Status, Distribution, and Phytoavailability of Heavy Metals and Metalloids in Soils Irrigated with Wastewater from Akaki River, Ethiopia: *Implications for environmental management of heavy metal/metalloid affected soils*.

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ABSTRACT

Soils are indispensable resources that afford production of agricultural and industrial crops as well as furnish manifold ecological services (as a filter, buffer, geochemical sink, and transformation system) and thus, safeguard the global ecosystem from the effect of pollution. However, being at the interface between the atmosphere and the earth's crust, soils are exposed to natural and anthropogenic inputs of heavy metals and metalloids which may entail potential environmental ramifications.

Akaki River, laden with untreated wastes from factories, commercial, public, and domestic utilities in Addis Ababa serves as irrigation water for vegetable farm on Eutric Fluvisol and Pelli–Eutric Vertisol that stretch along its bank at Akaki. Besides, livestock forage on the fallow when considerable part of the farm is inundated during high rains. Albeit the human and environmental health risks associated with heavy metal/metalloid buildup in soils, potential impact of wastewater irrigation has received little consideration. Consequently, saving a few prefatory works, huge information gap exists regarding systematic and comparative study on behavior, availability, simultaneous uptake, and translocation of array of heavy metals/metalloids from Vertisol and Fluvisol irrigated with untreated wastewater. Despite the significance of phytoremediation techniques as low–tech and potentially cheap *in situ* treatment alternatives to costly conventional remediation of contaminants, no prior study has been performed to screen candidate plants from mining landscape for phytoremediation of soils with polymetallic contamination.

In an attempt to address the above dearth of information, a series of studies involving green house and field experiments on the study soils as well as complementary field surveys on uncontaminated soils and contaminated mine spoils were carried out in order: (1) to catch on the levels of heavy metals/metalloids in uncontaminated soils; (2) to appraise the status of heavy metals/metalloids and distinguish their forms of retention in contaminated soils; (3) to assess the phytoavailability, uptake, within–plant distribution and potential risk of heavy metals/metalloids in forage plants grown on contaminated Vertisol and Fluvisol under controlled and actual field conditions and; (4) to assess the potential utility of plants collected from mineral landscape for phytoremediation of heavy metal affected soils.

The study on the status of uncontaminated soils (Vertisol, Fluvisol, Solonetz, Andosol, and Nitosol) established that all but Zn (>50 mg kg^{-1}: Vertisol, Andosol, and Nitosol) had lower mean heavy metal/metalloid levels than the corresponding common values for soils. In

contrast, attributable largely to wastewater irrigation for the last few decades, the average contents of Cr, Ni, Co, Cu, Zn, V, Hg and As of contaminated Vertisol and Fluvisol as well as Pb and Se in contaminated Fluvisol surpassed the respective mean + 2sd of their uncontaminated counterparts. Besides, sequential extraction of contaminated Vertisol and Fluvisol demonstrated that considerable proportions of the total levels of most heavy metals/metalloids in contaminated Vertisol and Fluvisol resided in non–residual fractions, and thus could be potentially mobile/phytoavailable. The green house and field studies on plant uptake and distribution of heavy metals/metalloids by forage plants grown on contaminated soils portrayed that the elements were highly phytoavailable and hence accumulated in the roots as well as remarkably translocated to the shoot. Thus, BCF > 1 (bioconcentration factor: root to soil concentration ratio) BCF >1 was observed for Cu, Zn, Cd, and Hg in grasses, while legumes had TF >1 for majority of heavy metals/metalloids. Besides, the mean levels of most heavy metals/metalloids of forage plants grown on contaminated soils were higher than the corresponding background levels for forage grasses and legumes. The study also demonstrated that soil type, kind of species, plant part, and their interactions significantly influenced ($p < 0.05$) uptake, translocation, sequestration of heavy metals/metalloids and thus, could govern their transfer through the food chain in the study area (and similar sites). Therefore, the data suggest that the potential environmental and health hazard could proceed from the use of fodder grasses/legumes, and cultivation of vegetables in soils with polymetallic and metalloid contamination.

Conversely, native plants grown on mine spoils tolerated the edaphic stressors (pH 3.9–6.6; organic carbon < 1.05%; total nitrogen < 0.12%, toxic metal levels reaching 307 mg kg^{-1}), produced considerable biomass (up to 228.4g, 178.3g, and 364.2g [dry weight] of shoot, root, and total, respectively) and sequestered appreciable levels of heavy metals (up to 740.45 mg kg^{-1}). Overall, with sound agronomic practice, the naturally capacity of these plants (extraction efficiency, EE = 176.90–25073µg, total plant levels = 211.37–28779.50µg $plant^{-1}$) could be developed for phytoremediation of soils affected by polymetallic pollution.

1. INTRODUCTION

1.1 Theoretical Viewpoint

1.1.1 Definition and Scope

The term "heavy metals" is often employed as a group name for metals and semimetals (metalloids) which have been related to contamination and potential toxicity or ecotoxicity (Duffus, 2002). Besides, heavy metals and metalloids under the present study belong to a group called "trace elements"— elements present at low concentrations (mg kg^{-1} or less in environmental materials (He *et al.,* 2005) with 76 elements all of which are recognized as potential pollutants (Davies, 1980). Moreover, among all chemical pollutants, trace elements are considered to have particular ecological, biological, or health significance (Pendias and Pendias, 2001).

In the present study, consequently, *heavy metals* (Cr, Co, Ni, Cu, Zn, Cd, Pb, Hg, and V) and *metalloids* (As, and Se) correspond to elements that occur in natural and perturbed environments in fairly small amounts, which, at reasonably higher concentrations, are reckoned toxic to living organisms (Adriano, 1986; Adriano, 2001). Correspondingly, attributable to their harmful properties, the term 'potentially toxic elements', has been increasingly employed for the study elements (Alloway, 1995b). Moreover, under some environmental conditions, these elements could accumulate to toxic levels and result in ecological damage (Freedman, 1989). Apart from those with no known physiological functions (Cd, Hg and Pb), Cu and Zn for higher plants; Co for N fixation in bacteria and algae; Se and V for E. coli and alga *Scenedesmus obliquus*; Cu, Co, Zn, Se, Cr, V, Ni and As in animals are required at small but essential concentrations (Adriano, 1986; 2001; Table 1.1). Consequently, this study is delimited to those environmentally significant elements mentioned above ascribed to their essential role and/or potential toxicity to humans, animals, or plants.

Table 1.1 Essentiality and potential effects of trace elements on plant[a], animal[a], and human nutrition. [a]Primarily refers to land based plants and animals.

Essential/beneficial to				Potential toxicity to			Comments
Element	*Plants*	*Animals*	*Humans*	*Plants*	*Animals*	*Humans*	
As	No	Yes	No	Yes	Yes	Yes	Phytotoxic before animal toxicity; similar geochemical behavior to P; carcinogenic; blackfoot disease (arsenicosis) in South Asia.
Cd	No	No	No	Yes	Yes	Yes	Narrow margin; bioaccumulative and phytotoxic; enriched in food chain; carcinogenic; *itai-itai* disease (Cd poisoning).
Co	Yes	Yes	Yes	Yes	Yes	Yes	Relatively phytotoxic; role in symbiotic N_2 fixation; carcinogenic
Cr	No	Yes	Yes	Yes		Yes	Cr^{6+} very toxic and mobile in soils; carcinogenic; Cr^{3+} relatively non toxic.
Cu	Yes	Yes	Yes	Yes			Easily complexed in soils; narrow margin in plants; immobile in soils.
Hg	No	No	No		Yes	Yes	Biomagnifies in aquatic food chain; a concern in newly established reservoirs; *Minamata* disease (Hg poisoning).
Ni	Yes	Yes	Yes	Yes	Yes	Yes	Very mobile in soils and plants; carcinogenic.
Pb	No	No	No	Yes	Yes	Yes	Relatively non phytotoxic; immobile in soils; human exposure to leaded gasoline, paint, and plumbing; young children most sensitive to Pb poisoning; a global social issue.
Se	Yes	Yes	Yes	Yes	<4 $mgkg^{-1}$		Narrow margin for animals (selenocosis); interact with other trace metals; similar geochemical behavior to S; Keshan and Kaschin-Beck diseases (Se deficiency).
V	Yes	Yes	No	Yes	Yes	Yes	Required by green algae; narrow margin and highly toxic in mammals; carcinogenic.
Zn	Yes	Yes	Yes	Yes			Wide margin; easily complexed in soils; similar geochemical behavior to Cd; may be lacking in some diets; relatively non toxic to mammals.

After Adriano (1986; 2001)

Albeit the wide use and popularity of the term *heavy metals* in environmental literature, it has recently been labeled as meaningless, confusing (Duffus, 2002), and geochemical bogeymen accountable for all manners of predicaments in the environmental world (Hodson, 2004). According to Duffus (2002), the limitation of the term stems from incongruity encountered in specifying heavy metals based on density, atomic weight, atomic number, and other chemical properties. The same author suggested alternative classification of metals for toxicity study based on the valence electron configuration (periodic table), Lewis acid behavior, or separate treatment of each metal species and compound in conformity with their chemical, biological, and toxicological properties. As per the categorizations suggested above, the study elements are regrouped into p–block (As, Se and Pb) and d–block (V, Cr, Co, Ni, Cu, Zn, Cd, and Hg), or Borderline (V, Cr, Co, Ni, Cu, and Zn) and Class B (Cd, Hg, and Pb^{2+}).

However, the classification of elements as Class A/hard, Class B/soft and Borderline/Intermediate class ions has also drawbacks (Duffus, 2002; Hodson, 2004). Accordingly, different authors may place the same metal ion into different classes (Duffus, 2002). Reported cases in point were, Cr^{3+}, Co^{3+} (Class A) (McBride, 1994; Ross, 1994a); Pb^{2+} (Borderline)(McBride, 1994; Pendias and Pendias, 2001; Ross, 1994a); Cd^{2+} (Borderline) (Pendias and Pendias, 2001; Ross, 1994a); As^{3+} (Class A) (Ross, 1994a); As^{3+} (Borderline) (Pendias and Pendias, 2001); Zn^{2+} (Class B), As and Se (ligand formers) (White, 2005). Apparently, conversely, based on the Goldschmidt's classification of elements by geochemical behavior (Alloway, 1995c; White, 2005), the heavy metals and metalloids under this study are arrayed as Lithophiles (Cr and V), Siderophiles (Co and Ni), and Chalcophiles (As, Cd, Cu, Pb, Se, Zn and Hg). Imperfections attributable to the difficulty to assess the order of complex stability of Borderline metals, lack of clear boundary between Borderline ions and Class B as well as limitation of the spdf grouping to highlight acceptably the considerable variations between the metal ions under each category have been indicated by Duffus (2002).

Notwithstanding the absence of other authoritative definition and/or collective name for environmentally important trace metals, the term *heavy metal* and *metalloid* in this study

represent element/s under respective p–block (As, Se and Pb) and d–block (V, Cr, Co, Ni, Cu, Zn, Cd, and Hg) or Borderline (V, Cr, Co, Ni, Cu, and Zn) and Class B (Cd, Hg, and Pb). Moreover, any references related to the potential environmental consequences of elements portray their mobility in response to changes in the environmental conditions. Included here are those easily mobile and potentially mobilizable operationally defined species/fractions and compounds of the elements associated with the different geochemical phases of the soils.

For the present study, *pollution* is defined as the presence of heavy metals/metalloids accumulation in soils to the levels that may pose risk to the receiving environment including human beings and their stocks. Conversely, c*ontamination* refers to the occurrence of elevated levels of heavy metals/metalloids in soils or other environmental substrates. For practical reasons, *trace metals* or *trace elements* is considered synonymous to *heavy metals/metalloids* considered in the study. On the other hand, *phytoavailability and uptake* denote the process of occurrence of *heavy metals/metalloids* in the mobile and easily mobilizeable forms and their eventual absorption by roots, in that order. Moreover, status denotes the condition of the soils in terms of concentration of elements studied, whereas distribution signifies the association of elements with different geochemical phases of the soils. Apart from this, the word 'distribution' is also used for vertical (depth–wise) distribution of elements in soils as well as distribution of the same in plant parts (above– and belowground).

1.1.2 Geochemical origins of heavy metals

The earth crust is the primal source of all the natural trace elements of the different environmental, geological, biological, or marine systems (Ward, 1995). Of the total 90 naturally occurring elements (Ward, 1995), 10 major elements (O, Si, Al, Fe, Ca, Na, K, Mg, Ti, and P) dominate (> 99%) the elemental composition of the earth crust (Alloway, 1995 c). Consequently, elements other than these are recognized as *trace elements* (Alloway, 1995c) and occur at levels < 0.1 % or 1000 mg kg^{-1} (Adriano, 1986; Adriano, 2001; Alloway, 1995c), with most existing at mean levels < 0.01 % or 100 mg kg^{-1} (Alloway, 1995c).

The trace element content of the soil parent material corresponds closely to that of the contributing rock types (Jenkins and Wyn Jones, 1980). Igneous and metamorphic rocks that account for 95% of the earth crust are the commonest natural sources of trace metals in soils (Ross, 1994b) (Table 1.2). Trace elements exist as trace constituents of the primary minerals in igneous rocks (Alloway, 1995c). Depending on the ionic charge, ionic radius, and electronegativity (Alloway, 1995c) in relation to the lattice geometry of possible host minerals (Jenkins and Wyn Jones, 1980), trace elements isomorphously substitute ions of major elements during crystallization of molten magma (Alloway, 1995c). Substitutions, for instance, that entail Pb^{2+} for K^{+} in silicates, Ni^{2+} for Fe^{2+} in pyrites, Ni^{2+} and Co^{2+} for Mg^{2+} in ultrabasic minerals (Ross, 1994b), Co, Zn and Cu for Mg/Fe^{2+} in ferromagnesian minerals (Jenkins and Wyn Jones, 1980), Cr^{3+} for Fe^{3+}, and Cr^{6+} for Al^{3+} in minerals of igneous rocks (Ross, 1994b) are possible (Table 1.3).

Table 1.2 Mean heavy metal contents of major rock types (mg kg^{-1}). Adapted from Alloway (1995b).

Element	Earth's crust	Igneous rocks			Sedimentary rocks		
		Ultramafic*	Mafic*	Granitic	Limestone	Sandstone	Shales*
As	1.5	1	1.5	1.5	1	1	13 (1-900)
Cd	0.1	0.12	0.13	0.09	0.028	0.05	0.22 (<240)
Co	20	110	35	1	0.1	0.3	19
Cr	100	2980	200	4	11	35	90 (<500)
Cu	50	42	90	13	5.5	30	39 (<300)
Hg	0.05	0.004	0.01	0.08	0.16	0.29	0.18
Ni	80	2000	150	0.5	7	9	68 (<300)
Pb	14	14	3	24	5.7	10	23 (<400)
Se	0.05	0.13	0.05	0.05	0.03	0.01	0.5 (<675)
V	160	40	250	72	45	20	130 (<2000)
Zn	75	58	100	52	20	30	120 (<1000)

Ultramafic rocks are also called 'ultrabasic', e.g., dunite, peridotite and serpentinite; Mafic rocks are also known as 'basic igneous rocks', e.g. basalt; 'Shales' also include clays.

Table 1.3 Trace metal constituents of common rocks and soil minerals

Ease of weathering	Mineral	Occurrence	Trace metal constituents
Easily weathered	Olivine	Igneous rocks	Ni, Co, Cu, Zn
↓	Hornblende	Widespread in igneous and metamorphic rocks	Ni, Co, Cu, Zn, V
↓	Augite	Ultrabasic and basic volcanic rocks	Ni, Co, Cu, Zn, Pb, V
↓	Biotite	Igneous and metamorphic rocks	Ni, Co, Cu, Zn, V
↓	Apatite		Pb
↓	Anorthite		Cu
↓	Andesine		Cu
↓	Oligoclase		Cu
↓	Albite		Cu
Moderately stable	Garnet		Cr
↓	Orthoclase	Acid igneous rocks	Cu
↓	Muscovite	Granites, Schists, glass	Cu, V
↓	Titanite		V
↓	Ilmenite		Cr, Ni, Co, V
Increasing mineral stability	Magnetite	Igneous and metamorphic rocks	Cr, Ni, Co, Zn, V

After Ross (1994b) and Alloway (1995b).

Sedimentary rocks, conversely, account for only 5% of the crust, of which 80% are shales, 15% sandstone, and 5% limestones (Davies, 1980; He *et al.,* 2005; Ross, 1994b; Thornton, 1981). As sedimentary rocks overlie most igneous formations (Alloway, 1995c; He *et al.,* 2005; Ross, 1994b; Thornton, 1981) accounting for 75% of the earth's surface (Alloway, 1995c; Ross, 1994b) and, thus, are recognized as much more important soil parent materials (Alloway, 1995c; Davies, 1980; Ross, 1994b) than igneous rocks (Alloway, 1995c). The levels of trace elements in sedimentary rocks pivot on the mineralogy and adsorptive properties of the sediment, the matrix, and amount of metals in the water in which sediments were deposited (Alloway, 1995c). Consequently, because of their capacity to adsorb metal ions (Alloway, 1995c), Shales usually accommodate

significant amount of trace metals (Alloway, 1995c; He *et al.,* 2005; Thornton, 1981) including Cu, Zn, Cd, and Pb (He *et al.,* 2005). In contrast, Sandstones which are largely composed of quartz grains with no trace constituents and inconsequential ability to adsorb metals often contain low quantities of trace metals (Alloway, 1995c).

Most of the heavy metals and metalloids considered in this study are Chalcophiles (As, Cd, Cu, Pb, Se, Zn, and Hg) that have a high affinity for sulfur and thus found in sulfide ores (Ross, 1994b; Table 1.4). Many of the non–ferrous metalliferous ores are sulfide minerals (Alloway, 1995c; Table 1.5) which contain environmentally and industrially important trace elements (Davies, 1980). Important minerals in this category include galena (PbS), sphalerite (ZnS), chalcopyrite ($CuFeS_2$)(Davies, 1980; Ross, 1994b), cinnabar (HgS), pentlandite ($(Ni, Fe)_9S_8$) (Ross, 1994b) as well as pyrite/marcasite (FeS_2), and arsenopyrite (FeAsS) (Davies, 1980). On the other hand, due to their geochemical similarity (comparable ionic structure and electronegativity), Zn and Cd occur together in complex sulfide and carbonate minerals (Ross, 1994b). Generally, most ore minerals incorporate several other metals as minor inclusions (Alloway, 1995c). In essence, minerals are rarely pure for they often contain intergrowths or inclusions of other minerals and hence, accompanying ‘guest’ metals (Davies, 1980; see Tables 1.4 and 1.5). The environmental implication of associations of the main and the ‘guest’ metals in the ores is the possibility of their simultaneous release during weathering.

Table 1.4 Trace metal constituents of selected sulfide minerals. Due to the presence of inclusions and intergrowths of associated minerals, estimates of trace constituents in the literature may not correspond to pure mineral specimens.

Mineral	***Trace constituents***
Galena (PbS)	As, Cr, Hg, Ni, Zn
Sphalerite (ZnS)	Cd, Co, Cu, Hg
Chalcopyrite ($CuFeS_2$)	Ni, Co, Se
Pyrite/Marcasite (FeS_2)	As, Co, Cu, Ni, Pb
Arsenopyrite (FeAsS)	Ni, Co

After Davies (1980).

Table 1.5 Common ore minerals of non–ferrous metals.

Metal	***Ore mineral***	***Associated heavy metals***
As	FeAsS, AsS, Cu ores	Cu, Hg
Cd	ZnS	Zn, Pb, Cu
Cr	$FeCr_2O_4$	Ni, Co
Cu	$CuFeS_2$, Cu_5FeS_4, Cu_2S, Cu_3AsS_4, CuS, Cu*	Zn, Cd, Pb, As, Se, Ni
Hg	HgS, Hg*, Zn ores	Se, Zn, Pb
Ni	$(Ni, Fe)_9S_8$, NiAs, $(Co, Ni)_3S_4$	Co, Cr, As, Se
Pb	PbS	Zn, Cu, Cd, Se
Se	Cu ores	As, Cu
Zn	ZnS	Cd, Cu, Pb, As, Se, Hg
Ag	Ag_2S, PbS	Cu, Zn, Pb, Se
Au	Au*, $AuTe_2$, $(Au, Ag)Te_2$	As, Se, Hg
Ba	$BaSO_4$	Pb, Zn
Mn	MnO_2	Co, Ni, Zn, Pb
Mo	MoS_2	Cu
Pt	Pt*, $PtAs_2$	Ni, Cu, Cr
Sb	Sb_2S_3, Ag_3SbS_3	Hg, As
U	U_3O_8	V, As, Se, Pb, Cu, Co

*Represents native metal deposits. After Alloway (1995b).

1.1.3 Natural sources and background levels of heavy metals/metalloids in soils

The primary sources of heavy metals/metalloids are the parent materials that are typically weathered bedrock or overburden transported by wind, water, or glaciation (local or exotic in origin) from which the soils are derived (Thornton, 1981). While the trace element content of the bedrock could be relevant for soils developed *in situ* (residual soils), the impact of the underlying rock may be either modified or completely masked in cases where the parent materials have been mixed or redistributed by alluvial transport, wind, or glacial activity (Thornton, 1981). Soils are rarely sedentary (derive directly from the solid rock), and hence, commonly are formed on materials transported by way of colluvial, fluvial, glacial, and aeoline processes (Jenkins and Wyn Jones, 1980). Consequently, when the parent material is known, the background content of trace elements in many soils, to certain extent, can be envisaged (Davies, 1980).

Initially, at early stages of weathering and pedogenic processes (Pendias and Pendias, 2001), the trace element composition of the soil will be inherited from geologic parent material (Jenkins and Wyn Jones, 1980; Pendias and Pendias, 2001). However, with time, the trace element status of the soil will diverge progressively due to the influence of pedogenic or soil forming processes (Jenkins and Wyn Jones, 1980; Pendias and Pendias, 2001). In this regard, the release of metals from the parent material by weathering as well as the translocation and accumulation of soil constituents that absorb metals, namely clays, hydrous oxides, and organic matter are important aspects of pedogenesis (Alloway, 1995c). Pedogenic processes that are involved in translocation of soil constituents include leaching, eluviation, salinization, calcification, podzolization, and ferralitization, gleying, and organic matter accumulation (Alloway, 1995c). Accordingly, pedogenic processes may lead to the mobilization and distribution of elements both within the soil profile and between neighboring soils (Thornton, 1981).

Conversely, weathering of minerals in the soil parent material entails hydration, hydrolysis, dissolution, oxidation, reduction, carbonation /carbonatization (Alloway, 1995c; Pendias and Pendias, 2001) and ion exchange (Alloway, 1995c). The extent to which elements are released by weathering hinges on the type of mineral (Thornton,

1981), intrinsic structural stability of the mineral (Alloway, 1995c; McBride, 1994; Thornton, 1981), temperature, specific surface area of a mineral, efficiency of removal of soluble weathering products (by precipitation, leaching, etc.) (Alloway, 1995c; McBride, 1994), pH, and presence of complexing ligands (organic acids, inorganic anions) (McBride, 1994). Thus, rates are high in the humid tropics and low in cold and/or dry environment (Alloway, 1995c; McBride, 1994). In an oxidizing and low pH weathering environment (McBride, 1994), relatively unstable ferromagnesian (olivine, orthopyroxene, clinopyroxene, amphibole, biotite) and sulfide minerals rapidly release their trace element content into the soil environment (Jenkins and Wyn Jones, 1980). Various easily weathered minerals from igneous rocks supply considerable amount of Ni, Co, Cu, Zn (He *et al.,* 2005; Ross, 1994b; Thornton, 1981) and Cr (He *et al.,* 2005) to soils. Likewise, Shales, derived from fine sediments of inorganic and/or organic origin, usually accommodate significant amount of trace metals (Alloway, 1995c; He *et al.,* 2005; Thornton, 1981) including Cu, Zn, Cd, and Pb (He *et al.,* 2005).

In contrast, typical granite that could consist of minerals relatively resistant to weathering (e.g., quartz, orthoclase, and muscovite) with low amount of trace metals, thus, may possibly release correspondingly small amount of the same to soils (Davies, 1980; Tables 1.2 and 1.3). Likewise, Sandstones are composed of minerals that are resistant to weathering and often contain low quantities of trace metals (Alloway, 1995c; Ross, 1994b). Consequently, soils derived from coarse-grained materials like sandstones, and acid igneous rocks such as rhyolites and granites are likely to contain comparatively small amount of trace metals than fine-grained sedimentary rocks and basic igneous rocks (He *et al.,* 2005; Thornton, 1981). Conversely, iron oxide minerals (magnetite: Zn, Co, Ni, Cr, V and ilmenite: Co, Ni, Cr, V) (Alloway, 1995c) are very stable (Table 1.3) and hence, retain their trace element complements against weathering (Jenkins and Wyn Jones, 1980). Apparently, it is likely for soils to have dissimilar availabilities of micronutrients (Co, Cu, and Zn) due to differing mineralogies of their respective parent rocks though both contain the same total trace element compositions (Jenkins and Wyn Jones, 1980).

The natural concentration ranges of most trace elements in soils are wide (Thornton, 1981). The normal abundance of particular element in earth material is commonly referred as *background* (He *et al.,* 2005; Thornton, 1981), and in soils, it varies depending on the nature of the parent material (Adriano, 1986; Adriano, 2001; He *et al.,* 2005; Ross, 1994b; Thornton, 1981) and on the process of weathering (Adriano, 1986; Adriano, 2001). Even in the absence of considerable inputs from external sources, variations in mineralogical and elemental composition of different rocks result in marked distinctions in the elemental composition as well as their corresponding levels of soils derived from different rocks (Alloway, 1995b; Table 1.6). In general, potentially toxic elements commonly exist in trace concentrations in the environment (Freedman, 1989; Tables 1.7).

Table 1.6 Typical ranges for background concentration (mg kg^{-1}) of trace elements in surface soils.

Element	***Mean[1]***	***Normal range in soil[2]***
As	2.5	0.1–40
Cd	0.25	0.01–2
Co	8	0.05–65
Cr	60	5–1500
Cu	15	2–250
Hg	0.05	0.01–0.5
Ni	20	2–750
Pb	20	2–300
Se	0.3	0.01–12
V	85	3–500
Zn	60	1–900

After [1]Ward (1995) and [2]Adriano (1986).

Table 1.7 Typical background concentrations (mg kg^{-1}) of toxic elements in selected environmental compartments

Element	[1]Fresh water	[1]River	[1]Sea water	[2]Marine fish	[1]Plant[a]	[1]Plant food stuffs[ab]	[2]Mammals		[1]Human				
							Muscle	*Bone*	*Kidney*	*Liver*	*Hair*	*Milk*	*Blood serum*
V	0.0005	0.001	0.0025	0.3	0.5 (0.1-2.5)	0.01 (0.001-0.7)	0.002-0.02	0.003-0.03	0.2	0.1	0.05	0.0008	0.00005
Cr	0.001	0.001	0.00005	0.03-2	0.2 (0.02-0.2)	0.05 (0.01-14)	<0.002-0.84	0.1-33	0.1	0.1	0.8	0.0015	0.0001
Co	0.00005	0.0002	0.00002	0.006-0.05	0.08 (0.03-0.6)	0.07 (0.008-0.2)	0.005-1	0.01-0.04	0.1	0.1	0.35	0.0005	0.0001
Ni	0.0005	0.0003	0.0005	0.1-4	1 (0.1-5)	0.8 (0.06-4)	1.2	<0.7	0.5	0.1	0.8	0.012	0.0003
Cu	0.003	0.005	0.002	0.7-15	5 (1-12)	4 (0.08-9)	10	1-26	14	20	20	0.28	1
Zn	0.015	0.02	0.01	9-80	30 (12-60)	25 (1.2-45)	240	75-170	150	250	180	1.5	0.9
As	0.0005	0.002	0.003	0.2-10	0.15 (0.009-1.5)	0.08 (0.003-0.3)	0.007-0.09	0.08-1.6	0.01	0.03	0.1	0.0005	0.001
Se	0.0002	0.0002	0.0001	0.2	0.06 (0.002-0.88)	0.03 (0.003-0.15)	0.4-1.9	1-9	0.5	2	2	0.018	0.09
Cd	0.00003	0.00002	0.0001	0.1-3	0.1 (0.02-0.5)	0.08 (0.008-0.3)	0.1-3.2	1.8	15	0.8	0.2	0.001	0.0003
Hg	0.00007	0.000007	0.00003	0.4	0.01 (0.001-0.04)	0.003 (0.002-0.04)	0.02-0.7	0.45	0.1	0.1	0.4	0.002	0.001
Pb	0.001	0.003	0.00003	0.001-15	1 (0.3-10)	0.7 (0.05-4)	0.2-3.3	3.6-30	3	5	1	0.01	0.003

[a] Designates mean (range) and [b] stands for Lettuce, Cabbage, beans, Corn and Cereals. After [1]Ward (1995) and [2]Freedman (1989).

On the other hand, the occurrence of surface or near surface mineralizations containing toxic elements could produce localized areas of contamination (Freedman and Hutchinson, 1981; Freedman, 1989). Soils in mineralized areas normally contain elevated concentrations of one or more of the metals (e.g., Cu, Pb, Zn, Cd and As: Thornton, 1981). Such soils could be developed over or in the dispersion halo (diffusion of traces of the mineral in rock) around ore bodies and mineral veins (fissures in rocks filled with minerals) or from transported overburden containing mineralized materials (Thornton, 1981). Typical cases are soils derived partly from ultramafic minerals particularly olivine, orthorhombic and monoclinic pyroxenes and hornblende and their secondary alteration products such as serpentine minerals, fibrous amphiboles and talc could often contain elevated levels of Cr (up to 25000 mg kg^{-1}), Ni (as high as 125000 mg kg^{-1}) and Co (Freedman and Hutchinson, 1981; Freedman, 1989).

Likewise, natural inputs to the atmosphere include volcanic outputs and outgassing [Hg(Freedman, 1989; Ross, 1994b), Ni, Pb (Ross, 1994b), As and Se(Freedman, 1989)], spontaneous combustion of carbonaceous materials (sulfide rich bituminous shales: As, Se, and V), forest fires (As, Se, Hg, and Cd) (Freedman and Hutchinson, 1981), eolithic dusts, and salt sprays (Pendias and Pendias, 2001; Ward, 1995). Such atmospheric depositions of trace elements, principally heavy metals, may ultimately reach the surface soil (Pendias and Pendias, 2001).

1.1.4 Anthropogenic inputs of heavy metals/metalloids in soils

A number of sources, both natural and anthropogenic, could impinge on the levels of trace elements in the environment (Adriano, 1986; Adriano, 2001; Ernst, 1998; He *et al.,* 2005; Pierzynski *et al.,* 2000; Thornton, 1981). While, natural geological and biological alterations of the earth crust are slow, man's input to the trace element composition of the biosphere has been dramatic and widespread in all components of the environment (Ward, 1995). Due to various anthropogenic inputs (Table 1.8), urban soils can contain higher levels of trace elements than the corresponding levels in their parent materials (He *et al.,* 2005). Consequently, ascribed to their proximity to pollution sources, urban soils

will commonly accommodate elevated soil trace element concentrations than those in rural areas (barring mining landscapes) (Pierzynski *et al.,* 2000). Similarly, urban and industrial diffuse sources of metals have been identified as primary cause of pollution of aquatic systems by toxic metals (Novotny, 1995). Increasingly higher quantities of some trace metals are being released into the environment by industrial processes (Ross, 1994b), agriculture (fertilizer, animal manure, pesticides, irrigation etc.)(Adriano, 2001; Ross, 1994b), metallurgy (mining, smelting, metal finishing, etc.), energy production (leaded gasoline, battery manufacturing, power plants, etc.), microelectronics (Adriano, 2001), and disposal of industrial and domestic refuses and waste materials (Adriano, 2001; Ross, 1994b).

Among major heavy metal/metalloid sources of anthropogenic origin, those with special significance to contamination of soils are considered there-under.

1.1.4.1 Metalliferous mining and smelting

On account of the impact covering fairly sizable areas with elevated levels of trace elements in soils (Freedman and Hutchinson, 1981; Freedman, 1989; He *et al.,* 2005; Pierzynski *et al.,* 2000), the mining and smelting activities have created enormous ecological damage (Pierzynski *et al.,* 2000). Serious metal contamination of many scattered, but often comparatively localized areas (Freedman and Hutchinson, 1981) is caused by dumping of metal–rich overburden, excavation wastes, metalliferous tailings (Freedman and Hutchinson, 1981; Freedman, 1989; He *et al.,* 2005; Pierzynski *et al.,* 2000) and liquors (Liu *et al.,* 2005; Williamson and Johnson, 1981). Conversely, smelter wastes include a molten waste material (slag) that is disposed of on land, atmospheric emission of SO_2 and other gases, and metal containing particulates to the atmosphere (Freedman, 1989). As the majority of ore-minerals accommodate various other metals as minor inclusions (Table 1.5), the soils in the neighborhood of mines and smelters are bound to be palpably contaminated by these metals and the major ore metals as well (Alloway, 1995c).

Table 1.8 Anthropogenic sources of toxic metals in the environment

1. *Metalliferous mining and smelting*
    a. Spoil heaps and tailings—contamination through weathering, wind erosion (As, Cd, Hg, Pb)
    b. Fluvially dispersed tailings—deposited on soil during flooding, river dredging, etc. (As, Cd, Hg, Pb)
    c. Transported ore separates—blown from conveyance onto soil (As, Cd, Hg, Pb)
    d. Smelting—contamination due to wind-blown dust, aerosol, from stack (As, Cd, Hg, Pb, Se)
    e. Iron and steel industry (Cu, Ni, Pb)
    f. Metal finishing (Zn, Cu, Ni, Cr, Cd)
2. *Industry*
    a. Plastics (Co, Cr, Cd, Hg)
    b. Textiles (Zn)
    c. Microelectronics (Cu, Ni, Cd, Zn)
    d. Wood preserving (Cu, Cr, As)
    e. Refineries (Pb, Ni, Cr)
3. *Atmospheric deposition*
    a. Urban/industrial sources, including incineration plants, refuse disposal (Cd, Cu, Pb, Hg, V)
    b. Pyrometallurgical industries (As, Cd, Cr, Cu, Ni, Pb, Zn)
    c. Automobile exhausts (Pb, Cr)
    d. Fossil fuel combustion including power stations (As, Pb, Se, V, Cd, Zn)
4. *Agriculture*
    a. Fertilizers (e.g., As, Cd, V, and Zn in some phosphatic fertilizers)
    b. Manures (e.g., As, and Cu in some pig and poultry manures, Zn in farmyard manure)
    c. Lime (Pb, As)
    d. Pesticides (Cu and Zn in fungicides, As and Pb used in orchards)
    e. Irrigation waters (Cd, Pb, Se)
    f. Corrosion of metals (e.g., galvanized and metal objects for fencing, troughs, etc. Pb, and Zn)
5. *Waste disposal on land*
    a. Sewage sludge (Cd, Cr, Cu, Hg, Ni, Pb, V, Zn)
    b. Leachate from landfill (As, Cd, Pb)
    c. Scrapheaps (Cd, Cr, Cu, Pb, Zn)
    d. Bonfires, coal ash, etc. (Cu, Pb)

After Ross (1994b).

With increasing demand for metals coupled with advances in mineral extraction technology (Alloway, 1995c), mining of lower grade ore bodies are being mined that

ultimately generate large amounts of waste (Alloway, 1995c; Williamson and Johnson, 1981) that are often discarded in land based impoundments or tips which may cover several square kilometers (Williamson and Johnson, 1981). Both metalliferous and coal mining produce large deposits of waste material as spoil heaps (Batty, 2005). Conversely, depending on the location and environmental considerations including recurrent earthquakes (Williamson and Johnson, 1981) or generation of acid mine drainage(Allan, 1995), tailings have been disposed of on aquatic ecosystems (fresh and marine)(Allan, 1995; Williamson and Johnson, 1981). Conversely, many of the non–ferrous ores (Alloway, 1995c) as well as iron pyrite and chalcopyrite in coal mine spoils are sulfide minerals that can generate sulfuric acid when exposed to water and air in wastes (Freedman and Hutchinson, 1981) which eventually leads to the release of associated metals (Adriano, 2001). Likewise, accidental failure of tailings dams has been responsible for many severe pollution events (Alloway, 1995c) including soil and river pollution as well as loss of over 500 lives around the world since 1970 (Liu *et al.,* 2005). Albeit the development of the mining and metal beneficiation industries into advanced operations, the fundamental causes of pollution remain unchanged (Allan, 1995).

Mine degraded landscape is commonly typified by steep unstable slopes, disruption of natural drainage systems, and absence of vegetation due to lack of nutrients, poor soil structure, acidity (Batty, 2005), drought, temperature fluctuations, absence of top soil, compaction (Wong, 2003) and/or metal toxicity (Batty, 2005; Wong, 2003). In such polluted soils, the levels of heavy metals can exceed the background levels by a factor of 100 to 1000 (He *et al.,* 2005). Consequently, development of vegetation on the waste can be limited to an early successional grassland-herb community or even worse, only a few pioneer species ever establish themselves in severely toxic metal levels (Freedman, 1989). In view of that, damaged land surface, tailings, waste rocks (Freitas *et al.,* 2004; Wong, 2003) with water infiltrating spoil heaps and cessation of pumping in underground workings can become source of air, water (Batty, 2005; Freitas *et al.,* 2004; Pratas *et al.,* 2005; Wong, 2003) and soil (Pratas *et al.,* 2005) pollution. Notwithstanding this, the exact character of the environmental problems related to mining pivot on the type of

material mined, processing methods, local geography and geology, and the time scale entailed (Batty, 2005).

1.1.4.2 Metallurgical industries

Soils can be affected by potential contaminants/pollutants from metallurgical industries. Important inputs of heavy from these sources include aerosol and dust emission, liquid effluents and waste dumps, which could pollute soils after deposition on soils/vegetation, through flooding/disposal on soils, and leaching to the underlying soil, respectively (Alloway, 1995c). As several heavy metals (Cr, Co, Ni, Cu, Zn, Cd, Pb, V and As) are employed in specialist alloys and steels, their manufacture and fabrication into machines and vehicles(Alloway, 1995c), their disposal after being used or recycling in scrap metal can cause contamination of the environment (Alloway, 1995c; Freedman, 1989).

1.1.4.3 Chemical and other manufacturing industries

The manufacture and/or use and disposal of chemicals and certain industrial products could be potential sources of heavy metal pollution of soils and other components of environment. Accordingly, the chlorine manufacture (Hg), batteries (Zn, Cd, Pb, Hg), pigments and paints (Cr, Co, Zn, Cd, Pb, Se, As), catalysts (Ni, Co), polymer stabilizers (Zn, Cd, Pb: incineration of plastics), printing and graphics (Cr, Zn, Cd, Pb, Se), Medical uses (Cu, Zn, Hg, Se, As), and additives in fuels and lubricants (Pb, Se) are considered environmentally important (Alloway, 1995c). Similarly, Cr, Co, Cu, Zn, Pb, Hg, Se, and As are used in the manufacture of semi-conductors, cables, contacts, and other electrical components where these heavy metals/metalloids could accidentally reach the soil during manufacturing process and/or from their disposal in waste (Alloway, 1995c).

1.1.4.4 Agricultural and horticultural practices

Heavy metal contamination of agricultural lands ascribed to different cultural practices has been well recognized (Freedman and Hutchinson, 1981). Various agricultural

activities involve very important non-point sources of metals for soils of many parts of the world, particularly in areas implementing intensive farming (Alloway, 1995c). Principal sources of metals comprise impurities in:

1. Fertilizers, principally phosphate fertilizers, may contain Cr, Zn, Cd, Pb, Cu, Ni, (Adriano, 1986; Adriano, 2001; Alloway, 1995c; Freedman and Hutchinson, 1981), V (Adriano, 1986; Adriano, 2001; Alloway, 1995c) and As (Pierzynski *et al.,* 2000; Table 1.9). Besides, micronutrient carriers are being employed to soils deficient in micronutrients (e.g., Cu, and Zn)(Adriano, 1986; Adriano, 2001). Nevertheless, data on metal loading rates for six agricultural watersheds in Canada revealed that fertilizer had much lower metal inputs than atmospheric loading, or the same derived from sludge or manure applications (Freedman and Hutchinson, 1981).

Table 1.9 Common ranges of heavy metal concentrations in fertilizers, farmyard manure, lime, and composts (mg kg^{-1})

Element	Phosphate fertilizers	Nitrate fertilizers	Farmyard manure	Lime	Composted refuse
As	2–1200	2.2–120	3–25	0.1–25	2–52
Cd	0.1–170	0.05–8.5	0.1–0.8	0.04–0.1	0.01–100
Co	1–12	5.4–12	0.3–24	0.4–3	—
Cr	66–245	3.2–19	1.1–55	10–15	1.8–410
Cu	1–300	—	2–172	2–125	13–3580
Hg	0.01–1.2	0.3–2.9	0.01–0.36	0.05	0.09–21
Ni	7–38	7–34	2.1–30	10–20	0.9–279
Pb	7–225	2–27	1.1–27	20–1250	1.3–2240
Se	0.5	—	2.4	0.08–0.1	—
V	2–1600	—	—	20	—
Zn	50–1450	1–42	15–566	10–450	82–5894

After Alloway (1995b).

Table 1.10 Metal containing pesticides recommended for use in Ontario, Canada from 1892-1975.

Common name of pesticide	***Metal composition of product***	***Years when recommended***	***Crops sprayed***	***Annual application (kg/hayr^{-1})***
A. Insecticides				
[1]Copper aceto–arsenite (Paris Green)	2.3% As, 39% Cu	1895–1920	Apples, peach, grape and cherries	0.8–1.7% As 0.2–0.4% Cu
>>	>>	1895–1957	Vegetables and small fruit (foliar and bait)	
[1]Calcium arsenate	0.8–26% As	1910–1953	Fruits and vegetables	2.0–2.5 As
[1]Lead arsenate	11–26% Pb 4.2–9.1% As	1910–1975	Apples	4.0–8.7 Pb 1.0–2.7 As
>>	>>	1910–1971	Cherries	
>>	>>	1910–1956	Peaches	
>>	>>	1910–1955	Vegetables	
[1]Mercuric chloride	6% Hg	1932–1954	Cruciferous crops	
[1]Zinc sulfate	20–30% Zn	1935–1955	Peaches	5.5–7.5 Zn
B. Fungicides				
[1]Copper sulfate-calcium salts	4–6% Cu	1892–1975	Fruit and vegetables	1.3–1.6% Cu
[1]Fixed copper salts[a]	2–56% Cu	1940–1975	>>	1.0–3.0% Cu
>>	>>	1948–1975	Fruit	
[2]Mancozeb	2% Zn	1966–1975	Fruit and vegetables	
[2]Methyl and phenyl mercuric salts	0.6–6% Hg	1932–1972	Seed treatment	
[2]Phenyl mercuric acetate	6% Hg	1954–1973	Apples	0.07–0.10% Hg
[2]Zineb and ziram	1–18% Zn	1947–1975	Vegetables	0.6–0.7% Zn
>>	>>	1957–1975	Fruit	
C. Top killers				
[1]Calcium arsenite	30% As	1930–1972	Vegetables	
[1]Sodium arsenite	26% As	1920–1972	>>	

After Adriano (1986) and Freedman and Hutchinson (1981).

[a] Various mixtures of copper chloride and/or copper sulfate with copper hydroxide.

[1] and [2] represent inorganic and metallic organics pesticides, in that order.

2. Liming materials [Limestone and Gypsum] could add Cd, Pb, As (Alloway, 1995c; Pierzynski *et al.,* 2000), Zn, V, Cu, Co (Adriano, 1986; Alloway, 1995c), Cr, Ni, Hg, and Se (Alloway, 1995c) to the soils;

3. Most of the metal containing pesticides have been employed in fruit orchards to control fungal pathogens and arthropods (Freedman, 1989). Determined by their type, repeated use of pesticides could furnish Cu, Zn, Pb, Hg, and As to agricultural soils (Adriano, 1986; Adriano, 2001; Alloway, 1995c; Freedman and Hutchinson, 1981; Freedman, 1989; He *et al.,* 2005; Table 1.10). Although the use of the above chemicals like lead arsenate, calcium arsenate, and copper sulfate have largely been replaced by organic pesticides (Freedman, 1989), fungicides with high Cu levels are still used in the USA for preventing citrus diseases (melanose, greasy spot, alternaria, and scab) (He *et al.,* 2005) ;

4. Wood preservative: As, Cu, Cr (Alloway, 1995c);

5. Corrosion of metal objects: Zn, and Cd (Alloway, 1995c);

6. Biosolids [sewage sludge, animal wastes, municipal solid waste, and some industrial waste such as paper pulp sludge] are employed as soil amendments in agricultural, forested, and reclaimed lands as the same furnish organic matter and plant nutrients (Adriano, 2001). However, dependent on the type and amount of urban and industrial discharges (Adriano, 2001; Pierzynski *et al.,* 2000) as well as rate/duration of application of sewage sludge, soil Cr, Ni, Cu, Zn, Cd, Pb (Adriano, 2001; Alloway, 1995c; He *et al.,* 2005), Hg, Se, and As levels are bound to increase (Adriano, 2001; Table 1.11). Likewise, conditional on the type of animal, and of the ration including the dietary supplement (e.g., 1% copper sulfate and copper iodized salts for pork and milk; up to 250 mg kg^{-1} Cu and 100-200 mg kg^{-1} Zn for swine and poultry: Adriano, 2001), manures can contain elevated levels of heavy metals (Adriano, 1986; Adriano, 2001; Alloway, 1995c; Davies, 1980; Table 1.9). Consequently, Cu and Zn levels in pig manure slurry

were 300-2000 mg kg^{-1} (mean 870 mg kg^{-1}) and 200-1500 mg kg^{-1} (mean 600 mg kg^{-1}), respectively (Alloway, 1995c) while application of Cu enriched pig/poultry manure at N fertilization rate could add 3-6 kg Cu ha^{-1} $year^{-1}$ (Adriano, 1986; Adriano, 2001). Furthermore, refuse derived compost can add Ni, Cu, Zn, Cd, and Pb to the soil (Alloway, 1995c; He *et al.,* 2005; Table 1.9).

Table 1.11 The range of trace elements (mg kg^{-1}) in sewage sludges reported in literature and maximum permitted concentrations in the European Union and USA.

Element	Range	Maximum permitted concentrations	
		EU	USA
As	3–30		
Cd	<1–3410	20–40	85
Co	1–260		
Cr	8–40600	600	3000
Cu	50–8000	1000–1750	4300
Hg	0.1–55	16–25	57
Ni	6–5300	300–400	420
Pb	29–3600	750–1200	840
Se	1–10		
Zn	91–49000	2500–4000	7500
V	15–80[1]	20–400[2]	15–92

After Alloway (1995b and references therein).
[1] and [2] denote ranges of mean values (of USA, UK, Canada, and New Zealand) and range for UK, respectively.

7. Irrigation: As water mobilizes and distributes trace elements, it is a chief source of trace element input to the food chain (Ward, 1995). Inputs of heavy metals through irrigation differ from one location to another. This is attributable to the contribution of a wide range of human activities (e.g., mining and ore processing, industrial processing [chemical, metal, alloys, chloro–alkali, petroleum], and domestic effluents), to the trace element pollution of the aquatic environments (Ward, 1995). Accordingly, wastewaters of domestic and industrial origin generally contain elevated levels of heavy metals than natural waters (He *et al.,* 2005). Moreover, selfsame elements listed under sewage sludge including Ni, Cu, Zn, Cd, and Pb (see number 6 above) are considered important potential threats if

present in wastewater (Adriano, 1986; Adriano, 2001). Ultimately, continual irrigation with wastewaters that have not been treated to remove heavy metals possibly will contribute to the accumulation of metals in soils (He *et al.,* 2005). Consequently, any project employing wastewater must check for trace elements (Table 1.12; compare with fresh water and river values in Table 1.7) for repeated application could eventually contaminate a soil, and may either turn it into non-productive or the product unusable (Ayers and Westcot, 1985).

Nonetheless, it is worthwhile to note that the amount of heavy metal/metalloid that may enter the soil environment hinges on the type and rate of the product/amendment employed as well as duration of the agricultural dealing. Apart from this, mainly in developed nations, some of the cultural practices (e.g., metal-based pesticides) may no longer be used due to environmental concerns (Adriano, 2001), or improved waste treatments could have significantly reduced the metal/metalloid inputs, or new products may have substituted for the previous materials etc. It is, conversely, axiomatic that land application of waste products, either as a beneficial reuse or as a disposal strategy, can lead to trace element contamination of soils (Pierzynski *et al.,* 2000).

1.1.4.5 Waste disposal on land

The disposal of household, municipal, and industrial wastes begets soil pollution by heavy metals in various ways (Alloway, 1995c). Consequently, temporary waste stockpiles can engender soil contamination, which may not be revealed until analysis at later time (Alloway, 1995c). Freedman and Hutchinson (1981) reviewed several reports on elevated soil metal concentrations (e.g., Cu, Pb, Ni, Zn) at sites that have served as dumps for municipal solid wastes, and suggested that agricultural use of reclaimed solid waste disposal sites should be accompanied with monitoring of the corresponding metal levels in edible plant tissues. Likewise, in poorly managed landfills, municipal solid waste can produce leachates enriched with various metals including Cd, Cu, Pb, and Zn that are capable of dispersing into the soil, ground waters, and surface waters (Alloway, 1995c).

Table 1.12 Surface and irrigation water quality criteria (mg kg^{-1}) for trace elements. [a]Maximum concentration is for good irrigation practice ($10000 m^3 ha^{-1} yr^{-1}$) on a continuous basis at one site. For higher water applications, the maximum values must be adjusted downwards.

