing the Bioconcentration factor (BCF), the translocation factor (TF) and enrichment factor (EF). The results showed that the BCF and EF were less than one for both marigold and alfalfa but the TF was greater than one in plants grown on soil amended with bioamendments. Therefore, they may be considered as Ni hyperaccumulators.
- ❖ Both marigold and alfalfa showed greater potential in tolerating and accumulating higher concentration Ni and therefore could be integrated along with bioamendments for phytoextraction and thus phytoremediation of Ni contaminated soil.

## **6.5 PHYTOREMEDIATION STRATEGY**

The results of the laboratory and pot experiments consistently have shown that the application of bioamendments to the Ni contaminated soil enhanced the bioavailability of Ni in the contaminated soil. The various mechanism and the role of bioamendments in phytoremediation of Ni contaminated soil are depicted in Fig. 6.1. After soil application, the bioamendments form complexes with fulvic acid and humic acid fractions of SOM. While fulvic acid complex would enhance the bioavailability by mobilization of Ni ions, the humic acid complex results in immobilization of Ni and enhances the adsorption of Ni ions on soil exchange sites. The enhanced bioavailability of Ni (as evident from the increase in water soluble and exchangeable Ni) facilitates greater absorption of Ni by plants. The higher Ni content of below ground biomass of marigold and alfalfa provide evidence for this. It is also expected that due to the decomposition of bioamendments various organic acids are produced resulting in reduction of soil pH. Low soil pH



**Fig. 6.1. Role of Bioamendments in phytoremediation of Nickel Contaminated soil**

solubilizes some of the insoluble Ni precipitates and would increase the bioavailability of Ni and thus greater removal of Ni by plants. The other benefits of bioamendments include improvement in soil physical characteristics and enhancement of soil cation exchange capacity besides adding nutrients to the soil, which favours better growth and biomass production of plants. Thus the study has demonstrated that a phytoremediation strategy could be developed by integrating bioamendments and hyperaccumulators (Marigold and Alfalfa) for remediation of Ni contaminated soil.

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