Element	Recommended maximum concentration[a] (mg L^{-1})	US EPA wastewater effluent limitation[b]		Surface water (FWPCA)[c]	Irrigation water[c]			Remarks[a]
					Continuous use		Short-term use	
		Maximum daily	Maximum monthly average		FWPCA any soil	NAS Coarse-textured soil	FWPCA Fine-textured soil	
As	0.1	0.084	0.072	0.05	1.0	0.1	10	Toxicity to plants varies widely, 12 mg/L for Sudan grass to <0.05 mg/L for rice
Cd	0.01	0.071	0.026	0.01	0.005	0.01	0.05	Toxic to beans, beets, and turnip at low levels (0.1 mg/L) in nutrient solutions. Due to its potential injury to humans caused by potential accumulation in soils and plants, conservative limits are suggested.
Co	0.05	—	—	—	0.2	0.05	10.0	Toxic to tomato plants at 0.1 mg/L in nutrient solution. It is inactivated by neutral and alkaline soils.
Cr	0.1	0.025	0.014	0.05	5.0	0.1	20.0	—
Cu	0.2	0.023	0.014	1.0	0.2	0.2	5.0	Toxic to various plants at 0.1-1 mg/L in nutrient solution.
Pb	5.0	0.057	0.032	0.05	5.0	5.0	20.0	Can inhibit cell growth at high levels.
Ni	0.2	—	—	—	0.5	0.2	2.0	Toxic to various plants at 0.5-1 mg/L in nutrient solution; reduced toxicity at neutral or alkaline pH.
Se	0.02	—	—	0.01	0.05	0.02	0.05	Toxic to plants at 0.025 mg/L and to toxic to livestock feeding on relatively high Se forage; essential to animals at low levels.
V	0.1	—	—	—	10.0	0.1	10.0	Toxic to many plants at rather low levels.
Zn	2.0	0.082	0.054	5.0	5.0	2.0	10.0	Toxic to many plants; low toxicity at pH>6
Hg	-	0.0023	0.0013	—	—	—	—	—

Adapted from Ayres and Westcot (1985)[a], He et al. (2005)[b], and Adriano (1986)[c].
NAS and FWPCA stand for national academy of science and federal water pollution control administration, in that order.

1.1.4.6 Atmospheric deposition

The wind movement is an important transport medium for metals from different sources (Alloway, 1995c). The gaseous and particulate contaminants are emitted from chimneys and exhausts or are deflated from soil heaps and are blown away by the wind eventually sink or washed by rain to the ground (Davies, 1980). The pattern of ground contamination pivots on the nature of the source, the size and density of the particle, changes in size and composition of the particle during transport and, in the case of gases, adsorption or solution process, and wind strength and direction (Davies, 1980). Generally, trace element or heavy metal contamination principally proceeds from atmospheric particles or particulates (Ward, 1995). Notwithstanding this, volatile elements (e.g., Hg, Se, and As) can be transported in gaseous form or enriched in particles, while other metals (e.g., Cu, Zn, and Pb) are transported only in the particle phase (Adriano, 2001). The size of the aerosol particle containing metals range from 5 nm to 20 μm, but most are between 0.1–10 μm in diameter and have an average residence time of 10–30 days (Alloway, 1995c).

Sources can be categorized as continuous point sources (chimneys and smelter stacks), line sources or linear pollution sources (motor vehicles), and area sources (urban and industrial areas, factory estates, and works complexes)(Davies, 1980). Environmentally important point sources for metal contamination of soils include emissions from primary and secondary smelters, metal refineries (Adriano, 1986; Adriano, 2001; Davies, 1980; Freedman and Hutchinson, 1981; Freedman, 1989; He *et al.,* 2005), and metal foundries (Freedman and Hutchinson, 1981; Freedman, 1989; He *et al.,* 2005). In addition, petroleum refining, cement production, chloro-alkali production (Ward, 1995), chemical production (Davies, 1980; Ward, 1995), power stations, and waste incinerators may serve as point sources of trace elements (Davies, 1980; Freedman and Hutchinson, 1981; He *et al.,* 2005; Ward, 1995). In most cases, industrial emissions of heavy metals comprise Cr, Ni, Cu, Zn, Cd, Pb, and V (Ward, 1995).

On the other hand, the use of motor vehicles and thus motor roads has been important line sources of heavy metals. Motor vehicle exhausts on busy highways could release Pb (leaded gasoline) (Adriano, 1986; Alloway, 1995c; Davies, 1980; Freedman and Hutchinson, 1981; Freedman, 1989; He *et al.,* 2005; Pierzynski *et al.,* 2000). Apart from this, other roadside metal contaminants with less-defined gradient than Pb including Zn, Cd, Cu, Ni (Adriano, 1986; Freedman and Hutchinson, 1981; Freedman, 1989), Cr, and V have been documented (Freedman and Hutchinson, 1981; Freedman, 1989). However, these metals might derive from wear of metallic automobile parts containing the metals (Ni, Cr, and V attrition of steel), wear of tires (Zn and Cd) (Pierzynski *et al.,* 2000), use of Ni as gasoline or oil additives (Freedman and Hutchinson, 1981; Freedman, 1989), and Cd (diesel fuel) (Pierzynski *et al.,* 2000). Conversely, as trace element contamination is a direct consequence of human activities, the problem gets worse in areas where people congregate-in towns and industrial areas where they could be affected by area source of contamination. Albeit the low intensity of many urban sources of pollution, their concentration, however, makes urban pollution by area source a serious environmental concern (Davies, 1980).

1.1.5 Sorption and partitioning of heavy metals in soils

Soils are recognized as sinks of metals (Das *et al.,* 1995; Evans, 1989; Ward, 1995), and thus are of paramount importance in the environmental cycling of trace elements (Ward, 1995). The accretion of metals in soils is ascribed to their surface–active mineral and humic constituents involved in reactions that govern retention of metals (Evans, 1989). Consequently, soils accumulate heavy metals at levels orders of magnitude higher than the corresponding levels in water and air (Navas and Lindhorfer, 2003). In order to assess the biogeochemical cycling of heavy metals, their distribution in soils is considered pivotal in environmental studies (Navas and Lindhorfer, 2003). Moreover, understanding the binding of metals with various soil phases and components is fundamental to define their behavior (Pendias and Pendias, 2001), which is requisite to appreciate their association with the biotic and abiotic components of the environment (Navas and Lindhorfer, 2003). In essence, due account should be taken for retention and release

reactions of the various species in the soil environment to predict the fate of heavy metals in soils (Selim and Amacher, 2001). Heavy metals in soils can be involved in a string of complex chemical and biological interactions including redox, surface and solution phase complexation, precipitation and dissolution, and volatilization (Selim and Amacher, 2001).

Due to the significant capacity of soils to remove ions from water (McBride, 1994), most trace elements undergo sorption process with the solid phases of the soil (Pierzynski *et al.,* 2000). Sorption is recognized as an important process determining the partitioning of metals between the aqueous and solid phases in soils (Adriano, 2001). Sorption, a general term employed when the retention mechanism (physical and chemical processes) at the surface is unknown (Sparks, 2003), corresponds to the retention of chemical constituents via transfer of ions from solution phase to solid phase (Adriano, 2001; McBride, 1994). Sorption may entail adsorption, surface precipitation, and polymerization [formation of small multinuclear inorganic species such as dimers or trimers] (Sparks, 2003) where the first two are major mechanisms involved in retention of metals by soils (Evans, 1989). Metal sorption in soils is influenced by type and amount of soil colloids (clay minerals, soil oxides, and organic matter), pH, ionic strength of the bathing solution (Impellitteri *et al.,* 2001; Morera *et al.,* 2001; Ross, 1994a), metal cation levels, the presence of competing metal cations, the presence of organic ligands, and nature of exchanging cations (Impellitteri *et al.,* 2001).

Adsorption typifies the removal of solute molecules from the solution and of solvent from the solid surface, and the attachment of the solute molecule to the surface (Sparks, 2003) involving the formation of either outer sphere or inner sphere complexes with the surfaces of mineral and organic constituents (Evans, 1989). While outer sphere complex entail non–specific electrostatic forces which reversibly retain cations on layer silicate clays and humus (McBride, 1994), inner sphere complexes result from short–range interactions that comprise a ligand exchange, covalent bonding, and hydrogen bonding (Sparks, 2003) with humus, on variable charge minerals (specific adsorption on oxides, allophane, etc) and edges of layer silicate clays (McBride, 1994). In the absence of high

pH (which favor hydrolysis), divalent (Cu^{2+}, Co^{2+}, Ni^{2+}, VO^{2+}, $Cr(OH)^{2+}$) and trivalent metal cations can exchange for other ions on layer silicates (Ross, 1994a) where the strength of attraction pivots on the charge and degree of hydration (Adriano, 2001; Evans, 1989; Ross, 1994a). In contrast, specific adsorption of metallic ions occurs most readily for metals that hydrolyze in water (Evans, 1989; Ross, 1994a) and for metals that form oxyanions (Evans, 1989).

Precipitation of new mineral phases (e.g., oxides, hydroxides, oxyhydroxides, carbonates, sulfides, phosphates, and silicates), conversely, could occur if the chemical composition of the soil solution becomes supersaturated with respect to insoluble precipitate (Evans, 1989). Precipitation of metals as secondary minerals commonly may occur when industrial and municipal wastes containing elevated levels of metals are applied to soil (Evans, 1989). Hydrolysis and dissolved complexation have a propensity to enhance solubility of heavy metals whilst precipitation and adsorption can hinder metal availability and transport (Bourg, 1995). Notwithstanding, pH and Eh have a bearing on the rate and direction of both adsorption/desorption and precipitation/dissolution processes (Ross, 1994a).

In soils and sediments, elements occur in several different forms that are associated with range of components (Tack and Verloo, 1995). Nowadays, it is generally established that the distribution, mobility, and bioavailability of heavy metals and radionuclides in the environment are governed not only by their total levels but also on the association form in the solid phase to which they are bound (Filgueiras *et al.,* 2002). It has been found that the chemical form or species (Table 1.13) of an element determines the potential environmental impact of the element (Christie, 1995; Navas and Lindhorfer, 2003), namely potential risk of metal mobilization and transfer to plants and other compartments (Karczewska, 1996). Consequently, essentials about the physicochemical forms are vital to get insight regarding the behavior and fate of metals such as mobility, pathways, and bioavailability (Tack and Verloo, 1995).

Table 1.13 Major chemical forms or species found in natural waters, soils and soil solutions

Trace element	*Chemical form or species*		
	Natural waters	***Soils***	***Soil solution***
Cr	$Cr(OH)_2(H2O)_4^+$, $CrO4_2^-$	Chromite [$FeCr_2O_4$]	$Cr(OH)^{2+}$, CrO_4^{2-}, CrO_3^{3-}
Co	Co^{2+}	Fe minerals, Mn oxides	Co^{2+}, Co^{3+}, $Co(OH)_3^-$
Ni	Ni^{2+}	Pentlandite [$(Ni, Fe)_9S_8$], Garnierite [$(Ni, Mg)_3Si_2O_5(OH)_4$]	Ni^{2+}, $NiOH^+$, $NNiO_2^{-2}$, $Ni(OH)_3^-$
Cu	Cu^{2+}, $Cu(OH)^+$, $Cu^{2+}SO4^{2-}$, $Cu^{2+}CO_3^{2-}$	Chalcocite [Cu_2S], Chalcopyrite [$CuFeS_2$], Bornite [Cu_5FeS_4]	Cu^{2+}, $Cu(OH)^+$, $Cu(OH)_2^0$, $CuCO_3$, $Cu(OH)_4^{2-}$, $Cu^{2+}CO_3^{2-}$, Cu^{2+}-humic or fulvic acids
Zn	$Zn(OH)^+$, $Zn(OH)_2$, $ZnCl^+$, $ZnCl_2$, $ZnCO_3$, Zn^{2+}	Sphalerite (ZnS)	Zn^{2+}, $ZnCl^+$, $Zn(OH)^+$, $Zn(OH)_2$, $ZnCO_3$, $[ZnCl_4]^{2-}$
As	$HASO_4^{2-}$ (<1nm,dissolved), As-fulvate chelates (1-10nm, dissolved)	AsO_4^{3-} [oxic], $AlAsO_4$, $FeAsO_4$, $Ca_3(AsO_4)_2$	AsO_2^-, AsO_4^{3-}, $H_2AsO_3^-$
Cd	$CdCl^+$, $CdCl_2$, $CdCl_3^-$, Cd^{2+}, Cd-fulvates or low molecular weight (1-10nm), Cd-organic chelates (>1μm)	CdS (reducing); CdO, $CdCO_3$, $Cd_3(PO4)_2$(oxic)	Cd^{2+}, $CdCl^+$, $CdOH^+$, $CdHCO_3^+$, $CdCl_3^-$, $CdCl_4^{2-}$, $Cd(OH)_3^-$, $Cd(OH)_4^{2-}$
Hg	$HgCl_2$, $HgCl_3^-$, $HgCl_4^{2-}$, HgOHCl, $Hg(OH)_2$ (<1nm), Hg-fulvate chelate (1-10 nm, dissolved), Hg-humic chelates (10-100nm, colloidal)	HgS (reducing); $HgCO_3$ (Oxic)	Hg_2^+, Hg^{2+}, $HgOH^+$, $HgCl_3^-$, $HgCl_4^{2-}$, CH_3Hg^+, $CH_3CH_2Hg^+$
Pb	Pb^{2+}, $PbCO_3$, $PbCl^+$, $PbCl_2$, $PbCl_3^-$, $Pb(OH)^+$, $Pb(OH)_2$, $Pb(OH)_3^-$, $Pb_3(OH)_4^{2+}$, $Pb_4(OH)_4^{4+}$, Pb-FeOOH (chemisorbed, 100-1000nm, colloidal), 2PbCO3.Pb(OH)2 (precipitate)	PbS, $PbSO_4$, $Pb(OH)_2$, $PbCO_3$, PbO, $Pb_3(PO_4)_2$	$Pb(OH)^+$, $Pb_4(OH)_4^{4+}$, Pb-organic complexes
V		Cationic/anionic oxides, hydroxy oxides	VO^{2+}, VO_4^{3-}, VO_3^-
Se	SeO_3^{2-}, SeO_4^{2-}		

Adapted from Ward (1995) and Ross (1994b).

The term "*speciation*" has no generally accepted definition (Tack and Verloo, 1995; Ure, 1991) and thus, speciation of heavy metals in soils could be defined in many ways (Ure, 1991). Speciation can be broadly defined as the identification and quantification of the different defined species, forms or phases in which the element of interest exists (Tack and Verloo, 1995; Ure, 1991). It could also denote the different physico–chemical forms of an element that constitute the total concentration in a given sample (Christie, 1995). Moreover, the species/forms/phases in soils can be specified: 1) functionally or by their role, representing plant available species or exchangeable; 2) operationally: by the reagents or the methods employed to identify, isolate, and quantify element bound to a given soil phase (Ure, 1991; Ure, 1995); and 3) as particular chemical compounds or oxidation states of an element (Ure, 1991).

Due to the complexity of possible reactions and usually unknown reaction kinetics in natural soil and sediment systems alike, studies of metal species distribution in solid phases are limited chiefly to operationally defined analytical procedures (Tack and Verloo, 1995). Of operationally defined procedures, sequential extraction methods are designed to isolate different soil phases and present enhanced phase specificity over single extractants (Ure, 1991). Albeit the problems of non–selectivity and readsorption, data from sequential extractions furnish qualitative evidence about the forms of association of trace metals, their bioavailability (Abollino *et al.,* 2005; Jeng and Singh, 1993; Ma and Rao, 1997; Morera *et al.,* 2001; Wasay *et al.,* 1998), their potential adverse effects on human health and on the environment (Abollino *et al.,* 2005). Consequently, sequential extraction methods that involve treatment of samples with reagents of increasing strength to dissolve metals associated with different solid phases (Abollino *et al.,* 2005; Voegelin *et al.,* 2003) which represent decreasing reactivity or mobility (Abollino *et al.,* 2005) have proved to be useful in the field of environmental sciences(Abollino *et al.,* 2005; Tack and Verloo, 1995).

Sequential extractions partition metals into operationally defined solid phases (Chlopecka, 1993; Morera *et al.,* 2001) that are instrumental for studying metal mobility and availability in soils (Ma and Rao, 1997). Heavy metals in soils can be associated with

different geochemical phases or forms of retention (Wasay *et al.,* 1998) which partition their total content into the respective levels contained in the different soil fractions. Accordingly, heavy metals associated with operationally defined geochemical phases can be categorized as exchangeable, bound to carbonates, bound to oxides of Fe and Mn (reducible), bound to organic matter (oxidizable), and lattice material/residual (Abollino *et al.,* 2005; Chlopecka, 1993; Gleyzes *et al.,* 2002; He *et al.,* 2005; Ma and Rao, 1997; Morera *et al.,* 2001; Navas and Lindhorfer, 2003). Natural and anthropogenic environmental changes on pH, Eh, organic matter decomposition, leaching and ion exchange processes, temperature, and microbial activity (Filgueiras *et al.,* 2002; Kennedy *et al.,* 1997) considerably affect the behavior of metallic pollutants (Filgueiras *et al.,* 2002) and hence, can cause their release from the solid to the liquid phase (Gleyzes *et al.,* 2002; Kennedy *et al.,* 1997). Apparently, change in ionic strength, change in pH (lower pH), reducing, and oxidizing conditions in soil can mobilize metals from exchangeable, carbonate, reducible, and oxidizable fractions, respectively (Dang *et al.,* 2002; Filgueiras *et al.,* 2002).

In unpolluted soils, residual and to lesser extent, oxide fractions are the most abundant pools of trace metals while exchangeable, carbonate, and oxide are important fractions for metals in polluted soils (Chlopecka, 1993). As a rule, heavy metals extracted from exchangeable fraction (which also includes water–soluble) are regarded as readily bioavailable (Chlopecka, 1993; He *et al.,* 2005; Ma and Rao, 1997) while those bound to carbonate, oxides, and organic matter could be potentially bioavailable (Chlopecka, 1993; He *et al.,* 2005). In to-to, sequential extraction data afford requisite information concerning the origin, mode of occurrence, biological and physicochemical availability, mobility, transport (Dang *et al.,* 2002; Filgueiras *et al.,* 2002) and biological effects of metals as well as their long-term impacts on the environment in relation to relatively changing soil properties such as pH, and Eh (Chlopecka, 1993).

1.1.6 The role of soil properties and plant factors on heavy metals'/metalloids' phytoavailability, uptake, and translocation to shoots.

Cardinal chemical processes determining the behavior and thus, bioavailability and root uptake of metals in soils are those [e.g., cation exchange, specific adsorption, and (co)precipitation] related with the control of metal concentrations and complexes in the soil solution (Alloway, 1995a). As a rule, the retention capacity of soils for most trace elements (saving Cr, As, V and Se which are commonly mobile under alkaline pH), increases with pH, with the expected maximum at around circumneutral pH (Adriano, 1986; Adriano, 2001). This is ascribed to the impact of pH on sorption by way of its influence on surface charge of layer silicates, organic matter (OM), and oxides of Fe and Al, as well as on the redox reactions, which could affect mobility and ultimately, bioavailability of metal ions (Adriano, 2001).

Likewise, adsorption of most heavy metals (barring V, and the metalloids Se and As) hinges on the density of negative charge on the surface of the soil colloids (Alloway, 1995a; Lasat, 2000), CEC, which may limit the solubility and mobility of metals rather than being a specific factor in the bioavailability of metals (Adriano, 1986; Adriano, 2001). Conversely, CEC, in turn, pivots mainly on OM, the type of clay, and oxides of Fe, Al, and Mn (Adriano, 1986; Adriano, 2001). Apart from its role in CEC, OM also adsorbs metals by forming chelate complexes (Adriano, 1986; Adriano, 2001; Alloway, 1995a). Low molecular weight organic ligands can form soluble complexes with metals and prevent them from being adsorbed or precipitated (Alloway, 1995a). On the other hand, the moisture content of the soils affects their retention capacity for trace metals (Adriano, 1986; Adriano, 2001) through changes in oxidation state of elements (e.g., Cr, Hg, Se, and As) depending on the redox conditions (Adriano, 2001). Under reducing conditions, sulfides of metals such as Cd, Zn, Ni, Co, Cu, and Pb can form (Adriano, 1986) are rather insoluble and thus significantly reduce their mobility and phytoavailability than would be expected under well-oxidized soils (Adriano, 1986; Adriano, 2001).

Apart from soil factors governing phytoavailability of heavy metals/metalloids, various plant factors have a bearing on the capacity of plants to accumulate the same. Whether toxic metals are available or not hinges on the form of the metal (Table 1.13 and 1.14) and the species of the plant (Ross, 1994a). Accordingly, food crops differ markedly in their sensitivity to superfluity of every trace element (Adriano, 1986) as well as in their relative uptake of metals (Ross, 1994c; Table 1.15). The genetic basis for relative variations in metal uptake between plant species/cultivars have long been recognized (Adriano, 1986; Alloway, 1995a). Such uptake variations could be ascribed to factors such as surface area of the root, root CEC, root exudates, and the rate of evapotranspiration (Alloway, 1995a).

Ross and Kaye (1994) pointed out that toxic metals are believed to enter cells with recourse to the same uptake process, namely passive uptake (via the apoplast or cell wall continuum) and active uptake (via symplast or protoplasmic space), which move essential micronutrient metal ions (Cu and Zn). While passive uptake involves ion diffusion driven by the ion concentration gradient, active ion transport processes across plasma membrane, which often occur against concentration gradient, require metabolic energy. Pendias and Pendias (2001) suggested that Pb and Ni are preferably absorbed passively, whereas Cu and Zn are taken up by active uptake process. However, when the levels of elements in soils exceed a threshold value for physiological barrier that alter the biological and structural properties of root cells, every element eventually is absorbed via passive process (Pendias and Pendias, 2001). In higher plants, solute cations move passively into the root cortical cell walls that form an apoplast — a hydrated free space continuum between the external bathing solution and the cortical cell membranes (Ross and Kaye, 1994). The matrix of cell wall which comprises cellulose, hemicellulose, pectins and glycoproteins carries charged groups (Mohr and Schopfer, 1995b; Ross and Kaye, 1994) /anchor ions on proteins and polymers containing glucuronic acid, and the net negative charge due to polyanionic pectins (Mohr and Schopfer, 1995b) enables the cell wall to reversibly bind considerable amount of cations (Ross and Kaye, 1994; Mohr and Schopfer, 1995b).

Table 1.14 Major chemical forms or species evolved during various industrial and anthropogenically derived processes.

Process	Metal form				
	Cd	***Pb***	***Hg***	***As***	***Cr***
Coal combustion	Cd(O),CdO, CdS	$PbCl_2$, PbO, PbS, Pb	$Hg^0_{(g)}$	As(O), As_2O_3	—
Oil combustion	Cd(O),CdO	PbO	$Hg^0_{(g)}$	As(O), As_2O_3, organic Arsenics	—
Non-ferrous metal production	CdO, CdS	PbO, $PbSO_4$, $PbO.PbSO_4$	—	As_2O_3	—
Iron and steel manufacture	CdO	PbO	—	—	—
Refuse incineration	Cd(O),CdO, $CdCl_2$	Pb(O), $PbOPbCl_2$,	$Hg^0_{(g)}$	As(O), As_2O_3	
Vehicular exhaust	—	PbBr2, PbBrCl, Pb(OH)Br, $(PbO)_2PbBrCl$, $(PbO)_2PbBr$. Changes to carbonates and oxides in soil.	—	—	—
Ferrochrome smelters	—	—	—	—	Cr^{6+}
Industrial emissions	CdO	PbO	—	—	Aerosols of Cr^{3+} and Cr^{6+}
Biochemical industries	—	—	$Hg^0_{(g)}$, $(CH_3)_2Hg$ produces soluble $Hg(OH)_2$ in rain	—	—

After Ross (1994b)

Conversely, as charged ions cannot move freely across the lipophilic plasma membrane (Lasat, 2000), metal ion transport into cells necessitates some form of carrier mechanism

involving membrane proteins and metabolic energy (active transport) (Lasat, 2000; Ross and Kaye, 1994). Active ion transport processes across plasma membrane entail ion specific carriers (Lasat, 2000; Ross and Kaye, 1994) consequent to the influence of the type, size, and valency of ion as well as the geometry of the carrier site on the transport processes (Ross and Kaye, 1994). Apart from this, physical adsorption of metals in the root cell wall diminishes the extent of their absorption into the cells to only a fraction of the total amount of ions associated with the root, which in turn, regulates subsequent translocation to the shoots (Lasat, 2000). Similarly, metals can be complexed and sequestered in cellular structures such as vacuoles that further control the quantity available for translocation to the shoots (Lasat, 2000). On the other hand, translocation — movement of metal containing sap from the root to the shoot — is chiefly governed by the root pressure (Lasat, 2000), and leaf transpiration processes (Alkorta *et al.,* 2004; Lasat, 2000; Liphadzi and Kirkham, 2005) as well as by metal binding ligands such as citrate and histidine (Alkorta *et al.,* 2004; Liphadzi and Kirkham, 2005). Subsequent to translocation to the leaves, metals can ultimately be reabsorbed from the sap into leaf cells (Lasat, 2000).

Accordingly, different species and cultivars regulate metal uptake at both the soil–root and root–shoot interfaces to a varying extent (Thornton, 1981). Besides, trace elements are often not distributed uniformly with in plants and hence, grain crops are expected to contribute inconsequential amount of trace elements to the diet than do leafy vegetables, subterranean crops, forages, and pastures (Adriano, 1986). The rate and degree of transport within plants pivots on the type of metal, the plant organ, and the age of the plant (Alloway, 1995a). In general, the trace element level of the whole plant tends to increase with increasing maturity, and seasonal influences are also important (Thornton, 1981). Over and above, cultural practices including application of inorganic fertilizers (Adriano, 1986; Thornton, 1981), animal manures, composts, sewage sludges, and irrigation as well as other soil conditions (e.g., ion interactions: macronutrient-micronutrient, trace element–trace element) and climate likely influence phytoavailability of trace elements (Adriano, 1986).

Table 1.15 Relative sensitivity to sludge-borne heavy metals and relative metal uptake by a range of crop plants growing on contaminated soils.

A. Relative sensitivity[1]			
Very sensitive	***Sensitive***	***Tolerant***	***Very tolerant***
Chard	Mustard	Cauliflower	Corn
Lettuce	Kale	Cucumber	Sudan grass
Red beet	Spinach	Zucchini squash	Smooth brome grass
Carrot	Broccoli	Flat pea	"Merlin" fescue grass
Turnip	Radish	Oat	
Peanut	Tomato	Orchard grass	
Ladino clover	Marigold	Japanese brome grass	
Alsike clover	Zigzag, Red, Kura and Crimson clover	Switch grass	
Crown vetch	Alfalfa	Redtop	
"Arc" alfalfa	Korean lespedeza	Buffel grass	
White sweet clover	Sericea lespedeza	Tall fescue	
Yellow sweet clover	Blue lupin	Red fescue	
Weeping love grass	Birds foot trefoil	Kentucky blue grass	
Lehman love grass	Hairy vetch		
Deer tongue	Soybean		
	Snap bean		
	Timothy		
	Colonial bent grass		
	Perennial rye grass		
	Creeping bent grass		

B. Relative metal uptake[2]	
Metal	***Relative uptake***
Cd	Lettuce>Radish>Carrot>Spinach>Cauliflower>Oat>Pea
Cd	Turnip, Spinach>Tomato, Lettuce>Swiss chard, Radish, Carrot>Corn>Fescue, Wheat, Field bean>Rice
Cd	Spinach>Lettuce>Beet root>Radish>Sweet corn>Spring green >Spring onion>leek> Cauliflower>Potato>Cabbage
Cd	Bermuda grass>Tall fescue>Alfalfa>White clover>Sudan grass
Pb	Spinach>Radish> Spring green, Lettuce> Cauliflower>Leek> Spring onion>Beet root>Cabbage>Potato>Sweet corn
Pb	Lettuce>Cabbage>Carrot>Radish
Zn	Lettuce>Kidney bean>Pea>Potato
Cu	Kidney bean>Lettuce>Pea>Potato
Ni	Radish>Lettuce>Cabbage>Carrot

Adapted from Adriano (1986)[1] and Ross (1994c)[2].

1.1.7 Physiological roles and toxicities of heavy metals/metalloids

Metals and their compounds are ubiquitous throughout the environment and are constantly being cycled through the same (Barbante and Cairns, 2002). While some of the same are essential for life, others can be toxic to living organisms at all concentrations (Barbante and Cairns, 2002; Tables 1.1, 1.16, and 1.17). In essence, trace elements are involved in key metabolic processes such as respiration, photosynthesis, fixation, and assimilation of some major nutrients (e.g., N, S)(Pendias and Pendias, 2001). Several enzymes contain one or more metals as tightly integrated components of the active center e.g., Zn^{2+} in lactate dehydrogenase and alcohol dehydrogenase while Cu^{2+} is associated with various oxidases (Mohr and Schopfer, 1995a). Consequently, the same are recognized to activate enzymes, as electron transfer system incorporated into metalloenzymes (Cu, Zn), catalyze valence changes in the substrate (Cu, Co), as well as could be associated with specific roles like in protection mechanisms of frost hardy and drought tolerant species (Pendias and Pendias, 2001; Table 1.18).

Table 1.16 The periodic table presenting elements recognized to be toxic and/or essential. After Barbante and Cairns (2002).

H																*He*
Li	*Be*											*B*	*C*	*N*	*O*	*F*	*Ne*
Na	*Mg*											*Al*	*Si*	*P*	*S*	*Cl*	*Ar*
K	*Ca*	*Sc*	*Ti*	*V*	*Cr*	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*	*Ga*	*Ge*	*As*	*Se*	*Br*	*Kr*
Rb	*Sr*	*Y*	*Zr*	*Nb*	*Mo*	*Tc*	*Ru*	*Rh*	*Pd*	*Ag*	*Cd*	*In*	*Sn*	*Sb*	*Te*	*I*	*Xe*
Cs	*Ba*	*La*	*Hf*	*Ta*	*W*	*Re*	*Os*	*Ir*	*Pt*	*Au*	*Hg*	*Tl*	*Pb*	*Bi*	*Po*	*At*	*Rn*
Fr	*Ra*	*Ac*															
		Ce	*Pr*	*Nd*	*Pm*	*Sm*	*Eu*	*Gd*	*Tb*	*Dy*	*Ho*	*Er*	*Tm*	*Yb*	*Lu*		
		Th	*P*	*U*	*Np*	*Pu*	*Am*	*Cm*	*Bk*	*Cf*	*Es*	*Fm*	*Md*	*No*	*Lr*		

Key

Hg	Toxic
O	Essential
Se	Essential and toxic

Table 1.17 Organisms most commonly affected by toxicities of selected trace elements After Pierzynski *et al*. (2000).

Element	**Vulnerable organisms**				
	Humans	***Animals***	***Aquatic organisms***	***Birds***	***Plants***
Cd	+	+	+	+	+
As, Pb, Hg, Cr, Se	+	+	+	+	
Cu, Ni, Zn			+		+
Co		+			

Table 1.18 Forms and principal functions of essential trace elements in plants.

Element	Constituent of	Involved in
As[a]	Phospholipid (in algae)	Metabolism of carbohydrates in algae and fungi
Co	Cobamide coenzyme	Symbiotic N_2 fixation, possibly also in non-nodulating plants, and valence changes stimulation synthesis of chlorophyll and proteins (?)
Cu	Various oxidases, plastocyanins, and ceniloplasmin	Oxidation, photosynthesis, protein and carbohydrate metabolism, possibly involved in symbiotic N_2 fixation and valence changes, and cell wall metabolism
Ni[a]	Enzyme urease (in *Canavalia* seeds)	Possibly in action of hydrogenase and translocation of N
Se[a]	Glycene reductase (in *Clostridium* cells) combined with cysteine and methionine	Can replace S in some plants
V[a]	Porphyrins, hemoproteins	Lipid metabolism, photosynthesis (in green algae), and possibly, in N_2 fixation
Zn	Anhydrases, dehydrogenases, proteinases, and peptidases	Carbohydrate, nucleic acid and lipid metabolism

[a] Indicates elements recognized to be essential for some groups or species and thus, their general essentiality require further verification. After Pendias and Pendias (2001).

Of heavy metals/metalloids under the present study, Ni, Co, Cu, Zn, and V are known as essential for plants (Alloway, 1995a; Pendias and Pendias, 2001) whereas Cr, Ni, Co, Cu, Zn, Se, V, and As are essential for animals (Adriano, 2001; Alloway, 1995a). Albeit their importance, some of the above metals/metalloids may not elicit deficiency problems (Alloway, 1995a) or are solely essential for some groups/a few species (Pendias and Pendias, 2001), and thus, only Cu, Zn (for both plants and animals), and Se (for animals) are unambiguously essential elements. As to the criteria of essentiality for plants (Adriano, 2001; Alloway, 1995a; Pendias and Pendias, 2001) and animals (Adriano, 2001; Alloway, 1995a), the specific biochemical roles of an essential element cannot be substituted by others and it has a direct effect on some aspect of growth and metabolism so that the plant/animal can neither grow nor complete some metabolic cycle.

Apart from the various roles of essential elements in biological systems, at higher concentrations they also have toxic effects to most plants (Turner, 1994; Pendias and Pendias, 2001). Pendias and Pendias (2001) indicated that trace element toxicity to plants is influenced by the electronegativity of divalent metals, solubility product of sulfides, stability of chelates, and bioavailability. Several metals form stable complexes with biomolecules, and consequently their presence in even small amounts can be detrimental to plants and animals (Evans, 1989). Heavy metals/metalloids under Class B (nitrogen or sulfur seeking; soft acids/bases) and to a certain extent, those belonging to Borderline or Intermediate category (see section 1.1.1) are expected to be more bioreactive, and thus, lead to biotoxicities (Adriano, 2001). Consequently, Ni, Co, Cu, Cd, Pb, and Hg (Table 1.17) are considered the most toxic metals for both higher plants and certain microorganisms (Alloway, 1995a; Pendias and Pendias, 2001). Biotoxicity induced by metal exposure is the effect of (bio)chemical reactions that disrupt biological processes (Adriano, 2001) at the cellular level involve (Ross and Kaye, 1994):

- Blocking functional groups of biologically important molecules, namely enzymes, polynucleotides (Adriano, 2001; Ross and Kaye, 1994; Turner, 1994), or transport systems for nutrient ions (Ross and Kaye, 1994);

- Displacing and/or substituting essential metal ions from biomolecules (Adriano, 2001; Ross and Kaye, 1994; Turner, 1994) and functional cellular units (Ross and Kaye, 1994; Turner, 1994);
- Denaturing and inactivating enzymes (Adriano, 2001; Ross and Kaye, 1994; Turner, 1994) and polynucleotides (Turner, 1994); and
- Disrupting cell and organelle membrane integrity (Ross and Kaye, 1994; Turner, 1994).

In higher plants, root cells are exposed to the deleterious effects mentioned above before other cells in the aboveground parts (Ross and Kaye, 1994). Depending on the type of plant/ecotype, soil, and environmental conditions, the above cellular damages could develop into specific injurious effects (visible/measurable toxicity symptoms) of metals on plant physiological processes such as:

- Inhibition of photosynthesis and respiration;
- Alteration of plant–water relations, causing water stress and wilting;
- Increased permeability of root cell plasma membrane, rendering roots less ion selective by changing cytoplasm pH, and plasma membrane electrical potential; and
- Adverse effects on the activities of metabolic enzymes (Ross and Kaye, 1994).

Gardea-Torresdey and his coworkers (2005) reviewed those heavy metals reported as putative causes for the disruption of the physiology and morphology of plants (Table 1.19). Accordingly, Cr, Ni, Cu, Pb, and Hg were found to affect surface area of the thylakoid membrane, electron transport, and enzymatic activity, or reduce chlorophyll production where the impact of Cr, Ni, Cu, Cd, and Pb could eventually decrease the growth of plants (Gardea-Torresdey *et al.,* 2005). Although visible symptoms of toxicity differ for every species and between individual plants, most common and non-specific symptoms of toxicity include chlorotic or brown points of leaves and leaf margins as well as brown–, stunted–, and coralloid roots (Pendias and Pendias, 2001; Table 1.19).

Table 1.19 General symptoms and main effects of trace elements toxicity in plants

Element	*Symptom*	*Effects*	*Sensitive crop*
As	Red-brown necrotic spots on old leaves, yellowing or browning of roots, depressed tillering, wilting of new leaves	—	Legumes, onion, spinach, cucumber, bromgrass, apricots, peaches
Cd	-Brown margins of leaves, chlorosis, reddish veins and petioles, curled leaves, and brown stunted roots. -Severe reduction in growth of roots, tops, and number of tillers (in rice). -Reduced conductivity of stem, caused by deterioration of xylem tissues. -Reduction of chlorophyll and carotenoids.	Decreases seed germination, lipid content, and plant growth; induces phytochelatins production	Legumes (bean, soybean), spinach, radish, carrots, and oats
Co	Interveinal chlorosis in new leaves followed by induced iron chlorosis and white leaf margins and tips, and damaged root tips	—	—
Cr	Chlorosis in new leaves, necrotic spots and purpling tissues, injured root growth	Decreases enzyme activity and plant growth; produces membrane damage, chlorosis and root damage	—
Cu	Dark green leaves followed by induced iron chlorosis, thick, short, or barbed-wire roots, depressed tillering. Changes in lipid content and losses of polypeptides involved in photochemical activities	Inhibits photosynthesis, plant growth and reproductive process; decreases thylakoid surface area	Cereals and legumes, spinach, citrus seedlings, and gladiolus
Hg	Severe stunting of seedlings and roots, leaf chlorosis and browning of leaf points	Decreases photosynthetic activity, water uptake and antioxidant enzymes; accumulated phenol and proline	Sugar beets, maize, and roses
Ni	Interveinal chlorosis (caused by Fe-induced deficiency) in new leaves, gray-green leaves, brown and stunted roots and plant growth	reduces seed germination, dry matter accumulation, protein production, chlorophyll and enzymes; increases free amino acids	Cereals
Pb	Dark-green leaves, wilting of older leaves, stunted foliage, and brown short roots	Decreases chlorophyll production and plant growth; increases superoxide dismutase	—
Se	Interveinal chlorosis or black spots at Se content at about 4 ppm, and complete bleaching or yellowing of younger leaves at higher Se content, pinkish spots on roots	—	—
Zn	Chlorotic and necrotic leaf tips, interveinal chlorosis in new leaves, retarded growth of entire plant, and injured roots resemble barbed wire	Reduces Ni toxicity and seed germination; increases plant growth and ATP/chlorophyll ratio	Cereals and spinach

Adapted from Pendias and Pendias (2001) and Gardea-Torresdey *et al*. (2005).

On the other hand, trace metals (Zn, 334; Cu, 99; Ni, 27; and Cd, 10 mg kg^{-1}, DW) inhibited N-fixation by half of white clover rhizobium bacteria, while 2–10 times higher (Cu, Zn, and Pb) reduced most of the biological activities in the forest litter/soil by 20–40% (Pendias and Pendias, 2001). In contrast, lower plants such as mosses, liverworts, lichens and fungi exhibited remarkable adaptation to toxic levels of certain trace elements (Pendias and Pendias, 2001).

1.1.8 Heavy metal tolerance in plants and potential utility of tolerant plants in phytoremediation

Plants have developed several cellular and subcellular mechanisms for the sequestration or detoxification of heavy metals (Liphadzi and Kirkham, 2005). Such mechanisms employed in common non–accumulator plants to regulate the homeostasis of intracellular ions embrace regulation of ion influx (stimulation of transporter activity at low intracellular ion supply or inhibition at high concentrations) and extrusion of intracellular ions back into external solutions (Lasat, 2000). Conversely, hyperaccumulator species have detoxification mechanisms such as Ni complexation by histidine (e.g., *Thlaspi goesingense*, Ni hyperaccumulator), Zn sequestration in the vacuole of the shoots (e.g., *Thlaspi caerulescens*, Zn hyperaccumulator) possibly through precipitation as Zn–phytate and binding to low molecular weight organic acids (Lasat, 2000).

Apart from this, antioxidants, chelators like metal binding peptides (e.g., metallothioneins and phytochelatins), organic acids, and amino acids are involved in metal detoxification by buffering cytosolic metal levels (Liphadzi and Kirkham, 2005). As to the same authors, several plants tolerate heavy–metal–induced oxidative stress of reactive oxygen species [e.g., superoxide radical (O^{2-}), hydroxyl radical (OH^{-}), and hydrogen peroxide (H_2O_2)], by producing metabolites such as ascorbate, glutathione, tocopherol and polyphenolics (tannins and lignin precursors) as well enzymatic scavengers of activated oxygen including superoxide dismutases, peroxidases, catalases, ascorbate peroxidases, glutathione S–transferases, and glutathione peroxidases. On the other hand, cell wall materials, particularly pectates, amino acids, and carboxylic acids have been suggested to

contribute to metals tolerance by way of restricting metal transport within plant and preventing metals from affecting sensitive components of the root (Ross and Kaye, 1994). Moreover, subcellular compartmentalization of metals in the cell (e.g., vacuole), which may involve cysteine–rich peptides (metallothioneins and phytochelatins) and organic acids including malate, citrate (Liphadzi and Kirkham, 2005; Ross and Kaye, 1994), oxalate, mustard oils and anthocyanins (Ross and Kaye, 1994), have been demonstrated for Ni, Zn, and Cd in the vacuoles of tolerant plants thriving on polluted soils (Liphadzi and Kirkham, 2005).

As the bulk majority of plants are sensitive to metal toxicity, most species are restricted to soils with low metal levels, and hence are recognized as *strict* or *obligate nonmetallophytes* (Pollard *et al.,* 2002). Nonetheless, certain plants can survive and even accumulate trace elements from soils contaminated by an array of these elements (Pendias and Pendias, 2001). In this regard, most of the evidence collected thus far suggests that only plants species containing the natural genetic variation for tolerance in their natural populations are capable to develop tolerant populations (Turner, 1994). The development of tolerant populations is ascribed to the selection pressure exerted by high levels of contamination in soil that favor the tolerant genotype with genetic variation that could exist already or may proceed from *de novo* by mutation following environmental alterations (Turner, 1994). Naturally, areas adjacent to the contaminated soil commonly support a diverse flora; however, comparatively few species appear to have the ability to colonize metalliferous soils (Turner, 1994).

Pollard and coworkers (2002) indicated that plants flourishing on metalliferous soils, which are branded as *metallophytes*, have been further categorized into quite a few often-overlapping ecological categories. As to the same authors, those taxa that grow exclusively on metalliferous soils [e.g., *Viola calminaria* (DC.) Lej., several subspecies of *Minuartia verna* (L.) Heirn.] are labeled as *strict metallophytes* (synonyms: *eumetallophytes*, *obligate metallophytes* or *absolute metallophytes*) and exhibit metal tolerance in all members of the taxa (species–wide tolerance). Conversely, several species [e.g., *Plantago lanceolata* L., *Mimulus guttatus* DC., and *Silene vulgaris*

(Moench) Garcke.] that have populations on both normal and metalliferous substrates are termed *facultative metallophytes* (Pollard *et al.,* 2002) or *pseudometallophytes* (Ginocchio and Baker, 2004; Pollard *et al.,* 2002). Although most populations of such species occur on nonmetalliferous soils and are, commonly, quite sensitive to metal toxicity (Pollard *et al.,* 2002), strong selection on metalliferous substrates favors metal-tolerant genotypes creating increase in the mean tolerance of the local populations (Pollard *et al.,* 2002; Turner, 1994) and thus metal tolerant "races" or "ecotypes" may establish (Pollard *et al.,* 2002).

On the other hand, a few facultative metallophytes namely, the non–accumulators *Typha latifolia* L., *Andropogon virginicus* L., and hyperaccumulator *Thlaspi montanum* that more often thrive on nonmetalliferous than metalliferous soils have been shown to possess high average levels of metal tolerance in all populations including those growing on soils without elevated metals (Pollard *et al.,* 2002). Such tolerance has often been termed as *constitutive/constitutional* (Pollard *et al.,* 2002; Turner, 1994) or species–wide tolerance, where the pattern of tolerance lacks intraspecific polymorphism (Pollard *et al.,* 2002). Likewise, some hyperaccumulator species such as *Thlaspi caerulescens*, *T. goesingense*, and *Arabidopsis halleri* are facultative metallophytes, which typically occur on metalliferous soils but their ecological range also encompasses populations flourishing on non–metalliferous substrates (Pollard *et al.,* 2002).

Pendias and Pendias (2001) indicated that the evolution of metal tolerance is expected to be fairly rapid in microorganisms and at times, in vascular plants as well. Nonetheless, the length of time of exposure to metals determines the degree of specialization of the metal resistant trait (Whiting *et al.,* 2004). Consequently, limited resistance could be exhibited in some plant populations ensuing a few years of metal entering the environment (e.g., around smelters) due to metal tolerant races, while highly specialized mechanisms of resistance or tolerance found in true metallophytes involve evolution for many thousands or millions of years on substrates derived from weathered mineral deposits (Whiting *et al.,* 2004).

A coordinated response of physiological mechanisms facilitates a plant to deal with the injurious effect of metal contamination or stress (Turner, 1994). Accordingly, survival of plants in environments contaminated with potentially toxic levels of heavy metals (Turner, 1994) can be achieved through either exclusion/avoidance (Pollard *et al.,* 2002; Turner, 1994), or accumulation (Pollard *et al.,* 2002) or true tolerance mechanisms (Turner, 1994). Exclusion mechanisms comprise blocking the movement of metals at the soil–root or root–shoot interface (Pollard *et al.,* 2002) employing mycorrhiza formation, alteration of membrane permeability, proliferation of roots in uncontaminated horizons, changes in the metal binding capacity of the cell wall or enhanced exudation of metal chelating substances to prevent excess uptake and heavy metal stress (Turner, 1994). Conversely, accumulation mechanisms entail allowing the transfer of metals into the shoot and eventually rendering them non–toxic by way of chemical binding, intracellular sequestration (Pollard *et al.,* 2002; Turner, 1994), alteration of cellular metabolism and membrane structure (Turner, 1994). Overall, the mechanisms of tolerance involve external factors (low solubility and mobility of cations around roots, effects of metal ion antagonisms) as well as internal factors (Pendias and Pendias, 2001). The internal factors, as to the selfsame authors, which are linked to real tolerance comprise several metabolic processes listed there-under:

1. Selective uptake of ions;
2. Diminished permeability of cell walls or other variations in the structure and functions of membranes;
3. Immobilization of ions in different organs (synthesis of immobilizing compound including the formation of minerals, and/or fixation by charged ligands);
4. Alteration in metabolic patterns—increased enzyme system that has been inhibited, or increased antagonistic metabolite, or reduced metabolic pathway bypassing an inhibited site, or decreased requirement for products of inhibited synthesis;
5. Adaptation to toxic metal replacement of a physiological metal in an enzyme;
6. Release of ions from plants by leaching from foliage, guttation, leaf shedding, and excretion from roots;

7. Release of volatile organic metal compounds (e.g., Hg, Pb), mainly as methylated metals that are toxic very toxic species to most organisms; and
8. Excretion from leaf tips in the form of salts.

The above tolerance mechanisms could impinge on the pattern of metal uptake strategies: 1) the "excluder" with a constant low shoot level until a critical concentration where toxicity and unrestricted metal transport occurs; 2) the "indicator" where internal concentrations reflect external concentrations; and 3) the accumulator with active concentration of metals (Baker and Walker, 1990; Turner, 1994). Based on the uptake strategies, most facultative metallophytes are metal excluders whereas hyperaccumulators can be considered as special and extreme case of the broader category of accumulators (Pollard *et al.,* 2002). Early studies on the metal tolerance employing metal–excluder species such as *Agrostis capillaris*, *Mimulus guttatus*, and *Silene vulgaris* revealed that clear intraspecific genetic polymorphism between nonmetallophyte and metallophyte populations were recognized where ecotypic tolerance is controlled by one or two major genes, mostly with modifier genes that could control the extent of tolerance (Pollard *et al.,* 2002). In contrast, comparatively little is known pertaining to the genetic basis of hyperaccumulation but some surveys have suggested that variation in metal tolerance (albeit species–wide tolerance) within hyperaccumulator species is expected to occur (Pollard *et al.,* 2002).

As a rule, metal accumulation has been documented as an extreme type of physiological response in heavy metal tolerance with different extent of metal accumulation ranging from a small elevation above "background" to an extreme response where a metal could constitute over 1% of the plant dry matter (Baker and Walker, 1990). The same authors also defined accumulators as those vascular plants capable of concentrating metals in the aboveground parts to a remarkable extent from a low or high substrate concentration, where a leaf to root metal concentration ratio exceeds unity. A term "hyperaccumulation" was coined by Brooks and his colleagues in 1977 (Macnair, 2003)], to identify an extreme form of accumulators, portrayed as "hyperaccumulators", in which the tissue metal concentration should exceed defined critical values (Macnair, 2003; Ross and

Kaye, 1994; Table 1.20). In general, typical concentration threshold for most metals in hyperaccumulators is 1000 mg kg^{-1} (0.1%) dry mass saving for Zn (10,000 mg kg^{-1}), Cd (100 mg kg^{-1}) (Brook *et al.,* 1998; Gardea-Torresdey *et al.,* 2005; Ginocchio and Baker, 2004; Plion-Smits, 2005; Pollard *et al.,* 2002), Cr (500 mg kg^{-1}) (Gardea-Torresdey *et al.,* 2005), and Hg (10 mg kg^{-1}) (Salt *et al.,* 1998). Such levels are 100-fold higher than those typically measured in nonaccumulator plants growing on the same substrate (Brook *et al.,* 1998; Plion-Smits, 2005; Salt *et al.,* 1998) or 100–1000 times higher levels than normal plants on background metal concentrations and about 10–100 fold higher than most other plants growing on metal-contaminated soils (Alkorta *et al.,* 2004). It appears that hyperaccumulation is an additional or alternative tolerance mechanism (sequestering in leaves or shoots) to those employed by non–accumulating species (largely sequestering in root vacuoles) thriving in the same toxic substrate (Macnair, 2003). The possible physiological connotation of metal hyperaccumulation might be as a tolerance strategy for high metal concentration in the soil (Pivetz, 2001; Pollard *et al.,* 2002). Nonetheless, as the relationship between metal tolerance and metal accumulation may not be similar for all metals and species, their exact association has not yet been fully resolved (Pollard *et al.,* 2002).

Table 1.20 Criteria for hyperaccumulator plants and number of hyperaccumulator plants in the world. After Ginocchio and Baker (2004).

Element	Shoot concentration (%, dry matter)	Number of taxa	Number of families
As	> 0.1	2	1
Cd	> 0.01	2	1
Co	> 0.1	26	11
Cu	> 0.1	35	15
Pb	> 0.1	14	7
Ni	> 0.1	317	37
Se	> 0.01	20	7
Zn	> 1.0	13	5

When compared against the total number of vascular plants which exceeds 300,000, only about 438 species are reported as metal hyperaccumulators (Ginocchio and Baker, 2004). Nevertheless, it is evident that the phenomenon has been established in a wide range of families (Baker and Walker, 1990; Cunningham and Ow, 1996; Ginocchio and Baker, 2004; Pollard *et al.,* 2002) and geographical areas involving a broad diversity of morphological, physiological, and ecological characteristics (Pollard *et al.,* 2002). Although most hyperaccumulators sequester Ni, plant species that can accumulate Cu, Co, Zn (Baker and Walker, 1990; Cobbett, 2003; Kumar *et al.,* 1995; Melendo *et al.,* 2002), Pb (Baker and Walker, 1990; Kumar *et al.,* 1995; Melendo *et al.,* 2002), Cd (Melendo *et al.,* 2002), Se (Cobbett, 2003; Kumar *et al.,* 1995) and As (Cobbett, 2003) have been identified. Consequently, due to their association with particular ore deposits, several metallophytes are employed in exploration for mineral deposits (Kumar *et al.,* 1995).

Conversely, there is a growing interest to exploit the capacity of metal accumulating plants for the development of plant–based technologies (Perez-de-Mora *et al.,* 2006), namely phytoremediation (including phytomining) that can be applied in situ and require low level of financial and technical input (Perez-de-Mora *et al.,* 2006). Notwithstanding this, the fundamental idea of phytoremediation is not a new concept (Cunningham *et al.,* 1995; Lasat, 2000; Salt *et al.,* 1998) in view of the fact that constructed wetlands, reed beds, and floating-plant systems have been common for treatment of some kinds of wastewater for many years (Cunningham *et al.,* 1995). Phytoremediation (synonyms: green remediation, botano-remediation, agroremediation, and vegetative remediation) is the use of plants to partially or substantially (Pivetz, 2001) remove, destroy, or sequester hazardous contaminants (Prasad, 2003) from media, such as soil (Prasad, 2003), sludge, sediment, ground water, surface water, wastewater (Pivetz, 2001), and air (Prasad, 2003). Accordingly, important plant attributes used as criteria during plant selection for phytoremediation include their potential to evaotranspirate groundwater, production of degradative enzymes, growth rate and yield, depth of root zone, and capacity to accumulation contaminants (Prasad, 2003).

The life cycle of plant has been recognized to have momentous influence on the chemical, physical, and biological processes that take place in its adjacent environs (Cunningham and Ow, 1996). Evidently, phytoremediation profits from the natural processes such as water and chemical acquisition, shoot and root growth (Cunningham and Ow, 1996; Pivetz, 2001), metabolism within the plant, and exudate release into the soil that ultimately leads to contaminant loss and the physical and biochemical impacts of the plant roots (Pivetz, 2001). According to the same author, phytoremediation involves various methods for contaminant degradation, removal (by way of accumulation or dissipation), or immobilization:

1. Degradation: destruction or alteration of organic contaminants.
    - *Rhizodegradation*: enhancement of biodegradation in the belowground root zone by microorganisms;
    - *Phytodegradation*: contaminant uptake and metabolism above- or belowground, within the root, stem, or leaves.
2. Accumulation: for containment or removal of organic and/or metal contaminants.
    - *Phytoextraction*: contaminant uptake and accumulation for removal;
    - *Rhizofiltration*: contaminant adsorption on roots for containment and/or removal.
3. Dissipation: for removal of organic and/or inorganic contaminants into the atmosphere.
    - *Phytovolatilization*: contaminant uptake and volatilization.
4. Immobilization: for containment of organic and/inorganic contaminants.
    - *Hydraulic control*: control of groundwater flow by plant uptake of water;
    - *Phytostabilization*: contaminant immobilization in the soil.

At present, the costs related to the environmental cleanup of contaminated sites is staggering, $25–50 billion a year worldwide (Plion-Smits, 2005). As biological processes are solar-driven (Cunningham *et al.,* 1995; Plion-Smits, 2005) pumping and filtering systems with perceptible loading, degrading, and fouling capacities (Cunningham *et al.,* 1995), phytoremediation is, on average, tenfold cheaper than engineering based remediation systems [e.g., soil excavation, soil washing, or burning, or pump and treat

systems](Plion-Smits, 2005). Besides, the treatment cost for cropping systems ranges from \$200 to 10,000 ha^{-1}(0.02–1.00 m^{-3} $year^{-1}$), which is several orders of magnitude cheaper than the corresponding costs entailed with physico-chemical remediation technologies (Cunningham *et al.,* 1995) such as soil flushing, pneumatic fracturing, solidification/ stabilization, vitrification, electrokinetics, chemical reduction/ oxidation, soil washing, and excavation, retrieval, and off-site disposal (Prasad, 2003). Moreover, as phytoremediation involves techniques that are often carried out in situ, it is a cost effective treatment (Cunningham *et al.,* 1995; Plion-Smits, 2005) and may reduce exposure of polluted substrates to humans, wildlife, and the environment (Plion-Smits, 2005). Apart from phytoremediation, phytomining (Table 1.21), the possibility of exploiting a low–grade ore bodies or mineralized soils (e.g., 0.35% Ni) using high biomass plants with high metal accumulation such as *Streptanthus polygaloides*, *Alyssum bertolonii*, and *Berkheya coddii* (Brook *et al.,* 1998; Plion-Smits, 2005) has been established for Ni (Brook *et al.,* 1998; Gardea-Torresdey *et al.,* 2005; Plion-Smits, 2005).

However, limited market share of phytoremediation (e.g., ca. \$100-150 million per year or 0.5% of the total remediation market in the U.S., and absence of significant commercial use in Europe), limited depth of treatment, and factors that affect availability, growth and uptake of pollutants (Plion-Smits, 2005) are among the potential hurdles that may impinge on the commercialization of phytoremediation. In spite of these limitations, phytoremediation could be suitable in cases where large surface areas of soils with comparatively immobile contaminants exist (Cunningham *et al.,* 1995). Besides, in order to commercialize phytoremediation, efforts were made in biotechnology to manipulate uptake and tolerance properties of several plant species. In this regard, increased metal tolerance in tobacco (*Nicotiana tobacum*) has been obtained through the expression of the mammalian genes for metal–binding proteins, methallothionein (Lasat, 2000). Likewise, transgenic plants (*Arabidopsis sp.* and tobacco) have been bioengineered to detoxify the methyl-mercury, a strong neurotoxic agent biosynthesized in Hg-contaminated soils, by means of expressing bacterial genes *mer*B and *mer*A which catalyze the protonolysis of the carbon-mercury bond to generate Hg^{2+} and that convert Hg^{2+} to Hg^{0} (a less toxic and volatile species), respectively (Lasat, 2000).

Table 1.21 Some hyperaccumulators with potential utility in phytomining.

Element	Species	Concentration[1] (mg kg^{-1}, dry matter)	Biomass (t ha^{-1} yr^{-1})
Cd	*Thlaspi caerulescens*	3000 (1)	4
Co	*Haumaniastrum robertii*	10200 (1)	4
Cu	*Haumaniastrum katangense*	8356 (1)	5
Pb	*Thlaspi rotundifolium subsp.*	8200 (5)	4
Mn	*Macadamia neurophylla*	55000 (400)	30
Ni	*Alyssum bertolonii*	13400 (2)	9
Ni	*Berkheya coddii*	17000 (2)	18
Se	*Astragalus pattersoni*	6000 (1)	5
Tl	*Iberis intermedia*	3070 (1)	8
U	*Atriplex confertifolia*	100 (0.5)	10
Zn	*Thlaspi caerulescens*	10000 (100)	4

[1] Concentrations are mean highest elemental values and those in brackets correspond to the values for the non-accumulator plants (after Brook *et al.,* 1998).

Table 1.22 Typical concentration of some trace metals in soils, the soil solution and in plants

	Soils					Plants		
			Average value in soil and maximum dissolved in the soil solution					
Element	Normal range in soil (mg kg^{-1}, dry weight)	Concentration in soil considered toxic (mg kg^{-1}, dry weight)	Average (mg kg^{-1}, dry weight)	Molar concentration at 10% soil moisture	Soil solution concentration considered as toxic (mg $liter^{-1}$)	Normal range in plant material (mg kg^{-1}, fresh weight)	Concentration in contaminated plants (mg kg^{-1}, dry weight)	Annual plant uptake (kg ha^{-1} yr^{-1})
Cr	5-1000	75-100	100	$10^{-1.72}$	0.001	0.03-15	5-30	-
Co	1-70	25-50	8	$10^{-2.87}$	0.01	0.05-0.5	15-50	0.0006
Ni	10-1000	100	40	$10^{-2.17}$	0.05	0.02-5	10-100	-
Cu	2-100	60-125	30	$10^{-2.33}$	0.03-0.3	4-15	20-100	0.006
Zn	10-300	70-400	50	$10^{-2.12}$	<0.005	8-400	100-400	0.01
Cd	0.01-7	3-8	0.06	$10^{-5.57}$	0.001	0.2-0.8	5-30	-
Hg	0.02-0.2	0.3-5	0.03	$10^{-5.83}$	0.001	0.005-0.5	1-3	-
Pb	2-200	100-400	10	$10^{-3.32}$	0.001	0.1-10	30-300	-

After Ross (1994b).

1.1.9 Heavy metals/metalloids in the food chain and their effects on ecosystem

All of the studied elements, though normally occur in trace concentrations (Tables 1.7 and 1.22), are potentially toxic, and thus, can affect the biotic component even at soil solution levels below 1 mg L^{-1} (Freedman, 1989). Consequently, the accumulations of heavy metals through the soil–plant–herbivore–carnivore food chain as well as the potential intensification of their effects have been well documented (Martin and Coughtrey, 1981). In the terrestrial ecosystems, soil followed by litter represents the major repository of the total ecosystem metal pool (Hughes, 1981; Martin and Coughtrey, 1981). Apparently, an early food chain effect could be envisaged to be associated with organisms inhabiting the soil–litter component of the ecosystem where invertebrates accumulate metals (exceeding the same in their food source) and eventually pose a potential hazard to carnivores feeding on them (Martin and Coughtrey, 1981). Correspondingly, among heavy metals (Cd, Ni, Pb, and Zn) in earthworms from roadsides and a mine site, there were biological concentrations (concentration of metal in earthworm to that in the top 10 cm of the soil) of Cd and Zn. It was, therefore, suggested that animals that fed on earthworms (e.g., *Xenopus laevis*, toads) for extended period could accumulate Pb and Zn to toxic levels (Martin and Coughtrey, 1981). Although there is some evidence for biological accumulation of metals through litter–detritivore, plant–herbivore, herbivore–carnivore stages, the extent to which this happens is influenced by the metal concerned (Martin and Coughtrey, 1981).

In terrestrial food chains, the mobility of heavy metals from herbivores to carnivores is governed by the site of accumulation within the body tissues of the herbivore. Consequently, Pb which associates with the kidney or the liver of vertebrates as well as calcified tissues of both vertebrates and invertebrates has little food chain accumulation, except in some carnivorous invertebrates and earthworms under specific circumstances (Hughes, 1981). On the other hand, due to lack of any tendency to associate with certain tissues and hence marked availability to consumers in virtue of being found in easily digested soft tissues (Hughes, 1981) or different organs (Martin and Coughtrey, 1981),

Cd has high mobility in terrestrial ecosystems (Hughes, 1981; Martin and Coughtrey, 1981).

In agroecosystems, food chain accumulations of heavy metals via soil–plant–herbivore system had toxic effects on grazing animals; sheep, horses, and poultry were most vulnerable while effects on cattle were less pronounced (Martin and Coughtrey, 1981). Besides, death of horses, cattle, and sheep close to the smelter has been reported (Martin and Coughtrey, 1981). As a rule, toxic effects of soil–plant–animal food chain accumulation of heavy metals could be governed by (Martin and Coughtrey, 1981):

1. the amount of dry matter intake (variable with the pasture);
2. species composition of forage;
3. fertilizer regime;
4. soil type and pH;
5. intake of trace elements from direct animal foods;
6. direct soil ingestion;
7. trace element interactions.

Some livestock such as sheep and cattle ingest soil particles equivalent to 14% and 2% of their diet (dry weight) which annually amount to 45 kg of soil for sheep (Adriano, 1986) and 450 kg of soil for cattle (Adriano, 1986; Martin and Coughtrey, 1981). Besides, herbage from grazed areas may contain up to 25% (DW) of soil which could serve as a source of Co, Se, and Zn (Martin and Coughtrey, 1981). Likewise, applied sewage or manure on pastures could be ingested (Adriano, 1986). Apart from death or injury to an individual organism, food chain accumulation of heavy metals can cause reduction in the breeding potential of a population (Martin and Coughtrey, 1981). Attributable to the diverse interactions, food chain accumulations of metals in sites contaminated with several elements may be less pronounced at the initial stage unless the normal ratios and balances between various metals have been disturbed (Martin and Coughtrey, 1981).

On the other hand, barring occupational exposure and inhalation, the chief route for the entry of trace elements into humans is via ingestion of foods of plant and animal origin (Adriano, 1986; Fig. 1.1). It follows that soil–plant–animal relationship is pivotal to

appreciate the potential transfer of heavy metals and metalloids to humankind. Plants are recognized as intermediate reservoirs through which trace elements from primary sources are transferred to other organisms (Adriano, 1986). Due to uptake of elements via root pathway, or through foliar and stem pathway or both, plant growth and quality principally hinge on the soil environment and the elements entailed (Adriano, 1986; Table 1.23). Correspondingly, as influenced by the source and pathway of trace elements to animals (Table 1.24), the growth and performance of animals mainly depends upon on the quantity and quality of plants or feedstuff (Adriano, 1986). In the nature of things, the trace element composition of animal and human beings or fluids (Table 1.7) is directly related to the trace element content and bioavailability in the soil or sediment–plant–animal–human food chain (Ward, 1995).

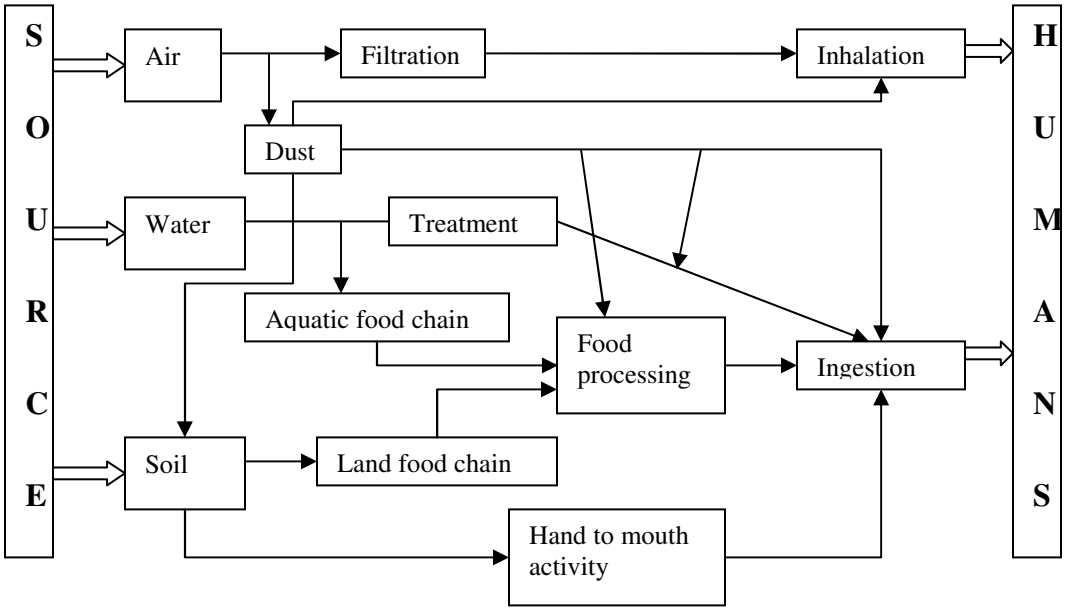


Fig. 1.1 Pathways of trace elements to humans. Source: Adriano (1986).

The first potential effect of heavy metals on the biotic component of ecosystem occurs at the destruent level that involves the organisms responsible for the decomposition of litter. A slower or less complete humification and eventually accumulation of incompletely decomposed litter are expected to take place (Martin and Coughtrey, 1981). Accordingly, lowered rates of litter decomposition have been directly related to elevated levels of Cd,

Zn, Pb, and to metals in combination (Ross and Kaye, 1994). Likewise, soil respiration, which is a general indicator of soil microbial activity, showed reduced CO_2 evolution due to high concentrations of Pb, Zn, Cu, Cd or Ni (Ross and Kaye, 1994). In addition, Ross and Kaye (1994) discussed that a 55% reduction in microbial biomass of an agricultural soil with 20 years of sludge application was ascribed to the presence of Cu (40–90 mg kg^{-1}) and Ni (5–10 mg kg^{-1}). Moreover, nitrogen mineralization (with greater inhibition in acidic [Hg, 73%] as well as alkaline soils [82%, Cu]) and nitrification processes could be affected by high metal levels (Ross and Kaye, 1994).

Table 1.23 Sources and pathways of trace elements to plants (Adriano, 1986).

	As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Se	V	Zn
I. Uptake by roots											
A. Soil or ground water	×	×	×	×	×	×	×	×	×	×	×
B. Fallout to soil from air pollution	×	×	×	×	×	×	×	×	×	×	×
C. Sewage sludge soil amendment	×	×	×		×	×	×	×	×		×
D. Biocides applied to soil and/or seed	×	×			×	×	×				×
E. Surface water contamination	×		×			×				×	
F. Fertilizers	×	×	×		×		×	×			×
G. Industrial pollution	×	×	×		×		×	×			×
II. Uptake by leaves and stems											
A. Pollutant fallout from industrial process	×	×	×	×	×	×	×	×	×		×
B. Pollutant fallout from auto emission						×					×
C. Biocide application to plants	×	×			×	×	×				×
D. Pollution fallout from incineration of fossil fuels and refuge	×	×	×	×	×	×	×	×	×	×	×

In soils heavily contaminated with Zn (Martin and Coughtrey, 1981), Cd and Pb (Ross and Kaye, 1994), a field study has indicated that total numbers of bacteria, actinomycetes, and fungi as well as densities of some invertebrate groups (e.g., nematodes and earthworms) were highly diminished (Martin and Coughtrey, 1981).

Apparently, this could be ascribed to the effect of heavy metals on microorganism diversity and activity ultimately influencing the production of essential enzymes. In a case where the ecosystem receives a continual input of heavy metals and eventually maintained sufficiently severe or long term contamination, a process which resembles a reversal of successional pattern may come about (Martin and Coughtrey, 1981). As a rule, the relative toxicities of trace metals to soil organisms increase in the sequence Pb < Zn < Cu < Cd < Hg (Ross and Kaye, 1994; Table 1.25).

Table 1.24 Sources and pathways of trace elements to animals (Adriano, 1986).

	As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Se	V	Zn
I. Terrestrial											
A. Breathing contaminated air		×	×	×	×	×	×	×	×	×	×
B. Eating contaminated plant or animal tissue	×	×	×	×	×	×	×	×	×	×	×
C. Drinking contaminated water	×	×	×	×	×	×	×	×	×	×	×
D. Licking and preening fur or feathers						×					
E. Receiving therapeutic drugs	×										
II. Aquatic											
A. Metal in water	×		×	×	×	×	×	×	×	×	×
B. Runoff and fallout	×	×	×		×	×	×	×	×	×	×
C. Sewage and industrial waste outfalls	×	×	×	×	×	×	×	×			×
D. Mine tailings or smelter waste leachate	×	×	×		×	×	×				×
E. Contaminated plants, animals or sediment	×	×	×	×	×	×	×	×	×	×	×
F. Biocides or runoff	×				×						×
G. Lead shot						×					

1.1.10 Biogeochemical cycles of heavy metals and metalloids

A biogeochemical cycle is a conceptual description of the mechanism by which an element or a compound is transformed within a defined system of interest, including the means through which the various forms are interchanged between the solid, liquid, and gaseous phases of that system (Pierzynski *et al.,* 2000). Biogeochemical cycling entails soil environment as well as geological materials, biological organisms, air, and waters, and such cycles are basically global in nature; we often view and attempt to manage them at a smaller scale (e.g., within a watershed, or even within a city, a farm, or an individual field) (Pierzynski *et al.,* 2000). Biogeochemical cycles summarize the major processes undergone by an element or compound at different scales and in different systems as well as furnish an overview of the environmental factors and management practices which control both transformations within a system and transport of an element or a compound to another system (Pierzynski *et al.,* 2000).

Table 1.25 Generalized patterns of metal toxicities recognized for some organisms.

Organism	Toxicity sequence
Protozoa (*Paramecium*)	Hg, Pb > Cu, Cd > Ni, Co > Zn
Annelids (Polychaete worm)	Hg > Cu > Zn > Pb > Cd
Vertebrata (Stickleback)	Hg > Cu > Pb > Cd > Zn > Ni > Cr
N-mineralizing bacteria	Hg > Cu > Cd > Pb > Cr > Zn = Ni
Algae (*Chlorella vulgaris*)	Hg > Cu > Cd > Cr > Zn > Ni > Co
Fungi	Hg > Cu > Cd > Cr > Ni > Pb > Co > Zn
Higher plants (Barley)	Hg > Pb > Cu > Cd > Cr > Ni > Zn

After Ross and Kaye (1994).

Adriano (2001) indicated that accumulation and transfer pathways for the biogeochemical cycles of metals in terrestrial ecosystems involve meteorologic vectors, geologic vectors, and biological flux. The meteorologic vectors (input and output) comprise airborne

particulate matter, aerosols, dissolved substances in precipitation, and gases (e.g., gaseous forms of certain metals like Pb, Se, Hg, etc.). On the other hand, geologic vectors entail dissolved and particulate matter moved by surface runoff as well subsurface drainage while biological flux represents the case when substances gathered by animals in one ecosystem are deposited in another (Adriano, 2001; Fig. 1.2). The elements in each ecosystem could exist in atmosphere (in aerosol or gaseous form found above- and belowground), organic (incorporated in living and dead biomass of plants and animals), bioavailable (free ionic or complexed forms in solution and those sorbed in exchange complex), and mineral (primary and secondary minerals of soils and rocks) compartments (Adriano, 2001). The biogeochemical cycling of elements, as to the same author, involves an exchange between the various components within the ecosystem whereas fluxes across ecosystem boundaries link individual ecosystems with the rest of the biosphere.

In agroecosystems, the relative importance of the different transfer pathways of trace elements in the two basic components (soil and crops) depends on the element, plant species, soil type, site characteristics, management practices, and climate (Adriano, 1986; 2001). There are two main routes of trace element inputs into agroecosystems: aerial (e.g., aerosols, particulate matter, resuspended and airborne dusts, etc.) and land (fertilizers, pesticides, solid waste, wastewaters, other soil amendments, etc.) (Adriano, 1986; 2001). Conversely, the output pathways can principally be through losses by removal of plant materials for food, feed stuff, and fiber, as well as leaching and erosion (Adriano, 1986; 2001). In contrast, the gaseous output pathway, saving those involving microbially mediated transformation such as Hg, Se, and As, is not significant (Adriano, 2001).

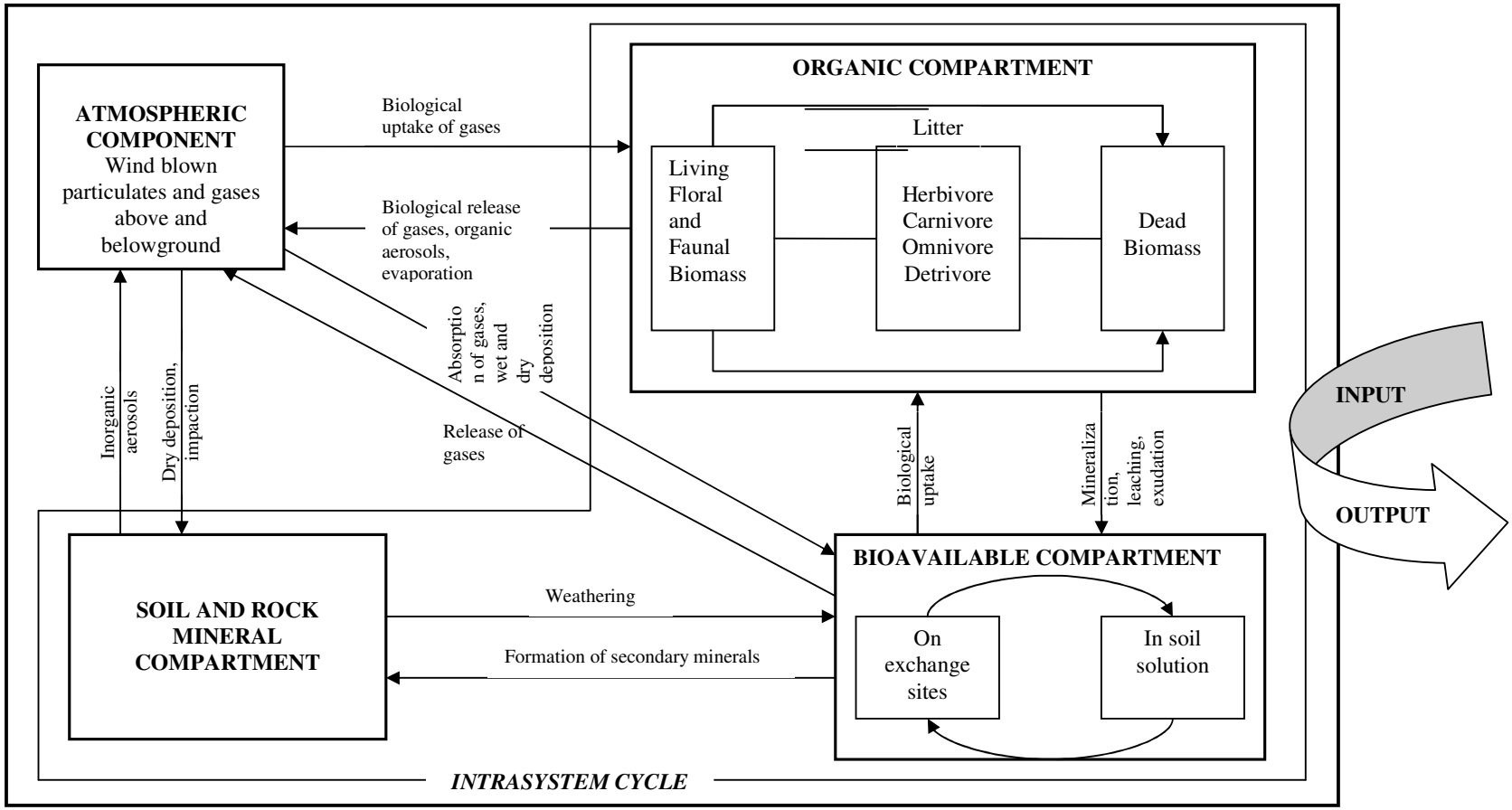


Fig. 1.2 A generalized model depicting elemental relationship in terrestrial ecosystems (Adriano, 2001)

As the soil solution is where plant roots and other organisms interact with essential and nonessential elements in the soil, it is the focal point of any soil element cycle (Pierzynski *et al.,* 2000). Plant uptake of trace elements from soils solution occurs from the soils solution and its composition reflects the interactions between soluble species and the solid phases (Pierzynski *et al.,* 2000). Ascribed to the dynamic changes in inputs and outputs, agroecosystems are in non-equilibrium state germane to elemental cycling, and hence an agroecosystem can either be enriched, sufficient, or deficient in one or more trace elements (Adriano, 1986, 2001). The soil–plant system plays a key buffering role on environmental cycling of trace elements, and eventually serves as an effective barrier against animal toxicity of trace metals like Ni and Cd (Adriano, 2001). Consequently, the concentrations of trace elements in the root zone are several orders of magnitude higher than the corresponding levels removed by plants during harvesting, indicating the long residence of pollutants in the soil system (Adriano, 2001).

In contrast to agroecosystems, atmospheric deposition is essentially the only input pathway in established forest ecosystems, and such aged and established forests often maintain an equilibrium state with respect to elemental cycling (Adriano, 1986, 2001). However, the various transfer pathways depend on similar factors that control cycling in the agroecosystems (Adriano, 1986, 2001). Likewise, leaching and rarely erosion take place in well–managed forests (Adriano, 1986; Adriano, 2001).

On the other hand, the most important processes determining the biogeochemical cycling of trace elements in freshwater ecosystems include (1) wet and dry deposition of acidic and neutralizing compounds, (2) dilution and concentration during runoff and evaporation, (3) terrestrial neutralization or mobilization due to weathering, (4) anthropogenic influences, (5) recharge and detention, and (6) buffering and geochemical reactions occurring within the lake (Adriano, 2001). Trace metal inputs to the lake are ultimately carried away by outflow or by deposition onto the sediment where they are buried. The transport and removal of metals from the water column depends on their complexation with the settling particulates (Fe and Mn oxides, organic matter, silicate minerals, and $CaCO_3$). Due to their large surface areas, Fe and Mn oxides are considered

as effective conveyers of metals, whereas the carbonate phase has inconsequential role as a carrier of the same (Adriano, 2001). Conversely, dissolved organic matter (DOC, product of the decomposition/degradation of organic detritus) with polyelectrolytic nature complexes with or chelates trace metal ions in natural waters and consequently alters the physicochemical states of trace metals in solution (Adriano, 2001). The same author also emphasized that such complexation capacity of DOC in natural systems governs the mobility, bioavailability, and toxicity of trace metals by means of controlling their speciation.

As part of the environment, metals and their compounds are incessantly cycled (Barbante and Cairns, 2002) which is believed to be linked to the biogeochemical cycles of major elements (Pendias and Pendias, 2001). Such important biogeochemical processes, however, are altered by humanity at unprecedented rate (Barbante and Cairns, 2002) by way of existing methods of processing energy and materials (Pendias and Pendias, 2001; van der Voet *et al.,* 2000). In the wake of unintended effects of human activities, pollution of the biosphere by trace elements would ensue (Pendias and Pendias, 2001) with the adverse effects, among other things, threats to human health, destruction of the ecosystem, economic loss by means of an array of mechanisms (van der Voet *et al.,* 2000).

1.2 Background and justification of the study

The soil is a key component of terrestrial ecosystems, both natural and agricultural, being essential for the growth of plants as well as the degradation, and recycling of dead biomass (Alloway, 1995a). Besides, soil has important ecological services as filter, buffer (controlling the transport of chemical elements and substances to the atmosphere, hydrosphere, and biota), geochemical sink (a reservoir pool in the biogeochemical cycles), and transformation system, thus protecting the global ecosystems from the effects of pollution (Pendias and Pendias, 2001). However, as soil occurs at the interface between atmosphere and the earth's crust, it is open to inputs of heavy metals from natural and anthropogenic sources (Alloway, 1995b). Moreover, soils has only finite

capacity for holding trace elements and thus, at levels which reach or exceed the (highest) capacity, potential environmental ramifications such as increased mobility of the elements could be anticipated (Adriano, 1986). Likewise, as the ecological functions of the soil including its vital role for survival of humanity, productivity, is not illimitable (Pendias and Pendias, 2001), such invaluable resource could be seriously affected. Apparently, maintenance of the above ecological and agricultural functions necessitates sound utilization of the resource, which depends, inter alia, on protection of the soil from those factors (human activities and natural causes) that may impinge on soil properties and the natural balance between soil and other components of ecosystem.

Human civilization increasingly relies on employment of various heavy metals and metalloids. This is attributable to the fundamental utility acquired from products directly or circuitously derived from heavy metals/metalloids such as electronic/electrical devices, automotive, ceramics, glass, paints, pigments, reagents, and alloys for various uses and such like (Daniel Fitamo *et al.,* 2007). Consequently, with increasing industrialization, great amounts of heavy metals have been excavated (Han *et al.,* 2002) where most human activities and industrial processes release the non–recovered heavy metals as wastes into the environment (Daniel Fitamo *et al.,* 2007). Heavy metals, as they stand out against a good number of organic pollutants, stockpile in the soil and other components of the ecosystem (Daniel Fitamo *et al.,* 2007).

Conversely, some of the heavy metals/metalloids under the present study are fundamental for normal growth and health of plants (e.g., Cu and Zn) (Adriano, 2001; Alloway, 1995a; He *et al.,* 2005), and animals (Cu, Zn, Co, and Se) (Alloway, 1995a; He *et al.,* 2005) including human beings (He *et al.,* 2005). Notwithstanding this, high concentrations of the beneficial (Alloway, 1995a; Pendias and Pendias, 2001) as well as non–essential elements (e.g., Pb, Hg, Cd, and As) in soils may constitute toxic effects on living organisms that entail oxidative and/or genotoxic mechanisms (Alkorta *et al.,* 2004). It follows that accumulation of trace elements, especially heavy metals, in the soil can restrict the function of the soil, give rise to toxicity of plants and contaminate the food chain (He *et al.,* 2005). On account of their immutable nature (Alkorta *et al.,* 2004;

Daniel Fitamo *et al.,* 2007) as well as associated human and environmental health risks (Bermond, 2001; Daniel Fitamo *et al.,* 2007), metals are a group of pollutants of much environmental concern (Alkorta *et al.,* 2004; Garbisu and Alkorta, 2001) and, hence, soil contamination has received global attention (Bermond, 2001; Daniel Fitamo *et al.,* 2007).

The development in agricultural, industrial, and urban sectors has precipitated considerable damage to the environment because of contamination of soils (Daniel Fitamo *et al.,* 2007; Morera *et al.,* 2001; Vangronsveld and Cunningham, 1998), sediments, and aquatic resources (Vangronsveld and Cunningham, 1998) with metals (Daniel Fitamo *et al.,* 2007; Morera *et al.,* 2001; Vangronsveld and Cunningham, 1998) and metalloids (Vangronsveld and Cunningham, 1998). Important anthropogenic sources of heavy metal pollution include, among other things, mining operations, aerosol deposition, fertilizer application, and irrigation with industrial and domestic wastewaters (Ahamuda *et al.,* 1999). Accordingly, main contributors to the metal burden in soils (Table 1.8) comprise discarded products as in scrapeheaps/landfills (As, Cr, Cu, Pb, and Zn), wastewater irrigation (Cr, Ni, Cu, Zn, Cd, Pb, Hg, Se, and As), metalliferous mining/ smelting (Cr, Ni, Cu, Zn, Cd, Pb, Se, Hg, and As), coal ashes (As, Cd, Pb, Hg, Ni, Se, V, and Zn) (Adriano 2001). Similarly, manufacturing (Cd, Cr, Cu, and Zn), electric power generation (As and Se), and domestic wastewater (As, Cd, Cr, Cu, Ni, and Zn) are primary sources of heavy metals to the aquatic systems (Adriano, 2001). Apart from direct application of wastes/amendments containing heavy metals/metalloids, flooding of liquid effluents from metallurgical industries (Alloway, 1995c) as well as irrigation of wastewaters from domestic and industrial sources (He *et al.,* 2005), are expected to contribute to heavy metal/metalloid accumulation in soils.

As natural healing process of heavy metal–affected soils could take centuries or even longer, human intervention is required to deal with the problem (Vangronsveld and Cunningham, 1998). Consequently, rehabilitation of metal contaminated sites involve innovative technologies that could economically remediate the substrates as well as regulation of pollution input (Garbisu and Alkorta, 2001) which are essential to restore the sites, keep them continually productive, and limit human exposure to toxic elements

(Liphadzi and Kirkham, 2005). However, metal–contaminated soils are notoriously hard to remediate (Alkorta *et al.,* 2004; Lasat, 2000). Besides, current remediation techniques applicable to heavy metal contaminated soils, which involve soil excavation and either landfilling or soil washing, followed by physical or chemical separation (Lasat, 2000), are expensive, environmentally invasive (Liphadzi and Kirkham, 2005; Vangronsveld and Cunningham, 1998), and labor intensive as well (Vangronsveld and Cunningham, 1998). In contrast, phytoremediation, the use of plants to extract, sequester, and/or detoxify pollutants, has been found to be an effective, non–intrusive, inexpensive, aesthetically pleasing, socially accepted technology (Alkorta *et al.,* 2004) to remediate diffusely polluted areas appears a very valid option (Garbisu and Alkorta, 2001; Barocsi *et al.,* 2003). Moreover, Phytoremediation has been successfully employed to remove toxic metals from sites where their levels posed a risk to human health (Barocsi *et al.,* 2003).

Considering size, population growth and industrialization, the last 100 years have seen tremendous expansion of Addis Ababa city, the metropolis. In contrast, the city has correspondingly low level of environmental awareness coupled with glaring neglect and poor economic standards, which eventually precipitated the deterioration of environmental quality due to inequitable waste collection and disposal systems (Tamiru Alemayehu *et al.,* 2005). Accordingly, the old as well as new factories, commercial, public, and domestic utilities in Addis Ababa release untreated wastes into any receiving environment found nearby (Tamiru Alemayehu *et al.,* 2005; Daniel Fitamo *et al.,* 2007). Consequently, such direct discharges of solid and liquid wastes into water bodies has remarkably increased the levels of potentially toxic elements and total coliforms in the streams, rivers, and shallow ground water reservoirs (Tamiru Alemayehu *et al.,* 2005).

The tributaries of Great Akaki and Little Akaki rivers, namely Kebena, Ginfile, Kechene, Kostre, Yeka and Kolfe streams, drain the city from north to south (Tamiru Alemayehu *et al.,* 2005). In view of that, the Akaki River was estimated to receive effluents from over 33 industries in Addis Ababa and hence, considered as the most highly polluted river in Ethiopia (Fisseha Itanna and Olsson, 2004). The reported levels of Co, Pb, and Zn in

surface water samples collected from Akaki River (Itanna and Olsson, 2004) exceed the natural levels (Ward, 1995) indicated for rivers. What is more, the liquid waste from Akaki Textile Factory has been employed as low–cost (gravity–driven) irrigation water until very recently. The concentrations of Cd, Co, Cr, Cu, Pb, Se, and Zn measured from samples taken at the effluent's entrance to the vegetable farms (Daniel Fitamo *et al.,* 2007) were still higher when weighed against the corresponding values for rivers shown by Ward (1995).

Essentially, heavy metals derived from various sources may ultimately reach the surface soil (Pendias and Pendias, 2001); typical cases in point include wastewater irrigation at Akaki. A study on irrigation water quality of major rivers in Addis Ababa (Fisseha Itanna *et al.,* 2004) indicated that over 360 hectares of vegetable farms in the city utilize untreated wastewater for irrigation. Compared to other farms at Kera, Kolfe, Mekanisa, and Peacock, conversely, the Pelli–Eutric Vertisol and Eutric Fluvisol at Akaki vegetable farm accommodated the higher total soil levels for some heavy metals (Fisseha Itanna and Olsson, 2004). Reuse of drain water for irrigation of crops has been suggested to elevate the trace element concentrations in soil profile and shallow ground water (Banuelos and Ajwa, 1999). At Akaki farm, on the other hand, during high rains that occur in the wet season, considerable parts of Fluvisol as well as adjacent portions of Vertisol are inundated. Consequently, livestock are allowed to forage on the leftover of vegetables and other herbaceous plants between vegetable growing periods, where the farm is left fallow due to inundation by the river.

Apart from pretty few works (Fisseha Itanna, 1998a; Fisseha Itanna, 1998b; Fisseha Itanna and Olsson, 2004; Fisseha Itanna *et al.,* 2003; Fisseha Itanna and Coulman, 2003), essential information on the potential mobility, phytoavailability, and simultaneous uptake of important heavy metals and metalloids as well as screening of plants for phytoremediation purpose (from heavy metal enriched environments such as those from mining landscape) is lacking. Consequently, information pertaining the status of heavy metals/metalloids in pristine vis–à–vis contaminated soils, their behavior and potential risks to humans and the environment, and possible ways of employing low–tech plant-

based alternative to deal with the heavy metal problems in Ethiopia is of paramount importance to formulate and implement sound environmental management system in heavy metal/metalloid affected soils.

1.3 Objectives

The principal objective of the present study was to assess the status, distribution, and phytoavailability of heavy metals and metalloids in soils irrigated with wastewater. The specific objectives, conversely, were those listed therunder:

1. To assess the heavy metal and metalloid levels and profile distribution of some uncontaminated soils (Paper I);
2. To evaluate the status of heavy metals and metalloids as well as distinguish their forms of retention and behavior in Vertisol and Fluvisol irrigated with wastewater (Paper II);
3. To determine the soil–root, root–shoot (Paper III) and soil–shoot (Paper IV) transfers of heavy metals / metalloids and evaluate the influence of plant (species and plant part) and soil factors (soil type, heavy metal or metalloid associated with the different geochemical phases of the soils, pH, and CEC) on the corresponding shoot/root levels of plants on contaminated soils under controlled (Paper III) and actual field conditions (Paper IV);
4. To screen plants from mine spoils in order to identify those with natural capacity to accumulate heavy metal/metalloid in their tissues and / or having relatively high biomass for phytoremediation of heavy metal affected soils (Paper V).

2. MATERIALS AND METHODS

2.1 General descriptions of the study sites

To acquire information for the first study, which was later used as a springboard for consecutive studies on contaminated soils (study II–V), five uncontaminated substrates were collected from Debre Zeit (8° 45′N, 38° 59′E), Ziway (8° 00′N, 38° 45′E), Bulbula (7° 40′N, 38° 40′E), Alaba (7°20′N, 38° 05′E), and Humbo (6° 50′ N, 37° 45′E). The soils at these locations were Vertisol (Debre Zeit), Fluvisol (Ziway), Solonetz (Bulbula), Andosol (Alaba), and Nitosol (Humbo) (Fig. 2.1). During the time of soil sampling, all the sites were under similar land use, free grazing plots, with out any other prominent vegetation cover of higher forms (i.e., trees or shrubs). By all appearances, therefore, the sites were selected from areas that were believed to have no previous history of contamination/pollution. As is presented in Table 2.1, the geology of the parent material of Fluvisol and Solonetz as well as Andosol and Nitosol are similar. Notwithstanding this, their eventual developments as different soil types portray the significance of other soil forming factors (climate, organisms, topography, and time) and ensuing pedogenic processes in determining the characteristics of these soils.

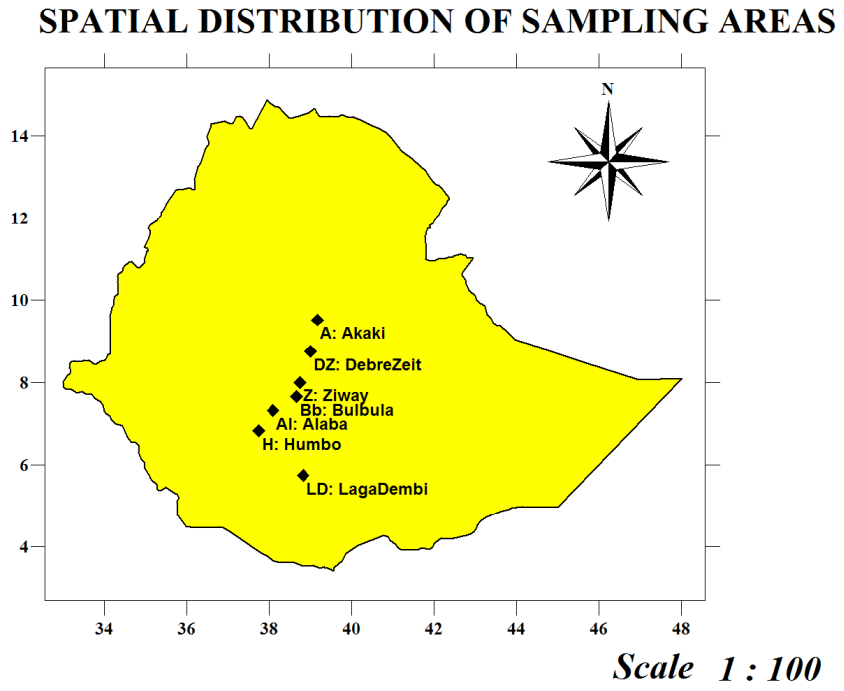


Fig. 2.1 Sampling areas of the study.

Three of the studies in the thesis were performed on soil samples collected from Akaki (study II and III) and the field experiment at the same site (study IV). Akaki (Fig. 2.1) is located at southeastern part of Addis Ababa (9°3′N Longitude and 39°10'E Latitude), Ethiopia. The altitude of the study area is 2290 m.a.s.l. The climate, as measured at the nearby station in Addis Ababa, is characterized by an annual temperature 16.6 °C, mean annual pressure of 767 millibars and yearly average precipitation of 1230 mm.

Table 2.1 Sampling site, soil type, and geology of the parent material of the study soils (study I). After King and Birchall (1975).

SITE	SOIL TYPE	GEOLOGY
Debre Zeit	Vertisol	Late tertiary volcanics
Ziway	Fluvisol	Lacustrine silt stone, sand stone and interbeded pumice and tuff
Bulbula	Solonetz	Lacustrine silt stone, sand stone and interbeded pumice and tuff
Alaba	Andosol	Ignimbrite, tuff, pumice, and interbeded scoriaceous lava
Humbo	Nitosol	Ignimbrite, tuff, pumice, and interbeded scoriaceous lava

Since the last few decades, farmers at Akaki have been growing vegetables such as cabbage, Swiss chard, onion, potato, and red beet on two agricultural soils, Eutric Fluvisol and Pelli-Eutric Vertisol, situated along the banks of Akaki River (Fisseha Itanna, 1998b). The vegetable farm, which is run by Fanta Farmers' Cooperatives, covers an area of about 15000 M^2. These soils have been irrigated year round with water from the Akaki River to support sizable cultivation of the vegetables that are bound to be put on the market at Addis Ababa. Addis Ababa city overlies the volcanic rocks that vary in composition from rhyolitic to basaltic (Tamiru Alemayehu *et al.,* 2005; Tamiru Alemayehu, 2006) with minor amounts of Quaternary alluvial sediments (Tamiru Alemayehu, 2006). The northern part of Addis Ababa is characterized mainly by the Entoto Silicic (rhyolitic and trachytic lava flows, unwelded tuffs, and ignimbrite), while the basaltic lava flows are primarily prevalent in the central and southern part of the city (Tamiru Alemayehu, 2006).

On the other hand, the final study (study V) was based on the plants and mine spoils found at Legadembi gold mine area. Legadembi Midroc Gold Mine is located in Oromiya Regional State (long. 5°45′N; lat. 38°50′E), Southern Ethiopia. The gold mine site

extends over 8.47 km^2 with an average elevation of 2000 m.a.s.l. and includes changes in the local relief of 300 m. The area receives an average yearly precipitation of 900 mm distributed over two seasons, March to May and August to October. Historical gold mine sites of placer deposits were established by government and private companies (including artisan miners) in Adola region (Legadembi and nearby gold bearing areas of the Southern Ethiopia) in 1930s (Worash Getaneh and Tamiru Alemayehu, 2006). Subsequent to the discovery of primary gold deposit in 1980s, Legadembi became an active gold mine site (Worash Getaneh and Tamiru Alemayehu, 2006).

Genesis of the primary gold deposit at Legadembi is shear zone–hosted quartz veins in the Neoproterozoic volcano–sedimentary succession of greenschist to amphibolite facies metamorphic rocks (Worash Getaneh and Tamiru Alemayehu, 2006; Solomon Tadesse *et al.,* 2003). The geochemical association of gold with sulfur minerals (sulfides and sulfosalts), namely pyrite, chalcopyrite, galena, and arsenopyrite (Worash Getaneh and Tamiru Alemayehu, 2006; Solomon Tadesse *et al.,* 2003) eventuated in elevated levels of chalcophilic heavy metals in soils, sediments, vegetation, and water bodies through natural or induced surface mineralization and / or dispersal to receiving environments.

2.2 Green house and field experiments

2.2.1 Green house experiment (Paper III)

The air–dried soil samples (2.5 kg/pot) were placed in plastic flowerpots (with a capacity of ca. 3 kg) and then planted with 20 seeds (later thinned to six plants per pot) of Oat (*Avena sativa)*, Alfalfa (*Medicago sativa*), Rhodes grass (*Chloris gayana*), Desmodium (*Desmodium unicinatum*), and Setaria (*Setaria sphacelata*) under glass house conditions. These locally growing (Rhodes and Setaria, indigenous; the rest introduced) plants were forage species that may subsist on certain levels of soil contamination.The glass house had a temperature of 35 ± 0.69 °C (max.) and 2.6 ± 0.42 °C (min.) with mean relative humidity of 50 %. Each of the treatment combinations (soil type and species) was arranged in a completely randomized factorial design with six replications. Every soil received urea and DAP, 0.14 g each (100 kg ha^{-1}), as fertilizer supplements. The soil

moisture was maintained at about 60 % of pot capacity by daily watering with tap water. Plants were harvested after 90 days (at the vegetative stage) and sorted into shoots and roots for subsequent analysis of heavy metal sequestration in the corresponding above– and below–ground parts.

2.2.2 Field experiment (Paper IV)

In order to investigate the soil–to shoot transfer of heavy metals / metalloids by plants under the natural conditions, a field study was instituted at Akaki on contaminated Vertisol and Fluvisol employing Oat (*Avena sativa*), Alfalfa (*Medicago sativa*), Rhodes (*Chloris gayana*), Clover [red] (*Trifolium pratense*), and Setaria (*Setaria sphacelata*). In this study, Randomized Complete Block Design (RCBD) with five plant species and six replications was used. Each species was sown in a plot consisting of six rows of 2 m length spaced 0.2 m apart. After 90 days, the above ground parts of the plants were harvested at the vegetative stage. For data on shoot heavy metal and metalloid concentrations, composite samples of each species in a block were prepared by bulking sub–samples randomly harvested from 50 cm length of the middle 4 rows on each plot.

2.3 Sampling and analyses

2.3.1 Soil sampling and analyses

Two soil sampling schemes (profile or horizon–based and depth–specific) with three replicates were implemented to evaluate the status, vertical distribution (Study I and II), and potential mobility/availability of heavy metals (Cr, Ni, Co, Cu, Zn, Cd, Pb, Hg, and V) and metalloids (Se and As) associated with the discrete soil phases (Study II). Accordingly, at each site (Akaki, Debre Zeit, Ziway, Bulbula, Alaba, and Humbo), soil samples were acquired for every horizon from a pit (2 m × 1 m) with 4–5 genetic horizons, and from a set–depth (0–20 cm and 30–50 cm) of three small pits (dia. ca. 0.5 m) situated at a distance of 20 m from the pit in three directions. In study I, soil core samples were taken from each horizon for bulk density determination. For the second study, both sampling methods (horizon and depth–specific) were performed on irrigated

farms located at 50 m and 100 m distance from Akaki River on Fluvisol and Vertisol, respectively. On the other hand, only surficial layers (0–20 cm) were sampled in the case of subsequent studies (Study III–V). For the pot–experiment (Study III) under the green house conditions, composite samples that were bulked from 10 samples of surface soil (0–20 cm) of the individual soil (Fluvisol and Vertisol) have been employed. Conversely, for the study on the nature of mine spoils (Study V), upper surface (0–20 cm) samples were collected from the foot of the tailing dam (the first of the three dams, TD^{a}), at Southern Waste Dump (SWD), and at Eastern Waste Dump (EWD) in three replicates. In all cases, about 3–4 kg of soil sample was collected from each horizon and/or set–depth.

Qualitatively assessment of the crystalline mineralogical components of soil samples from the first study was carried out with recourse to X–ray diffractometry. The silt and clay fractions were separated by wet sieving over a 75 microns sieve without any chemical pre–treatment. The oven–dried fine fraction of each sample was dry sieved through a 56 microns mesh size. The random orientation of the samples for X–ray analysis was secured by way of the sample cup and technique for sideward packing as described by Bystrom-Asklund (Bystrom-Asklund, 1966). Firmly packed sample with smooth and planar surface was then placed in the goniometer (PW 1050/80) for X–ray analysis (Phillips PW 1710). The X–ray diffractometer was powered by high voltage generator (40kV and 24 mA) and emitted X–rays from a Cu–tube (the anode) giving characteristic X–ray radiation with a wavelength of ca. 1.54 Å. In order to homogenize the Cu–Kα radiation, a 0.01mm thick Ni–filter was used together with a divergence slit of 1°. Samples were scanned from 2–40 degrees 2Θ with the step scanning speed of 0.02° 2Θ /sec. All runs were set by Phillips computer software, APD (automatic powder diffraction).

In the laboratory, all the samples were dried at ambient temperature, crushed by hand, and then sifted through a 2 mm sieve. The pH was determined potentiometrically in a suspension prepared from 8g of mineral soil and 20ml of 0.01M $CaCl_2$, in a ratio of 1:2.5(w/v). For studies I–IV, organic carbon and total nitrogen were analyzed with recourse to elemental analyzer (LECO CNS–1000) whereas the same parameters were

determined according to Walkely and Black, and Kjeldahl methods, respectively, in study V. The base cations were determined after extraction of 15 g of soil with 1 M NH_4OAC at pH 7.0, by ICP–AES (Jobin Yvon JY 24) whilst the level of exchangeable acidity was ascertained from similar extract (1 M KCl) through titration with 0.1 M NaOH. The cation exchange capacity (CEC) was established as the sum of the equivalent of exchangeable acidity and exchangeable cations. Subsequent to dispersion with 0.05 M $Na_4P_2O_7$ (sodium pyrophosphate) and over night shaking, particle size distribution was determined by hydrometer method. For profile samples of Solonetz, Fluvisol, and Andosol assayed in study I, particle size analysis was performed following the procedure in Barona and Romero (Barona and Romero, 1997) and consequently two fractions were considered for practical reasons: fine fractions (< 0.08 mm, clay and silt) and sand fraction (0.08–2 mm, fine and medium sand). The total carbonate content was assessed by the Passon volumetric method (Wahnschaffe, 1924) (studies I–IV), and employing Bernard Calcimeter (study V). Available P (Olsen) was measured based on the standard procedure (Olsen *et al.,* 1954). For samples taken from mine spoils (study V), electrical conductivity was assessed on extract from saturated soil paste (1:5 soil/H_2O mixture).

In studies I–IV, total acid digestion was performed with 7 M HNO_3 on 2.5 g of mineral soil on a heating block for 2 hours at 120° C (with only inconsequential modification to the Swedish Standard for metal content in water, sludge, and sediment, SS 028 150). The levels of As, Cd, Co, Cr, Cu, Ni, Pb, Se, Zn, V, Hg were determined with ICP–MS (Perkin Elmer 6100-DRC). For heavy metal analyses of the mine spoils (study V), mixed acid (HNO_3–HCl–HF mixture, 5:5:4 v/v) digestion was carried out on 1 g of soil and total heavy metal contents (Cr, Ni, Co, Cu, Zn, Cd, and Pb) was determined with atomic absorption spectrometer (AA–220 Varian spectra plus).

The sequential extraction was performed according to the ideas indicated by McLaren and Crawford (1973), and Eloskkary and Lag (1978) [both cited in Alriksson, 1998], which involved five extraction steps. Accordingly, the scheme operationally defined five chemical forms/species/fractions of the heavy metals that were expected to be influenced by an assortment of environmental conditions (e.g., ionic strength/ion exchange, pH,

redox potential, organic matter decomposition, microbial activity, temperature, and leaching): exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter, and the residual phase. Apart from the extractants employed, the chemical forms or associations are similar to the corresponding fractions indicated by related studies (e.g., Das *et al.,* 1995; Stalikas *et al.,* 1999; Tessier *et al.,* 1979). Extractions were carried out with 5 g of mineral soil from each sample that entailed the separation of the above five fractions with the extractants listed in Table 2.2.

Table 2.2 The extraction conditions and instrumentation used for the sequential extraction method (according to the established methods by the Soil Science Department, Swedish University of Agricultural Sciences).

Fraction	***Operational definition***	***Instrumentation***
1. Exchangeable	50ml of 1M NH_4NO_3, pH 7, 1 hour shaking, 20:1	ICP-MS[1]
2.Carbonate bound	50 ml of 0.5 M NH_4OAc + 0.02 M EDTA, pH 4.65, 1 hour shaking, 10:1	»
3. Reducible	100 ml of 0.175 M $(NH_4)_2C_2O_4$/ O.1M $H_2C_2O_4$, pH 3.25, boiling for 15 minutes, occasional agitation, 40:1	»
4. Oxidizable	50 ml 0.1 $MNa_4P_2O_7$, shaking overnight, 20:1.	GFAAS[2], FFAAS[3]
5.Residual	Total minus sum of the extractable (1 to 4)	Not determined
6. Total	50 ml of 7 M HNO_3, 20:1*	ICP-MS[1]

** 2.5 g of soil was employed for the determination of the total heavy metal levels*
[1] Perkin Elmer 6100-DRC
[2] Perkin Elmer Zeeman 4100 ZL (for the analysis of Cd, Pb, and Cr)
[3] Perkin Elmer AAnalyst 300 (for the measurement of Cu, Ni, Co and Zn)

To authenticate the data quality of the total and sequential extraction, internal reference materials with recognized concentration of the above said heavy metals and duplicate of the reagent blank samples were employed. The relative standard deviation characterizing the precision and bias of analytical results was less than 10%.

2.3.2 Plant sampling and analyses

Plant sampling involved collection of various species such as Oat, Alfalfa, Rhodes grass, Setaria (Study III and IV), Desmodium (Study III), and Clover [red] (Study IV), as well

as other 37 species that belong to 19 families (Study V). Subsequently, the shoots (Study III–V) and roots (Study III and V) of plant materials were analyzed for heavy metal and metalloid sequestration in the corresponding above– and below–ground parts. Two sampling strategies involving 1) all plants found in each pot (Study III) and plot (Study IV), and 2) systematic selection of plants (Study V) were employed during collection of plants. In the latter case (Study V), three individuals per species were picked based on their health (with no apparent growth depressions), coverage, soil color, and texture, and carefully excavated to remove the root along with the shoot from each site. For trees and shrubs under the same study, conversely, twigs were collected. Plant identification (Study V) was carried out at the National Herbarium located in Science Faculty, Addis Ababa University.

After collection, the harvested plant parts were thoroughly washed with tap water, and rinsed with deionized water, and later oven dried (at 60°C for 48 h, Study III and IV; at 80°C for two days, Study V) and finally ground in an agate mortar and pestle to pass a 1 mm nylon sieve. For plants collected in study V, dry weights were measured consequent to drying. After total acid digestion (15 ml of concentrated 14 M HNO_3, Study III and IV; concentrated HNO_3–$HClO_4$ mixture [2.5:1, v/v], Study V) of 1 g of each plant material on heating block, plant analyses of As, Cd, Co, Cr, Cu, Ni, Pb, Se, Zn, V, and Hg were carried out (ICP–MS, Perkin Elmer 6100–DRC; atomic absorption spectrometer, AA–220 Varian spectra plus). Due to low sensitivity of the atomic absorption spectrometer, only Cr, Ni, Co, Cu, Zn. Cd, and Pb were analyzed in plant (and soil) samples collected in study V.

Data quality for both soil and plant materials was verified with recourse to internal reference materials with recognized concentration of the above elements and duplicate of the reagent blank samples. The relative standard deviation characterizing the precision and bias of analytical results was less than 10%.

2.4 Data analysis

Study I

Based on the physicochemical analysis, statistical description of selected physical and chemical parameters of the soils and graphical summary of the distribution of heavy metals in the soils were carried out. Comparison of the stocks of heavy metals (mg m^{-3}) in the five soils were computed with recourse to the summation of the amounts in respective horizons, each calculated from the bulk density (kg m^{-3}) and total concentration of heavy metals (mg kg^{-1})measured. Apart from these, a Principal Component Analysis (PCA) with scores, loadings, and Pearson Correlations were performed to evaluate the inherent relationships among heavy metal contents and between heavy metals and main soil characteristics.

Study II

For the surface (0–20 cm) and subsurface (30–50 cm) samples collected from each soil, arithmetic means, standard deviations, and the coefficients of variation of the heavy metal contents were ascertained. To assess the effect of depth and soil type on heavy metal concentrations on surface and subsurface samples, a one way analysis of variance was performed. For the same samples, correlation analysis was carried out to assess the associations between the total heavy metals and major soil variables, as well as the total levels of heavy metals and the proportions of the total concentrations in the five geochemical phases of the soils. Moreover, to appreciate the contribution of the separate phases in the soil system to the total level of heavy metals, portrayal of the relative proportion (percentage of the total heavy metal associated with each phase) was presented. On the other hand, from the total contents of heavy metals measured for the pit samples, the pattern of heavy metal levels with depth in the study soils was graphically represented.

Study III

To establish the validity of the assumption of equal group variances for each group formed by categorical independents, Levene's test for homogeneity of variance was carried out. To assess the capacity of the study plants to accumulate and / or translocate heavy metals, arithmetic means (for contents in the soils and plant parts), BCF, and TF were established. Furthermore, to appreciate the main and interaction effects of the soil type, species, and the plant parts (above– and below–ground) on the levels of Cr, Co, Ni,

Cu, Zn, Cd, Pb, Hg, Se, V, and As in the study plants, multiple analysis of variance (MANOVA) was performed using multivariate general linear model (GLM). In view of that, the four multivariate tests entailed Wilk's test, Lawley–Hotelling test, Pillai's test and Roy's largest root test for each term in the model. A one–way analysis of variance was performed to see the effect of plant part and soil type on the heavy metal levels of each species. Moreover, to recognize those soil fractions and selected soil parameters, which contribute to the availability of heavy metals observed in the plant parts, stepwise regression analysis was executed.

Study IV

Levene's test for homogeneity of variance was carried out to ascertain the assumption of equal group variances across the groups formed by categorical independents. Furthermore, to appreciate the influence of the soil type, and species as well as their interaction on the shoot levels of Cr, Co, Ni, Cu, Zn, Cd, Pb, Hg, Se, V, and As in the study plants, multiple analysis of variance (MANOVA) was performed with multivariate general linear model (GLM). To assess the capacity of the plants to translocate heavy metals and metalloids, arithmetic means (for contents in the soils and shoots) and TC were established. A one–way analysis of variance was performed to see the effect of soil type on the heavy metal contents in the shoot of each species. Moreover, to recognize the effect of those soil fractions and selected soil parameters on the availability of heavy metals in the shoot, stepwise regression analysis was executed.

Study V

To assess the capacity of the study plants to accumulate and translocate heavy metals, BCF (bioconcentration factor, a ratio of root/soil concentration), TF (translocation factor, shoot/root concentration ratio), and TC (transfer coefficient, a concentration ratio of aboveground tissue/soil) were established. One-way analysis of variance was performed to compare the means (levels: shoot, root, and soil; values: BCF, TF, and TC) for Cr, Ni, Co, Cu, Zn. Cd, and Pb between the sites. For those cases where the overall F test has demonstrated at least one significant difference existed, differences between the means was assessed by way of Tukey–HSD test at $p < 0.05$ level. Besides, in order to evaluate the association between heavy metals, linear regression analysis was carried out between

their levels (shoot, root, and soil), and their respective values of BCF, TF, and TC at every site.

3. RESULTS AND DISCUSSION

3.1 Heavy metals in uncontaminated soils (Paper I)

3.1.1 Geology and properties of uncontaminated soils

The geology of the rocks underlying Vertisol is marked by late tertiary volcanics (Table 2.1) with the main geological formations of ignimbrite, pumice, ash, rhyolite domes with intercalation of basaltic flows. On the other hand, Fluvisol and Solonetz overlie lacustrine silt stone, sand stone and interbeded pumice and tuff (Table 2.1). As engendered by parching of the big lake that had once occupied the floor of rift valley during the pluvial periods, lacustrine deposits commonly occur in the rift valley. Such lacustrine sediments embrace silts, clays, diatomites, and volcanoclastic sediments. Apart from the predominance of silts and clays as lacustrine sediments, considerable outcrop of diatomite is apparent between Lakes Shalla and Abiyata. Conversely, ignimbrite, tuff, pumice, and interbeded scoriaceous lava portray the underlying geological materials of Andosol and Nitosol (Table 2.1).

Rift pyroclastic formation represented by typical ignimbrite, sillar, and layered pumice is prevalent and hence extends over most part of the rift floor. In contrast to the typical ignimbrite which is hard and welded rock, sillar ignimbrite with a coarse and poorly welded kind of ignimbrite rich in big pumice fragments features area in the environs of Lake Shalla, western shore of Lake Langano, and east Ziway. Moreover, a layered pumice formation (one of the rift pyroclastic formations) has generally thin layer formed by unwelded small–sized pumice. In effect, thin layers of lacustrine deposit, ashes, and paleosoils could characterize soil horizons under such environmental settings (see layers of Solonez Table 3.2).

As is presented in Tables 3.1 and 3.2, the carbonate content, CEC, pH, clay, and fine fractions (clay and silt) of the horizons from uncontaminated soils more or less revealed an increasing trend with depth. Below 115 cm in Solonetz, on the other hand, an alternating pattern in the magnitude of the same parameters was observed. Such an

arrangement appeared to have been caused by the presence of thin (only 2–5 cm thick) ash layers regularly alternating with layers containing considerably higher amount of fine fractions (see Table 3.2). Organic carbon (OC) for the soils, in contrast, generally had a decreasing tendency down the soil profiles; a layer > 173 cm of Solonetz, however, revealed comparable OC level to the top layer and thus, might be old buried surface layer (see Tables 3.1 and 3.2). Of the uncontaminated soils, Vertisol had the highest amount of organic carbon per cubic meter of soil (26476.92 mg m^{-3}).

In the face of the potential influence ascribed to pedogenic consequences, soils developed on parent materials derived from similar geologic materials appeared to retain certain similitude with respect to some soil properties (Tables 3.1 and 3.2). Apart from this, the total trace element content of uncontaminated soil in rural situation could practically be the same as that of the rocks from which the soil parent material was derived during weathering (Purves, 1985).

Table 3.1 Some chemical and physical properties in the soil profiles of Nitosol and Vertisol.

Soil type	Depth(cm)	Organic carbon (%)	Calcium carbonate (%)	CEC (cmol $_{(+)}$/kg)	pH	[a]Clay (%)	[b]Silt (%)	[c]Sand (%)
Nitosol	0-14	0.64	0	9.28	4.79	42.1	18.2	39.7
	14-50	0.86	0	9.46	4.88	56	14.6	29.4
	50-97	0.26	0.01	9.24	4.84	40	22	38
	97	0.22	0.01	10.35	4.88	39.4	24.1	36.5
Vertisol	0-40	1.33	0	45.74	6.32	65.5	28.5	6
	40-84	1.24	0.06	50.43	6.35	64.2	29.7	6.1
	84-118	1.02	0.66	55.34	6.16	68	26	6
	>118	0.23	4.33	59.19	6.84	70.7	23.5	5.8

[a]Clay <0.002 mm; [b]Silt 0.002-0.06 mm; [c]Sand 0.06-2 mm

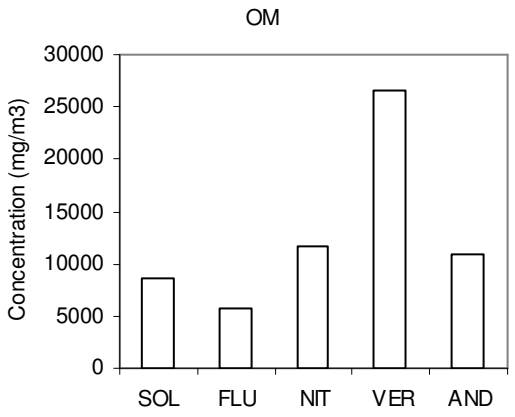
Table 3.2 A pick of some chemical and physical parameters of Solonetz, Fluvisol, and Andosol with soil depth.

Soil type	*Depth (cm)*	*Organic carbon (%)*	*$CaCO_3$ (%)*	*CEC* ($cmol_{(+)}/kg$)	*pH*	*Coarse fraction (>0.08mm) (%)*	*Fine fraction (< 0.08mm) (%)*
Solonetz	0-20	0.7	4.61	19.57	8.47	29.9	70.1
	20-31	0.51	4.25	20.66	7.9	10.9	89.1
	31-115	0.69	11.67	35.48	9.95	16.2	83.8
	115-117	0	5.79	24.43	10.24	64.7	35.3
	117-132	0.57	5.82	49.39	10.57	33.6	66.4
	132-134	0	0.35	28.22	9.77	59.6	40.4
	134-148	0.12	7.36	57.42	10.65	7.9	92.1
	148-153	0	0.23	6.51	8.52	73.2	26.8
	153-168	0.07	8.95	56.97	10.56	9.9	90.1
	168-173	0.03	0.12	9.13	8.51	77.3	22.7
	>173	0.65	11.08	56.21	10.34	13.6	86.4
Fluvisol	0-20	0.72	1.65	24.69	7.47	65	35
	20-44	0.41	1.84	28.21	7.75	61.8	38.2
	44-112	0.08	5.71	26.12	8.1	68.5	31.5
	112-167	0.06	2.23	23.8	8.12	32.4	67.6
	>167	0.2	1.26	31.62	8.82	16.1	83.9
Andosol	0-24	0.99	0	12.77	5.73	50.6	49.4
	24-74	0.66	0	12.68	5.4	43.2	56.8
	74-121	0.21	0.27	40.76	6.78	66.7	33.3
	>121	0.19	0.27	41.52	7.02	63.8	36.2

3.1.2 Status and profile distributions of heavy metals in uncontaminated soils

Zinc

The concentrations of Zn in Vertisol (102.1–117 mg kg^{-1}), Andosol (90.2–162 mg kg^{-1}), and Nitosol (52.3–75.3 mg kg^{-1}) as well as in upper layers of Fluvisol (50.6 and 51.7 mg kg^{-1}, 0–44 cm), and the clayey layers (>117 cm) in Solonetz (62.5–91.7 mg kg^{-1}) were higher than the reported average [50 mg kg^{-1}: (Abollino *et al.,* 2002; Adriano, 1986; Adriano, 2001; Angelone and Bini, 1992; Kiekens, 1995; McLean and Bledsoe, 1992)] for soils (Figs 3.2–3.6). Apart from this, Vertisol (133576.3 mg m^{-3}) followed by Andosol (113730.6 mg m^{-3}) had the highest total amount of Zn to a depth of a meter (Fig. 3.1).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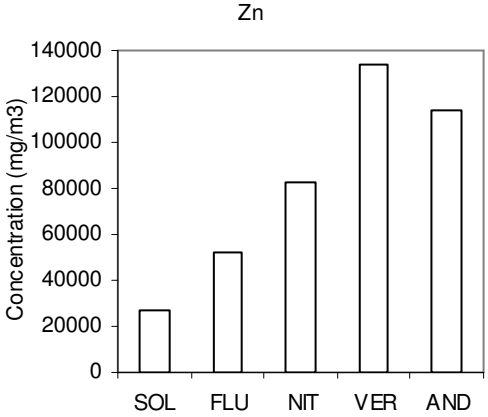
FLU
NIT
VER
AND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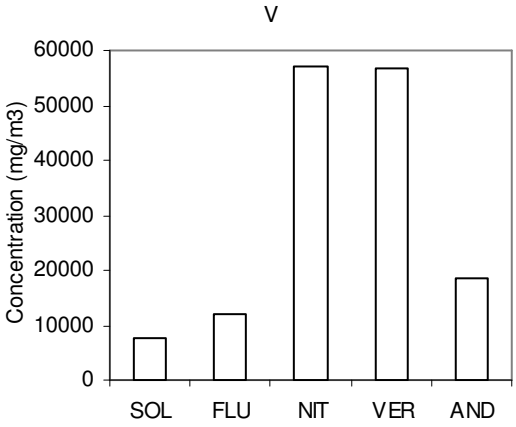
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VER
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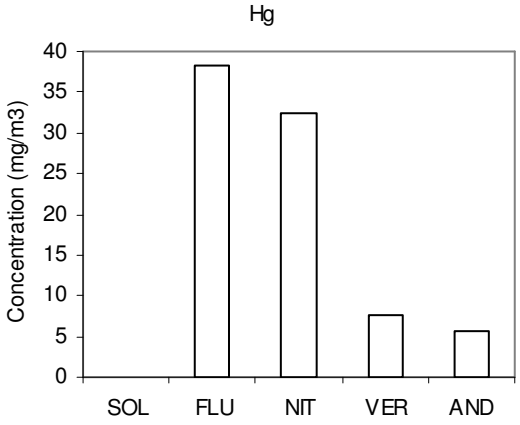
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10
5
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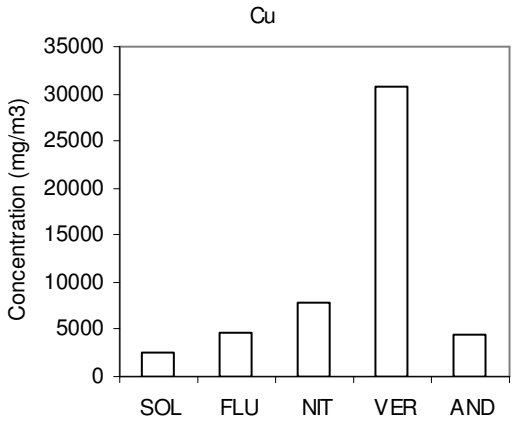
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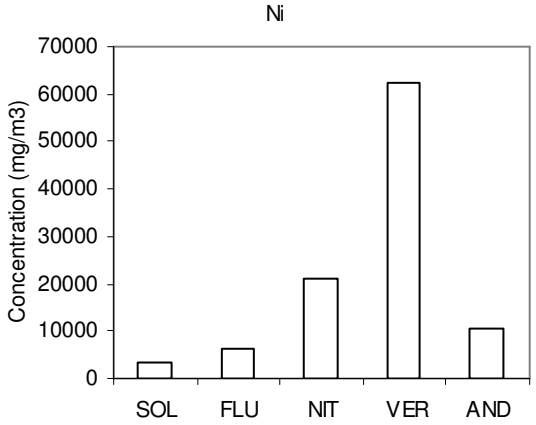
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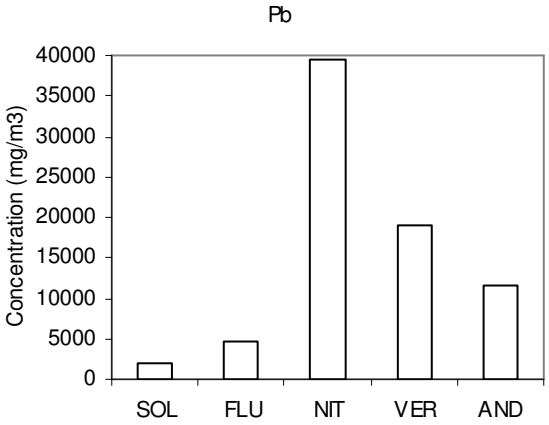
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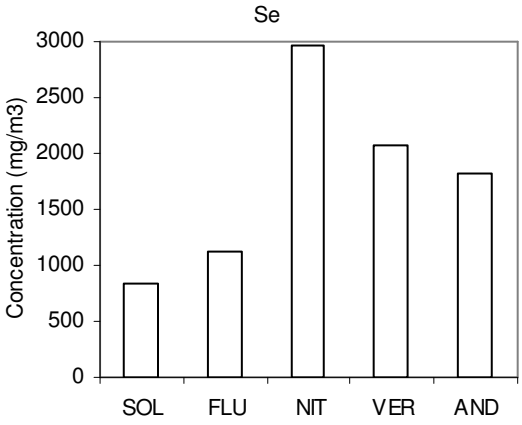
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1000
500
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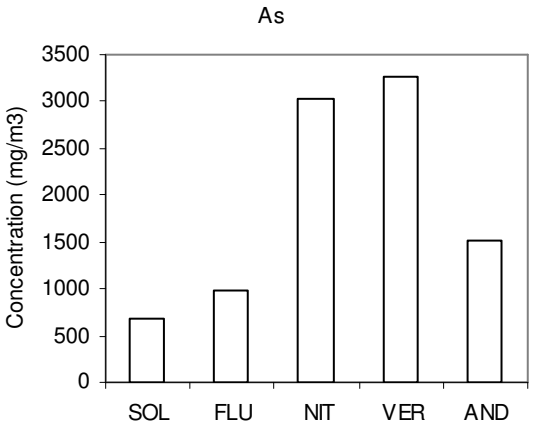
FLU
NIT
VER
AND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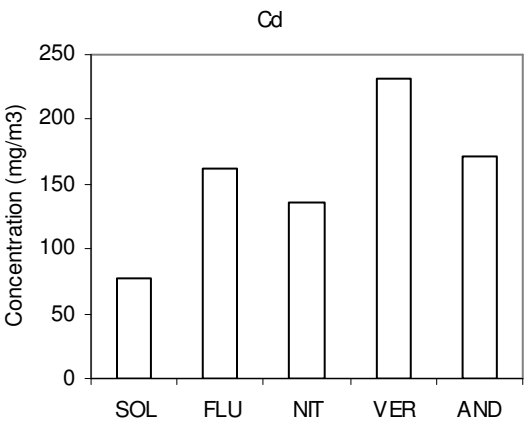
FLU
NIT
VER
AND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100
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FLU
NIT
VER
AND

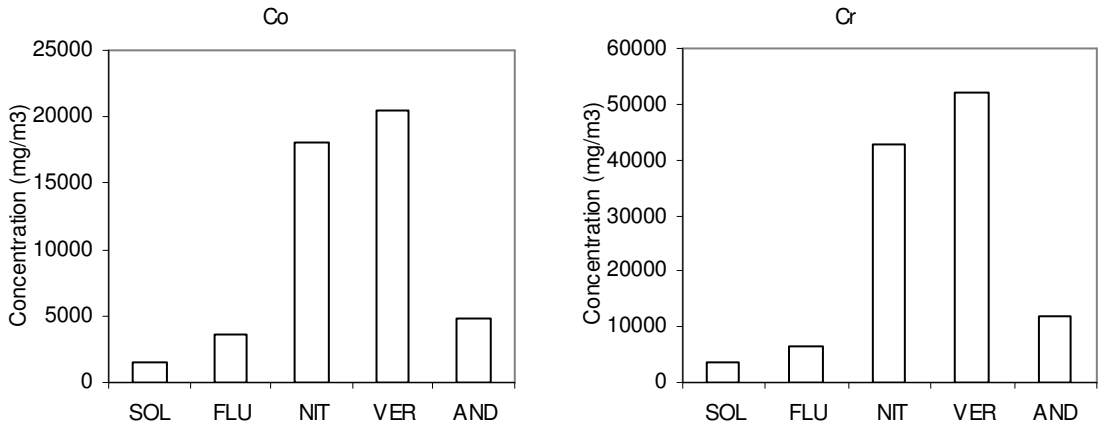

Fig 3.1 Comparisons of the stock (mg m^{-3}) of organic matter, heavy metals and metalloids in Solonetz (SOL), Fluvisol (FLU), Nitosol (NIT), Vertisol (VER), and Andosol (AND).

The underlying layers of Solonetz (Fig. 3.4) and Andosol (Fig.3.5) contained perceptibly higher Zn level than the overlying layers. On the other hand, upper layers of Fluvisol (Fig. 3.3) and Nitosol (Fig. 3.6) accommodated palpably higher Zn levels while a more or less uniform distribution of Zn was observed in Vertisol (Fig. 3.2). The distribution trends of Zn in the soils were consistent with the reported irregularity (Adriano, 1986; 2001) in the distribution pattern of Zn in the profile. Besides, organic carbon and clay showed strong ($p < 0.01$) positive associations with Zn levels of the soils (Table 3.3). The bonding of Zn by soil organic matter eventually leads to its accumulation in higher percentage in mineral soils (Pendias and Pendias, 2001). Conversely, Pendias and Pendias (2001) reported that the clay fraction regulates up to 60% of Zn distribution in soils.

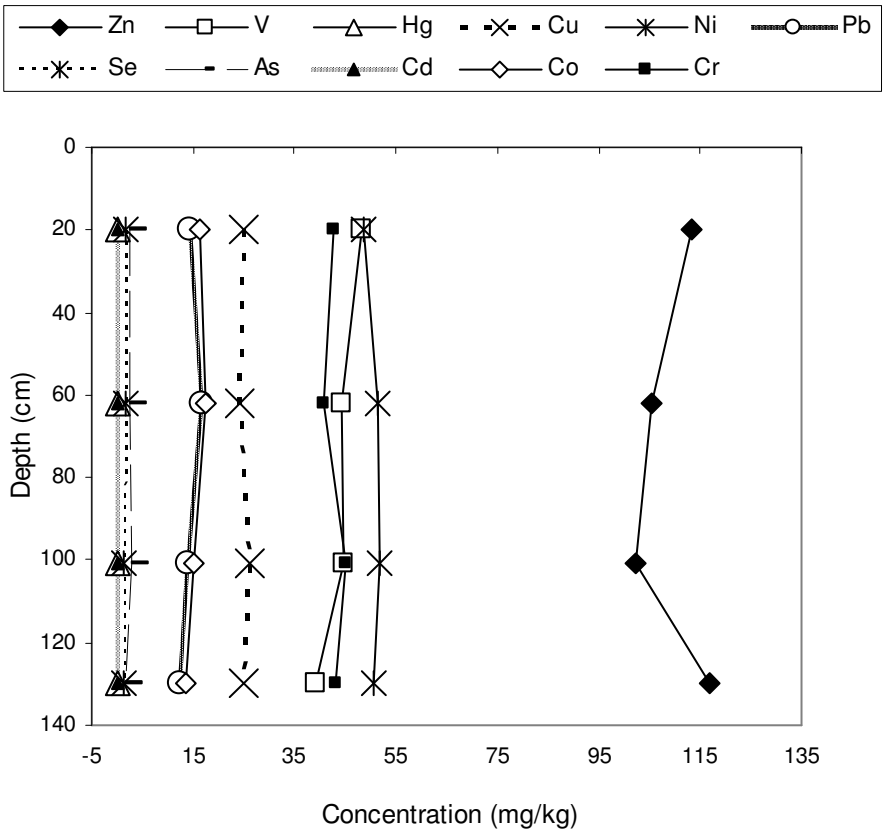

Fig. 3.2 Variation in amount of heavy metals with soil depth in Vertisol, Debre Zeit.

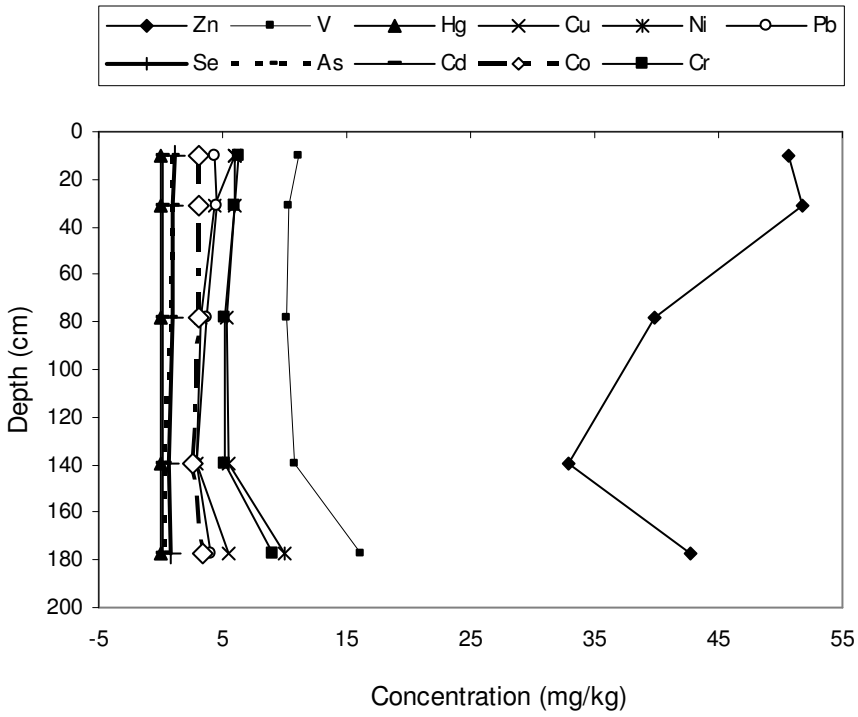

Fig. 3.3 Levels of heavy metals with soil depth in Fluvisol, Ziway.

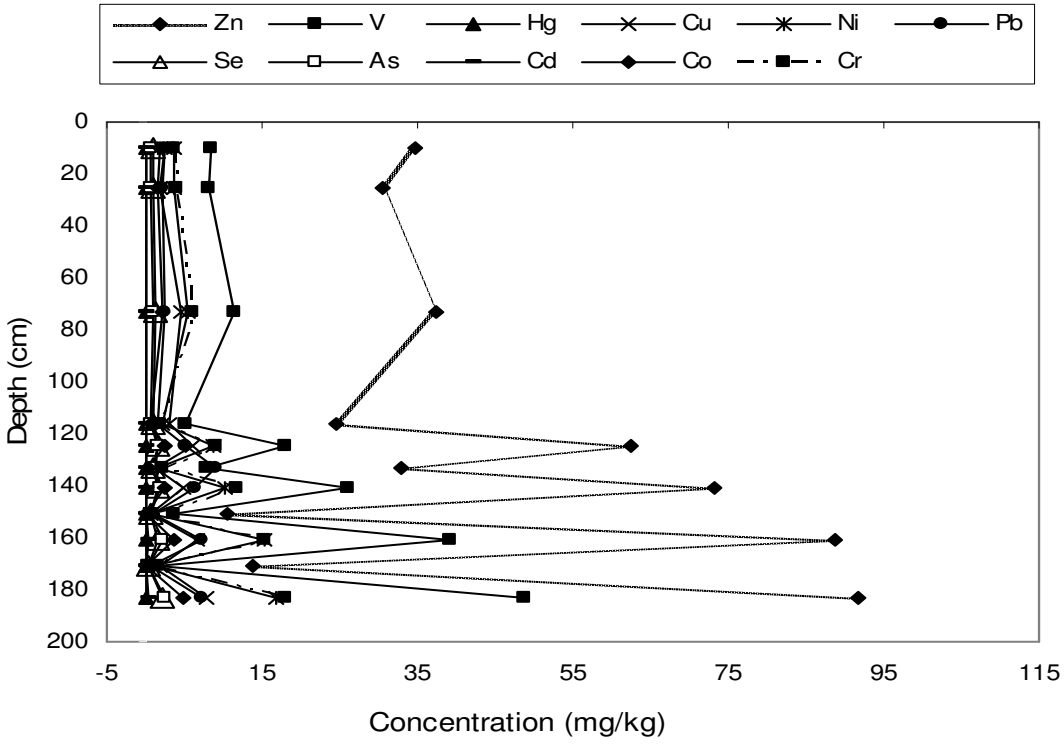

Fig 3.4 Variations in heavy metal/metalloid levels with depth in Solonetz, Bulbula

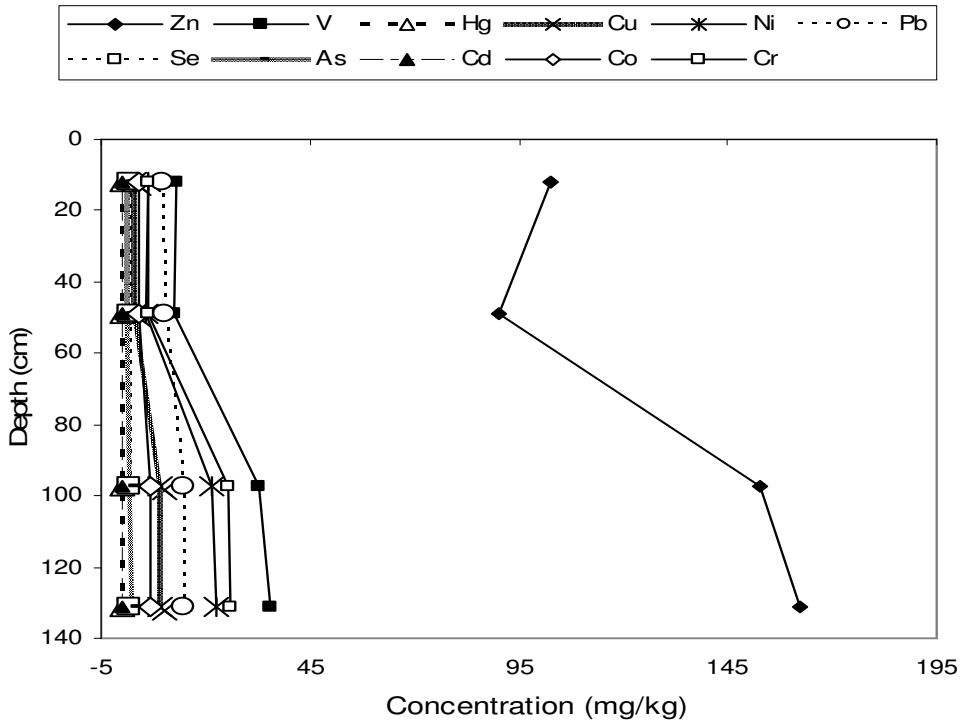

Fig. 3.5 Vertical distribution of heavy metals/metalloids in Andosol, Alaba.

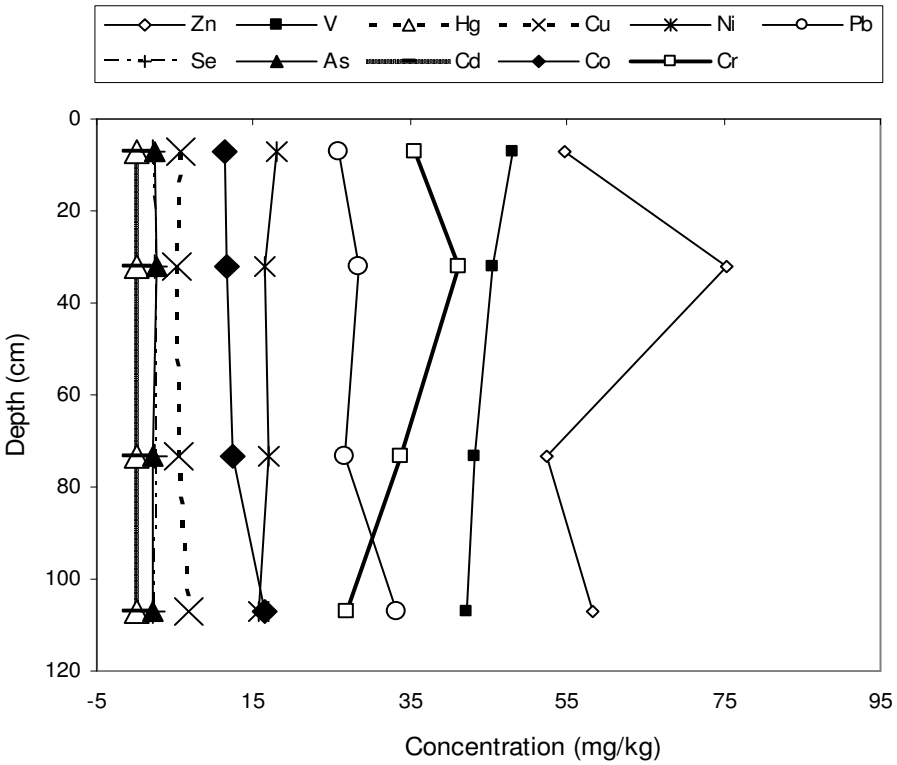

Fig. 3.6 Heavy metals distribution with depth in Nitosol, Humbo.

Besides, the precipitation of zinc as carbonate, nucleation of zinc hydroxide on clay surfaces (McLean and Bledsoe, 1992; Pendias and Pendias, 2001), physical entrapment in clay lattice wedge zones (Kiekens, 1995; McLean and Bledsoe, 1992; Pendias and Pendias, 2001), especially in montmorillonite (Pendias and Pendias, 2001) could be possible mechanisms for the status of Zn in Solonetz and Vertisol. Conversely, an adsorption of heavy metals on variable negative–charge sites (e.g., parallel depth–wise increasing trends between Zn [Fig. 3.5] and CEC [Table 3.2] of Andosol), and selective adsorption of heavy metal cations at discrete sites (e.g., Si–OH or Al–OH) on poorly crystalline materials (allophane and imogolite) (McBride, 1989) may contribute to retention of Zn in this soil (Wada, 1989).

Vanadium

Solonetz had the lowest (1.57 mg kg^{-1}) as well as the highest (48.66 mg kg^{-1}) V contents (Fig. 3.4). Solonetz and Saline alkali soils in Chad (55–100mg kg^{-1}), Madagascar, and New Caledonia (90 mg kg^{-1}) are documented to have plentiful levels of V (Aubert and Pinta, 1977). Apart from this, the surface layers of Nitosol (47.99 mg kg^{-1}) and Vertisol (48.40 mg kg^{-1}) contained considerable amounts of the element and thus, the soils had the highest V quantities per cubic meter as well (Nitosol: 57252.92 mg m^{-3}; Vertisol: 56780.06 mg m^{-3}). The V levels of the soils (Figs. 3.2–3.6) were within the common range (18–115 mg kg^{-1}: Pendias and Pendias, 2001) of V worldwide and were lower than the average contents of V for soils of arid, semiarid (which are often >100 mg kg^{-1}), and tropical humid (80–90 mg kg^{-1}) (Aubert and Pinta, 1977). Variations in amount of V in soils are indicated to reflect the levels of V inherited from parent materials (Pendias and Pendias, 2001).

As is presented in Table 3.3, V exhibited strong positive association with clay ($p < 0.01$) and organic carbon ($p < 0.05$), but negatively correlated with pH ($r = –0.617$; $p < 0.01$). As a rule, V concentration pivots on the soil texture where the level increases with increasing clay content (Aubert and Pinta, 1977). Besides, the same authors made known that in ferralitic soils of Dahomey, the distribution of V in different horizons proceeds

along with that of clay. On the other hand, Pendias and Pendias (2001) indicated that V display marked association with organic matter. Moreover, Pendias and Pendias (2001) indicated that V could be adsorbed or incorporated in Fe oxides. Low pH favors adsorption of V (both vanadate anion and vanadyl cation) on oxides and silicates as well as enhances the chemical reduction of vanadate anion to vanadyl cation (VO^{2+}, which behaves much like Cu^{2+} and complexes with organic matter) by humus resulting in lower mobility (McBride, 1994).

In his review of the distribution pattern of total soil V, Adriano (1986; 2001) discussed that V is more or less uniformly distributed in the profile (see also Pendias and Pendias, 2001) despite the tendency to accumulate in the surface. In the main, similar distribution was observed in Nitosol (Fig. 3.6) and to some extent in Vertisol (Fig. 3.2) as well. On the other hand, a notable increasing trend of total V content with soil depth was seen in Andosol (Fig. 3.5) and, to considerable extent in Fluvisol (Fig. 3.3) and Solonetz (Fig. 3.4) as well.

Table 3.3 Pearson correlation coefficients for the heavy metals and major soil variables in the soils under this study

	Zn	V	Hg	Cu	Ni	Pb	Se	As	Cd	Co	Cr	CEC	pH	CaCO3	Org-C	Sand	Silt
V	0.535*																
Hg	0.238	-0.117															
Cu	0.713**	0.708**	-0.203														
Ni	0.736**	0.822**	-0.159	0.979**													
Pb	0.271	0.785**	0.086	0.168	0.337												
Se	0.440	0.793**	0.228	0.258	0.428	0.942**											
As	0.672**	0.969**	0.016	0.728**	0.839**	0.771**	0.822**										
Cd	0.856**	0.397	0.054	0.753**	0.698**	0.031	0.152	0.488*									
Co	0.673**	0.969**	-0.101	0.842**	0.924**	0.659**	0.709**	0.963**	0.561*								
Cr	0.552*	0.993**	-0.149	0.767**	0.865**	0.736**	0.746**	0.962**	0.437	0.985**							
CEC	0.476*	0.348	-0.294	0.887**	0.789**	-0.257	-0.170	0.362	0.645**	0.519*	0.432						
pH	-0.449*	-0.617**	-0.341	-0.087	-0.242	-0.845**	-0.843**	-0.641**	-0.184	-0.537*	-0.561*	0.352					
CaCO3	-0.696**	-0.586*	-0.192	-0.365	-0.453*	-0.611**	-0.633**	-0.635**	-0.528*	-0.605**	-0.563**	0.038	0.781**				
Org-C	0.806**	0.513*	-0.036	0.776**	0.764**	0.158	0.247	0.626**	0.736**	0.643**	0.551*	0.611**	-0.187	-0.612**			
Sand	-0.594**	-0.736**	0.030	-0.723**	-0.806**	-0.350	-0.500*	-0.774**	-0.356	-0.778**	-0.744**	-0.511*	0.287	0.320	-0.587**		
Silt	-0.216	-0.617**	0.144	-0.258	-0.319	-0.702**	-0.569**	-0.527*	-0.313	-0.556*	-0.604**	0.047	0.630**	0.603**	-0.128	-0.042	
Clay	0.612**	0.981**	-0.115	0.742**	0.847**	0.725**	0.761**	0.953**	0.484*	0.976**	0.978**	0.380	-0.628**	-0.639**	0.552*	-0.774**	-0.600**

** and * represent significance level at 1% and 5%, respectively.

Mercury

Of all the heavy metals assessed at all sites, mercury (Hg) was found in smallest amounts; with the range extending from below detection limit(in various layers at all sites) to 0.118 mg kg^{-1} in 0–14cm of Nitosol. Consequently, Hg levels were within the common range of 0.01–0.3 mg kg^{-1} (McLean and Bledsoe, 1992) and the top layers had Hg concentrations lower than the mean content of the same (< 0.4 mg kg^{-1}) reported for surface soils worldwide (Pendias and Pendias, 2001). The stock of Hg in a cubic meter of Fluvisol (38.34 mg m^{-3}) and Nitosol (32.39 mg m^{-3}) were fairly higher than the corresponding quantities in the other three soils (Fig. 3.1).

Upper layers of Nitosol, Vertisol, Andosol, and Fluvisol as well as underlying horizons of Solonetz accommodated relatively higher Hg (Figs. 3.2–3.6). Hg in soils is associated with organic carbon and sulfur levels, which explain higher concentration of the metal in surface soils at several times than in underlying layers (Pendias and Pendias, 2001). Sorption of all Hg compounds has been shown to exhibit positive correlations with organic carbon and CEC (Pendias and Pendias, 2001). In addition, a study on the distribution of Hg in profiles of virgin soils illustrated that at a neutral pH (pH > 6) the preponderance of HgOCl and $Hg(OH)_2$ (rather than $HgCl_2$), intimates the undisputed co-variation between the Hg level and iron (Steinnes, 1995). Moreover, some researchers also suggest that $Hg(OH)_2$ is the preferred sorbed species (Pendias and Pendias, 2001).

Copper

The copper concentration of the soils had a spread from 0.7 mg kg^{-1} (148–153 cm, Solonetz), to 26.1 mg kg^{-1} (84–118 cm, Vertisol). Compared to the average level of Cu (20 mg kg^{-1}) for world soils (Aubert and Pinta, 1977; Angelone and Bini, 1992), common values (20–30 mg kg^{-1}) reported for rural soils (Abollino, *et al.,* 2002), 30 mg kg^{-1} for world soils (Adriano, 1986; 2001; McLean and Bledsoe, 1992; Baker and Senft, 1995), or 13–24 mg kg^{-1} for surface soils at the world scale (Pendias and Pendias, 2001) the soils had lower Cu contents. Essentially, variation in Cu content, for most part, is due to different levels of the metal in parent rocks and, to certain extent, to the types of soils typifying the variation in climatic zones and geographic region (Aubert and Pinta, 1977).

Cu displayed significant ($p < 0.01$) positive correlations with clay, organic carbon, and CEC (Table 3.3). Of all soils, on the other hand, Vertisol contained the maximum amount of Cu per cubic meter (30676.22 mg, Fig. 3.1). The clay fraction considerably influences the status of Cu in the soils (Pendias and Pendias, 2001). Accordingly, in agreement with the present study, Vertisols are rich in terms of their Cu content (Aubert and Pinta, 1977) and the adsorption of Cu by soil minerals pivots on the surface charge of the adsorbents (Pendias and Pendias, 2001). Conversely, surficial accumulation of Cu is indicated as typical feature of the metal distribution in soil profiles (Pendias and Pendias, 2001).

Besides, surface layer of Fluvisol (5.95 mg kg^{-1}) as well as underlying layers of Solonetz (7.76 mg kg^{-1}), Nitosol (6.80 mg kg^{-1}), Vertisol (26.07 mg kg^{-1}), and Andosol (9.18 mg kg^{-1}) showed slightly higher Cu levels (Figs. 3.2–3.6). Apart from this, more or less uniform distribution of Cu was revealed, particularly, in Nitosol (Fig. 3.6) and Vertisol (Fig. 3.2). As is pointed out by Pendias and Pendias (2001), this reflects that Cu is rather immobile element owing to the various interactions with mineral and organic components of the soil. As with the level, the distribution of total Cu in the soil profile is also bound to vary depending on the soil type and the nature of the parent material (Adriano, 1986; 2001). Nonetheless, the profile distribution could also be a reflection of the alterations by several pedological processes (Adriano, 1986; 2001), namely physical, chemical, and biological by their attributes (Adriano, 1986). Accordingly, rather uniform distribution could be observed in those soils developed in–situ (on igneous rock or sandstone), slight variation in freely drained soils, or considerable accumulation in the B–horizons (due to depletion in surficial horizons) (Adriano, 1986; 2001).

Nickel

The nickel concentrations of the soils were spread between 0.8 mg kg^{-1} (148–153 cm, Solonetz) and 52 mg kg^{-1} (84–118cm, Vertisol). These Ni concentrations (except Vertisol) were below the grand mean concentration (22 mg kg^{-1}) for the world soils (Pendias and Pendias, 2001), and 20 mg kg^{-1} (Angelone and Bini, 1992; McGrath, 1995; Adriano, 2001). On the other hand, rather higher levels of Ni levels (50 mg kg^{-1}) for soils by other reports (e.g., Adriano, 1986; McLean and Bledsoe 1992; and Abollino *et al.,*

2002) and for Vertisol from arid and semiarid regions (Aubert and Pinta, 1977) were more or less comparable to Ni in Vertisol (Fig. 3.2). Conversely, Vertisol contained the highest quantity Ni per m^{-3} (62361.20 mg, Fig. 3.1). As a rule, the Ni status of soils primarily hinges on the Ni content of the parent rocks (Aubert and Pinta, 1977; Pendias and Pendias, 2001). However, the distribution of the same in soils is also governed by other factors, inter alia, soil type, extent of soil development, fine fraction, metal sesquioxides and humus content (Aubert and Pinta, 1977).

Ni displayed significant ($p < 0.01$) positive association with % clay, CEC, and % organic carbon (Table 3.3). The important influence of the clay fraction in determining the Ni level is indicated for clay rich (McGrath, 1995; Pendias and Pendias, 2001) and loamy soils (Pendias and Pendias, 2001). As engendered by the geology, pedogenic processes (McGrath, 1995; Pendias and Pendias, 2001), and ensuing association of the metal either to organic matter or to amorphous oxides (Pendias and Pendias, 2001), the surface, or the subsoil could be comparatively enriched, or may even have uniform Ni distribution (McGrath, 1995). Various reports on the distribution of total Ni indicated that there is no distinguishable pattern in the profile of non–polluted soils (Adriano, 1986; 2001). Consequently, comparable to the observation made in a comprehensive survey in California (Adriano, 1986; 2001), four patterns were observed: fairly uniform distribution (Vertisol, Fig 3.2; Nitosol, Fig. 3.6), increasing total content with depth (Andosol, Fig. 3.5), decreasing to a certain depth followed by increasing concentrations (Fluvisol, Fig. 3.3), increasing pattern to a certain depth, followed by an alternatively increasing (in layers rich in finer fractions) and decreasing (in an ash layers) concentrations (Solonetz; Fig. 3.4).

Lead

The lead contents of the soils ranged from 0.6 mg kg^{-1} (168–173 cm, Solonetz) to 33.3 mg kg^{-1} (>97cm, Nitosol). The spread of the Pb content was within the typical range of the metal in normal soils, <10 mg kg^{-1} to 10 % in ore materials (Adriano, 1986; 2001). In contrast, mainly the Pb levels in Nitosol (Fig. 3.6), but also that of Vertisol (Fig. 3.2), and underlying layers of Andosol (Fig. 3.5) exceed the mean soil Pb content (10 mg kg^{-1}) for

world soils (Angelone and Bini, 1992) and selected average for soils (McLean and Bledsoe, 1992). Nevertheless, when compared to the reported average value (25 mg kg^{-1}) for surface layers (Pendias and Pendias, 2001), the surficial concentrations of these soils (2.5–25.8 mg kg^{-1}) were low. The natural variations in Pb contents of soils reflect the strong association between the Pb level and the composition of the bedrock (Pendias and Pendias, 2001).

The total Pb content of Nitosol (25.81–33.28 mg kg^{-1}) and the amount of the metal in a cubic meter (39552.80 mg, Fig. 3.1) were the highest in the study (Fig. 3.6). Apart from this, Pb displayed marked association with clay fractions (Table 3.3). According to Pendias and Pendias (2001), the level of Pb in this soil is governed by the association of Pb to clay minerals, Mn oxides, Fe and Al hydroxides, and organic matter. The Pb concentrations in Fluvisol (Fig. 3.3) and Vertisol (Fig. 3.2) revealed imperceptibly reducing (more or less unvarying) trend with depth and while modest increments in Pb levels were found in underlying layers of Solonetz (with fine fractions, Fig. 3.4), Nitosol (Fig. 3.6) and Andosol (Fig. 3.5). In mineral soils with low Pb content, however, no decreasing trend with depth is reported (Adriano, 1986; 2001). Conversely, in tropical soils, Pb displays a propensity to increase with corresponding increase in clay content, relative to the depth of the horizon (Aubert and Pinta, 1977).

Selenium

The selenium concentrations of the soils ranged from 0.5 (168–173 cm, Solonetz) to 2.34 mg kg^{-1} (14–50 cm, Nitosol). Compared to the selected average (0.3 mg kg^{-1}) for soils (McLean and Bledsoe, 1992), and average concentration (0.4 mg kg^{-1}) for the world soils (Adriano, 2001), soils from all sites contained high Se concentrations. In essence, the level of Se in the parent rocks have a strong bearing on the level of the same in soils (Aubert and Pinta. 1977). Nonetheless, the pitch of weathering and leaching processes of the parent materials determine the eventual Se composition of the soils derived from parent materials (Neal, 1995).

For some measure, Nitosol contained relatively high (2.20–2.34 mg kg^{-1}, see Fig. 3.6) levels of Se as well as had the highest amount (2962.71 mg m^{-3}) of the same per unit volume (Fig. 3.1). Besides, Vertisol and Andosol accommodated considerable quantity of Se in their upper layers (Fig. 3.2, 3.6 and 3.1). Conversely, Se contents revealed significant ($p < 0.01$) positive and negative relationships with clay fractions and pH, respectively (Table 3.3). Se is mainly associated with clay minerals (Neal, 1995; Pendias and Pendias, 2001), oxides and hydroxides of Fe (Neal, 1995; Pendias and Pendias, 2001; Pezzarossa and Petruzzelli, 2001) and Mn, and organic carbon (Pezzarossa and Petruzzelli, 2001). Apart from redox status, the concentration and the different physicochemical forms of Se in soil are influenced by pH (McLean and Bledsoe, 1992). Consequently, increased sorption of selenite at low pH (Pezzarossa and Petruzzelli, 2001) could be the underlying reason for the observed negative correlation between pH and Se content of the soils (Table 3.3). Although the relative proportions of adsorbing surfaces vary with the level of adsorbent in soils, the selenite (preferred adsorbing species of Se) adsorption by clay minerals pivots more on the pH than the type of layer associated (Neal, 1995). Besides, small, though imperceptible, increase in Se content beneath surface (at 14–50cm) in Nitosol was observed (Fig. 3.6). As to Pendias and Pendias (2001), this in part could be explained by the pedogenic increase in B–horizon through the fixation by organic matter, Fe and Mn hydroxides, and clay minerals (see Table 3.1 for higher levels of organic carbon and clay in Bt–horizon, 14–50 cm).

Apart from insignificant increases at the surface in Fluvisol (0–20cm, Fig. 3.3), in the second layers of Nitosol (14–50cm, Fig.3.6) and Vertisol (40–84cm, Fig. 3.2), and in layers rich in fine fractions in Solonetz (Table 3.2, Fig. 3.4), nearly uniform distribution of Se contents were observed (see Figs. 3.2–3.6). In the soil profile, the distribution of total Se is governed by the scores of soil factors, namely parent material, organic matter, pH, Fe oxide, and to a certain extent, rain fall (Adriano, 1986; 2001).

Arsenic

The arsenic levels in the soils varied between 0.367 (112–167cm, Fluvisol) to 2.78 mg kg^{-1} (84–118cm, Vertisol). Seen in the light of the average levels of As (5–10

mg kg^{-1}) in uncontaminated soils (Aswathanarayana, 1999) with mean values rarely exceeding 10 mg kg^{-1} (Adriano, 1986) and the range (1–40 mg kg^{-1}) for the natural levels with the lower half being the value for the majority of the soils (O'Neill, 1995), the study soils contained lower amounts of As. In essence, the amount of As in the soil is governed by the levels contained in the source rock (O'Neill, 1995).

Vertisol (3272.42 mg m^{-3}) followed by Nitosol (3033.69 mg m^{-3}) contained higher As contents per unit volume than other soils (Fig. 3.1). As contents revealed significant ($p <$ 0.01) positive relationship with clay and organic carbon fractions, but correlated negatively with pH (Table 3.3). As adsorbs of on clay minerals, oxides and hydroxides of Fe and Al, and organic matter, which in turn, is determined by pH—high at low pH and vice versa (Matera and Le Hecho, 2001). Accordingly, the As levels showed tendency to increase in the B layers of Nitosol (2.77 mg kg^{-1}, Fig 3.6) and Vertisol (2.78mg kg^{-1}, Fig 3.2) in relation to both overlying and underlying layers. Such trend may be the reflection of the downward movement of As on one hand, and strong sorption by clays, and hydroxides (Pendias and Pendias, 2001), on the other. In Fluvisol, on the other hand, the As levels in the top two layers (0–44 cm) were comparatively higher (over two folds) than the underlying layers (below 112 cm). In contrast, As levels in Andosol depicted a tendency to increase with depth (Fig. 3.5). Similar downward movement of As with leaching water is reported by a recent work in coarse textured soils (Adriano, 1986, 2001). The vertical arrangement of As is determined by its behavior that proceeds from the soil oxidation state (Pendias and Pendias, 2001). Aswathanarayana (1999) pointed out that under oxic condition, As in arsenate form $(AsO_4)^{3-}$, is strongly sorbed onto clays, and Fe–Mn oxides/ hydroxides.

Cadmium

Both the minimum (0.1 mg kg^{-1} and maximum (0.26 mg kg^{-1}) Cd concentrations were observed in Solonetz. Although soils derived from different rocks vary in their content of Cd (Adriano, 1986), certain ranges, namely 0.06–1.1 mg kg^{-1} (Alloway, 1995; Pendias and Pendias, 2001), 0.1–1 mg kg^{-1} (Aswathanarayana, 1999), and 0.01–2 mg kg^{-1} (Abollino *et al.,* 2002) are reported as common Cd levels in soils. Apart from these, the

mean abundance of Cd in soil is indicated to be in the order of 0.3 mg kg^{-1} (Adriano, 1986, 2001; Angelone and Bini, 1992). Evidently, the Cd concentrations for all soils under the study were found to be within the aforementioned ranges.

Conversely, Cd revealed significant positive correlations with organic carbon, CEC, and clay fractions (Table 3.3). Slight increases in Cd levels were observed in underlying layers of Solonetz (those layers rich with fine fractions), Vertisol, Andosol, and Nitosol (Fig. 3.2–3.6) while the same phenomenon was found in the those upper layers with relatively high organic carbon content (Tables 3.1 and 3.2). Notwithstanding this, more or less uniform Cd level was found throughout the soil profiles (Figs. 3.2–3.6). This was consistent with the distribution of the same in the soil profile when the concentration is below 1 mg kg^{-1} (Adriano, 1986). The significance of CEC (Adriano, 1986; 2001), organic matter (Alloway, 1995), pH, and $CaCO_3$ (Adriano, 1986; 2001; Alloway, 1995), are well recognized in adsorption of Cd in soil matrix. Besides, Vertisol (231.47 mg) followed by Andosol (171.60 mg), and Fluvisol (161.71 mg) have accommodated high cadmium amounts per cubic meter (Fig. 3.1). Apart from competitive adsorption by clays (McLean and Bledsoe, 1992; Pendias and Pendias, 2001), sorption study (Pendias and Pendias, 2001) revealed that the soil has high affinity for Cd at pH 6 (Tables 3.1 and 3.2 for the pH of Vertisol and Andosol), which is due to increasing surface negative charges with increasing pH (Alloway, 1995).

Cobalt

The total Co levels of the soils varied from 0.11 mg kg^{-1} (148–153 cm, Solonetz) to 17.32 mg kg^{-1} (40–84 cm, Vertisol), and fell within a reported common range of 2–40 mg kg^{-1} (Adriano, 1986). On the other hand, Vertisol (20458.05 mg) followed by Nitosol (18092.12 mg) contained high Co levels per m^{-3} (Fig. 3.1). Consistent with the present study, similar works on Vertisols in Australia, India, Chad, and Central African Republic showed that the soil accommodated high amount of Co (Aubert and Pinta, 1977). The level of Co and its variation in soils largely is governed by the composition of the parent material (Aubert and Pinta, 1977; Smith and Paterson, 1995; and Pendias and Pendias, 2001). Apart from this, the level and distribution of Co in soil profiles are influenced

considerably by the pedological processes (Smith and Paterson, 1995; Pendias and Pendias, 2001). Among soil factors, the status of Co in the soils is determined by texture, pH, and soil moisture content (Sillanpaa, 1972).

The Co contents in the soils revealed positive associations with clay and organic fractions ($p < 0.01$) as well as CEC ($p < 0.05$). The essential association between Co level and amount of clay and organic matter in determining the distribution of Co in soils has also been cited by similar works (Aubert and Pinta, 1977; Smith and Paterson, 1995; Pendias and Pendias, 2001). In addition, earlier reports (Sillanpaa, 1972) indicated that a linear positive association was found between the clay contents and the level of Co in Pakistani soils. Similar to the report by Aubert and Pinta (1977), approximately uniform distribution of Co was found in the profiles. Nevertheless, small increase in Co levels were found in the surface upper layers of Vertisol (17.32 mg kg^{-1}) and underlying layers of Solonetz (4.83 mg kg^{-1}), Fluvisol (3.38 mg kg^{-1}), Nitosol (16.37 mg kg^{-1}), and Andosol (6.76 mg kg^{-1}) (Figs 3.2–3.6). It is found in most soils that the Co distribution follows the spread of clay, Fe and Mn minerals (Pendias and Pendias, 2001). The significant contribution of montmorillonitic clays in governing the aforesaid distribution lies in their higher sorption capacity and the relative ease of release of Co (Pendias and Pendias, 2001).

Chromium

Total chromium in the soils varied between 0.5 mg kg^{-1} (168–173 cm, Fluvisol) and 44.92 mg kg^{-1} (84–118 cm, Vertisol). On the whole, the values for the total Cr of the soils were lower than the reported values for world soils of 200 mg kg^{-1} (Angelone and Bini, 1992), selected average (100 mg kg^{-1}) for soils (McLean and Bledsoe, 1992), a mean range of 100–300 mg kg^{-1} (Aubert and Pinta, 1977), and a world average of 84 mg kg^{-1} (McGrath, 1995). As a rule, the level of Cr in the soil is predominantly determined by the nature of the parent rocks (Aubert and Pinta, 1977; McGrath, 1995). Nonetheless, the pedogenic characteristics (soil types), climatic conditions, and other factors have considerable influence on the total content of chromium in tropical soils (Aubert and Pinta, 1977).

Compared to other soils under this study, Vertisol followed by Nitosol contained higher amount of total Cr in their horizons (40.88–44.92 mg kg^{-1} and 26.85–41.23 mg kg^{-1}) (Figs. 3.2 and 3.6) as well as in a m^{-3} soil (52068.22 mg and 42861.32 mg, Fig. 3.1). Similarly, Vertisols from Madagascar, Chad, and Central African Republic (Aubert and Pinta, 1977) were found to have high Cr content. Saving Nitosol, the vertical distribution of total Cr in the profile exhibited relatively higher concentration in underlying layers (Figs. 3.2–3.6). Comparable trends of Cr accumulations with soil depth are reported for various soils (Adriano, 1986; 2001). Besides, Cr of the soils showed positive associations with clay (strong, $p < 0.01$) and organic fractions ($p < 0.05$), but displayed significant ($p < 0.05$) negative correlations with pH (Table 3.3). In the distribution of Cr in a profile, which usually follows the distribution pattern of humus in the soil, the soil texture also has a strong bearing on the content of chromium in the horizons (Aubert and Pinta, 1977). This is revealed by studies in tropical (Aubert and Pinta, 1977) and some temperate (McGrath, 1995) soils alike, where positive association is found between Cr and clay contents. Conversely, oxidation of Cr^{3+} to Cr^{6+} at pH > 5 (McGrath, 1995) may occur in oxidized subsoil with lower organic matter and comparatively higher Mn oxide (soils with high oxidative capacity) and this, in turn, induces the mobility and leaching of Cr^{6+} (Adriano, 2001).

3.1.3 Relationships among soil types, and between heavy metals and main soil variables

Principal Component Analysis (PCA)

The Fluvisol and Solonetz scored high on the negative PC1 whereas Vertisol was positioned at the highest positive score on the same component (Fig. 3.7). Evidently, there was a trend that spread the scores of the five soils along the PC1. In the score plot, Fluvisol and Solonetz were grouped together due to their exhibited similarity with regard to some of the soil variables. Consequently, PC1 had something related to the soil types. On the other hand, the score plot with respect to the PC2 indicated that Nitosol stood highest and Vertisol the lowest, with the other three soils scattered in between these two. The first and the second principal components described 59% and 19.5% of the variation

in the data, in that order. As can be seen from the loading plot (Fig. 3.8), principal component 1 contrasted between the total heavy metal content (barring mercury), organic carbon, clay content, and CEC in one hand, and the level of sand, silt, carbonate, and pH on the other. Conversely, on the principal component 2, mainly the CEC, Pb, and Se content differentiated the soil types.

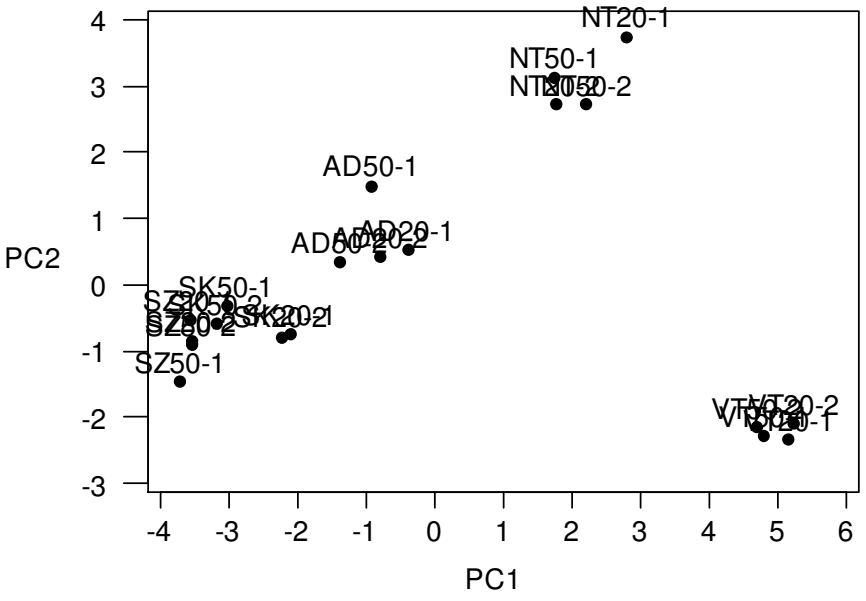

Fig. 3.7 Score plots for the surface (20: 0–20cm) and subsurface (50: 30–50cm) samples from the five sites depicting grouping of the different soil types. SZ = Solonetz, SK= Fluvisol, NT = Nitosol, VT = Vertisol, AD = Andosol.

The total heavy metal content (saving mercury), %clay, %organic carbon, and CEC were parameters with positive loading on the PC1, and hence were located in the right side of the plot (Fig. 3.8). On the other hand, %sand, %silt, %carbonate, and pH were those parameters with the negative loading on PC1. It is therefore lucid that those parameters with large positive and negative loadings contributed strongly to PC1. The comparison of the score plot with the loading plot displayed the underlying reasons for the distribution of the scores by way of the associated parameters. Accordingly, the positive scores on

PC1 of Nitosol and Vertisol could be explained by the corresponding positive loadings of the heavy metal content (except mercury), %clay, and %organic carbon. On the other hand, the grouping of Fluvisol and Solonetz had its roots on the related parameters, namely %carbonate, pH, and %silt. In addition, between Andosol and Fluvisol (and to some extent to Solonetz) certain relation was represented through the parameter %sand. Moreover, the position of Andosol in the score plot corresponded with the variable for the total mercury in the loading plot (Fig. 3.7 and 3.8).

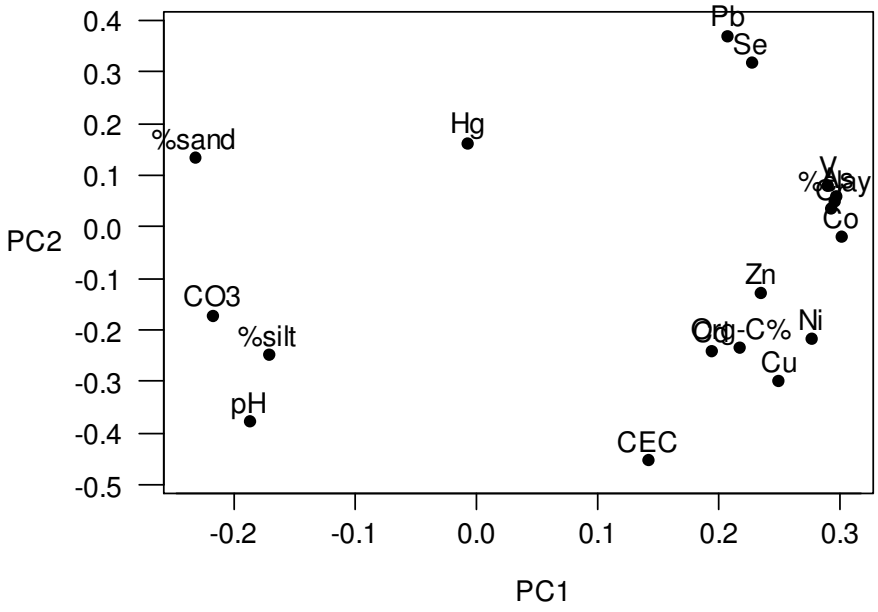

Fig. 3.8 The loading plot of heavy metals, metalloids, and major soil variables contributing to the grouping of soil types revealed in score plots.

On the whole, Vertisol and Nitosol contained higher total heavy metal (Figs. 3.1-3.6, Fig. 3.8) and clay content (Vertisol: 64.2-70.7%, Nitosol: 39.4-56%; Fig. 3.8) than the other soils under the study. However, compared to Nitosol, Vertisol had relatively higher value for the variables organic carbon (Vertisol: 0.23-1.33%, Nitosol: 0.22-0.86%) and CEC

(Vertisol: 45.74-59.19 cmol $_{(+)}$/kg, Nitosol: 9.28-10.35 cmol $_{(+)}$/kg) whereas Nitosol showed higher scores for the total lead, selenium and to certain extent to mercury (Fig. 3.8). As can be seen from the relative positions on the corresponding score and loading plot (Fig. 3.7 and 3.8), Vertisol was considerably low in % sand; but also low value for % carbonate and pH. The Nitosol, on the other hand, displayed lower values for % carbonate, % silt, and pH. Similarly, the diagonal relationship between the scores and the variables also revealed the trend that Fluvisol and Solonetz displayed lower values for the variables of total Pb, Se, V, Co, Cr (Fig. 7), and %clay. Consequently, the PCA markedly laid bare that these soil types were also distinct with respect to their heavy metal contents.

3.1.4 The mineralogical composition of the study soils

The overall intensities of the XRD peaks were relatively low, which thus indicated that a major part of the sample was characterized by X–ray amorphous properties. Despite the very weak intensity of the XRD peaks, certain patterns about the mineralogical distribution could be deciphered.

Solonetz

The X–ray diffractogram of Solonetz, which has developed on dry lacustrine sediments, is presented in Appendices 1–4. The surficial layer (0–20 cm, Appendices 1 and 2) indicated distinct peaks at 3.34 and 4.25 Å that represented quartz. The second most prominent peak at 3.03 Ǻ (Appendix 2), on the other hand, revealed calcite. Besides, potassium feldspar (possibly microcline) and plagioclase were shown with weak (which could have been a reflection of their depletion through chemical weathering) and broad peaks at 3.24 and 3.20 Ǻ, respectively (Appendix 2). Conversely, crushed sample of the surficial layer (0–20 cm) revealed quite strong and distinct peak at 3.20 Ǻ with a shoulder at 3.18 Ǻ (Appendix 1), which could be interpreted as plagioclase feldspar.

On the other hand, in the subsequent layers (20–31 and 31–115 cm, Appendix 2), similar mineralogical distribution was exhibited with quartz (4.25 and 3.34 Ǻ), K–feldspar (3.24 Ǻ), and calcite (3.03 Ǻ). Calcite was found to be relatively conspicuous on the diagram

(Appendix 2). Besides, there is an indication, though apparently small, of other minerals with trace intensity peaks that were masked by peaks of the above-mentioned minerals. One such possibility, though no strong indication on the XRD, is the existence of natron, whose peak might have coincided with calcite. In such case, it can be assumed that there could be high sodium content in this sample. The premise for the existence of natron sounds plausible since the area where the samples were collected was once covered by the big lake and still a soda lake (Lake Abiyata) is found at a few kilometer distances from the sampling point. However, further verification by XRD is required to ensure the presence of natron on the soda–lake samples. By all appearances, there could be a complex environment where calcite and natron coexist. Conversely, in the layer at the depth of 31–115 cm, a peak at 3.22 Å represents plagioclases. Apart from this, the shoulder of a peak at 3.03 Å at this depth could be an indication of the contribution from olivines and pyroxenes. Moreover, the calcite displayed more prominence down to this depth, and in the underlying thin ash layer (115–117 cm) as well (Appendix 3).

The depth below 115 cm was characterized by alternating ash layers and layers rich with fine fraction (< 0.08 mm). Accordingly, the mineralogical composition in the ash layers (115–117, 132–134, 148–153, and 168–173 cm) were dominated by quartz, but calcite and feldspar were found in 115–117 cm and 132–134 cm, respectively (Appendices 3 and 4). On the other hand, largely calcite and quartz, but also feldspars were conceived to be major minerals in those layers with high level of fine fraction (117–132, 134-148, 153-168, and > 173 cm). Apart from this, in the depth range of 117–132 cm (Appendix 3), the peaks at 9.94 Å (mica), and 4.45 Å (probably from disordered kaolinite or halloysite) represented some phyllosilicates.

It is notable that in the layer at 132–134 cm, apparent depletion of calcite and concomitant enhancement of feldspars and traces of phyllosilicates were observed (Appendix 3). In the layer below (134–148 cm), on the other hand, the conceivable change exhibited on the calcite peak, 3.03 Å to 3.00 Å, possibly represents olivines and /or pyroxenes (Appendix 3). Recognizable contrast in mineralogical distributions could be captured from the X–ray diffractogram of the layer at 148–153 cm (Appendix 4). The

XRD of this layer was dominated by peaks from feldspars (e.g., at 3.21 and 6.47 Å), including both microcline and calcium bearing plagioclases. In addition, the peak at 8.46 Å of the same layer could be considered as a clear indication of the presence of amphibole, presumably hornblende. Conversely, the three deepest layers (below 153 cm) displayed (Appendix 4) low content of quartz and had characteristic peaks at 3.00 Å, which could indicate olivines or pyroxenes. Moreover, at these layers the feldspar content was shown to diminish.

Fluvisol

Plagioclase with slightly higher anorthite content (a strong peak at 3.20Å), calcite (at 3.03 Å), quartz (at 4.25 and 3.34 Å), traces of kaolinite or halloysite (at 4.48 Å), and a faint trace of mica (10.4 Å) were detected in the surficial layer (0–20cm) of Fluvisol (Appendix 5). Conversely, plagioclase with high sodium content, oligoclase, with distinct peak at 3.18 Å and a perceptible peak of calcite (3.02 Å) typified the layer at the depth of 20–44 cm. In the subsequent layer (44–112 cm), however, the prominence of calcite was portrayed in the XRD at 3.02 Å. On the other hand, below 112 cm, microcline, plagioclases, and quartz displayed equivalent intensities while that of calcite appeared to be depleted (Appendix 5). Despite the pitfalls of the present method to analyze phyllosilicates, weaker peaks at 14 Å, however, could indicate the occurrence of vermiculite or smectite. In addition, a peak at 4.47 Å indicated kaolinite of low crystallinity.

Nitosol

As it is depicted in Appendix 6, the mineralogical composition of Nitosol was characterized by quartz at 4.25 and 3.34Å as well as feldspars at 6.47, 3.74, 3.24, and 3.20 Å. Apart from this, there was low content of kaolinite depicted by the peaks 7.27, and 4.45 Å. However, the possibility that the peaks could have also originated from halloysite cannot be ruled out. Imperceptible rise neighboring 10 Å may be regarded as a signal for the existence of trace amount of mica. The XRD also exposed that the feldspars in the upper most layer (0–14 cm) were depleted (apparently by chemical weathering) in

comparison to the underlying layers (Appendix 6). Below 14 cm, however, parallel mineralogical distributions were observed.

Vertisol

In Vertisol, peaks from feldspars, potassium feldspar at 3.24 as well as plagioclase at 3.20 Å characterized the mineralogical composition (Appendix 7). A broad and weak peak around 15 Å exhibited the presence of phyllosilicates (Appendix 7). On the other hand, a rather distinct peak at 4.47 Å, which may indicate disordered kaolinite or halloysite, featured the assembly of minerals to the depth of 118 cm. Apart from its similar mineralogical composition to the overlying layers, a distinct peak for calcite at 3.02 Å (Appendix 7) characterizes the horizon below 118 cm. Evidently, to acquire requisite information on this clay rich soil, further analysis on the clay fraction is basic to bring the clay minerals to light.

Andosol

In the main, Andosol contained quartz as a dominant mineral in all of its horizons that was represented by peaks at 4.25 and 3.34 Å (Appendix 8). Besides, K–feldspar (microcline, 3.24 Å) together with plagioclases could be seen (3.21 and 3.19 Å). Moreover, there were traces indicating the existence of disordered kaolinite or halloysite (4.45 Å) and mica or illite (10.0 Å) (Appendix 8). The overall intensities have been relatively low, indicating that a major part of the sample might have X–ray amorphous character. This is often the case when pyroclastic material is involved.

3.1.5 Heavy metal sources of uncontaminated soils

In the whole, most of the minerogenic materials in the study were X–ray amorphous (Appendix 1–8). Besides, the mineralogical composition found in all the soils bore witness that the soils are heavily weathered with only few primary minerals.

With respect to the importance of these minerals as potential sources for heavy metals in the soil, at least calcite is indicated to involve in sorption of Zn and Cd (Doner and Lynn,

1989). Evidently, such attribute could significantly contribute to the comparatively higher contents of zinc and cadmium in those layers with considerable calcite levels (e.g., Solonetz). However, the contribution from calcite appears to be negligible for other soils. Similarly, Huang (1989) pointed out that Pb and Cu may substitute for potassium in K–feldspar whereas Co, Cu, and Pb are often practically present in plagioclases. Albeit the quite small quantity of these trace elements in plagioclases, the ubiquity, and relatively lower resistance to weathering of these minerals could make them these minerals important reserves of the above heavy metals (Huang, 1989). As indicated in Table 1.3, different heavy metals could be associated with primary minerals such as olivines, pyroxenes, and hornblende. Moreover, the phyllosilicates, depending on their levels in a given soil (Appendices 1–8, Table 3.1) appeared to have positively contributed to the status of heavy metals (Figs. 3.1–3.6) by their ability to adsorb cations.

3.2 Heavy metals and metalloids in contaminated Fluvisol and Vertisol (Paper II)

3.2.1 Total heavy metal/metalloid levels and profile distributions

3.2.1.1 Soil properties

The results from the horizon samples of Vertisol showed that the pH, carbonate, and organic carbon largely displayed a decreasing trend with depth (Table 3.4). The clay content and CEC exhibited an opposite trend with increasing values down to 90 cm, followed by descending tendency downwards. In Fluvisol, on the other hand, carbonate content (saving slight increase between 74–129 cms) and organic carbon diminished with depth whereas CEC, %clay and to a considerable extent pH, rendered a propensity to build up with increasing depth (Table 3.4). Likewise, the set–depth samples, on average, displayed corresponding trends depth wise (Table 3.4).

Table 3.4 Selected soil physical and chemical properties of the study soils.

Soil type	Depth (cm)	Org-C (%)	CaCO3 (%)	BS (%)	CEC (cmol+/kg)	pH ($CaCl_2$)	Clay (%)	Silt (%)	Sand (%)
1. Horizon samples									
Vertisol	0–25	1.63	1.75	100	57.94	7.74	57	28	15
	25–90	0.99	0.41	100	61.57	7.76	67	21	12
	90–147	1.15	0.18	100	56.73	7.36	64	22	14
	>147	0.65	0.10	100	47.12	6.83	53	31	16
Fluvisol	0–15	0.54	0.23	100	29.42	6.82	18	14	68
	15–40	1.26	0.14	100	33.58	6.69	26	21	53
	40–74	0.89	0.10	100	29.32	6.74	25	19	56
	74–129	0.62	0.31	100	34.92	7.02	28	23	49
	>129	0.56	0.14	100	37.35	6.85	34	23	43
2. Set-depth samples									
Vertisol	0–20	1.44	0.73	100	58.94	7.25	60	26	14
	30–50	1.25	0.63	100	61.56	7.86	64	25	11
Fluvisol	0–20	0.65	0.42	100	25.83	6.81	14	12	74
	30–50	0.83	0.34	100	27.27	6.8	18	12	70

3.2.1.2 General soil distribution facet of the different heavy metals and metalloids

Of all the heavy metals analyzed in this study, Zn (93.5–138.9 mg kg^{-1}, Vertisol; 85.4–113.9 mg kg^{-1}, Fluvisol), followed by V (78–97.3 mg kg^{-1}, Vertisol; 60.4–114.2 mg kg^{-1}, Fluvisol) had the highest concentrations observed in both sampling schemes (Table 3.5; Figures 3.9 and 3.10). On the other hand, Hg with its concentration stretching between 0.02–0.36 mg kg^{-1} in Vertisol (Table 3.5 and Figure 3.9) and a relatively wider range of 0.03–0.59 mg kg^{-1} in the Fluvisol (Table 3.5 and Figure 3.10) was found to be the least abundant. Overall, the abundance of heavy metals in Vertisol decrease in the order: Zn > V > Cr > Ni > Cu > Co > Pb > As > Se > Cd > Hg while in Fluvisol the sequence follows: Zn > V > Ni > Co > Cr > Pb > Cu > As > Se > Cd > Hg. Thus, despite the difference in the corresponding amounts, the heavy metals in the soils displayed considerable similarity in their relative abundances.

Overall, an apparent similarity was observed in the vertical distribution of As, Pb, Co and to some degree Se and Zn in the soils (Figures 3.9 and 3.10). Comparatively higher

concentrations of Pb, Se, Zn, Cd, and Hg in both soils (Figures 3.9 and 3.10), but also Cr, Cu, Ni, and Co, in Vertisol (Figure 3.9) were observed in top layers of the soils. Conversely, As, Co, and V in both oils (Figures 3.9 and 3.10), as well as Cr, Cu, Ni, and Hg (only 74–129 cm) in Fluvisol (Figure 3.10) were observed to predominate in the underlying layers of the respective soils. Apparently, the organic carbon and clay fraction (Tables 3.4, 3.5 and 3.6) could determine the distribution of the above elements in the soils.

Table 3.5 Mean (n = 3) of the total heavy metal levels (ppm) in surface and sub-surface samples of contaminated and uncontaminated soils.

Soil Type	Depth (cm)	Parameter	As	Cd	Co	Cr	Cu	Ni	Pb	Se	Zn	V	Hg
Vertisol (Contamd.)	0-20	Mean ±sd	2.81±0.14	0.18±0.01	28±0.3	75.9±2.25	37.47±0.53	65.59±0.95	13.97±0.93	1.74±0.11	138.9±7.1	87.36±4.81	0.11±0.02
		CV	4.98	5.55	1.07	2.96	1.41	1.44	6.66	6.32	5.11	5.5	18.18
>>	30-50	Mean ±sd	2.78±0.08	0.16±0.008	27.35±0.85	80.42±2.36	36.29±1.74	69.69±1.51	13.21±0.62	1.83±0.21	135.7±10.9	90.57±2.6	0.09±0.03
		CV	2.88	5	3.11	2.93	4.79	2.17	4.69	11.47	8.03	2.87	33.33
Fluvisol (Contamd.)	0-20	Mean ±sd	3.05±0.87	0.13±0.01	27.44±5.25	23.76±5.28	16.21±3.25	32.68±6.48	24.33±2.93	2..58±0.12	109±8.4	63.53±17.4	0.12±0.12
		CV	28.52	7.69	19.13	22.22	20.05	19.83	12.04	4.65	7.71	27.39	100
>>	30-50	Mean ±sd	3.21±0.44	0.12±0.02	28.07±2.08	28.97±4.12	21..51±3.34	36.66±4	19.49±3.85	2..39±0.38	113.1±1	72.6±12.54	0.08±0.06
		CV	13.71	16.67	7.41	14.22	15.53	10.91	19.75	15.9	0.88	17.27	75
Vertisol (Uncontamd.)	0-20	Mean ±sd	2.68±0.04	0.21±0.01	16.81±0.02	44.76±0.19	24.75±0.12	51.1±1.16	14.49±0.42	1.76±0.004	104±9.9	50.79±0.45	0.01±0.001
		CV	1.49	4.76	0.12	0.42	0.48	2.27	2.9	0..23	9.52	0.89	10
>>	30-50	Mean ±sd	2.43±0.01	0.21±0.003	16.45±0.02	41.37±0.37	23.7±0.16	49.55±1.54	13.77±0.39	1.69±0.02	113.35±7.71	45.74±0.19	BDL[1]
		CV	0.41	1.43	0.12	0.89	0.67	3.11	2.83	1.18	6.8	0.41	
Fluvisol (Uncontamd.)	0-20	Mean ±sd	0.91±0.02	0.15±0.008	2.96±0.03	5.73±0.07	5.65±0.07	5.6±0.09	3.91±0.11	1.00±0.05	51±0.57	9.28±0.04	BDL[1]
		CV	2..2	5.33	1.01	1.22	1.24	1.61	2.81	5	1.11	0.43	
>>	30-50	Mean ±sd	0.73±0.1	0.14±0.004	2.84±0.02	5.47±0.08	4.97±0.1	5.6±0.04	3.7±0.07	1.07±0.09	42.95±0.5	9.22±0.04	0.01±0.001
		CV	13.7	2.86	0.7	1.46	2.01	0.71	1.89	8.41	1.16	0.43	10

Contamd and Uncontamd represent soils irrigated with wastewater and rainfed, in that order. [1] BDL stands for concentrations below detection limits.

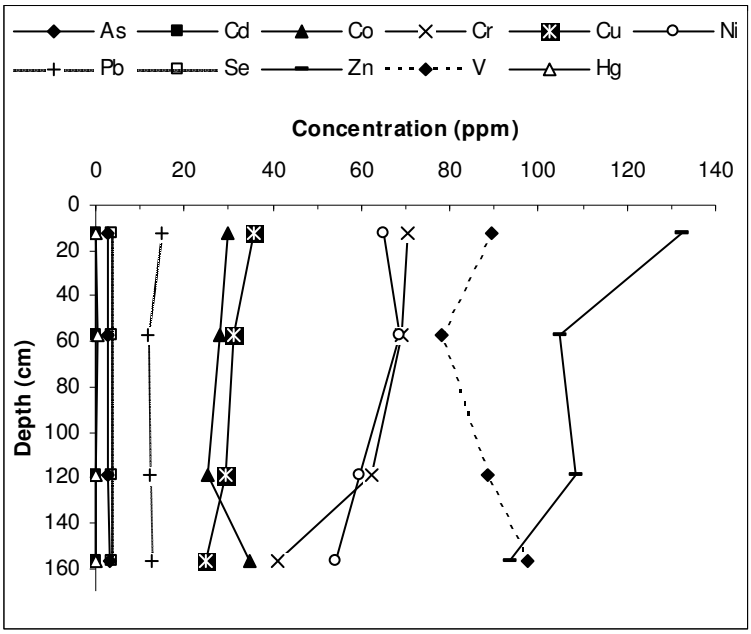

Fig. 3.9 Profile distribution of heavy metals/metalloids in contaminated Vertisol, Akaki.

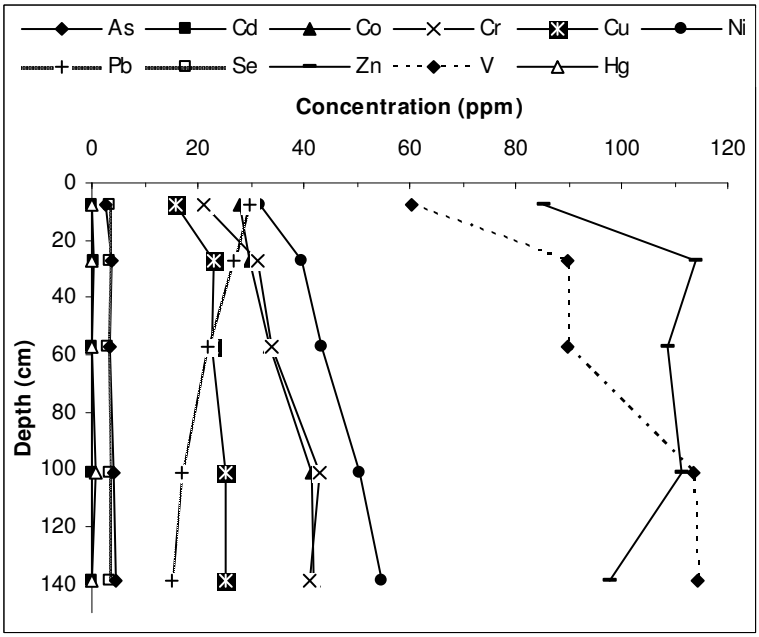

Fig. 3.10 Vertical distribution of heavy metals/metalloids in contaminated Fluvisol, Akaki.

Over and above, in depth–specific (0–20 and 30–50 cm) samples of Vertisol, the depth appeared to have significant effect on the average total level of Ni, Cr, and Cd ($P < 0.05$). In contrast, no significant differences in the mean contents of other heavy metals in Vertisol as well as for all heavy metals in Fluvisol were found for the above depths. Evidently, the mean levels of heavy metals in the study soils are more or less unaffected by the set–depth. Conversely, when the same element from the two soils is compared, the majority of the elements were found to have significant differences at both 0–20 cm ($p < 0.001$ for Cr, Cu, Ni; $p < 0.01$ for Pb, Se, Zn, and V; $p < 0.05$ for Cd) and 30–50 cm ($p < 0.001$ for Cr, Ni; $p < 0.01$ for Cu; and $p < 0.05$ for Cd and Zn) depths. When the average of the selfsame depths is considered, Vertisol accommodated comparatively higher levels of Cr, Cu, Ni, Zn, V, and Cd, while high contents of Pb and Se were observed in Fluvisol. Alternatively, comparable levels of Co and Hg were found in either soil. By all appearances, given the same type of waste water application, the significance differences in the total contents of the above elements of the two soils, at least partly, could reflect the associated differences in the corresponding soil properties (see Table 3.4).

3.2.1.3 Heavy metal/metalloid levels and association with the soil properties

Based on the geochemical behavior indicated in the classification by Goldschmidt (White, 2005), the heavy metals in this study were grouped as Lithophiles (Cr and V), Siderophiles (Co and Ni), and Chalcophiles (As, Cd, Cu, Pb, Se, Zn and Hg).

Lithophiles

Although the average levels of Cr in studied soils (Table 3.5) were below the mean levels indicated for soils of 100–300 mg kg^{-1} (Aubert and Pinta, 1977), Cr values of Vertisol were twice the reported normal average concentration for soils of 40 mg kg^{-1} (Adriano, 2001) or higher than the global average of 54 mg kg^{-1} (Pendias and Pendias, 2001). On the other hand, the average V contents of the soils (63.53–90.57 mg kg^{-1}) were in the upper range of 18–115 mg kg^{-1} (Pendias and Pendias, 2001) reported as the worldwide mean V contents of soils. On the other hand, the average Cr and V concentrations of the

soils were exceedingly higher than the corresponding levels reported for similar but uncontaminated soils at another sites in Ethiopia (Table 3.5).

Strong positive relationships ($p < 0.05$) were observed between Lithophiles (Cr and V) and clay content in Vertisol, and with organic carbon and CEC in Fluvisol (Tables 3.6 and 3.7). It is affirmed that organic matter and clays are key soil attributes regulating the status of Lithophiles in soils (Adriano, 2001; Pendias and Pendias, 2001). Additionally, the oxidation state of Cr, redox potential, and oxides of Fe and Mn, for the major part, determine the mobility, solubility, transformation, speciation, and uptake of Cr in soils (Adriano, 2001).

Siderophiles

The mean soil values of Siderophiles calculated to a set–depth of 50 cm (Co: about 28 mg kg^{-1}, both soils; Ni: 65.59–69.69 mg kg^{-1}, Vertisol and 32.68–36.66 mg kg^{-1}, Fluvisol) of the present study (Table 3.5) markedly surpassed the world average soil contents for Co (10–15 mg kg^{-1}: Smith and Paterson, 1995; Adriano, 2001) and Ni (20 mg kg^{-1}: Angelone and Bini, 1992; McGrath, 1995; Adriano, 2001; 22 mg kg^{-1}: Pendias and Pendias, 2001). Likewise, the average siderophile contents of the contaminated Vertisol and Fluvisol (Table 3.5) were considerably higher than the same observed for similar but uncontaminated soils (Co: 16.75 and 3.12 for Vertisol and Fluvisol, in that order; Ni: 48.75 and 6.02 for Vertisol and Fluvisol, respectively. See Figs. 3.2–3.6).

Table 3.6 Correlation coefficients for total heavy metal levels and selected soil parameters of Vertisol (depth-specific samples).

	As	Cd	Co	Cr	Cu	Ni	Pb	Se	Zn	V	Hg	pH	%$CaCO_3$	%Org-C	%Clay	%Silt	%Sand
pH	-0.802*	-0.874*	-0.471	0.059	-0.508	0.338	-0.888**	0.615	-0.580	-0.397	0.033						
%$CaCO_3$	-0.684	-0.095	0.124	-0.724	0.012	-0.463	-0.389	0.544	-0.341	-0.826*	0.784*	0.476					
%Org-C	0.935**	0.868*	0.141	0.022	0.742*	-0.326	0.987**	-0.774*	0.852*	0.547	0.071	-0.912**	-0.522				
%Clay	0.362	-0.283	-0.356	0.830*	-0.164	0.816*	0.016	-0.015	0.207	0.795*	-0.351	0.092	-0.502	0.096			
%Silt	-0.497	0.245	0.656	-0.823*	-0.068	-0.650	-0.143	0.365	-0.432	-0.843*	0.207	-0.064	0.467	-0.183	-0.867*		
%Sand	-0.276	0.276	0.202	-0.768*	0.249	-0.821*	0.039	-0.133	-0.096	-0.713	0.384	-0.097	0.477	-0.052	-0.978**	0.743*	
CEC	-0.708	-0.970*	-0.500	0.316	-0.650	0.567	-0.907**	0.565	-0.612	-0.188	-0.206	0.961**	0.257	-0.895**	0.262	-0.224	-0.258

Table 3.7 Correlation coefficients for total heavy metal levels and selected soil parameters of Fluvisol (depth-specific samples).

	As	Cd	Co	Cr	Cu	Ni	Pb	Se	Zn	V	Hg	pH	%$CaCO_3$	%Org-C	%Clay	%Silt	%Sand
pH	-0.303	0.258	-0.345	-0.133	-0.038	-0.189	-0.227	-0.422	-0.126	-0.102	0.864*						
%$CaCO_3$	-0.697	-0.210	-0.638	-0.769*	-0.769*	-0.716	-0.215	-0.213	-0.506	-0.649	0.867*	0.568					
%Org-C	0.967**	0.333	0.962**	0.942**	0.856*	0.995**	0.170	-0.714	0.574	0.987**	-0.599	-0.251	-0.708				
%Clay	0.324	-0.190	0.207	0.695	0.808*	0.511	-0.284	-0.435	0.212	0.428	-0.457	-0.104	-0.709	0.441			
%Silt	-0.252	-0.812*	-0.273	-0.226	-0.258	-0.289	-0.127	0.402	-0.566	-0.403	-0.393	-0.600	-0.022	-0.268	0.146		
%Sand	-0.151	0.529	-0.045	-0.469	-0.547	-0.288	0.292	0.174	0.085	-0.167	0.556	0.361	0.594	-0.240	-0.891**	-0.580	
CEC	0.901**	0.487	0.886**	0.898**	0.830*	0.948**	0.157	-0.864*	0.542	0.977**	-0.305	0.092	-0.506	0.938**	0.349	-0.517	-0.050

*** and * denote significant correlations at the 0.01and 0.05 levels, in that order.*

Siderophiles in the Fluvisol showed strong positive associations ($p < 0.01$) with organic carbon, and CEC but exhibited weak to moderately positive relations with clay (Table 3.7). In the case of Vertisol, on the other hand, only Ni revealed significant ($p < 0.05$) association with the clay (Table 3.6). A number of factors influence the status of Siderophiles in soils. Among these, pH, texture (Sillanpaa, 1972; Adriano, 2001), organic matter (Smith and Paterson, 1995; Adriano, 2001), and hydrous oxides of Fe and Mn (McGrath, 1995; Adriano, 2001) are indicated to have a bearing on the soil Siderophile levels. Likewise, moisture content (Sillanpaa, 1972) influences Co levels in soils, while clay content (McGrath, 1995), and CEC (Adriano, 2001) play important role in the retention of Ni.

Chalcophiles

The concentration of As, Cd, Cu (Table 3.5) were lower than the reported average soil contents of As (10 mg kg^{-1}: Adriano, 2001), Cd (0.06–1.1mg kg^{-1}: Alloway, 1995; Pendias and Pendias, 2001), and Cu (30 mg kg^{-1}: Baker and Senft, 1995; Adriano, 2001). On the other hand, the soils had comparatively higher levels of Pb, Se, Zn, and Hg than indicated for Pb (10mg kg^{-1}: Angelone and Bini, 1992; McLean and Bledsoe, 1992), Se (0.3 mg kg^{-1}: McLean and Bledsoe, 1992; 0.4 mg kg^{-1}: Adriano, 2001), Zn (50 mg kg^{-1}: Angelone and Bini, 1992; McLean and Bledsoe, 1992; Kiekens, 1995; Abollino *et al.,* 2002; Adriano, 1986; 2001), and Hg (0.03 mg kg^{-1}: McLean and Bledsoe, 1992; Steinnes, 1995). Apart from this, the mean Cu levels of contaminated Vertisol (37 mg kg^{-1}) were comparatively higher than 20mg kg^{-1} and 13–24mg kg^{-1} reported by Aubert and Pinta (1977) and Pendias and Pendias (2001), in that order.

All the Chalcophiles, saving Se and Hg (which somewhat associated positively with pH and / or carbonate levels) exhibited a marked positive correlation with the organic carbon contents of the study soils. It is notable, however, that the positive relationships between the same were more prominent in Vertisol than Fluvisol (Tables 3.6 and 3.7). In addition, as influenced possibly by organic carbon contents (and / or with pH), CEC associated negatively (Vertisol) or positively (Fluvisol) with the heavy metals under this category. Apparently, mainly organic carbon, but also CEC could have an impact on the total levels

of most Chalcophiles in the study soils. It has been indicated that organic matter is important in determining the status of As (Pendias and Pendias, 2001), Cd, Cu (Adriano, 2001), Pb, and Zn (Adriano, 1986; 2001) in soils. CEC, on the other hand, is reported to have influence on the sorption of Cd, Cu (Adriano, 2001), and Se (Pezzarossa and Petruzzelli, 2001). Moreover, adsorption of Se is governed by the pH (Adriano, 2001, Pezzarossa and Petruzzelli, 2001) and $CaCO_3$ (Pezzarossa and Petruzzelli, 2001), while particle size distribution, redox potential, pH (Steinnes, 1995; Adriano, 2001), and soil type (Adriano, 2001) are of paramount significance in the retention of Hg. Over and above, the content and distribution of Zn (Adriano 1986; 2001), and As (Pendias and Pendias, 2001) draw a parallel with clay content of soils.

3.2.2 Relative proportion (percentage) of heavy metal association with the soil fractions

Variations in distribution of heavy metals among soil components, for the most part, are attributable to the chemical properties of heavy metals and characteristic features of the soil (Ma and Rao, 1997; Olajire *et al.,* 2003). Despite the difference in the origin of heavy metals (geogenic, pedogenic, or anthropogenic), assessment of the availability or lability of heavy metals could reflect their mobility, uptake by plants, and extractability by chemical treatments (McBride, 1994). In this regard, the sequential extraction is instrumental in the assessment of potential mobility and bioavailability of heavy metals in soils. Acquisition of such information is vital in deciphering the chemical behavior and fate of heavy metals in soils, and for establishing environmental guidelines of their potential hazards / toxicities (Ma and Rao, 1997; Olajire *et al.,* 2003).

In general, the sequential extraction procedure provides operationally defined species or fractions characterized by decreasing solubility. Based on the premise that bioavailability is associated to solubility (Ma and Rao, 1997; Olajire *et al.,* 2003), heavy metals in this study are expected to display lability which decrease in the order: exchangeable > carbonate > reducible > oxidizable > residual. Likewise, heavy metals in non-residual fractions (exchangeable + carbonate + reducible + oxidizable) could further be postulated to be more bioavailable than those in the residual fraction (Lu *et al.,* 2003).

Arsenic

In this study, due to analytical intricacy connected with determining the intensely colored alkaline extract with the Flame Atom–Absorption method (Ladonin, 2002), no data were generated for As, Se, V, and Hg from the oxidizable fractions. Irrespective of this, As exhibited marked tendency to associate with reducible fraction, followed by residual and carbonate fractions. The relative proportion of As in exchangeable fraction was < 0.3 % of total As for both soils.

The reducible fraction, on average to 50 cm, accounted for 67 % (Vertisol), and 54.81 % (Fluvisol) of total As (Table 3.9). In soils and sediments, As is known for its noticeable affinity to oxidic surfaces (Adriano, 2001). Consequently, despite variations among soils, As (V) and As (III) sorption appears to be related to the oxide content of soils (Adriano, 2001; Pendias and Pendias, 2001). Evidently, consistent with these reports, the reducible fractions have strongly represented their capacity to bind As. On the other hand, considerable portions of total As were retained in the residual fractions (Table 3.9).

The non–residual fractions, on the average, accounted for 74.6 %, and 60.8 %, of the total As, in Vertisol and Fluvisol, in that order. Apparently, As is expected to present a relatively higher mobility and bioavailability, with Vertisol slightly higher than Fluvisol.

Cadmium

The largest proportion of the total Cd was associated with carbonate and residual fractions (Table 3.9). The average Cd in carbonate fraction was 48 % in Vertisol, and 49 % in Fluvisol. Likewise, this phase correlated well with total Cd (Table 3.8). It would seem that the carbonate fraction of the study soils has been an important geochemical phase, which could considerably determine the behavior of Cd. Rule (1998) stated that the carbonate phase could be an important sink for trace metals at circumneutral pH. Apart from this, Alloway (1995) pointed out that at low Cd levels (below 1µmol/g), calcite manifested a marked tendency to sorb Cd.

The residual fraction, on the other hand, accommodated about 30 % of the total Cd in the study soils. As a rule, the percentages of metals in residual fraction are higher in native or uncontaminated soils than in soils contaminated with soluble forms of contaminants (Rule, 1998). Nonetheless, a study of the influence of sewage sludge application on the phase distribution of metals showed only small deviation in the proportion of Cd in the residual fraction (Rule, 1998).

A traceable percentage of total Cd was extracted from reducible and oxidizable fractions (Table 3.9). The Cd in the former phase approximates the moderate proportion of total Cd associated with reducible fraction for both contaminated and uncontaminated soils reported by Rule (1998). Conversely, as Cd may not form stable organic complexes (Olajire *et al.,* 2003); the association of Cd with organic matter is commonly low (Rule, 1998).

In the studied soils, non–residual Cd fractions made up a substantial proportion of the total Cd. Among the non–residual fractions, the sum of exchangeable and carbonate fractions (nearly half of the total Cd of the soils) could further portray the extent of bioavailability of Cd. Adriano (2001) stated that Cd uptake by cabbage plants was perceptibly regulated by the level of Cd associated with the exchangeable and carbonate fractions rather than the total Cd content in the soil. In general, the contribution of different forms of Cd decreases in the sequence: carbonate > residual > reducible > oxidizable > exchangeable. Saving the order of Cd association in the first two fractions i.e. residual > carbonate, comparable Cd abundance was reported for contaminated (sludge treated) soils (Rule, 1998).

Table 3.8 Correlation coefficients between concentration of heavy metals in different fractions and total heavy metal contents.

*** and * represent significant correlations at the 0.01and 0.05 levels, correspondingly.*

Heavy metal	Fraction of heavy metal	Vertisol	Fluvisol
As	Exchangeable	-0.433	0.726
	Carbonate	0.756*	0.658
	Reducible	0.509	0.514
	Residual	0.851*	0.995**
Cd	Exchangeable	0.899**	-0.144
	Carbonate	0.843*	0.868*
	Reducible	0.492	0.295
	Oxidizable	0.58	0.068
	Residual	-0.083	0.644
Co	Exchangeable	0.454	0.04
	Carbonate	0.619	0.793*
	Reducible	-0.16	0.261
	Residual	0.028	0.427
Cr	Carbonate	-0.54	0.8*
	Reducible	-0.513	0.365
	Oxidizable	-0.447	0.644
	Residual	0.797*	0.89 **
Cu	Exchangeable	0.522	0.481
	Carbonate	0.897**	0.953 **
	Reducible	0.573	-0.158
	Oxidizable	-0.384	0.588
	Residual	0.033	0.875 *
Ni	Exchangeable	-0.532	-0.053
	Carbonate	0.237	0.949**
	Reducible	-0.511	-0.071
	Oxidizable	-0.78	0.914**
	Residual	0.86*	0.747*
Pb	Exchangeable	0.16	-0.112
	Carbonate	0.855*	0.914**
	Reducible	0.675	0.813
	Oxidizable	-0.121	-0.135
	Residual	0.229	0.169
Se	Exchangeable	-0.237	0.413
	Carbonate	-0.366	-0.611
	Reducible	0.266	-0.281
	Residual	0.985**	0.989**
Zn	Exchangeable	0.913**	-0.009
	Carbonate	0.638	-0.297
	Reducible	0.834*	-0.209

	Oxidizable	0.54	0.711
Heavy metal	**Fraction of heavy metal**	**Vertisol**	**Fluvisol**
	Residual	-0.318	0.447
V	Exchangeable	-0.055	0.615
	Carbonate	-0.363	0.623
	Reducible	0.924**	0.5
	Residual	0.94**	0.986**
Hg	Exchangeable	0.336	-0.094
	Carbonate	-0.832	0.417
	Reducible	0.128	-0.057
	Residual	0.765*	0.969**

Table 3.9 Percent of the mean (n = 3) partitioning and standard deviation of heavy metals in soil fractions of Vertisol and Fluvisol, Akaki.

Soil type	Fraction	Depth(cm)	As	Cd	Co	Cr	Cu	Ni	Pb	Se	Zn	V	Hg
Vertisol	Exchangeable	0-20	0.19±0.04	1.1±0.3	0.0	0.0	0.2±0.1	0.1±0.01	0.0	1.0±0.6	0.02±0.01	0.1±0.0	2.5±2.2
Vertisol	Exchangeable	30-50	0.16±0.04	0.4±0.3	0.0	0.0	0.17±0.1	0.1±0.02	0.0	0.8±0.5	0.02±0.02	0.1±0.01	7.1±4.2
Fluvisol	Exchangeable	0-20	0.1±0.01	0.6±0.1	0.01±0.01	0.0	0.0	0.2±0.03	0.0	0.3±0.2	0.01±0.01	0.02±0.01	3.4±1.2
Fluvisol	Exchangeable	30-50	0.12±0.03	1.0±0.9	0.0	0.0	0.0	0.03±0.03	0.0	0.0	0.01±0.02	0.03±0.01	14.1±35.9
Vertisol	Carbonate bound	0-20	7.7±0.36	49.5±0.9	47.7±1.0	0.6±0.02	30.4±0.8	23.2±1.4	34.0±1.2	20.5±2.6	8.3±0.02	21.0±0.9	0.4±0.4
Vertisol	Carbonate bound	30-50	6.9±0.34	46.6±5.9	46.4±6.1	0.5±0.1	27.1±2.3	23.1±3.1	29.0±2.8	19.2±2.7	6.8±1.2	18.2±2.0	2.2±3.7
Fluvisol	Carbonate bound	0-20	5.9±1.54	46.8±1.3	39.6±5.1	0.6±0.12	29.6±4.18	22.6±1.9	54.8±4.0	8.4±1.2	17.9±2.4	10.5±1.2	1.0±1.7
Fluvisol	Carbonate bound	30-50	5.8±0.4	51.5±1.7	39.4±2.1	0.6±0.04	28.9±0.7	21.9±0.5	46.6±6.2	9.3±2.0	16.8±3.7	10.1±1.1	1.0±2.5
Vertisol	Reducible	0-20	63.8±5.3	10.8±0.7	31.6±0.1	20.1±1.1	31.3±0.4	21.3±0.8	52.0±2.8	14.5±0.8	23.6±5.5	42.1±3.4	33.7±21.8
Vertisol	Reducible	30-50	70.4±4.2	11.9±3.6	34.5±5.7	18.2±2.4	32.8±4.0	19.3±2.7	53.4±2.2	16.3±2.1	22.1±7.2	43.5±3.0	1.2±3.6
Fluvisol	Reducible	0-20	57.6±15.5	19.4±1.9	47.0±2.7	31.6±9.4	34.9±4.0	30.9±3.7	39.9±3.3	11.4±2.5	40.2±5.7	50.4±10.6	20.2±42.7
Fluvisol	Reducible	30-50	52.0±7.8	6.7±6.5	37.2±15.8	31.0±4.8	23.8±12.2	21.6±5.5	40.9±6.1	12.0±2.6	25.8±17.3	41.8±6.9	1.4±3.3
Vertisol	Oxidizable	0-20	ND[1]	7.4±1.6	BDL[2]	22.2±4.8	14.3±3.4	13.4±1.2	6.4±1.5	ND[1]	19.7±2.3	ND[1]	ND[1]
Vertisol	Oxidizable	30-50	ND[1]	10.8±1.6	BDL[2]	20.5±5.9	15.9±4.7	11.9±1.1	6.4±0.8	ND[1]	18.8±1.3	ND[1]	ND[1]
Fluvisol	Oxidizable	0-20	ND[1]	6.5±1.4	BDL[2]	28.6±7.5	15.1±2.8	17.3±2.9	2.7±0.6	ND[1]	10.7±2.9	ND[1]	ND[1]
Fluvisol	Oxidizable	30-50	ND[1]	7.6±3.3	BDL[2]	26.6±4.9	13.6±3.1	19.5±4.5	4.1±1.9	ND[1]	12.7±3.7	ND[1]	ND[1]
Vertisol	Residual	0-20	28.3±4.9	31.4±1.6	20.6±1.1	57.2±6.0	23.7±3.1	42.0±1.0	7.6±3.1	63.9±3.9	49.1±3.2	36.8±4.4	63.3±24.4
Vertisol	Residual	30-50	22.6±4.4	30.4±8.6	19.1±11.1	60.8±8.4	24.0±8.9	45.7±6.2	11.0±4.9	63.7±4.5	52.3±9.4	38.1±4.6	89.4±4.2
Fluvisol	Residual	0-20	36.4±17.0	26.8±1.96	13.3±7.8	39.2±14.8	20.4±10.6	29.1±3.9	2.6±0.2	80.0±3.4	31.1±5.4	39.0±11.8	75.3±41.1
Fluvisol	Residual	30-50	42.0±7.8	33.2±8.7	23.3±13.8	41.8±7.4	33.5±15.1	36.9±6.4	8.3±6.6	78.8±4.6	44.7±16.3	48.1±7.0	83.5±34.3

[1]ND = Not determined; [2] BDL = below detection limit; 0.0 = below 0.01 %

Cobalt

Co was mainly associated with carbonate (47 %, Vertisol and 39 %, Fluvisol) and reducible fractions (avg. ca 33 %, Vertisol and 42 %, Fluvisol). Conversely, Co was not detected (below 10 ppb) in the organic fraction. However, considerable amount of total Co resides in the residual fraction, which might also include certain amount Co from organic fraction.

At the circumneutral pH, the carbonate phase serves as a sink to trace metals (Rule, 1998). As shown in Table 3.8, the carbonate fractions showed positive associations with total Co levels. The sorption capacity of Co in soils was reported to reveal high correlation with Co status (Adriano, 2001).

The observed proportion of total Co in reducible fraction could be attributable to the chemisorption of Co on oxidic surfaces. The accumulation of Co in hydrous oxides of Fe and Mn of soils was reported (Adriano, 2001). The notable affinity exhibited by Fe oxides for selective adsorption of Co could account for their association with Co (Pendias and Pendias, 2001). In spite of that, in certain soils enriched with Mn minerals, the association of Co with Mn would display a domineering effect over other factors, which determine Co distribution (Pendias and Pendias, 2001). In view of that, Mn oxides are fundamental in the chemistry of soil Co (Adriano, 2001).

The exchangeable fraction constituted very low proportion (< 0.01 %) of the total Co. Notwithstanding, the levels of Co associated with the non–residual fractions (primarily, carbonate + reducible) were substantial.

Chromium

Most of the chromium was associated with residual, oxidizable, and reducible geochemical fractions of the studied soils (Table 3.9). However, the soils exhibited palpable differences in the distribution of total Cr in the aforesaid fractions. On the other hand, no detectable amount was extracted from the exchangeable phases, while very low (< 0.6 %) proportion of total Cr was associated with carbonate fractions (Table 3.8). Apparently, such low proportion of Cr in the exchangeable and carbonate fractions could suggest the existence of Cr–bearing minerals.

Redox status, presence of electron donor or acceptors, oxidation state, pH, soil minerals, competing ions, complexing agents and similar others are conceived to govern the behavior of Cr in soils (Adriano, 2001). The pH–influenced adsorption of Cr by organic matter and Fe, Mn, Al oxides (Adriano, 2001) could account for the observed associations of Cr with the corresponding fractions (see Tables 3.8 and 3.9). The strong positive relationship found between the residual fractions and total Cr levels (Table 3.8) may reflect the importance of natural inputs to the measure of Cr in the soils.

Similar studies revealed that Fe–Mn oxides (Stalikas *et al.,* 1999), or Fe–oxides (Ahumuda, 1999) and oxidizable fraction (Ahumuda, 1999; and Stalikas *et al.,* 1999), were the principal fractions with higher proportions of total Cr. In agreement with the present study, the same authors also indicated that the proportions of total Cr extracted from exchangeable and carbonate fractions were relatively low. The association of Cr with the different geochemical fractions or phases, as a result, followed the order of residual > oxidizable, reducible >> carbonate > exchangeable.

Copper

The bulk of total Cu was associated with non–residual fractions (Table 3.9). The greater part of the non–residual fractions constituted Cu extracted from reducible (average, 31.5 %, Vertisol; 29.4 %, Fluvisol) and carbonate (averaging, 28.8 %, Vertisol; and 29.3 %, Fluvisol) fractions. On the other hand, the Cu in oxidizable fraction (about 15 % of the total Cu of the study soils), was also important. Conversely, the residual fraction accounted for nearly a quarter of the total Cu in these soils (Table 3.9). In the whole, the average percentage of Cu association with different geochemical fractions in the study soils was in the order of reducible > carbonate > residual > oxidizable >> exchangeable. Similar trend in the order of Cu abundance associated with these phases was reported for contaminated soils (Rule, 1998).

It has been indicated that the organic matter, oxides of S, Fe and Mn (Adriano, 2001), clay minerals (montmorillonite, vermiculite, imogolite), phosphates, (Pendias, and Pendias, 2001), and carbonates (Adriano, 2001; Pendias and Pendias, 2001) govern

adsorption of Cu in soils. Of these, the distribution of Cu among soil constituents is largely influenced by organic matter and oxides of Fe and Mn (Adriano, 2001).

Despite the comparatively low level of the organic carbon levels observed (Table 3.4), considerable proportion of total Cu were found associated with the oxidizable phase (Table 3.9). Moreover, organically bound Cu is more phytoavailable than Cu associated with the inorganic precipitates and residual fraction. Conversely, apart from Pb, Cu is the most strongly adsorbed of all transition metals on Fe and Al oxides, and oxyhydroxides (Adriano, 2001). Consequently, consistent with the present study, Cu associated with Fe and Mn oxides could comprise the highest fractions of the total Cu in soils (Adriano, 2001).

At neutral to alkaline pH, the dominant hydrolysis product ($Cu(OH)_2$) (Adriano, 2001), may be extracted as hydroxide phase by the reagents of carbonate fraction (Rule, 1998). Apparently, such account could underlie the observed contribution of the carbonate phase in sequestering substantial percentage of total Cu (Tables 3.8 and 3.9).

As with the other heavy metals, the Cu in exchangeable fraction was pretty small, < 0.5 %. This could be attributed to the important influence of pH towards specific adsorption of Cu as ions or complexes above certain pH (e.g., 5.5). Rule (1998) stated that the Cu in exchangeable was often below 1%, with the range of 0.2–1 % for nine contaminated soils.

Nickel

As a rule, Ni, resembling Cr, displayed a general tendency to associate more to the residual fraction (Table 3.9). These results are consistent with observations of McGrath (1995), Adriano (2001) and Olajire et al. (2003 and references therein). The residual fraction exhibited strong positive correlation with the total Ni (Table 3.8) and clay contents (r = 0.895) of the soils. Adriano (2001) expressed that Ni level in various soil fractions show a propensity to increase with decreasing particle size.

The reducible, carbonate and oxidizable fractions were important phases for Ni (Table 3.9). In agreement with the present study (Table 3.9), other reports elucidated that the

reducible fraction accommodated about 20 % of total Ni (McGrath, 1995; and Adriano, 2001). Moreover, Ni is indicated to have association with Fe and Mn oxides, organic matter (McLean and Bledsoe, 1992; and Pendias and Pendias, 2001), carbonates (Pendias and Pendias, 2001), and clay minerals (McLean and Bledsoe, 1992). Accordingly, Ni could be present at specific adsorption sites, adsorbed or occluded into the sesquioxides, fixed within the clay lattice, or bound in organic residues and microorganisms while it exists in complexed form with organic and inorganic ligands (Adriano, 2001).

The distribution of the Ni in different soil phases in Vertisol was in the order of residual >> carbonate > reducible > oxidizable >> exchangeable. A corresponding order for Fluvisol, on the other hand, was in the following order: residual > reducible > carbonate > oxidizable >> exchangeable. Similar sequences were observed for the abundance of Ni in the same geochemical phases of contaminated (sludge–treated) soils (Rule, 1998). Similar with other heavy metals, the Ni in the exchangeable fraction was extremely low.

Of non-residual fractions, Ni associated with the reducible phase appears to be in the most available form for plants (Pendias, Pendias, 2001). Apparently, this may enhance the potential availability of Ni in the non–residual fractions.

Lead

It is notable that non–residual fractions contained large proportions of total Pb (Table 3.9). Similar observation of Pb association with these fractions (Olajire *et al.,* 2003) is reported. The reducible and carbonate fractions contributed to the bulk of these fractions, while only < 7 % of the total Pb was the share of the oxidizable fraction. Likewise, proportions of Pb from the organic fraction were observed to be the most minuscule (Olajire *et al.,* 2003). The reducible and carbonate fractions also revealed strong relations with total Pb (Table 3.8).

Pb associates with carbonates, Fe and Al hydroxides, Mn oxides clay minerals, organic matter (McLean and Bledsoe, 1992; Pendias and Pendias, 2001), and Mn oxides (Pendias and Pendias, 2001). On the other hand, at pH values above 6, Pb either could be adsorbed on clay surfaces or may possibly form lead carbonate

(McLean and Bledsoe, 1992). As with the latter case, Pb may be concentrated in calcium carbonate particles (Pendias and Pendias, 2001). Conversely, the negligible exchangeable Pb may intimate the important influence of pH in determining the nature of soil reactions, which, in turn determine the bioavailability of Pb.

The distributions of total Pb over the different geochemical phases appeared to have been affected by inherent features of the soil properties. Accordingly, the order of the dominant fractions of Pb in Vertisol was reducible > carbonate >> residual > oxidizable >> exchangeable, while the sequence for Fluvisol followed: carbonate > reducible >> residual > oxidizable >> exchangeable.

Despite the moderate levels of total Pb in the study soils, the potential bioavailability of Pb (as reflected from the proportion of non–residual Pb, with considerable contribution from the carbonate phase) may be of interest environmentally.

Selenium

The proportion of total Se contained in residual fraction (but actually, residual + organic fractions) accounted for the major portion of the total Se (Table 3.9), and the same fractions correlated well with total Se (Table 3.8). On the other hand, the proportions of total Se associated with reducible (average, 15.4 %, Vertisol; 12 %, Fluvisol) and carbonate (average, 20 %, Vertisol; 9 %, Fluvisol) fractions were important.

In soils, the level and chemical form of Se is regulated by pH, redox status, and soil composition (McLean and Bledsoe, 1992). Moreover, these and other plant–related factors also determine the solubility and eventually the bioavailability of Se in soils and sediments (Adriano, 2001). Selenite (Se^{4+}) is suggested to be a preferred Se species for sorption in a wide range of pH than selenate (Se^{6+}) (Pezzarossa and Petruzzelli, 2001). Clay minerals, particularly montmorillonite, as well as Fe oxide preferentially adsorb selenites (Pendias and Pendias, 2001). Besides, H_2SeO_3 is a weak acid, and its salt are less soluble than the more strong acid H_2SeO_4 (Pezzarossa and Petruzzelli, 2001). It is thus apparent that selenite may not be more bioavailable than selenate (Adriano, 2001; Pezzarossa and Petruzzelli, 2001). By all appearances, as dictated by the pH, certain level of selenate may perhaps exist as mobile form of Se

in these soils. By virtue of its relatively higher pH (and hence lower sorption) coupled with the level of potentially available proportions of total Se, Vertisol seemed to have a potential to render more bioavailable Se than Fluvisol. Nevertheless, the relatively lower clay content, and the total Se level (Table 3.5) may perhaps contribute for the release of Se in Fluvisol.

Zinc

The residual fractions of both soils accommodated sizeable proportions of the total Zn, but the contribution was most prominent (ca. 50 % of the total) in the Vertisol (Table 3.9). The proportions of total Zn sequestered in the non–residual fractions were, however, substantial (avg. ca. 50 % and 62 % of the total Zn in Vertisol and Fluvisol, respectively). The major portions of the non-residual fractions (Table 3.9) were attributed to the reducible, followed by either the oxidizable (Vertisol) or carbonate (Fluvisol) phases. As indicated by Kiekens (1995), the colloidal phase of the soil, namely the clay minerals, hydrated metal oxides, and organic matter could have been important in the observed associations of Zn with these fractions. Besides, the formation of Zn carbonates (Kiekens, 1995; Adriano, 2001), and /or Zn sorption by carbonates (McLean and Bledsoe, 1992; Adriano, 2001) may account for Zn extracted from carbonate fraction.

The proportion of total Zn, on average, followed the order of residual > reducible > oxidizable > carbonate >> exchangeable and residual > reducible > carbonate > oxidizable >> exchangeable in Vertisol and Fluvisol, correspondingly. Zn from background and contaminated soils (Rule, 1998) exhibited similar distribution sequences to Vertisol and Fluvisol, in that order. As a rule, Zn in soils associates with the reducible and clay fractions (Adriano, 2001; Pendias and Pendias, 2001). The reported percentages of the total Zn in the reducible fraction (Rule 1998), 18–39 % (non–contaminated) and 34–44 % (contaminated), effectively agree with the proportions of Zn observed for the same fraction in Vertisol and Fluvisol, respectively.

Compared to other heavy metals, Zn is rated as readily soluble in soils (Pendias and Pendias, 2001). Apart from the role of Zn–organic complexes and complex anions, mobilization from basic carbonates and oxides by decomposing plant material is

indicated important in bioavailability of Zn (Pendias and Pendias, 2001). However, the pH determines bioavailability as the solubility drops with the increase in pH (Adriano, 2001). By all appearances, the formation of Zn–organic complexes in slightly alkaline soils (McBride, 1994), pH and non–residual fractions may play significant role in the phytoavailability of Zn of the study soils.

Vanadium

Even with out taking V in the oxidizable fraction into account (due to analytical difficulty), the non-residual fractions accommodated more than half of the total V in the study soils (Table 3.9). Of these fractions, V was mostly concentrated in the reducible phase of the soils, averaging 42.8 and 46.1 % of the V found in Vertisol and Fluvisol, respectively. The substitution of V, namely V^{3+} (McBride, 1994) for Fe^{3+} in the minerals of Fe oxides (McBride, 1994; Adriano, 2001) may explain the association observed with reducible fraction (Table 3.8). On the other hand, significant proportions of total V (19.6 % in Vertisol and 10.3 % in Fluvisol) were extracted from the carbonate fraction. As indicated by Rule (1998), the carbonate phase could be an essential sink for trace metals at circumneutral pH.

A considerable fraction of V contained in Fe oxides, and in clay minerals has been reported as mobile and hence phytoavailable (Pendias and Pendias, 2001 and references therein). Greater amounts of V (especially in the roots) were found to accumulate in those plants developing on soils with high V levels (Adriano, 2001). Besides, the pH dependent uptake of V by barley roots was indicated to display a linear function of V concentration (Pendias and Pendias, 2001). Moreover, as influenced by the redox potential, very high mobility is expected at neutral to alkaline pH (McBride, 1994).

The sizeable V contents (Table 3.5), considerable proportions of V in the reducible fractions (Table 3.9), and the neutral to alkaline pH (Table 3.4) may contribute to the V uptake of the plants growing on the study soils. Nevertheless, the soil type (Adriano, 2001) mainly determines the plant uptake of V. Apparently, depending on the soil associated attributes, the redox status, and plant factors, the study soils may exhibit certain variations in the availability of V and the ensuing uptake of the same by the plants.

Mercury

The combined percentages of the residual and oxidizable fractions accommodated the majority of Hg in the study soils (Table 3.9). These residual fractions (which also contain oxidizable fractions) displayed significant associations with their corresponding total Hg levels (Table 3.8). It is, however, difficult to decipher the amount of Hg contained in the mineral matrix, and the contribution of the oxidizable fraction from the available data. Despite this, each fraction is expected to contribute significantly to the Hg levels of the soils. With regard to organic matter, it is reported that it has a capacity to retain Hg (Adriano, 2001; Pendias and Pendias, 2001) and hence, Hg accumulation in soils exhibits a tendency to associate with organic matter level (McBride, 1994; Pendias and Pendias, 2001).

The reducible fraction, on the other hand, accounted for a considerable proportion (17.4 %, Vertisol and 11.7 %, Fluvisol) of the total Hg in the soils. The sorption of Hg on Fe oxides has previously been reported (Steinnes, 1995; Adriano, 2001; Pendias and Pendias, 2001). Furthermore, in soils with neutral pH (pH > 5), the participation of Hg^{2+} on oxides of Fe is indicated to be more efficient (Steinnes, 1995). Conversely, it is notable that the exchangeable fractions accounted for relatively higher amount (4.85 %, Vertisol; 8.74 %, Fluvisol) of the total Hg in the soils. Besides, moderately positive relationships were observed between these phases and total contents of the respective soils (Table 3.8). In the nature of things, the exchangeable phase epitomizes the mobile and bioavailable Hg fraction.

Relatively small proportions, averaging only about a percent, of their respective total amounts of Hg were extracted from carbonate fraction of the soils (Table 3.9). This could be attributed to the fact that Hg^{2+}, a soft acid, bonds preferentially to soft bases than hard base like carbonate. In the neutral to alkaline pH, precipitation of $Hg(OH)_2$ may significantly diminish the activity of Hg^{2+} (McBride, 1994). The neutral molecule, $Hg(OH)_2$, however, may have high solubility, which eventually could preclude its precipitation (McBride, 1994) or could be the preferred sorbed species (Pendias and Pendias, 2001).

3.2.3 Assessment of contamination and potential mobility of heavy metals/metalloids

The total heavy metal contents of the contaminated soils, when compared with uncontaminated soils, could impart an important clue about the contribution of anthropogenic inputs of heavy metals to the soil environment. Such comparison of the heavy metal levels may render certain indication about the impending threat of heavy metal buildup on a given environment.

Compared to the uncontaminated Vertisol at Debre Zeit (Figs. 3.2–3.6), high Co, Cr, Cu, Ni, Zn, V, and Hg; while fairly comparable amounts of As, Cd, Pb, and Se, were observed in the contaminated Vertisol. Likewise, saving comparable levels of Cd, the contaminated Fluvisol revealed comparatively lofty levels of all heavy metals than the uncontaminated Fluvisol from Ziway (Fig. 3.3). An earlier study at Akaki (Fisseha Itanna, 1998a), on the other hand, conveyed comparable levels of Cd, Cu, Ni, Zn, Hg and to certain extent of As, Co and Cr amounts. Similarly, the average Cd, and Zn contents reported for Fluvisol (Fisseha Itanna, 1998a) were also comparable to the corresponding values observed for the same soil under this study.

With recourse to the natural background values and + 2 standard deviations (Lu *et al.,* 2003), pollution criteria of the study area could possibly be made, provided, the background / baseline levels have been aptly deciphered and instituted. In the absence of any preceding studies related to baseline levels of heavy metals in soils, however, one might be tempted to see the deviation (taking the above criterion as a yardstick) in the heavy metal levels of soils irrigated with wastewater against those from the same but unpolluted soils. In view of that, the average levels of As, Co, Cr, Cu, Ni, Zn, V, Hg (in both soils), Pb and Se (Fluvisol) exceeded the corresponding levels + 2 standard deviations observed for the same soils from the previous study (Figs. 3.2–3.6). Apart from certain variations attributed to local pedological and / or geological consequences, these elements appeared to have been accumulated due to anthropogenic activities at the study site. Manta et al. (2002) remarked that higher concentrations coupled with high standard deviations are symptoms of anthropogenic influences on the natural levels of elements. Conversely, the Cd levels of both soils

and Pb, Se of Vertisol were below the above criterion, which thus suggests that these metals have been derived mainly from the natural sources.

In general, the majority of the heavy metals of the present study were extracted from non–residual fractions. Apparently, such association could indicate their potential bioavailability. Notwithstanding this, the mobility factor, a relative index of metal mobility based on proportions of the most weakly bound fractions (Olajire *et al.,* 2003 and references therein), is indicated to be instrumental in this regard. Consequently, heavy metals with relatively enhanced lability decrease with the sequence of mobility factor: Cd>Co>Pb>Cu>Ni>Se>V in Vertisol, and Pb>Cd>Co>Cu>Ni>Zn for Fluvisol.

3.3 Heavy metal uptake and distribution in plants growing on contaminated soils (Paper III and IV)

3.3.1 Heavy metal/metalloid uptake and translocation to the shoot under controlled condition (green house)

3.3.1.1 Soil characterization and heavy metal status of the soils

As is presented in Table 3.10, certain variations in the physical and chemical characteristics were observed between the two soils. Accordingly, significantly higher levels of CEC (60.23 $cmol_{(+)}$/kg, $p < 0.001$), clay (61%, $p < 0.001$), and silt (25%, $p < 0.001$) contents and to some extent organic carbon (1.4%, $p < 0.05$) were observed in Vertisol than Fluvisol. On the other hand, the significantly higher ($p < 0.001$) sand fraction (72%) essentially features the texture of Fluvisol (Table 3.10). Apart from this, both soils had similar (100%) base saturation percents. In both soils, Zn (85.4–138.9 mg kg^{-1}) and Hg (0.02–0.59 mg kg^{-1}) had the highest and the lowest concentrations, respectively (Table 3.5; Figs. 3.9–3.10). On the average, Vertisol accommodated relatively higher levels of Cr, Ni, Cu, Zn, Cd, and V whereas the levels of Pb, Se and As were comparatively higher in Fluvisol. Conversely, despite the difference in the corresponding amounts, the heavy metals/metalloids in the soils displayed considerable similarity in their relative abundances (see also section 3.2.1.2). As Figs 3.9 and 3.10 show, comparatively higher concentrations of Pb, Se,

Zn, Cd, and Hg were found in upper layers of both soils (Figures 3.9 and 3.10). Likewise, top layers of Vertisol accommodated fairly higher levels of Cr, Cu, Ni, and Co (Figure 3.9).

In general, the majority of the heavy metals of the present study were extracted from non-residual fractions. Albeit the small amounts of heavy metals extracted from exchangeable fractions (<14%, Table 3.9), Hg (2.5–14.1%) followed by Cd (0.4–1.1%) and Se (0.3–1%) dominated the same in both soils. On the other hand, mainly Cd (46.6–51.5%), Pb (29–54.8%), and Co (39.4–47.7%) but also Cu (27.1–30.4%) and Ni (21.9–23.2%) exhibited discernible affinity for the carbonate fractions of the soils. Alternatively, principally As (52–70.4%), Pb (40–53.4%), V (41.8–50.4%), and Co (31.6–47 %) and also Cu (23.8–34.9%), Zn (22.1–40.2%), Cr (18.2–31.6%), and Ni (19.3–30.9%) displayed marked association with reducible fraction of the soils. Moreover, Cr (20.5–28.6%), Zn (10.7–19.7%), Ni (11.9–19.5%), and Cu (13.6–15.9%) in both soils were those heavy metals with high selectivity for oxidizable fraction. The marked associations of most heavy metals/metalloids with non-residual fractions (Table 3.9) could display their potential bioavailability or mobility.

Table 3.10 Selected physical and chemical properties of the study soils.

Soil type	Depth (cm)	Org-C (%)	CaCO3 (%)	BS (%)	CEC ($cmol_+/kg$)	pH ($CaCl_2$)	Clay (%)	Silt (%)	Sand (%)
Vertisol	0-20	1.39	0.72	100	60.23	7.55	61	25	14
Fluvisol	0-20	0.74	0.40	100	26.55	6.80	16	12	72

3.3.1.2 Heavy metal levels (dry weight) and their distribution in plant parts

Zn was the heavy metal with the highest shoot (28.23–89.09 mg kg^{-1}) and root levels (24.49–124 mg kg^{-1}) in the plants on both soils with the maximum values corresponding to Desmodium (shoot: Vertisol) and Setaria (root: Fluvisol). Likewise, Setaria from both soils and Alfalfa from Fluvisol accommodated fairly higher and lower levels of heavy metals, respectively (Table 3.11). Alfalfa, however, had considerable amount of most heavy metals when grown on Vertisol (Table 3.11). Factors that control the concentration and speciation of metal in the soil solution, the movement of metal from the bulk soil to the root surface and into the root, and the

translocation from the root to the shoot determine the amount of metal absorbed by the plants (Alloway, 1995a). Conversely, variations in the capacity to absorb, accumulate, and tolerate heavy metals between plant species and cultivars are genetically controlled (Alloway, 1995a).

In general, relatively higher average root concentrations than the corresponding levels in the shoot marked the distribution of the majority of heavy metals in the plant parts (Oat, Rhodes, and Setaria from both soils; Desmodium, Fluvisol) (Table 3.11). Such trend is consistent with other works reported for Cr (McGrath, 1995; Adriano, 2001; Gardea-Torresdy *et al.,* 2004; Pendias and Pendias, 2001), Ni, Co (Adriano, 2001), Cu (Adriano, 2001; Gardea-Torresdey *et al.,* 2004; Pendias and Pendias, 2001; Sheldon and Menzies, 2005), Zn (Pendias and Pendias, 2001), Cd (McBride, 1994; Pendias and Pendias, 2001), Pb (McBride, 1994; Adriano, 2001; Finster, 2004; Panich-Pat *et al.,* 2004; Chandra Sekhar *et al.,* 2005), V (Edwards *et al.,* 1995; Adriano, 2001), As (O'Neill, 1995; Adriano, 2001). Immobilization in the root tissues is attributable to complex formation (Pendias and Pendias, 2001) that reversibly bind (e.g., Cd) considerable amount of cations (Ross and Kaye, 1994; Mohr and Schopfer, 1995b) and specific adsorption (e.g., Cu) on the cell walls of the root free space (Baker and Senft, 1995) as well as the precipitation (e.g., Pb and V) or insoluble complexation of with xylem elements (e.g., Pb) (Adriano, 2001).

On the other hand, the mean levels of most heavy metals in the shoots of Alfalfa (on either soil) and Desmodium (Vertisol) were higher than the corresponding levels in the roots (Table 3.11). Under certain conditions, translocation of Ni (McGrath, 1995), Co (Adriano, 2001), Zn (Alloway, 1995a; Adriano, 2001; Pendias and Pendias, 2001; Chiu et al., 2006), Cd (Alloway, 1995a; Alloway, 1995b; Adriano, 2001; Pendias and Pendias, 2001), Pb, and As (Pendias and Pendias, 2001) have been reported to result in higher shoot concentrations than the same in the root. In addition, the shoot levels of Cr (Alfalfa and Desmodium on Vertisol), Ni (Alfalfa on Vertisol), Cu (Alfalfa on Vertisol), and V (Alfalfa, Desmodium and Setaria on Vertisol) (Table 3.11) were in the excessive/phytotoxic range indicated for above ground plant part (Pendias and Pendias, 2001). The levels of Cr (all plants), Co (Alfalfa, Desmodium and Setaria on Vertisol), Cd (Setaria on Vertisol), and V (Alfalfa and Desmodium on Fluvisol) in the shoots exceeded the corresponding normal levels shown in Pendias and Pendias

(2001). Apart from this, elevated Ni (Desmodium on Vertisol), Zn (Alfalfa, Desmodium and Setaria on both soils), and Hg (Setaria on Fluvisol) in the shoots (Table 3.11) were close to those phytotoxic levels indicated by Pendias and Pendias (2001). The shoots of the studied plants had rather higher concentrations of Zn, and Cu (but to certain extent Se as well) than those plant species used in phytoremediation reported by Banuelos and Ajwa (1999). The nature of the distribution and accumulation of each element within plants largely depends on the type of plant as well as the electrochemical variables of elements (Pendias and Pendias, 2001).

Table 3.11 Mean (n = 6) heavy metals contents (mg kg^{-1} DM) of plant parts and ± standard deviations.

Plant	Soil		Cr	Ni	Co	Cu	Zn	Cd	Pb	Hg	Se	V	As
Oat	CV	Shoot	0.43 ±0.09	3.61 ±0.62	0.16 ±0.05	7.95 ±1.33	46.11 ±3.53	0.05 ±0.01	0.14 ±0.03	0.09 ±0.01	0.12 ±0.03	0.1 ±0.03	0.01 ±0.00
		Root	5.95 ±1.23	4.9 ±0.97	1.53 ±0.35	18.71 ±3.01	79.21 ±11.96	0.1 ±0.01	0.67 ±0.15	0.02 ±0.01	0.41 ±0.05	5.77 ±1.14	0.22 ±0.06
Oat	CF	Shoot	1.25 ±0.40	1.78 ±0.38	0.14 ±0.04	5.28 ±1.01	36.44 ±5.42	0.03 ±0.01	0.13 ±0.05	0.04 ±0.01	0.03 ±0.01	0.08 ±0.02	0.01 ±0.00
		Root	5.56 ±1.14	5.74 ±1.12	2.91 ±0.90	19.43 ±2.10	92.99 ±9.24	0.09 ±0.02	2.00 ±0.42	0.13 ±0.05	0.58 ±0.10	8.52 ±1.54	0.35 ±0.06
Alfalfa	CV	Shoot	22.31 ±3.5	12.81 ±1.73	4.82 ±0.74	19.73 ±1.99	68.28 ±2.25	0.05 ±0.00	2.84 ±0.93	0.06 ±0.02	1.17 ±0.40	15.76 ±2.59	0.76 ±0.02
		Root	1.72 ±0.35	1.82 ±0.17	0.31 ±0.10	9.72 ±0.90	24.49 ±1.49	0.02 ±0.00	0.18 ±0.05	0.01 ±0.00	0.08 ±0.02	1.29 ±0.34	0.05 ±0.01
Alfalfa	CF	Shoot	1.39 ±0.25	1.31 ±0.24	0.51 ±0.12	15.1 ±1.26	61.2 ±5.21	0.03 ±0.01	0.80 ±0.12	0.06 ±0.02	0.09 ±0.03	1.71 ±0.27	0.11 ±0.03
		Root	0.63 ±0.14	1.42 ±0.08	0.24 ±0.04	8.51 ±2.06	24.91 ±1.56	0.01 ±0.00	0.19 ±0.08	0.13 ±0.05	0.05 ±0.01	0.74 ±0.17	0.03 ±0.02
Rhodes	CV	Shoot	1.18 ±0.35	1.19 ±0.02	0.18 ±0.02	4.09 ±0.05	28.23 ±1.99	0.06 ±0.01	0.31 ±0.06	0.07 ±0.02	0.07 ±0.02	0.47 ±0.09	0.03 ±0.00
		Root	7.44 ±0.28	7.70 ±0.72	2.31 ±0.45	19.06 ±2.23	90.04 ±8.16	0.16 ±0.02	1.17 ±0.27	0.04 ±0.01	0.42 ±0.07	8.11 ±1.44	0.33 ±0.03
Rhodes	CF	Shoot	0.78 ±0.04	0.99 ±0.07	0.16 ±0.04	3.92 ±0.77	30.37 ±2.83	0.05 ±0.02	0.21 ±0.03	0.10 ±0.06	0.03 ±0.01	0.25 ±0.04	0.03 ±0.01
		Root	8.38 ±0.62	8.22 ±0.50	4.39 ±0.15	24.74 ±1.40	107.3 ±7.27	0.17 ±0.06	4.03 ±0.15	0.06 ±0.02	1.02 ±0.29	13.4 ±1.21	0.58 ±0.07
Desmodium	CV	Shoot	5.33 ±0.47	8.12 ±0.61	1.6 ±0.36	9.51 ±0.09	89.09 ±3.55	0.17 ±0.04	1.41 ±0.35	0.11 ±0.02	0.36 ±0.12	5.54 ±0.68	0.3 ±005
		Root	4.73 ±0.39	6.4 ±0.38	0.9 ±0.10	23.93 ±1.19	34.29 ±2.61	0.07 ±0.01	0.42 ±0.08	0.05 ±0.02	0.28 ±0.07	14.6 ±1.74	0.22 ±0.03
Desmodium	CF	Shoot	1.65 ±0.23	2.66 ±0.41	0.68 ±0.15	8.35 ±0.14	70.26 ±3.20	0.09 ±0.01	1.09 ±0.21	0.09 ±0.01	0.14 ±0.06	2.19 ±0.08	0.14 ±0.04
		Root	4.12 ±0.66	5.17 ±0.80	1.93 ±0.14	19.29 ±1.21	49.06 ±2.13	0.13 ±0.05	1.47 ±1.05	0.04 ±0.01	0.32 ±0.11	9.56 ±0.37	0.36 ±0.09
Setaria	CV	Shoot	4.28 ±0.56	4.03 ±0.37	1.20 ±0.08	11.51 ±0.45	77.29 ±4.19	0.28 ±0.01	1.10 ±0.08	0.07 ±0.02	0.11 ±0.02	4.69 ±0.35	0.35 ±0.03
		Root	18.32 ±0.48	16.45 ±0.62	4.98 ±0.21	35.42 ±1.21	87.19 ±0.61	0.31 ±0.00	2.58 ±0.09	0.02 ±0.01	0.78 ±0.06	33.62 ±0.73	1.01 ±0.04
Setaria	CF	Shoot	1.5 ±0.68	1.64 ±0.46	0.72 ±0.19	9.8 ±2.37	84.41 ±4.74	0.03 ±0.01	0.48 ±0.16	0.33 ±0.05	0.08 ±0.01	0.93 ±0.11	0.08 ±0.01
		Root	15.8 ±2.30	15.55 ±1.60	9.77 ±1.16	29.08 ±1.07	123.56 ±10.74	0.22 ±0.01	8.68 ±1.11	0.02 ±0.00	1.5 ±0.22	26.7 ±1.87	1.18 ±0.57

Moreover, the age of the plant and the plant organ are deemed important in regulating the rate and the level of movement of heavy metals within plants (Alloway, 1995a). In view of that, some of the heavy metal levels in the above plants showed significant differences ($p < 0.05$) between the above– and below–ground parts (Table 3.12).

Table 3.12 The p values established for the significant difference ($p < 0.05$) between mean heavy metal levels in the above– and below–ground plant parts of the study plants.

Plant type	Soil type	Cr	Ni	Co	Cu	Zn	Cd	Pb	Hg	Se	V	As
Oat	Vertisol	0.003		0.002		0.034	0.004	0.049		0.001	0.001	0.025
	Fluvisol	0.042	0.041	0.023	0.005	0.034		0.024		0.019	0.032	0.031
Alfalfa	Vertisol				0.027	0.024			0.020			
	Fluvisol	0.025			0.009	0.002		0.003				0.018
Rhodes	Vertisol		0.039		0.035	0.002	0.002					
	Fluvisol				0.001	0.009						
Desmodium	Vertisol					0.002	0.038					
	Fluvisol					0.049			0.006			
Setaria	Vertisol		0.000					0.000			0.000	0.000
	Fluvisol		0.028	0.020	0.003		0.000			0.034	0.040	0.028

Compared with the reported background average concentrations of Cr (McGrath, 1995; Pendias and Pendias, 2001), Ni, (Adriano, 2001; Pendias and Pendias, 2001), Co (Pendias and Pendias, 2001), Cu (Adriano, 2001; Pendias and Pendias, 2001), Zn, Pb, Hg (Pendias and Pendias, 2001), Se (Neal, 1995), and V (Adriano, 2001) in forage grasses and legumes, the corresponding levels observed in the plants (Cr, Ni, Co, Cu, Zn, Se, and V in all plants; Pb in Alfalfa: Vertisol, Rhodes: Fluvisol, and Setaria on both soils; Hg in Oat, Alfalfa, Rhodes and Setaria on Fluvisol) were higher (Table 3.11). Besides, the levels of Cr (Alfalfa: Vertisol; Setaria on both soils), Ni (in Oat, Rhodes, and Setaria), Zn (in Oat, Rhodes, and Setaria), and V (in all plants) were comparable to the concentrations of Cr (McGrath, 1995), Ni , Zn (Pendias and Pendias, 2001), and V (Pendias and Pendias, 2001; < 38 mg kg^{-1}: Edwards *et al.,* 1995) reported for (similar) plants grown on contaminated soils. On the other hand, the concentrations of Cd and As of the plants (Table 3.11) fell under the background ranges of Cd (Pendias and Pendias, 2001) and As (Adriano, 2001; Pendias and Pendias, 2001) in grasses and legumes/ plants.

On the other hand, individuals of the same species display traceable variations in heavy metal levels of their root or shoot between the soils (Table 3.11). Of these, however, only Cr (root, Alfalfa; shoot, Setaria), Ni (root, Alfalfa; shoot, Oat and Setaria), Cd (root, Setaria; shoot, Oat and Desmodium; Setaria), Pb (shoot, Setaria), Se (shoot, Oat and Rhodes), V (shoot, Rhodes and Setaria), and As (shoot, Setaria) had significant differences ($p < 0.05$) between the soils. McBride (1994) suggested that the soil might possibly affect translocation of elements with in the plants; because mobility in plants is sensitive to the specific chemical form absorbed from soil solution. In agreement with the above statement, Pendias and Pendias (2001) also indicated that the electrochemical properties of elements determine the transport of trace elements among plant organs.

3.3.1.3 Bioconcentration factor (BCF), and translocation factor (TF) for heavy metals and metalloids in the plants

BCF, a ratio that relates the heavy metal levels of the root with the soil, renders an important estimate on a plant's potential to accumulate heavy metals from the soil (Yoon *et al.,* 2006). BCF values have always been used to represent the accumulation of chemicals in organisms that live in a contaminated environment (Wang *et al.,* 2004). In this study, Cu (Oat, Rhodes, and Desmodium, Setaria: Fluvisol), Hg (Oat and Alfalfa: Fluvisol), Cd (Rhodes: Fluvisol; Setaria from both soils), and Zn (Setaria: Fluvisol) had BCF > 1 (Table 3.13). Therefore, the above species with BCF > 1 may have potentials to accumulate higher levels of the heavy metal in their roots relative to the corresponding levels in the respective soils. In this regard, grasses appeared to excel legumes in the relative sequestration of the above heavy metals. In agreement with this observation, Yoon *et al*. (2006) reported that four out of the five species with the highest root accumulations of heavy metals were grasses. The same authors reported that plants with high BCF and low TF (ratio of heavy metal concentration of plant shoot to root) have the potential for phytostabilization, which can be employed to reduce migration of contaminants in soils. Of the grasses, on the other hand, Setaria had the highest root concentration of most heavy metals from either soil, and hence, its potential is worthy of further research.

On the other hand, Zn (Desmodium: Fluvisol), Hg (Oat: Vertisol; Desmodium: Fluvisol; Rhodes and Setaria on both soils), and most of the heavy metals (Desmodium: Vertisol; Alfalfa on both soils) displayed TF > 1 (Table 3.13). Compared to the grasses, therefore, the legumes depicted a general inclination to accumulate most heavy metals in their shoots particularly when grown in Vertisol (Table 3.13). Consequently, the observed tendency by legumes to translocate heavy metals from their roots to shoots justifies additional investigation to assess their efficacy to remove heavy metals from moderately contaminated soils.

Table 3.13 Mean (n = 6) heavy metals contents (mg kg^{-1} DM) of the soils, with their relative accumulation and translocation in the plants and ± standard deviations.

Plant	Soil		Cr	Ni	Co	Cu	Zn	Cd	Pb	Hg	Se	V	As
Oat	CV	BCF	0.08 ±0.02	0.07 ±0.02	0.05 ±0.01	0.50 ±0.12	0.57 ±0.09	0.56 ±0.06	0.05 ±0.01	0.19 ±0.07	0.24 ±0.03	0.07 ±0.01	0.08 ±0.02
		TF	0.07 ±0.02	0.75 ±0.12	0.10 ±0.02	0.46 ±0.10	0.58 ±0.13	0.50 ±0.15	0.21 ±0.03	**3.98** ±0.92	0.29 ±0.05	0.02 ±0.00	0.06 ±0.02
		[1]Soil	75.90 ±2.25	65.59 ±0.95	28.00 ±0.30	37.48 ±0.53	138.90 ±7.10	0.18 ±0.01	13.97 ±0.93	0.11 ±0.02	1.74 ±0.11	87.36 ±4.81	2.81 ±0.14
Oat	CF	BCF	0.23 ±0.07	0.18 ±0.06	0.11 ±0.03	**1.20** ±0.25	0.85 ±0.18	0.67 ±0.17	0.08 ±0.02	**1.07** ±0.30	0.22 ±0.05	0.13 ±0.05	0.11 ±0.04
		TF	0.23 ±0.08	0.34 ±0.11	0.05 ±0.01	0.27 ±0.04	0.41 ±0.09	0.34 ±0.12	0.07 ±0.04	0.82 ±0.24	0.05 ±0.02	0.01 ±0.00	0.04 ±0.01
		[2]Soil	23.76 ±5.29	32.68 ±6.48	27.44 ±5.25	16.21 ±3.25	109.00 ±8.40	0.13 ±0.01	24.33 ±2.93	0.12 ±0.12	2.58 ±0.12	63.53 ±17.3	3.05 ±0.87
Alfalfa	CV	BCF	0.02 ±0.00	0.03 ±0.00	0.01 ±0.00	0.26 ±0.02	0.18 ±0.01	0.11 ±0.01	0.01 ±0.00	0.14 ±0.05	0.05 ±0.01	0.01 ±0.00	0.02 ±0.01
		TF	**16.47** ±0.47	**7.28** ±0.29	**16.04** ±0.28	**2.03** ±0.13	**2.79** ±0.15	**3.09** ±0.24	**19.41** ±0.28	**6.00** ±0.19	**19.47** ±0.47	**12.26** ±0.19	**19.43** ±0.43
Alfalfa	CF	BCF	0.03 ±0.01	0.04 ±0.00	0.01 ±0.00	0.53 ±0.13	0.23 ±0.01	0.07 ±0.01	0.01 ±0.00	**1.07** ±0.27	0.02 ±0.00	0.01 ±0.00	0.01 ±0.01
		TF	**2.26** ±0.58	0.92 ±0.27	**2.13** ±0.63	**1.82** ±0.26	**2.47** ±0.41	**3.17** ±0.61	**4.57** ±0.53	2.93 ±0.51	**2.05** ±0.35	**2.31** ±0.50	**10.92** ±1.47
Rhodes	CV	BCF	0.10 ±0.05	0.12 ±0.06	0.08 ±0.04	0.51 ±0.13	0.65 ±0.09	0.88 ±0.10	0.08 ±0.03	0.34 ±0.09	0.24 ±0.08	0.09 ±0.02	0.12 ±0.04
		TF	0.24 ±0.12	0.18 ±0.08	0.10 ±0.04	0.24 ±0.08	0.32 ±0.09	0.40 ±0.06	0.31 ±0.10	**2.31** ±1.00	0.22 ±0.09	0.07 ±0.03	0.10 ±0.04
Rhodes	CF	BCF	0.35 ±0.11	0.25 ±0.08	0.16 ±0.07	**1.53** ±0.26	0.98 ±0.16	**1.24** ±0.21	0.17 ±0.05	0.52 ±0.19	0.39 ±0.12	0.21 ±0.10	0.19 ±0.09
		TF	0.12 ±0.05	0.17 ±0.09	0.05 ±0.02	0.16 ±0.02	0.30 ±0.09	0.31 ±0.04	0.09 ±0.04	**1.67** ±0.45	0.06 ±0.02	0.03 ±0.01	0.05 ±0.02
Desmodium	CV	BCF	0.06 ±0.01	0.10 ±0.04	0.03 ±0.00	0.64 ±0.19	0.25 ±0.05	0.39 ±0.11	0.03 ±0.01	0.50 ±0.13	0.16 ±0.04	0.17 ±0.11	0.08 ±0.01
		TF	**1.14** ±0.35	**1.30** ±0.25	**1.69** ±0.45	0.51 ±0.23	**2.64** ±0.27	**2.74** ±0.51	**3.36** ±0.58	**2.24** ±0.64	**1.35** ±0.41	0.35 ±0.14	**1.47** ±0.42
Desmodium	CF	BCF	0.17 ±0.04	0.16 ±0.06	0.07 ±0.02	**1.19** ±0.17	0.45 ±0.05	0.99 ±0.11	0.06 ±0.02	0.33 ±0.07	0.12 ±0.04	0.15 ±0.05	0.12 ±0.03
		TF	0.45 ±0.19	0.60 ±0.21	0.41 ±0.17	0.47 ±0.17	**1.44** ±0.10	0.73 ±0.24	1.08 ±0.27	**2.26** ±0.25	0.53 ±0.21	0.27 ±0.13	0.48 ±0.19
Setaria	CV	BCF	0.25 ±0.01	0.27 ±0.03	0.21 ±0.05	0.98 ±0.06	0.65 ±0.04	**2.04** ±0.14	0.24 ±0.07	0.37 ±0.08	0.49 ±0.06	0.41 ±0.04	0.65 ±0.11
		TF	0.29 ±0.10	0.35 ±0.15	0.25 ±0.02	0.36 ±0.05	0.94 ±0.18	0.93 ±0.12	0.43 ±0.04	**2.41** ±0.25	0.41 ±0.16	0.17 ±0.05	0.26 ±0.14
Setaria	CF	BCF	0.66 ±0.18	0.48 ±0.09	0.36 ±0.07	**1.79** ±0.22	**1.13** ±0.20	**1.64** ±0.10	0.36 ±0.11	0.20 ±0.05	0.58 ±0.13	0.42 ±0.10	0.39 ±0.09
		TF	0.14 ±0.04	0.11 ±0.03	0.07 ±0.02	0.34 ±0.03	0.72 ±0.12	0.15 ±0.08	0.07 ±0.05	**13.25** ±1.45	0.06 ±0.02	0.03 ±0.01	0.07 ±0.03

NB. *CV stands for contaminated Vertisol while CF denotes contaminated Fluvisol.*
[1]soil *and* [2]soil *correspond to heavy metal levels of CV and CF, in that order.*

BCF or **bioconcentration factor** = *Heavy metal or metalloid concentration in root* / *Heavy metal or metalloid concentration in soil*

TF or **translocation factor** = *Heavy metal or metalloid concentration in shoot* / *Heavy metal or metalloid concentration in root*

Values of **BCF** *and* **TF > 1** *are indicated with* **boldface**.

3.3.1.4 The essence of main and interaction effects on heavy metal levels of the plants

The MANOVA in the balanced design as well as GLM executed for the main (soil type, species, plant part) and interaction effects (1st order: soil type × species, soil type × plant part, species × plant part; 2nd order: soil type × species × plant part) revealed significant differences ($p < 0.001$) for the response variables (heavy metal levels) of their corresponding groups. On the other hand, the univariate output on each heavy metal for the above said effects threw further light on the nature of the overall effects determined by the omnibus F–test. Consequently, soil type (Cd), species (Co, Cu, Zn, Cd, Pb, V, and As), plant part (Ni, Co, Cu, Zn, Cd, Pb, Se, V, and As), soil type × species (Cd), soil type × plant part (Co, Zn, Cd, Pb, and Se), species × plant part (Cr, Ni, Co, Cu, Zn, Cd, Pb, Se, V, and As), soil type × species × plant part (Cd) had significantly ($p < 0.05$) affected the contents of the indicated heavy metals in the plants.

In general, heavy metal/metalloid sequestration of the plants averaged over the soil types and plant parts decreases in the order of Setaria > Desmodium > Rhodes > Alfalfa > Oat. In agreement with the overall distribution of the elements in the plant parts indicated in section 3.2, the roots of plants contained significantly ($p < 0.05$) higher levels of most heavy metals/metalloids than the same found in the aboveground parts. However, the effect of plant part on plant heavy metal/metalloid levels varied depending on the type of species considered (or put it another way, the effect of species type on plant heavy metal/metalloid levels varied depending on the plant part considered). Notable cases in this regard were the association of highest mean shoot and root heavy metal/metalloid levels to Alfalfa and Setaria, respectively. The variations in the effect of species on heavy metal/metalloid uptake and accumulation were higher in the case of roots than were for shoots. On the other hand, the mean heavy metal/metalloid contents of the plant parts were somewhat comparable when the plants were grown on Vertisol than was case for similar plants from Fluvisol. By and large, Vertisol promoted shoot translocation of most of the heavy metals/metalloids, whereas Fluvisol appeared to encourage root accumulation. Consequently, for the study area as well as similar sites, plant part, type of species and their interactions (including soil type × plant part) could govern heavy metal uptake, translocation, sequestration and ultimately, transfer through the food chain.

3.3.1.5 Relationship between heavy metal levels associated with the different soil phases / fractions, and the corresponding amounts in the shoot and root.

In general, the non–residual fractions (exchangeable, carbonate bound, reducible, and oxidizable) contributed significantly to the uptake of heavy metals and metalloids by the plants and hence the resultant levels in either plant parts (see Table 3.14). Likewise, mainly CEC but to some extent the pH as well, appeared to have influence on the availability of heavy metals. Previous investigation (Daniel Fitamo *et al.,* 2007) on the same soils revealed that considerable proportions of the total levels of heavy metals were extracted from the non-residual fractions, and CEC and pH were key factors in the distribution of heavy metals among these fractions. Moreover, the antagonistic (competition for the same adsorption sites in the soils or for absorption at the root surface and translocation to the shoot) or synergistic interactions of heavy metals with each other and other nutrient elements in the soils and /or plants are expected to affect heavy metal levels and distribution within plants.

Chromium

Among the variables tested for the soil–plant transfer of Cr, exchangeable, pH and CEC in Vertisol (R^2 adj. 83.5–89.9%, $p < 0.01$); and carbonate, reducible, oxidizable and CEC in Fluvisol (R^2 adj. 82.2–91.6%, $p < 0.05$) were important (Table 3.14). Mobility, solubility, transformation, speciation, and uptake of Cr is governed by pH, oxidation state of Cr, presence of reducing (organic matter, Fe^{2+}, and sulfides) and oxidizing materials (high–valency Mn oxides), oxides of Fe and Mn and redox potential (Adriano, 2001). The occurrence of Cr^{6+} as chromate (CrO_4^{2-}) at neutral pH (McLean and Bledsoe, 1992; Adriano, 2001) or at pH > 6 (McGrath, 1995) and non-specific adsorption of Cr^{6+} on anion exchange sites at neutral pH (McLean and Bledsoe, 1992) enhances the mobility and availability of Cr (McBride, 1994; Adriano, 2001). Similarly, complexation of Cr^{3+} with soluble organic acids (e.g., citric acid, DTPA, fulvic acids, and soil extracts of water–soluble organic matter) above pH 5.5 (McGrath, 1995; Adriano, 2001), limited adsorption of the anionic form of Cr as CEC increases, could contribute to Cr uptake. In contrast, the formation of insoluble Cr^{3+} precipitate and / or poor translocation (Adriano, 2001) might have impinged on the shoot Cr levels.

Nickel

Fractions of the total Ni associated with exchangeable, carbonate, reducible, and oxidizable phases as well as CEC and pH (to a lesser extent), explained the variation in the Ni levels of the plants (R^2 adj. 84.8–93%, $p < 0.05$; Table 3.14). Ni bioavailability is influenced by total levels, pH, CEC, hydrous oxides, organic matter, texture, and mineralogy of the soil (Adriano, 2001). Apart from the residual fraction, considerable proportions of the total Ni could be extracted from carbonate, reducible (Fe–Mn oxides), and organic fractions through sequential extraction of contaminated soils (McGrath, 1995; Adriano, 2001; Daniel Fitamo *et al.,* 2007). Ni complexed by dissolved organic matter with increase in pH (McLean and Bledsoe, 1992; McBride, 1994; Adriano, 2001; Pendias and Pendias, 2001) can be plant available. Besides, there are reports that exchangeable and plant–available Ni increase in poorly drained soils (McGrath, 1995) which, thus, might occur in irrigated soils of the study area.

Table 3.14 Stepwise regression equations relating heavy metal levels in plant parts with the corresponding levels in the five soil fractions and the two soil variables.

Soil type	Plant part	Heavy metal	Stepwise regression equation	R^2 adjusted %	P value[a]
Vertisol	Shoot	Cr	**Cr = - 0.853 + 120 E + 0.147pH**	**83.5**	**0.003****
Vertisol	Root	Cr	**Cr = - 4.15 + 323 E + 0.0934 CEC+ 0.555 pH**	**89.9**	**0.001****
Fluvisol	Shoot	Cr	**Cr = 2.68 + 0.298 Ox - 17.1 C + 0.218 Red**	**82.2**	**0.017***
Fluvisol	Root	Cr	**Cr = - 3.91 + 0.241 CEC - 0.0742 Res**	**91.6**	**0.006****
Vertisol	Shoot	Ni	**Ni = - 23.4 + 1.53 C - 0.0736 Res + 0.683 pH + 1.41 CEC**	**93.0**	**0.010***
Vertisol	Root	Ni	**Ni = - 14.8 + 177 Ex + 2.29 Red**	**85.6**	**0.025***
Fluvisol	Shoot	Ni	**Ni = - 2.69 - 0.211 C + 0.0261 Ox - 0.0285 Res + 0.233 CEC**	**90.9**	**0.018***
Fluvisol	Root	Ni	**Ni = 96.5 + 7.29 CEC + 11.5 C + 1.02 Red + 0.926 Ox**	**84.8**	**0.027***
Vertisol	Shoot	Co	**Co = 1.33 + 14.2 E - 0.0475 C - 0.0131 R**	**75.2**	**0.027***
Vertisol	Root	Co	**Co = 9.01 - 0.204 R - 0.971 pH + 0.0766 CEC**	**94.9**	**0.011***
Fluvisol	Shoot	Co	**Co = 2.39 + 357 E - 0.120 C - 0.0129 R**	**88.1**	**0.024***
Fluvisol	Root	Co	**Co = - 15.3 - 0.292 C + 0.00655 R + 2.02 pH + 0.226 CEC**	**78.7**	**0.046***
Vertisol	Shoot	Cu	**Cu = 37.2 - 3.14 Ox**	**75.4**	**0.016***
Vertisol	Root	Cu	**Cu = - 32.0 + 10.0 Ox + 0.0405 E**	**72.7**	**0.019***
Fluvisol	Shoot	Cu	**Cu = - 32.0 - 0.150 Red + 2.76 pH + 0.880 CEC**	**92.0**	**0.000****
Fluvisol	Root	Cu	**Cu = - 12.8 - 1.16 C - 2.36 Red + 1.84 CEC**	**95.4**	**0.004****
Vertisol	Shoot	Zn	**Zn = 50.3 + 338 E + 1.73 Ox**	**72.4**	**0.037***
Vertisol	Root	Zn	**Zn = 104 + 23.1 C - 3.94 Red - 21.6 pH**	**91.8**	**0.000****
Fluvisol	Shoot	Zn	**Zn = 151 + 1.93 Ox + 4.36 CEC**	**83.4**	**0.031***
Fluvisol	Root	Zn	**Zn = - 425 + 2.56 Red + 15.8 Ox + 23.0 CEC**	**94.3**	**0.002****
Vertisol	Shoot	Cd	**Cd = 0.0744 - 1.50 Red**	**64.7**	**0.038***
Vertisol	Root	Cd	**Cd = 0.0350 + 67.9 E**	**61.8**	**0.045***
Fluvisol	Shoot	Cd	**Cd = 0.197 + 1.51 Ox - 0.00612 CEC**	**95.9**	**0.004****
Fluvisol	Root	Cd	**Cd = 0.738 - 1.14 Red + 3.88 Ox - 0.0223 CEC**	**90.6**	**0.002****
Vertisol	Shoot	Pb	**Pb = - 111 + 12.1 Ox + 1.60 CEC**	**74.0**	**0.019***
Vertisol	Root	Pb	**Pb = 0.367 + 0.464 E**	**82.6**	**0.008****
Fluvisol	Shoot	Pb	**Pb = 2.19+ 57.4 E - 0.0412 C- 0.133 Red + 0.195 Ox**	**87.1**	**0.012***
Fluvisol	Root	Pb	**Pb = - 6.52 + 20.7 Ox**	**72.0**	**0.020***
Vertisol	Root	Hg	**Hg = 0.329 + 0.258 E - 0.571 Res + 0.0637 pH**	**91.0**	**0.003****

Fluvisol	Shoot	Hg	**Hg = 0.214 + 7.74 E - 32.0 C - 1.81 Red**	**84.6**	**0.003****
Fluvisol	Root	Hg	**Hg = - 0.0199 - 2.19 C + 0.0670 Red + 0.00175 CEC**	**90.5**	**0.021***
Vertisol	Shoot	Se	**Se = 0.114 + 0.118 E - 0.00605 Res**	**67.5**	**0.040***
Vertisol	Root	Se	**Se = 0.764 + 0.504 E - 0.0114 Res + 0.00277 pH**	**89.1**	**0.002****
Fluvisol	Shoot	Se	**Se = 1.16 + 2.59 Red**	**90.1**	**0.003****
Fluvisol	Root	Se	**Se = 9.78 + 24.7 Red - 0.327 Res**	**93.3**	**0.013****
Vertisol	Shoot	V	**V = 26.4 - 0.625 Res**	**73.0**	**0.034***
Vertisol	Root	V	**V = 37.6 + 0.0604 Red + 18.8 pH**	**92.1**	**0.004****
Fluvisol	Shoot	V	**V = 0.755 - 3.95 E + 0.0480 Red**	**84.6**	**0.023***
Fluvisol	Root	V	**V = 101 - 3.34 C + 0.224 Red**	**73.2**	**0.019***
Vertisol	Shoot	As	**As = 0.129 + 20.1 E**	**64.7**	**0.042***
Vertisol	Root	As	**As = 0.180 + 28.0 E**	**75.0**	**0.038***
Fluvisol	Shoot	As	**As = - 0.456 - 1.21 C +0.0405 Red + 0.0318 CEC**	**94.0**	**0.008****
Fluvisol	Root	As	**As = 1.58 - 0.812 Res**	**79.1**	**0.011***

NB. ***Symbols employed above as predictor variables represent heavy metals extracted from the different geochemical phases/fractions of the soils (E = exchangeable; C = carbonate; Red = reducible; Ox = oxidizable and Res = residual phases) and the two soil variables, pH and CEC.***

[a] *****, and * denote significance at 1 and 5 per cent, respectively.***

Cobalt

The exchangeable, carbonate, and reducible fractions as well as pH and CEC were important factors that accounted for variations in Co levels of the plant parts (R^2 adj. 75.2–94.9%, $p < 0.05$; Table 3.14). Smith and Paterson (1995) suggested that the Co uptake of plants is a function of the concentration of Co in the soil solution and on the exchange sites of the CEC. Likewise, the Co status can be influenced by soil pH (Smith and Paterson, 1995; Adriano, 2001), levels Mn and Fe oxides, and moisture (Adriano, 2001). Diminished uptake of Co could arise due to enhanced chemisorption on oxides (the reducible fraction) and precipitation of $Co(OH)_2$ (the carbonate phase) with increase in pH (McBride, 1994). However, soluble organic complexes of Co at higher pH (McBride, 1994; Pendias and Pendias, 2001), higher amount of extractable Co in poorly drained soils and the rhizosphere pH (which can differ from the bulk by two units) (Smith and Paterson, 1995), possibly will enhance availability. The association of Co in the study soils mainly with the carbonate and reducible fractions (Daniel Fitamo *et al.,* 2007), may suggest the significance of the pH and Eh in determining the uptake of Co from the same.

Copper

In Vertisol, the oxidizable and exchangeable fractions were prominent in relating soil and plant Cu levels (R^2 adj. 72.7–75.4%, $p < 0.05$; Table 3.14). On the other hand, carbonate, reducible, pH, and CEC were important factors in describing the association between soil and plant Cu concentrations of Fluvisol (R^2 adj. 92–95.4%, $p < 0.05$; Table 3.14). Adriano (2001) suggested that Cu in soil solution (i.e., free and

complexed) and exchangeable form is believed to be labile fractions while those specifically adsorbed on organic matter and oxide surfaces are semilabile. The pH increase enhances complexation of Cu with soluble organic matter (McBride, 1994; Adriano, 2001) and removal of Al^{3+} from exchange sites (through hydrolysis) that creates additional sites for exchangeable Cu (Adriano, 2001). Conversely, Cu bound to soluble organic matter (see Table 4) is regarded as more phytoavailable than from carbonate and residual fractions (Adriano, 2001).

Zinc

The exchangeable, carbonate, reducible, oxidizable, pH, and CEC were important soil factors relating soil Zn with the corresponding levels in the plant parts (R^2 adj. 72.4–94.3%, $p < 0.05$; Table 3.14). The plant available fractions of Zn in soils largely entail the soluble, exchangeable (Kiekens, 1995; Adriano, 2001), complexed forms (Kiekens, 1995). Besides, Zn in the specifically adsorbed cation form (reducible and oxidizable fractions) may be mobilized (Pendias and Pendias, 2001). In harmony with the highest plant Zn levels observed (Table 3.11), Zn is regarded as readily soluble relative to other heavy metals (Pendias and Pendias, 2001).

Cadmium

The exchangeable, reducible, oxidizable and CEC were key soil factors in explaining the variability of plant Cd levels (R^2 adj. 61.8–95.9%, $p < 0.05$; Table 3.14). Marked reduction of Cd solubility under reducing conditions (Alloway, 1995b; Adriano, 2001), soil type, the mineralogy of the clay fraction (Adriano, 2001), and the influence rhizospheric pH appeared important in the uptake of Cd from the reducible fraction (Table 3.14). Low molecular weight organic acids (e.g., oxalic, citric, malate, fumarate, succinate, etc.) that exhibit some ability to desorb Cd from soils (Adriano, 2001) may also contribute to plant Cd. Consistent with the negative coefficients of CEC in this study, Alloway (1995b) and Adriano (2001) suggested that the Cd content of plants is inversely proportional to the CEC of the soil.

Lead

The Pb contents of the plant parts were related to the corresponding Pb levels associated with exchangeable, carbonate, reducible, and oxidizable fractions in the soils (R^2 adj. 72–87.1%, $p < 0.05$; Table 3.14). It has been indicated that Pb sorption

on montmorillonite entails cation exchange processes (Pendias and Pendias, 2001). Albeit the potential immobilization of Pb by CEC (Davies, 1995; Adriano, 2001), competitive adsorption of prevalent cations such as Ca^{2+} and / or other divalent heavy metals may release Pb from exchange site, and hence contribute to Pb uptake by plants. Likewise, certain soil and plant factors (e.g., low P, organic ligands) are believed to encourage root uptake and translocation to the shoot (Pendias and Pendias, 2001). Apart from poor Pb translocation to the shoot (McBride, 1994; Adriano, 2001; Pendias and Pendias, 2001), the redox potential (which affects the Pb in the reducible fractions) (Adriano, 2001) and precipitation as the carbonate and hydroxide (McBride, 1994) might affect the uptake and shoot Pb levels of plants.

Mercury

In general, most of the factors appeared to have influence on the relationships between soil and plant Hg levels (R^2 adj. 84.6–91%, $p < 0.05$; Table 3.14). However, the general portrayals related to mobility (and hence availability) are challenging to depict due to the complex chemistry of Hg (McBride, 1994). Notwithstanding this, the behavior of Hg in soils pivots mainly on its form, which is the product of various soil factors; and of the initial levels of Hg and other important ions like Cl^{-1} (Pendias and Pendias, 2001). Consequently, the chemical speciation of the dominant form of Hg in soils, Hg (II), principally has a bearing on the bioavailability and subsequent transport in plants (Adriano, 2001). The carbonate and reducible fractions may immobilize Hg through precipitation of Hg as $Hg(OH)_2$ at the neutral to alkaline pH (McBride, 1994) and adsorption of Hg^{2+} in neutral soils by iron oxides (Steinnes, 1995) . However, the suggested release of Hg due to the dissolution processes under the reduction of Hg oxides (Pendias and Pendias, 2001) might contribute to Hg uptake from the same fractions.

Selenium

The variations in Se levels of the study plants were explained by the exchangeable (in both soils), carbonate, and reducible fractions as well as CEC (Fluvisol), and pH (Vertisol) of the soils (R^2 adj. 67.5–93.3%, $p < 0.05$). The solubility and bioavailability of Se in soils are governed by pH (Neal, 1995; Adriano, 2001; Pendias and Pendias, 2001), $CaCO_3$ content (Pendias and Pendias, 2001; Pezzarossa and Petruzzeli, 2001), Fe oxide content (Adriano, 2001; Pendias and Pendias, 2001;

Pezzarossa and Petruzzelli, 2001), CEC (Pezzarossa and Petruzzelli, 2001), redox potential (Adriano, 2001) and plant factors (Neal, 1995; Adriano, 2001). Weak exchangeable adsorption of selenate onto oxides (exchangeable Se on reducible fraction), and other minerals at neutral to alkaline pH (McBride, 1994; Adriano, 2001) and thus, the ensuing high Se mobility (McBride, 1994) could enhance Se uptake and translocation (see Table 3.14). On the other hand, the residual fractions may intimate the importance of non–residual fractions for the plant Se levels.

Vanadium

The reducible (both soils), exchangeable, carbonate (Fluvisol), pH, and residual (Vertisol) were important soil factors in relating soil and plant V levels (R^2 adj. 73–92.1%, $p < 0.05$; Table 3.14). In general, the soil type (Edwards *et al.,* 1995; Adriano, 2001), redox potential (McBride, 1994; Edwards *et al.,* 1995), pH, and organic matter content of the soil (Edwards *et al.,* 1995) influence bioavailability of V in soils. The potential supply of plant available V by the Fe oxides, possible uptake of oxyanions of V^{5+} (VO^{3-}, HVO_4^{2-}) at the neutral to alkaline pH (Pendias and Pendias, 2001), root immobilization and precipitation of sparingly soluble V compounds (Edwards *et al.,* 1995) might depict the observed V uptake and translocation.

Arsenic

The As levels in the plant parts grown on Vertisol were related to exchangeable (R^2 adj. 64.7–75%, $p < 0.05$), while for those from Fluvisol, carbonate, reducible, residual, and CEC (R^2 adj. 79.1–94%, $p < 0.05$) were found important (Table 3.14). The mobility and eventually the availability of As is mainly influenced by changes in pH and redox potential of the medium, sorption by clay minerals, and Fe and Al oxides (O'Neill, 1995; Adriano, 2001; Matera and Le Hécho, 2001; Pendias and Pendias, 2001). Apart from this, adsorption of As (V) on calcite (Adriano, 2001; Matera and Le Hécho, 2001; Pendias and Pendias, 2001), which increases from pH 6-10 has been indicated (Adriano, 2001; Matera and Le Hécho, 2001). The reducible fractions of the study soils (Daniel Fitamo *et al.,* 2007) and similar others (Adriano, 2001) accommodate the bulk of total As, which could be released upon hydrolysis under the reduction of the soil potential (Adriano, 2001; Pendias and Pendias, 2001). Besides, the possible release of As from anion exchange sites with increase in CEC may ultimately contribute to plant uptake. However, availability might be affected by

competing ions (O'Neill, 1995; Adriano, 2001; Matera and Le Hécho, 2001) and adsorption of As (V) on calcite (Adriano, 2001; Matera and Le Hécho, 2001).

3.3.2 Heavy metal/metalloid uptake and translocation to the shoot under natural condition (field experiment)

3.3.2.1 Heavy metal levels in the shoot of plants

Even with out considering the root, which naturally accommodates disproportionately higher fraction of the total amount of heavy metal in plants (Tables 3.11 and 3.13), relatively higher levels (see Table 3.15) than the reported natural levels (Cr: 0.02–0.2, Ni: 0.1–1.7, Co: 0.10–0.57, Se: < 0.09 mg kg^{-1} Pendias and Pendias, 2001; V: 1 mg kg^{-1} Adriano, 2001) were observed in the shoots. Besides, the levels of Ni (3.78–13.68 mg kg^{-1}: for all plants), Zn (Clover: 83.58, Setaria: 60 mg kg^{-1}), and V (Oat: 8.90, Clover: 16.05 mg kg^{-1}) of shoots on Vertisol were comparable to those plants from contaminated soils (V: Edwards *et al.,* 1995; Ni, and Zn: Pendias and Pendias, 2001). Apart from this, the average levels of Cr, Ni, Cu, and Zn of plants (Table 3.15), were comparatively higher than the mean values (1.0, 4.0, 9.5, and 33.5 mg kg^{-1}, respectively) of the same in aerial parts of vegetation on a highly disturbed soil with polymetallic pollution (Remon *et al.,* 2005). Over and above, the levels of Cr (16.61 mg kg^{-1}), Ni (13.68 mg kg^{-1}), and Cu (23.34 mg kg^{-1}) in Clover were higher than the reported values (Vandecasteele *et al.,* 2005) for two Salix clones from contaminated sediments. Similar to the previous glass house study (see section 3.3.1), most of the heavy metals in the soils were highly phytoavailable and showed remarkable transfer to the shoot (see Table 3.15). The most notable in this regard were the highest levels of most heavy metals in Clover and Setaria grown on Vertisol.

Table 3.15 The average (n = 6) concentration (±SD) of heavy metals in the shoot, soil (mg kg^{-1}, DM), and the translocation coefficients of heavy metals in the study plants.

Plant		Cr	Ni	Co	Cu	Zn	Cd	Pb	Hg	Se	V	As
Oat	CV	9.94 ±1.63	7.48 ±1.01	2.70 ±0.51	7.90 ±1.54	33.15 ±3.83	0.05 ±0.01	1.69 ±0.29	0.01 ±0.00	0.55 ±0.13	8.90 ±1.19	0.54 ±0.14
	[1]Soil	75.90 ±2.25	65.59 ±0.95	28.00 ±0.30	37.48 ±0.53	138.90 ±7.10	0.18 ±0.01	13.97 ±0.93	0.11 ±0.02	1.74 ±0.11	87.36 ±4.81	2.81 ±0.14
	TC	**0.13** ±0.05	0.11 ±0.03	**0.10** ±0.02	0.21 ±0.04	0.24 ±0.07	0.26 ±0.03	**0.12** ±0.04	0.07 ±0.02	0.31 ±0.10	0.10 ±0.03	**0.19** ±0.05
Oat	CF	1.30 ±0.15	2.14 ±0.18	0.83 ±0.19	4.60 ±1.11	33.31 ±5.22	0.03 ±0.01	0.56 ±0.10	0.01 ±0.00	0.14 ±0.03	2.50 ±0.11	0.13 ±0.04
	[2]Soil	23.76 ±5.29	32.68 ±6.48	27.44 ±5.25	16.21 ±3.25	109.00 ±8.40	0.13 ±0.01	24.33 ±2.93	0.12 ±0.12	2.58 ±0.12	63.53 ±17.3	3.05 ±0.87
	TC	0.06 ±0.01	0.07 ±0.02	0.03 ±0.01	0.28 ±0.10	0.31 ±0.09	0.22 ±0.05	0.02 ±0.00	0.11 ±0.02	0.05 ±0.01	0.04 ±0.01	0.04 ±0.01
Alfalfa	CV	6.64 ±0.29	5.16 ±0.58	2.00 ±0.09	9.86 ±0.83	28.45 ±0.73	0.04 ±0.00	1.34 ±0.03	0.01 ±0.00	0.48 ±0.02	6.72 ±0.25	0.43 ±0.02
	TC	0.09 ±0.00	0.08 ±0.01	0.07 ±0.01	0.26 ±0.02	0.20 ±0.01	0.24 ±0.02	**0.10** ±0.03	0.08 ±0.01	0.27 ±0.00	0.08 ±0.00	**0.15** ±0.01
Alfalfa	CF	0.94 ±0.11	0.97 ±0.24	0.41 ±0.11	7.58 ±0.43	19.95 ±3.83	0.03 ±0.01	0.55 ±0.17	0.01 ±0.00	0.09 ±0.02	1.43 ±0.31	0.10 ±0.02
	TC	0.04 ±0.01	0.03 ±0.01	0.01 ±0.00	0.47 ±0.03	0.18 ±0.04	0.21 ±0.04	0.02 ±0.01	0.06 ±0.01	0.04 ±0.02	0.02 ±0.01	0.03 ±0.01
Rhodes	CV	4.34 ±1.12	3.78 ±0.67	1.28 ±0.14	5.69 ±1.26	27.96 ±3.24	0.13 ±0.05	0.80 ±0.14	0.02 ±0.01	0.39 ±0.12	3.91 ±0.58	0.24 ±0.06
	TC	0.06 ±0.02	0.06 ±0.02	0.05 ±0.01	0.15 ±0.03	0.20 ±0.08	0.70 ±0.25	0.06 ±0.02	0.18 ±0.04	0.23 ±0.06	0.04 ±0.01	0.09 ±0.02
Rhodes	CF	2.77 ±0.66	3.03 ±0.29	1.35 ±0.23	6.02 ±0.56	44.91 ±4.36	0.07 ±0.02	0.90 ±0.15	0.01 ±0.00	0.23 ±0.05	4.24 ±0.23	0.24 ±0.10
	TC	**0.12** ±0.03	0.09 ±0.02	0.05 ±0.01	0.37 ±0.03	0.41 ±0.13	0.50 ±0.14	0.04 ±0.01	0.08 ±0.01	0.09 ±0.02	0.07 ±0.03	0.08 ±0.02
Clover	CV	16.61 ±2.43	13.68 ±2.17	5.12 ±0.84	23.34 ±1.89	83.58 ±6.69	0.06 ±0.02	2.80 ±0.34	0.02 ±0.01	0.94 ±0.18	16.05 ±2.07	1.01 ±0.23
	TC	**0.22** ±0.07	0.21 ±0.06	**0.18** ±0.05	0.62 ±0.13	0.60 ±0.14	0.35 ±0.08	**0.20** ±0.05	**0.15** ±0.04	0.54 ±0.10	0.18 ±0.06	**0.36** ±0.12
Clover	CF	0.96 ±0.15	1.82 ±0.43	0.69 ±0.08	20.34 ±2.84	61.82 ±10.47	0.03 ±0.00	0.55 ±0.12	0.01 ±0.00	0.09 ±0.03	1.50 ±0.35	0.10 ±0.02
	TC	0.04 ±0.01	0.06 ±0.01	0.03 ±0.00	1.25 ±0.18	0.57 ±0.10	0.21 ±0.02	0.02 ±0.00	0.06 ±0.01	0.04 ±0.01	0.02 ±0.01	0.03 ±0.01
Setaria	CV	7.47 ±1.00	5.67 ±0.33	2.16 ±0.35	11.38 ±0.44	59.87 ±4.65	0.17 ±0.02	1.22 ±0.25	0.03 ±0.00	0.42 ±0.15	6.68 ±1.33	0.39 ±0.09
	TC	**0.10** ±0.03	0.09 ±0.02	0.08 ±0.01	0.30 ±0.01	0.43 ±0.08	0.92 ±0.09	0.09 ±0.03	**0.24** ±0.07	0.24 ±0.05	0.08 ±0.02	**0.14** ±0.03
Setaria	CF	3.08 ±0.72	3.03 ±0.76	1.41 ±0.15	9.40 ±0.92	52.65 ±9.92	0.12 ±0.02	1.11 ±0.42	0.01 ±0.00	0.22 ±0.06	4.69 ±0.72	0.31 ±0.07
	TC	**0.13** ±0.03	0.09 ±0.01	0.05 ±0.01	0.58 ±0.06	0.48 ±0.09	0.87 ±0.12	0.05 ±0.01	0.06 ±0.02	0.09 ±0.02	0.07 ±0.01	**0.10** ±0.02

NB. *CV and CF stand for average shoot levels of heavy metal/metalloid of plants grown on contaminated Vertisol and Fluvisol, respectively.*
[1]soil *and* [2]soil *correspond to heavy metal levels of CV and CF, in that order.*
The TC values are based on the ratio of heavy metal levels in the shoot to the corresponding levels in the soil. TC values in **bold** depict mean values above those reported by Alloway (1995b).

Apart from Alfalfa, conversely, the shoot levels of most heavy metals under the present experiment were comparatively higher than the corresponding levels for the same species and soil from the preceding glass house study. Of these, those with

significant differences ($p < 0.05$) in mean heavy metal levels of the shoot between these studies are presented in Table 3.16. As the roots of container–grown plants are exclusively exposed to contaminated soil, higher uptake of metals than from the same soil in the field is expected (Alloway, 1995b). However, factors related to transpiration intensity (Pendias and Pendias, 2001) and / or specific and synergistic effects of factors including wind speed, temperature, humidity, water supply (Kozlowski *et al.,* 1991) and related factors (passive uptake, pH, and Eh) in the field could have played a role for relatively higher levels of heavy metals. Likewise, local variations in availability of heavy metals between blocks on the same soil in the field might be important. Accordingly, the strong significant differences ($p < 0.001$) found for mean shoot levels of Cr (Setaria, Fluvisol), Ni (Oat on both soils; Alfalfa and Rhodes on Vertisol), Co (Alfalfa, Vertisol), Cd (Alfalfa, Vertisol), Pb (Alfalfa, Fluvisol; Clover and Setaria, Vertisol), Se (Oat and Alfalfa, Vertisol), and As (Oat, Vertisol) might partly corroborate for the higher uptake in the field. Similar to the glass house study (see section 3.3.1), on the other hand, Oat (Cd), Clover (Cd), and Setaria (Ni, Co, Cu, Cd) had shoot levels that exhibited significant variations ($p < 0.05$) between the soils.

Table 3.16 The *p* values established for mean (n = 6) differences between heavy metal levels in the shoots of plants of the present and the preceding greenhouse studies.

Plant type	Soil type	Cr	Ni	Co	Cu	Zn	Cd	Pb	Hg	Se	V	As
Oat	Fluvisol								0.009			
Alfalfa	Vertisol								0.011			
Alfalfa	Fluvisol				0.001	0.001			0.006			
Rhodes	Vertisol								0.039			
Rhodes	Fluvisol			0.013	0.018			0.002		0.003	0.013	0.026
Clover/Desmodium[1]	Vertisol				0.011		0.016		0.003			
Clover/Desmodium[1]	Fluvisol				0.002		0.000	0.017	0.000			
Setaria	Vertisol			0.007		0.047	0.000			0.024		
Setaria	Fluvisol		0.02	0.047			0.003				0.003	0.005

[1]Due to failure of Desmodium to grow on the experimental site, Clover was used instead.

3.3.2.2 Soil–plant transfer coefficients (TC) of heavy metals

The transfer coefficient (Alloway, 1995) or enrichment coefficient (Yanqun *et al.,* 2004), a ratio of metal concentration in the aboveground tissue to the corresponding level in the soil (Alloway, 1995; Yanqun *et al.,* 2004), can be employed to assess heavy metal accumulation of plants (Yanqun *et al.,* 2004) or as indicator of bioavailability (Adriano, 2001). Compared to the general order of the TC indicated by Alloway (1995b), higher TC values (0.12–0.36, see Table 3.15) were observed for Cr (Oat, Vertisol; Rhodes, Fluvisol; Clover, Vertisol; and Setaria, Fluvisol), Co (Clover, Vertisol), Pb (Oat and Clover, Vertisol), Hg (Clover and Setaria, Vertisol) and As (Oat, Alfalfa, Clover, and Setaria on Vertisol). In addition, Cr (Setaria, Vertisol), Co (Oat, Vertisol), Pb (Alfalfa, Vertisol), and As (Setaria, Fluvisol) had values (0.10) similar to the upper limits indicated by Alloway (1995b). Likewise, Alfalfa (Vertisol: Cr, Co, Pb, Hg, As; Fluvisol: Hg), Desmodium (Vertisol: Pb: Fluvisol: Hg), and Setaria (Hg on both soils and As, Vertisol) under the glass house condition (see section 3.3.1) had higher TC values than indicated by the above author.

In general, the TC values of the plants on both soils decreased in the order of Cd > Cu > Zn > Se > Cr > As > Ni > Hg > Pb > V > Co, with comparatively higher values on Vertisol. The higher TC values of Cu, Cd, Zn, and Se (Table 3.15; see section 3.3.1) confirmed their (relatively) enhanced bioavailability as well as translocation to the shoot. Zn, Cd (Alloway, 1995b; Adriano, 2001) and Se (Alloway, 1995b; Pendias and Pendias, 2001) were labeled as elements that can be readily translocated to aboveground parts; Ni, Co, and Cu were intermediate; and Cr, Pb, and Hg were the least translocated (Alloway, 1995b).

Clover (Vertisol) and Setaria (Fluvisol) presented the highest values of transfer coefficients for most of the heavy metals (Table 3.15). Moreover, Rhodes on Fluvisol, while Oat and Alfalfa on Vertisol showed relatively higher TC values for most heavy metals (Table 3.15). Generally, the plants (saving Rhodes) exhibited palpable disposition to transfer relatively more heavy metals to shoot (higher TC) when grown on Vertisol (Table 3.15). However, this tendency seemed more pronounced on legumes (Alfalfa and Clover) than grasses (Oat and Setaria). Correspondingly, higher TC and TF (root to shoot translocation factor) values of most heavy metals were

found (see section 3.3.1) for similar plants grown under glass house conditions on Vertisol. As a rule, the same species could display different responses to metals in the soil depending on the local conditions including soil type, mean air temperature, and elevation above sea level (Edwards *et al.,* 1998). By all appearances, Clover and Oat on Vertisol, Rhodes on Fluvisol and Setaria on both soils appeared to be effective in the transfer of heavy metals / metalloids from soil to shoot.

3.3.2.3 Influence of the main and interaction effects on heavy metal levels in the shoot

Multivariate test on the main (soil type, and species) and their interaction effects on heavy metal levels of the shoot revealed that soil type (p = 0.005) and species (p = 0.000) were important. The average levels of heavy metals/metalloids in the shoots averaged over the species types were higher for the plants grown on Vertisol than those from Fluvisol. In this regard, the soil type particularly had significant impact on the levels of Cr (p = 0.004), Ni (p = 0.003), Co (p = 0.009), Cu (p = 0.032), Cd (p = 0.004), Pb (p = 0.017), Se (p = 0.003), V (p = 0.013) and As (p = 0.012). Conversely, species type impinged on the content of Cu (p = 0.000), Zn (p = 0.000), and Cd (p = 0.000) where Clover followed by Setaria and Oat had the highest mean levels (averaged over the soil types) of the elements. In contrast, the interaction effects (soil type × species) did not have significant effects on heavy metal/metalloid contents of the shoots. As with similar plants under greenhouse condition (see section 3.3.1.3), Vertisol promoted soil–shoot transfer of most of the heavy metals/metalloids

The nature of distribution and accumulation of each element within plants largely depends on the type of plant (Pendias and Pendias, 2001). Apart from the type of metal, this entails the age of the plant, and the plant organ that are deemed important in regulating the rate and the level of movement of heavy metals within plants (Alloway, 1995a). Conversely, McBride (1994) suggested that the soil might possibly affect translocation of elements with in the plants; because mobility in plants is sensitive to the specific chemical form absorbed from soil solution. In agreement with the above statement, Pendias and Pendias (2001) also indicated that the electrochemical properties of elements determine the transport of trace elements among plant organs.

3.3.2.4 Contribution of the different soil fractions and soil factors to heavy metal/metalloid sequestration in the shoot

Chromium

The variations in the Cr levels in the shoots of the plants might be explained by way of the corresponding levels extracted from the carbonate, reducible, oxidizable fractions as well as the pH and CEC of Fluvisol as predictor variables (R^2 adj. 70.8–90%, $p < 0.05$; Table 3.17). Similar variables were important in relating soil–plant transfer of Cr on Fluvisol under controlled environment (section 3.3.1). The mobility, solubility, transformation, speciation, and uptake of Cr is influenced by the pH, oxidation state of the Cr, presence of reducing (e.g., organic matter, Fe^{2+}, and sulfides) and oxidizing materials (e.g., high–valency Mn oxides), oxides of Fe and Mn, and redox potential (Adriano, 2001; Adriano, 2001).

The predominance of weakly adsorbing and highly bioavailable form (CrO_4^{2-}) (McBride, 1994) at neutral pH (McLean and Bledsoe, 1992; McBride, 1994; Adriano, 2001) with faster uptake than Cr^{3+} (Han *et al.,* 2004), complexation of Cr^{3+} with soluble organic acids above pH 5.5 (McGrath, 1995; Adriano, 2001), acidification and redox changes at the rhizosphere (Alloway, 1995b) might enhance Cr uptake. Conversely, the formation of insoluble Cr^{3+} precipitate at about pH over 4.5 (Adriano, 2001), specific adsorption of Cr^{3+} to humic acid, clay and Fe and Mn oxides (see section 3.3.1), reduction of Cr^{6+} to Cr^{3+} (Han *et al.,* 2004), may diminish uptake and translocation to the shoot.

Nickel

The carbonate, reducible, and oxidizable fractions as well as the pH and CEC appeared to be important variables in describing shoot Ni levels of the plants (R^2 adj. 70.5–91%, $p < 0.05$; Table 3.17). It was found that the carbonate and oxidizable fractions as well as the CEC were central variables in the soil-plant transfer of Ni under the glass house conditions (section 3.3.1). Sizeable proportions of the total amount of Ni were extracted from the carbonate, reducible, and oxidizable fractions of contaminated soils (McGrath, 1995; Adriano, 2001; Daniel Fitamo *et al.,* 2007).

The potential availability of Ni associated with the oxides of Fe and Mn (Pendias and Pendias, 2001) and modification of soil pH by roots and fungi at the rhizosphere (Pendias and Pendias, 2001) might enhance plant uptake. Apart from the plant and pedological factors, the pH appears fundamental influencing Ni uptake by plants (Pendias and Pendias, 2001). Conversely, Ni could become less available to plants as CEC decreases (McGrath, 1995) and Ni is immobilized by organic matter (Adriano, 2001).

Cobalt

Similar to the greenhouse study (section 3.3.1), the exchangeable, carbonate, reducible, pH and CEC were important factors linking the shoot Co levels with the corresponding levels in the soils (R^2 adj. 58.1–91.8%, $p < 0.05$; Table 3.17). Uptake of Co by plants is governed by the Co concentration in the soil solution (Pendias and Pendias, 2001; Smith and Paterson, 1995), on the exchange sites (Smith and Paterson, 1995), and the mobile Co content of the soil (Pendias and Pendias, 2001). Besides, soil properties like the amount of Mn and Fe (Adriano, 2001), the soil pH and moisture (Adriano, 2001; Smith and Paterson, 1995) are important factors determining the Co status of plants (Adriano, 2001). While poor drainage may release elevated level of Co (Smith and Paterson, 1995), acidification at the pH at the rhizosphere (Alloway, 1995b) could bring certain dissolution of Co precipitate (possibly $Co(OH)_2$) that eventually might contribute to the uptake of Co. By all appearances, the natures of the soils, the management practice (e.g., irrigation), the plant factors, namely plant species and type of organ (Adriano, 2001), as well as their interaction appeared relevant in describing the observed soil–plant transfer of Co.

Table 3.17 Shoot heavy metal levels (n = 6) as function of some soil properties and / or heavy metal / metalloid associated with the geochemical phases of the soils.

Species	Soil type	Heavy metal	Stepwise regression equation	R^2 adjusted	P value
Oat	Fluvisol	Cr	**Cr = - 43.6 + 34.5 C - 1.50 O + 5.81 pH + 0.409 CEC**	**90.0%**	**0.012**
Alfalfa	Fluvisol	Cr	**Cr = 3.12 + 0.0490 O - 0.0953 CEC**	**71.1%**	**0.022**
Rhodes	Fluvisol	Cr	**Cr = - 7.86 + 0.447 O + 0.278 CEC**	**89.4%**	**0.016**
Clover	Fluvisol	Cr	**Cr = 2.08 + 0.0246 O - 0.0491 CEC**	**70.8%**	**0.037**
Setaria	Fluvisol	Cr	**Cr = - 0.873 - 38.1 C + 0.425 R + 0.781 O + 0.0279 CEC**	**90.0%**	**0.001**
Alfalfa	Vertisol	Ni	**Ni = - 36.3 + 4.65 R - 2.62 O**	**70.5%**	**0.034**
Alfalfa	Fluvisol	Ni	**Ni = 2.87 + 0.0721 C - 0.0926 CEC**	**88.7%**	**0.018**
Rhodes	Vertisol	Ni	**Ni = - 18.9 + 0.908 R + 1.36 pH**	**71.0%**	**0.023**
Rhodes	Fluvisol	Ni	**Ni = - 27.1 + 1.76 C + 0.478 R + 2.98 pH - 0.303 CEC**	**91.0%**	**0.002**
Clover	Fluvisol	Ni	**Ni = - 0.544 + 1.24 C + 0.280 R - 0.367 CEC**	**87.1%**	**0.017**
Oat	Fluvisol	Co	**Co = 9.01 + 350 E - 0.204 R - 0.971 pH**	**88.1%**	**0.011**
Alfalfa	Vertisol	Co	**Co = - 43.1 + 1.66 C + 3.11 pH**	**73.2%**	**0.021**
Alfalfa	Fluvisol	Co	**Co = 5.57 + 0.0932 C - 0.00157 R - 0.629 pH - 0.0711 CEC**	**77.0%**	**0.011**
Rhodes	Vertisol	Co	**Co = 14.6 + 26935 E + 0.171 C + 6.10 pH - 1.03 CEC**	**89.7%**	**0.034**
Rhodes	Fluvisol	Co	**Co = - 3.34 + 0.387 C + 0.0426 R**	**84.5%**	**0.028**
Clover	Vertisol	Co	**Co = 83.0 + 129698 E + 26.1 pH - 4.60 CEC**	**91.8%**	**0.049**
Clover	Fluvisol	Co	**Co = 2.36 + 14.1 E - 0.0106 R - 0.171 pH - 0.0160 CEC**	**78.2%**	**0.019**
Setaria	Fluvisol	Co	**Co = 2.39 - 0.0368 CEC**	**58.1%**	**0.048**
Oat	Fluvisol	Cu	**Cu = 15.9 - 1.87 C - 1.15 R + 4.31 O - 0.239 CEC**	**87.9%**	**0.024**
Alfalfa	Vertisol	Cu	**Cu = - 8.48 + 3.29 O**	**76.7%**	**0.014**
Alfalfa	Fluvisol	Cu	**Cu = 17.6 + 0.0594 C - 0.975 pH - 0.138 CEC**	**91.9%**	**0.048**
Rhodes	Vertisol	Cu	**Cu = 1.19 + 0.807 O**	**78.5%**	**0.012**
Rhodes	Fluvisol	Cu	**Cu = 9.78 - 0.715 C - 0.422 R + 1.67 O - 0.0764 CEC**	**91.9%**	**0.021**
Clover	Vertisol	Cu	**Cu = -9.5 2 + 2.77 R**	**87.9%**	**0.020**
Clover	Fluvisol	Cu	**Cu = 6.30 + 6.10 C + 3.27 R - 13.9 O**	**92.0%**	**0.048**
Setaria	Vertisol	Cu	**Cu = 11.0 + 0.0330 R + 0.409 O + 0.299 pH**	**89.2%**	**0.005**
Setaria	Fluvisol	Cu	**Cu = 13.2 - 1.40 O**	**74 .4%**	**0.021**
Oat	Vertisol	Cu	**Cu = - 22.1 + 1.07 ECRO**	**57.5%**	**0.045**
Oat	Fluvisol	Zn	**Zn = 109 - 2.87 CEC**	**89.1%**	**0.003**
Alfalfa	Fluvisol	Zn	**Zn = - 34.1 + 194 E - 1.89 C - 0.886 O + 14.6 pH**	**79.6%**	**0.041**
Rhodes	Fluvisol	Zn	**Zn = 146 - 3.80 CEC**	**76.4%**	**0.014**
Clover	Fluvisol	Zn	**Zn = 2.12 + 5.33 E + 5.19 C + 2.42 O - 4.03 pH**	**85.7%**	**0.036**
Setaria	Vertisol	Zn	**Zn = 99.3 + 362 E - 1.13 O - 2.28 pH**	**92.9%**	**0.042**
Setaria	Fluvisol	Zn	**Zn = 84.8 - 0.220 R - 1.85 O**	**78.5%**	**0.046**
Oat	Fluvisol	Cd	**Cd = 0.0688 - 0.00149 CEC**	**74.5%**	**0.020**
Alfalfa	Vertisol	Cd	**Cd = - 0.132 + 2.03 C - 5.45 R + 7.27 O**	**90.7%**	**0.032**
Alfalfa	Fluvisol	Cd	**Cd = 0.169 + 5.90 E + 0.509 C + 0.185 R - 0.0268 pH**	**85.0%**	**0.001**
Clover	Fluvisol	Cd	**Cd = 0.0134 + 0.00716 R - 0.225 O + 0.000630 CEC**	**79.8%**	**0.001**
Setaria	Fluvisol	Cd	**Cd = - 0.00071 + 5.00 E + 0.175 R + 0.00413 CEC**	**87.4%**	**0.016**
Oat	Fluvisol	Pb	**Pb = 35.2 + 0.383 C - 0.725 R + 2.32 O - 5.03 pH**	**79.8%**	**0.029**
Alfalfa	Fluvisol	Pb	**Pb = 1.42 - 0.0340 C - 0.665 O**	**86.2%**	**0.024**
Rhodes	Fluvisol	Pb	**Pb = - 10.5 - 0.138 C + 0.236 R - 1.11 O + 1.72 pH**	**88.6%**	**0.008**
Clover	Fluvisol	Pb	**Pb = 1.17 - 0.0109 C - 0.00607 R - 0.368 O - 0.00688 CEC**	**88.0%**	**0.001**
Setaria	Fluvisol	Pb	**Pb = 30.8 + 713 E - 0.0863 C - 4.25 pH**	**87.3%**	**0.027**
Oat	Vertisol	Hg	**Hg = 0.0126 - 2.80 C - 0.126 R**	**65.5%**	**0.028**
Oat	Fluvisol	Hg	**Hg = 0.000344 + 0.414 E + 3.56 C + 0.0856 R**	**75.1%**	**0.029**
Alfalfa	Vertisol	Hg	**Hg = - 0.106 + 0.281 E + 0.287 R + 0.0143 pH**	**94.2%**	**0.007**
Alfalfa	Fluvisol	Hg	**Hg = 0.00589 + 0.0121 E + 0.715 C**	**90.8%**	**0.013**
Rhodes	Vertisol	Hg	**Hg = - 0.198 + 0.529 ECR + 0.0251 pH**	**81.2%**	**0.008**
Rhodes	Fluvisol	Hg	**Hg = - 0.00265 + 0.676 E + 5.12 C + 0.144 R**	**76.2%**	**0.023**
Clover	Vertisol	Hg	**Hg = - 0.141 - 4.88 C + 0.00267 CEC**	**71.1%**	**0.027**
Clover	Fluvisol	Hg	**Hg = - 0.00533 + 0.00842 R + 0.000462 CEC**	**91.6%**	**0.011**
Setaria	Fluvisol	Hg	**Hg = 0.0139 + 0.499 E + 1.00 C + 0.123 R**	**88.1%**	**0.011**
Oat	Fluvisol	Se	**Se = - 1.94 + 21.8 EC - 0.105 CEC**	**69.7%**	**0.024**
Alfalfa	Fluvisol	Se	**Se = - 0.756 + 8.62 EC - 0.0402 CEC**	**68.4%**	**0.025**
Rhodes	Fluvisol	Se	**Se = - 0.067 + 1.00 R**	**59.8%**	**0.044**
Clover	Fluvisol	Se	**Se = - 0.399 + 3.10 E + 2.97 C - 0.594 R**	**93.6%**	**0.038**
Setaria	Fluvisol	Se	**Se = 2.03 - 14.2 EC + 0.0511 CEC**	**67.2%**	**0.047**
Oat	Fluvisol	V	**V = - 21.2 + 643 E - 2.50 R + 9.86 pH + 1.77 CEC**	**87.9%**	**0.009**
Alfalfa	Vertisol	V	**V = - 230 + 4.25 ECR**	**58.1%**	**0.048**
Alfalfa	Fluvisol	V	**V = 3.80 - 0.338 C**	**59.8%**	**0.044**
Rhodes	Vertisol	V	**V = - 69.8 + 1.33 ECR**	**78.3%**	**0.016**
Rhodes	Fluvisol	V	**V = - 13.1 + 121 E + 0.386 R + 0.112 CEC**	**94.0%**	**0.036**
Clover	Fluvisol	V	**V = 10.1 + 0.152 C + 0.0239 R + 0.890 pH + 0.165 CEC**	**82.0%**	**0.003**
Alfalfa	Vertisol	As	**As = - 6.45 + 435 E + 2.57 R**	**67.0%**	**0.028**

Alfalfa	Fluvisol	As	**As = 0.602 - 1.20 C - 0.165 R**	**93.4%**	**0.008**
Rhodes	Vertisol	As	**As = - 0.447 + 144 E**	**63.7%**	**0.029**
Rhodes	Fluvisol	As	**As = - 0.800 + 0.213 R + 0.0254 CEC**	**78.8%**	**0.045**
Clover	Fluvisol	As	**As = 0.495 - 0.773 C - 0.148 R**	**77.5%**	**0.049**
Setaria	Fluvisol	As	**As = - 1.90 - 4.65 C - 0.278 R + 0.395 pH + 0.0320 CEC**	**89.9%**	**0.021**

NB. Symbols employed above as predictor variables represent heavy metals extracted from the different geochemical phases/fractions of the soils (E = exchangeable; C = carbonate; R = reducible; and O = oxidizable fraction) while the symbol combinations (EC, ECR, and ECRO) denote the sum of heavy metals associated with respective fractions.

Copper

The carbonate, reducible, oxidizable fractions as well as the pH and CEC had important effect in the soil–shoot transfer of Cu (R^2 adj. 57.5–92%, $p < 0.05$; Table 3.17). Apart from the exchangeable fraction, selfsame variables were consequential for soil–shoot transfer of Cu under the glass house condition (section 3.3.1). Similar to the plants on Vertisol (section 3.3.1), the oxidizable fraction was important for shoot Cu status of the plants from both soils (Table 3.17). It has been reported that organically bound Cu is more phytoavailable than Cu from other fractions (Adriano, 2001).

In neutral and alkaline solutions soluble complexes of Cu^{2+}, most importantly carbonate (McBride, 1994; Adriano, 2001; Pendias and Pendias, 2001), hydroxy, and organic matter complexes (McBride, 1994), the reduction of pH at the rhizosphere may possibly play a role in the uptake of Cu. In contrast, formation of hydroxy–carbonate (McBride, 1994), and / or Cu complexation with selective complexing groups like amines and polyphenols (McBride, 1994) may result in diminished uptake of Cu. On the other hand, Cu sorption capacity (or diminished availability of Cu) of soils may follow closely the extent of the CEC, the levels of oxides of Fe and Mn, and organic matter in the soil (Adriano, 2001).

Zinc

The exchangeable, carbonate, oxidizable, pH, and CEC were basic variables that appeared to have effect on the level of Zn in the shoots (R^2 adj. 76.4–92.9%, $p < 0.05$; Table 3.17). Saving reducible fraction, selfsame fractions and soil variables were important in Zn uptake and translocation in similar plants under controlled environment (section 3.3.1). It has been suggested that plant available fractions of Zn in soils largely entail the soluble, exchangeable (Kiekens, 1995; Adriano, 2001; Pendias and Pendias, 2001), and complexed forms (Kiekens, 1995). Comparatively,

Zn is regarded as readily soluble relative to other heavy metals (Pendias and Pendias, 2001). Consistent with this premise, the Zn levels were highest in the present study (Table 3.15) and in similar plants grown under the glass house condition (section 3.3.1).

Zn sorption by carbonates, precipitation of Zn hydroxide or carbonates, or formation of insoluble calcium zincate, adsorption by insoluble soil organic matter (Adriano, 2001), pH dependent retention of Zn, selective adsorption of Zn on Ca exchange sites (Pendias and Pendias, 2001) could result in limited availability / translocation of Zn.

Cadmium

The Cd levels in the shoots of the plants may perhaps be estimated by a linear combination of two or more of the exchangeable, carbonate, reducible, oxidizable, pH, and CEC as predictor variables (R^2 adj. 74.5–90.7%, $p < 0.05$; Table 3.17). Saving carbonate fraction and pH, identical fractions and soil variable were important in Cd uptake and translocation in similar plants under controlled environment (section 3.3.1). Adriano (2001) suggested that the bioavailability of Cd to crop plants could be more accurately predicted with recourse to the bioavailable extractants. The competition of divalent cations (e.g., Ca^{2+}, and Zn^{2+}) with Cd adsorption on permanent charge clays, low molecular weight organic acids at the rhizosphere (Adriano, 2001), and acidification attributable to the metabolic activity of roots, microorganisms and other living organisms (McBride, 1994) could facilitate uptake. On the other hand, adsorption of Cd by amorphous oxyhydroxides of Fe and Mn (in soils with low Eh–high pH), adsorption on CEC, chelate formation (Adriano, 2001) and in regularly waterlogged soils (McBride, 1994), limited availability is expected.

Lead

The shoot Pb contents of the plants were related to the Pb associated with the exchangeable, carbonate, reducible and oxidizable fractions (section 3.3.1), as well as to selected soil variables (pH, and CEC) of Fluvisol (R^2 adj. 79.8–88.6%, $p < 0.05$; Table 3.17). Albeit the low solubility of Pb in soil, certain soil and plant factors are recognized to facilitate both the uptake of Pb by roots and its translocation to the shoot (Pendias and Pendias, 2001). Correspondingly, potential translocation of Pb

from the root into the shoot of edibles grown in contaminated residential soils has been confirmed (Finster *et al.,* 2004).

Complexation with humic acids (Davies, 1995), higher pH, precipitation as carbonate, hydroxide, or phosphate (McLean and Bledsoe, 1992; McBride, 1994; Pendias and Pendias, 2001), chemisorption on oxides and silicate clays (McLean and Bledsoe, 1992; McBride, 1994), and CEC (Davies, 1995) could reduce uptake. Conversely, complexation with dissolved organic carbon (DOC) or fulvic acids (Adriano, 2001), acidification and change in redox potential at the rhizosphere (Alloway, 1995b) might contribute to the uptake and translocation of Pb.

Mercury

The exchangeable, carbonate, reducible, pH, and CEC were important soil parameters for the description of Hg accumulation in the shoots of plants (R^2 adj. 65.5–94.2%, $p < 0.05$; Table 3.17). Similar parameters (saving the carbonate and the pH) were relevant for soil–shoot transfer of Hg of plants under glass house conditions (section 3.3.1). Hg and its compounds are absorbed by roots and translocated to other plant parts (Adriano 2001; Pendias and Pendias, 2001).

General account regarding Hg mobility (and hence its phytoavailability) is taxing to make due to of the complex chemistry of Hg (McBride, 1994). Notwithstanding this, the level of Hg transfer to foliage, hinges on array of factors including pH, redox potential (Steinnes, 1995; Adriano, 2001), the Hg species, type of ions on the exchange complex, and plant species (Adriano, 2001). Besides, the release of Hg through the dissolution processes and under the reduction of Hg oxides is suggested (Pendias and Pendias, 2001). In contrast, precipitation of Hg as $Hg(OH)_2$ at the neutral to alkaline pH (McBride, 1994), might limit uptake.

Selenium

The exchangeable, carbonate, and reducible were key soil fractions in the description of soil–shoot transfer of Se (R^2 adj. 59.8–93.6%, $p < 0.05$; Table 3.17). Likewise, comparable results were reported for other plants grown under controlled conditions (section 3.3.1). Apart from this, it is notable that statistically significant predictions of

shoot Se levels were possible for most cases when combination of the above variables were used than individual variable (Table 3.17).

It has been indicated that numerous factors could impinge on the uptake and accumulation of Se by plants (Neal, 1995). Accordingly, the bioavailability of Se hinges on CaCO3 content, CEC (Pezzarossa and Petruzzelli, 2001), redox potential (Adriano, 2001; Pendias and Pendias, 2001), Fe oxide content (Adriano, 2001; Pendias and Pendias, 2001; Pezzarossa and Petruzzelli, 2001), as well as plant–related factors (Neal, 1995; Adriano, 2001; Pezzarossa and Petruzzelli, 2001). Besides, the occurrence of mobile form of Se (SeO_4^{2-}) at circumneutral pH (Adriano, 2001) that adsorbs non–specifically (Neal, 1995) and the possibility of competition by other anions (mainly PO_4^{3-}) for the exchange complex in soils (Adriano, 2001), may augment uptake. Consequently, the soil and plant factors may interact under certain conditions to enhance or reduce Se translocation from soil to plants (Neal, 1995).

Vanadium

The exchangeable, carbonate, reducible, pH, and CEC were soil variables that related the levels of V in the soil and shoot (R^2 adj. 58.1–94%, $p < 0.05$; Table 3.17). Saving pH, this finding agrees well with reported soil factors (see section 3.3.1) for the soil-shoot transfer of V under the glass house study. It has been indicated that plant uptake (Adriano, 2001) and toxicity (Edwards *et al.,* 1995) of V are influenced primarily on soil type (Edwards *et al.,* 1995; Adriano, 2001). Edwards *et al*. (1995) suggested that pH, redox potential, and organic matter content are among important edaphic factors expected to have great bearing on V uptake by plants.

Mobility and bioavailability of V at neutral to alkaline pH (McBride, 1994) where the anionic forms of V (VO^{3-}, HVO_4^{2-}) prevail, the supply of V from Fe oxides (Pendias and Pendias 2001), acidification and redox changes at rhizosphere (Alloway, 1995b), V release from anion exchange with opposite relationship with CEC may effectively portray potential sources of V for plant uptake. On the other hand, the possible immobilization of V in the root due to precipitation of sparingly V compounds (Edwards *et al.,* 1995) may also affect soil–shoot V relationship.

Arsenic

The exchangeable, carbonate, reducible, pH, and CEC were key variables in the description of soil-shoot transfer of As (R^2 adj. 63.7–93.4%, $p < 0.05$; Table 3.17). Similar variables (barring pH) were reported pivotal for the same process under controlled conditions (see section 3.3.1). Mobility and bioavailability pivot on chemical form of As, oxides and hydroxides of Fe and Al, competing ions (O'Neill, 1995; Adriano, 2001; Matera and Le Hécho, 2001), pH, redox potential (McLean and Bledsoe, 1992; O'Neill, 1995; Adriano, 2001), soil texture (Adriano, 2001), clay minerals (O'Neill, 1995; Adriano, 2001), and plant factors (Adriano, 2001).

The release of As^{3+} and As^{5+} via dissolution of Fe and Mn oxides after flooding (McBride, 1994), As on the exchangeable fraction and circuitous contribution of CEC, and enhanced uptake at higher pH (O'Neill, 1995) might be important for soil–shoot transfer. Conversely, reduced solubility of As^{3+}/Fe^{3+} precipitate with pH increase, adsorption of As (V) on calcite at pH 6–10 (Matera and Le Hécho, 2001), and As accumulation in the root (O'Neill, 1995; Adriano, 2001) could affect the relationship between As in the soil and shoot.

3.4 Identification and selection of plants naturally growing on mine spoils for phytoremediation of heavy metal affected soils (Paper V)

3.4.1 Soil properties and levels of heavy metals in surface layer of the mine spoils

As presented in Table 3.18, the mean $pH_{(KCl)}$ at Southern Waste Dump (SWD) was extremely acidic (3.93), while that of Eastern Dump (ED) was moderately acidic (5.57). Conversely, the dried tailings material (TD^{b}) and the soil affected by seepage and previously spilled over tailings material (TD^{a}) had slightly acidic pHs (6.2 and 6.6, respectively). Generation of acidity on metalliferous mine spoils could be attributable to the weathering of sulphides (Williamson and Johnson, 1981). On the other hand, the use of sodium cyanide during gold processing that results in alkaline effluent (Allan, 1995), may contribute to the relatively higher pH found in the TD soils. The strongly alkaline pH (8.5) of the liquid waste at the tailings dam (Worash Getaneh and Tamiru Alemayehu, 2006) corroborates the potential impact of the alkaline effluent on its environs. In essence, soil pH determines soil reactions that

affect release (desorption, dissolution, complexation with dissolved organic matter) and sorption (adsorption, surface precipitation, polymerization: see Sparks, 2003 p. 133) of heavy metals. Consequently, mobility and uptake of heavy metals including those that are fundamental for normal growth and health of plants (Cu, and Zn), other nutrient elements (Ca, Mg, K, Na, N, P), and non-essential heavy metals (Cd, Pb) may possibly be influenced by pH.

The organic carbon levels of the spoil materials were generally low (0.17–1.05%). The lower organic carbon is consistent with reported values (< 0.5%, which corresponds to ED and SWD, Table 3.18) for other mine spoils (Álvarez *et al.,* 2003). On the other hand, their total nitrogen and C/N ratio (Table 3.18) fell under the normal range for surface mineral soils of 0.02-0.5% and 8–15, in that order (Brady and Weil, 1996). Besides, as is indicated for most soils (Brady and Weil, 1996), the CEC of the mine spoils revealed an increasing trend with pH (Table 3.18). Moreover, available phosphorous levels of the mine spoils (2.06–4.9 mg kg^{-1}) were relatively higher than the expected concentrations (about 0.01 % of the total P: Brady and Weil, 1996) for most soils. Likewise, the mean values of the exchangeable cations (Table 3.18) were slightly higher than the critical values specified by Álvarez et al. (2003).

The average levels of Cu, Ni (saving TD^{a}), Co, Pb (TD^{b}) and Cr in the mine spoils (Table 3.19) were in the range of values reported as toxic levels for soils by Ross (1994b). Apart from this, some sampling spots on the spoil materials had Zn (> 70 mg kg^{-1}: ED), and Pb (> 100mg kg^{-1}: SWD) levels considered as toxic soil concentrations (Ross, 1994b).

Table 3.18 Mean (n = 3) and SD for the physicochemical characteristics of soils at the mine dumps, the foot of the tailings dam and the solid tailings material.

Parameter	Tailings dam[a]	Eastern waste dump	Southern waste dump	Tailings dam[b]
pH (H_2O)	7.7±0.8	7.1±0.2	5.1±0.3	7.9±0.3
pH (KCl)	6.6±0.8	5.67±0.7	3.9±0.2	6.2±0.2
EC (dS/m)	1.3±0.6	0.13±0.05	0.14±0.03	0.6±0.1
Organic C (%)	1.05±0.6 (low)	0.47±0.07 (v. low)	0.31±0.09 (v. low)	0.13±0.01 (v. low)
Total N (%)	0.12±0.06 (medium)	0.05±0.01 (low)	0.03±0.01 (low)	0.02±0.0 (low)
C/N	9±1.4	10±3	11.3±2	8.3±0.3
% Sand	40±17	55±1	44±5.3	48±4
% Silt	40±8.5	35±1	36±3.5	44±2
% Clay	20±8	10±0	20±2	8±0.0
$CaCO_3$ (%)	0.17±0.05	0.08±0.006	Trace	2.35±0.3
Na ($cmol_{(+)}$/kg)	1.65±0.5	0.10±0.01	0.20±0.08	0.67±0.2
K ($cmol_{(+)}$/kg)	1.56±0.6	0.31±0.07	0.19±0.01	0.41±0.1
Ca ($cmol_{(+)}$/kg)	10.88±2	6.1±1.5	1.79±1	7.34±1.4
Mg ($cmol_{(+)}$/kg)	1.33±0.5	3.36±1	2.65±0.9	0.41±0
Base saturation (%)	98±2	95.4±5	70±5.8	94±3.7
Exch acidity ($cmol_{(+)}$/kg)	Trace	Trace	1.28±0.8	Trace
Exch Al ($cmol_{(+)}$/kg)	Trace	Trace	0.76±0.2	Trace
CEC ($cmol_{(+)}$/kg)	15.76±3.2	10.3±2.6	6.89±1.5	9.38±1.8
Available P (ppm)	2.06±0.06 (v. low)	2.82±0.4 (v. low)	3.65±0.2 (v. low)	4.9±0.3 (low)

[a] Soil from the foot of the tailings dam with over spilt tailings material and seepage from the dam.
[b] Dried tailings sampled from the dam.
NB. Exch symbolizes exchangeable

Table 3.19 Average (with SD for n = 3) and range of total concentration (mg kg^{-1}) of heavy metals in soils at the mine dumps, the foot of the tailings dam and the tailings material.

Heavy metal	Tailings dam[a]	Eastern waste dump	Southern waste dump	Tailings dam[b]
Cr	173±2.5 (171–175.8)	168±88 (95–266)	150±66 (99–225)	230.5±70 (159.9–301)
Ni	86±6 (80–92)	102.3±9 (92–109)	188.7±104.9 (107–307)	152.7±10 (142.3–163)
Co	59±1 (58–60)	59.3±6 (53–65)	55.3±16 (45–74)	57.5±14.4 (43–72)
Cu	84.5±14.5 (70–99)	56±8.8 (46–63)	77±10.8 (68–89)	145.2±6.8 (138.4–152)
Zn	56.79±13 (43.6–70)	77.76±38.9 (45.7–121)	51.83±20 (28.3–65.5)	34.79±2.8 (31.9–37.6)
Cd	1.69±0.16 (1.53–1.85)	2.26±0.97 (1.3–3.3)	2.11±0.09 (2.02–2.2)	2.17±0.78 (1.4–2.96)
Pb	34.5±4.5 (30–39)	5.4±3.4 (2.96–9.3)	82.98±76.7 (17.28–167.4)	132±11 (121–143)

[a] Soil from the foot of the tailings dam with over spilt tailings material and seepage from the dam.
[b] Dried tailings sampled from the dam.

3.4.2 Plant species growing on the mine spoils

In this study, 37 species that belong to 19 families were collected from the three sites (Table 3.20). Among the plant families, Asteraceae (30%), Poaceae (22%), Solanaceae (11%), Cyperaceae (8%), Euphorbiaceae (8%), Leguminosae-Papilionoideae (5%), and Tiliaceae (5%) represented the majority of the species. At every site, conversely, Asteraceae and Poaceae followed by either Cyperaceae (57%, TD[a]), by Solanaceae (72%, ED), or by Euphorbiaceae (45%, SWD) were prominent. Moreover, *Vernonia myriantha*, *Tagetus minuta*, *Panicum maximum*, *Hyparrhenia filipendula*, *Solanum indicum*, *Croton macrostachyus*, and *Trifolium acaule* were species common to more than one site. On account of the relatively high species number observed, more plant specimen (21 species, 12 families) were collected from TD[a] than other sites.

3.4.3 Uptake, distribution, extraction efficiency, and total plant accumulations of heavy metals by plants on the mine spoils.

Variations in shoot and root heavy metal levels were observed among the different species on the same site and between individuals of the same species growing on different substrates (Table 3.20). In most cases, the heavy metals displayed a tendency to accumulate in root than shoot. However, depending on the species as well as the type of heavy metal, comparable or even higher shoot levels were observed.

The levels of Cu on plants collected from the 3 mine spoils ranged from 5.41–56.4 and 2.12–116.06 mg kg^{-1} for shoot and root, respectively. *Solanum indicum* from SWD and *Cyperus sp.* grown on TD[a] had the highest shoot and root Cu levels, in that order (Table 3.20). Besides, *Laggera alata* (high shoot Cu and the highest TF, 20.3), *Ageratum conyzoides* (high shoot and root Cu), *S. indicum* (high shoot Cu), and *Phragmites karka* (high root Cu) from TD[a] were among important plants with desirable traits of Cu uptake and distribution (Table 3.20). In general, many plants contained comparatively higher shoot or root Cu levels (Table 3.20) than those values reported (5–25 mg kg^{-1}: Álvarez *et al.,* 2003) as common Cu levels for plants from metalliferous soils. A representative range of 20–100 mg kg^{-1} is reported for plants contaminated with Cu (Ross, 1994b), which also includes excessive / toxic Cu levels for aerial plant part (Pendias and Pendias, 2001) and in tissues of most crops (Adriano, 2001).

On the other hand, the potential worth of a plant for heavy metal extraction from contaminated soils (extraction efficiency, EE) hinges on the product of the metal concentration and dry weight of the harvestable tissue or shoot (Bech *et al.,* 2002). Besides, taking the extraction of heavy metals by roots into account and combining it with shoot extraction (total plant heavy metal levels = shoot level × shoot biomass + root level × root biomass), the plant's capacity for both phytoextraction and phytostabilization could be deciphered. Accordingly, *Datura stramonium*, *Ocimum urticifolium*, *Laggera pterodonta*, *Hyparrhenia filipendula* and *Panicum maximum* from ED with the EE of 3955.77–10245.11 µg and total plant heavy metal levels of 5446.55–10839.83 µg $plant^{-1}$ displayed their potential to abstract sizeable amounts of Cu from the mine spoils. Apart from this, *Vetiveria zizanioides* and *Hyparrhenia*

filipendula from SWD had the highest root extraction of Cu (6709 and 4473 µg, respectively) with moderate translocation to their shoots (2040 and 2697 µg, in that order) as well.

On the other hand, the root and shoot levels of Ni were high (Table 3.20) and fell under the typical range 10–100 mg kg^{-1} reported for Ni contaminated plants (Ross, 1994), which may produce phytotoxicity to most plant species (Adriano, 2001). Besides, the shoot Ni levels of most plants (mainly those from ED and SWD) were comparable to Ni concentrations in the above ground tissues of serpentine species [20–100 mg kg^{-1}: Freitas *et al*., 2004)]. Plants collected from TD[a] revealed significantly lower ($p < 0.001$) mean shoot Ni levels than the other sites while the root levels between sites were not significantly different. Apart from the influence of species / cultivars (Adriano, 2001) and pedological factors, Ni uptake by plants principally depends on the pH, with higher uptake at acidic pH (Pendias and Pendias, 2001). *Trifolium acaule* (97.4 mg kg^{-1}) and *Panicum maximum* (114.41 mg kg^{-1}) both grown on ED had the highest shoot and root Ni levels, in that order. Apart from these, other plants as well contained considerable amount of Ni in their shoot (> 50 mg kg^{-1}: *Laggera pterodonta*, *Persicaria senegalensis* (ED); *T. acaule*, *Vernonia myriantha*, *Solanum indicum*, *Tagetus minuta*, SWD) and roots (> 80 mg kg^{-1}: *Conyza stricta*, *Cyperus sp*., *Ageratum conyzoides*, TD[a]; *Hyparrhenia filipendula*, ED and SWD). *T. acaule* (EE 14250 µg; 24565 µg $plant^{-1}$), *H. filipendula* (EE: 10484 µg; 21401 µg $plant^{-1}$), *D. stramonium* (EE: 9968 µg; 10673 µg $plant^{-1}$), *L. pterodonta* (EE: 7476 µg; 9798 µg $plant^{-1}$), and *P. maximum* (EE: 6656 µg; 10923 µg $plant^{-1}$) from ED were the most effective species in removing Ni from soil. It has been indicated that some species from Leguminosae (where *T. acaule* belongs) had capacity to tolerate and hyperaccumulate Ni (Pendias and Pendias, 2001).

Most species in this study (Table 3.20) contained relatively higher and / or comparable Co levels to those values indicated as excessive (15–50 mg kg^{-1}, Ross, 1994; Pendias and Pendias, 2001) and generally phytotoxic Co levels in plants (Pendias and Pendias, 2001). *Chenopodium ambrosioides* (43 mg kg^{-1}) and *Ageratum conyzoides* (131 mg kg^{-1}) both from TD[a] accumulated the highest shoot and root Co levels, respectively (Table 3). However, *H. filipendula* (ED) due to its higher biomass (shoot: 214.4, root: 127.5 g) presented the highest EE (6240.33 µg) as well as root

accumulation (5238.34 µg) and thus, was a plant with the highest Co accretion (11478.66 µg plant^{-1}). Besides, *Schoenoplectus corymbosus*, *O. urticifolium* (TD^{a}); *P. maximum* and *T. acaule* (ED) with EE 3632.51-4972.21µg and total plant Co accumulation of 5129.71–8096 µg plant^{-1} showed important potential of abstracting Co from their substrates. The shoot averages and the TC values of Co for plants from SWD and were significantly lower ($p < 0.001$) than the same for plants from the other sites. Conversely, the mean root Co levels and the BCF values of plants on TD^{a} were significantly higher ($p < 0.001$) than the same for plants from other mine spoils (Table 3). The differences in the levels of Co on the exchange sites, soil drainage status (Smith and Paterson, 1995) and variations in Co uptake due to plant factors might be the underlying reasons for the above differences.

Zn concentration in the plants varied from 11.65–740.45 mg kg^{-1}, with the highest level being observed in the roots of *Equisetum ramosissimum* from TD (Table 3.20). In addition, the roots of *Cyperus sp*, *Ageratum conyzoides* (TD^{a}); *D. stramonium* (ED); *Vetiveria zizanioides* and *S. indicum* (SWD) contained considerable amount of Zn (107–180 mg kg^{-1}). Conversely, sizable amounts of Zn (> 100 mg kg^{-1}) were also found in the shoots of *E. ramosissimum*, *C. stricta*, *Ocimum urticifolium* (TD^{a}); *D. stramonium* (ED); and *T. minuta* (SWD). Zn concentrations in the range of 100–400 mg kg^{-1} are reported as phytotoxic (Ross, 1994; Pendias and Pendias, 2001). Among those species with high Zn concentration and / or biomass, *D. stramonium* (EE: 25074 µg; 28780 µg plant^{-1}) followed by *O. urticifolium* (EE: 15186 µg; 17348 µg plant^{-1}) had the highest capacity to abstract soil Zn. Apart from this, *H. filipendula* (ED and SWD), *Helichrysum odoratissiumum* (ED), *C. stricta* (TD^{a}), and *T. minuta* (ED and SWD) showed important potential to accumulate Zn (EE: 5538–9183 µg; 5950–20360 µg plant^{-1}). Although higher Zn values were observed in the roots of some plants, mostly Zn was translocated to the shoots as reflected by relatively higher average TF (1.24–1.30) and TC (0.64–1.14) values (Table 3.20). Zn, and Cd (Adriano, 2001; Alloway, 1995) were rated as elements that can be readily translocated to aboveground parts; Ni, Co, and Cu were intermediate; and Cr, and Pb were the least translocated (Alloway, 1995b).

The Pb contents of the plants ranged from 1.22–73.31 mg kg^{-1}, with the highest value corresponding to the shoots of *A. conyzoides* from TD^{a} (Table 3.20). Besides, *Schinus*

molle and *H. filipendula* from SWD contained 53.18 and 49.64 mg kg^{-1} of Pb in their shoots, respectively. On the other hand, *Persicaria senegalensis* from ED had the maximum root Pb (72.41 mg kg^{-1}). Excessive or toxic range for plants affected by Pb ranged from 30–300 mg kg^{-1} (Rose, 1994; Pendias and Pendias, 2001). Although the Pb levels in the roots of plants were relatively higher than shoots (Table 3.20), the apparent variations were not significant ($P > 0.05$). Conversely, plants from ED displayed significantly higher ($p < 0.001$) mean BCF (3.87) and TC (1.70) than the corresponding values for plants from other mine soils. Adriano (2001) stated that soil type (mainly the clay content), pH, and redox potential (Eh) determine the Pb levels in the plant parts. The same author also indicated that higher Pb concentrations in soil solutions and eventually higher shoot Pb were reported for plants grown on growth chamber with the lowest clay and organic matter content. The clay content of the mine spoil at ED was significantly lower ($p < 0.05$) than the same in other spoils (Table 3.18). When the biomass values were considered, *H. filipendula* (ED and SWD), *A. conyzoides* (TD), and *V. zizanioides* (SWD) exhibited the highest EE (2486–7069 µg) and total Pb (4054–13448 µg $plant^{-1}$). *V. zizanioides* is reported to tolerate heavy metals and found to accommodate elevated Pb concentration mostly in the roots (Chantachon *et al.,* 2004).

Table 3.20 Biomass (DM, g dry weight), heavy metal levels within plant parts (mg kg^{-1}, dry weight), accumulation and translocation of heavy metals in plants growing adjacent to the tailings dam and on the dump sites.

1. Tailing dam		DM	Cu	Ni	Co	Zn	Pb	Cd	Cr
Equisetum ramosissimum Desf. (Equistaceae)	Shoot	12.5	14.73	16.83	11.81	114.07	28.13	2.95	4.7
	Root	8.3	69.28	23.28	52.26	740.45	25.01	2.79	93.81
	BCF		0.8	0.3	0.9	**13.0**	0.7	**1.7**	0.5
	TF		0.21	0.72	0.22	0.15	**1.12**	**1.1**	0.05
	TC		0.2	0.2	**0.2**	2.0	**0.8**	1.7	0.03
Cyperus distans L.f. (Cyperaceae)	Shoot	9.7	12.60	16.14	13.69	95.11	16.31	2.73	44.52
	Root	8.3	26.30	22.65	21.42	54.41	23.54	3.73	58.68
	BCF		0.31	0.26	0.36	0.96	0.68	**2.21**	0.34
	TF		0.48	0.71	0.64	**1.75**	0.69	0.73	0.76
	TC		0.15	0.19	**0.23**	1.67	**0.47**	1.62	**0.26**
Conyza stricta Willid. (Asteraceae)	Shoot	51.7	29.13	27.98	17.99	124.99	21.44	1.69	12.94
	Root	19.7	71.20	86.31	48.57	71.59	30.29	1.74	88.46
	BCF		0.84	**1.00**	0.82	**1.26**	0.88	**1.03**	0.51
	TF		0.41	0.32	0.37	**1.75**	0.71	0.97	0.15
	TC		0.35	0.33	**0.31**	2.20	**0.62**	1.00	0.07
Panicum maximum Jacq. (Poaceae)	Shoot	18.3	13.04	11.53	13.42	47.96	10.50	0.63	14.48
	Root	14.1	9.43	45.34	10.92	34.47	21.74	1.89	52.88
	BCF		0.11	0.53	0.19	0.61	0.63	**1.12**	0.31
	TF		**1.38**	0.25	**1.23**	**1.39**	0.48	0.33	0.27
	TC		0.15	0.13	**0.23**	0.84	**0.31**	0.37	0.08
Chenopodium ambrosiodes L. (Chenopodiaceae)	Shoot	36.5	28.35	43.77	43.01	93.08	18.15	2.34	27.56
	Root	13.5	39.22	49.42	43.01	77.78	32.61	1.83	54.78
	BCF		0.47	0.58	0.73	**1.37**	0.95	**1.08**	0.32
	TF		0.72	0.89	**1.00**	**1.20**	0.56	**1.28**	0.50
	TC		0.34	0.51	**0.73**	1.64	**0.53**	1.39	**0.16**
Cyperus sp. (Cyperaceae)	Shoot	25.9	16.34	16.27	14.99	64.82	5.64	1.78	44.39
	Root	29.3	116.06	99.48	99.52	106.83	32.25	0.01	122.61
	BCF		**1.38**	**1.16**	**1.69**	**1.88**	0.94	0.01	0.71
	TF		0.14	0.16	0.15	0.61	0.17	**178**	0.36
	TC		0.19	0.19	**0.25**	1.14	**0.16**	1.05	**0.26**
Laggera alata (D. Don) Oliv. (Asteraceae)	Shoot	12.8	42.95	27.14	31.60	92.15	24.68	1.18	36.14
	Root	3	2.12	9.41	16.77	26.15	14.97	0.59	16.18
	BCF		0.03	0.11	0.28	0.46	0.44	0.35	0.09
	TF		**20.29**	**2.89**	**1.88**	**3.52**	**1.65**	**2.00**	**2.23**
	TC		0.51	0.32	**0.54**	1.62	**0.72**	0.70	**0.21**
Ageratum conyzoides L. (Asteraceae)	Shoot	48.3	42.95	29.25	37.03	11.65	73.31	1.20	43.47
	Root	18.9	112.79	92.96	131.04	179.89	48.87	2.44	160.50
	BCF		**1.34**	**1.08**	**2.22**	**3.17**	**1.42**	**1.45**	0.93
	TF		0.38	0.31	0.28	0.06	**1.50**	0.49	0.27
	TC		0.51	0.34	**0.63**	0.21	**2.13**	0.71	**0.25**
Solanum indicum L. (Solanaceae)	Shoot	14.5	48.87	23.69	36.59	96.53	13.37	1.88	34.93
	Root	5.3	48.72	47.52	63.71	78.24	20.16	2.42	67.74
	BCF		0.58	0.55	**1.08**	**1.38**	0.59	**1.43**	0.39
	TF		**1.00**	0.50	0.57	**1.23**	0.66	0.78	0.52
	TC		0.58	0.28	**0.62**	1.70	**0.39**	1.11	**0.20**
Hyparrhenia filipendula (Hochst.) Stapf. (Poaceae)	Shoot	79.1	7.89	13.88	20.76	29.67	14.10	1.25	29.28
	Root	46.7	63.74	61.94	77.16	51.37	20.75	1.25	103.31
	BCF		0.76	0.72	**1.31**	0.90	0.60	0.74	0.60
	TF		0.12	0.22	0.27	0.58	0.68	**1.00**	0.28
	TC		0.09	0.16	**0.35**	0.52	**0.41**	0.74	**0.17**
Ocimum urticifolium Roth. (Lamiaceae)	Shoot	118.7	44.82	25.11	35.16	127.94	14.99	1.80	52.73
	Root	39	22.56	13.65	24.51	55.43	23.24	1.84	36.75
	BCF		0.27	0.16	0.42	0.98	0.68	**1.09**	0.21

	TF		**2.00**	**1.84**	**1.43**	**2.31**	0.64	1.00	**1.43**
	TC		0.53	0.30	**0.60**	**2.25**	**0.44**	**1.10**	**0.30**
Helinus integrifolius (Lam.) O. Kuntze (Rhamnaceae)	Shoot	93.6	21.37	25.53	36.04	36.42	7.49	1.89	52.18
	Root	29.1	24.56	34.93	46.64	24.00	12.71	1.18	63.43
	BCF		0.29	0.41	0.79	0.42	0.37	0.70	0.37
	TF		0.87	0.73	0.77	**1.52**	0.59	**1.60**	0.82
	TC		0.25	0.30	**0.61**	0.64	**0.22**	1.12	**0.30**
Bothriocline schimperi Oliv. and Hiern ex Benth. (Asteraceae)	Shoot	54.7	38.34	20.63	31.50	82.68	5.04	1.21	42.3
	Root	94.7	37.05	29.67	43.73	48.47	8.52	1.79	46.51
	BCF		0.44	0.35	0.74	0.85	0.25	**1.06**	0.27
	TF		**1.03**	0.70	0.72	**1.71**	0.59	0.68	**1.15**
	TC		0.45	0.24	**0.53**	1.46	**0.15**	0.72	**0.31**
[a]*Dodonaea angustifolia* L.f. (Sapindaceae)	Shoot	75	13.09	21.17	23.94	49.89	6.42	1.80	10.77
	Root	13	9.47	12.69	15.99	24.16	4.40	0.92	9.22
	BCF		0.11	0.15	0.27	0.43	0.13	0.54	0.05
	TF		**1.38**	**1.67**	**1.50**	**2.06**	**1.46**	**1.96**	**1.17**
	TC		0.16	0.25	0.41	0.88	0.19	1.07	0.06
Phrgmites karka (Retz.) Steud. (Poaceae)	Shoot	25.8	14.54	15.76	24.07	56.37	6.45	1.21	9.03
	Root	14.5	80.16	50.24	61.39	51.54	20.39	2.36	56.08
	BCF		0.95	0.59	**1.04**	0.91	0.59	**1.40**	0.32
	TF		0.18	0.31	0.39	**1.09**	0.32	0.51	0.16
	TC		0.17	0.18	**0.41**	0.99	**0.19**	0.72	0.05
Schoenoplectus corymbosus (Roem. and Schult.) Rayn. (Cyperaceae)	Shoot	146.3	12.57	21.45	33.99	31.3	3.62	1.21	15.17
	Root	43.4	27.45	32.88	71.99	29.39	9.72	1.26	18.84
	BCF		0.33	0.38	**1.22**	0.52	0.28	0.75	0.11
	TF		0.46	0.65	0.47	**1.06**	0.37	0.96	0.81
	TC		0.15	0.25	**0.58**	0.55	0.11	0.72	0.09
Pavonia urens Cav. (Malvaceae)	Shoot	64.2	14.89	16.14	24.58	38.16	13.22	1.23	8.01
	Root	37.7	23.88	20.96	34.00	30.12	9.89	1.18	25.50
	BCF		0.28	0.24	0.58	0.53	0.29	0.70	0.15
	TF		0.62	0.77	0.72	**1.27**	**1.34**	**1.04**	0.31
	TC		0.18	0.19	**0.42**	0.67	**0.38**	0.73	0.05
Triumfetta tomentosa Boj. (Tiliaceae)	Shoot	37.7	13.15	32.79	25.40	65.65	6.48	1.64	11.97
	Root	16	7.35	26.58	20.11	33.14	6.90	1.16	6.38
	BCF		0.09	0.31	0.34	0.58	0.20	0.69	0.04
	TF		**1.79**	**1.23**	**1.26**	**1.98**	0.94	**1.41**	**1.88**
	TC		0.16	0.38	**0.43**	1.16	**0.19**	0.97	0.07
Sporobolus pyramidialis P. Beauv. (Poaceae)	Shoot	77.8	5.41	14.77	17.56	22.75	4.47	0.93	8.45
	Root	62.3	36.82	44.20	51.54	41.6	11.10	1.32	52.42
	BCF		0.44	0.51	0.87	0.73	0.32	0.78	0.30
	TF		0.15	0.33	0.34	0.55	0.40	0.70	0.16
	TC		0.06	0.17	**0.30**	0.40	**0.13**	0.55	0.05
Ipomea obscura (L.) Ker. Gawi (Convovulaceae)	Shoot	67.2	9.74	16.95	22.58	37.94	7.11	1.00	11.45
	Root	6	23.56	26.80	37.97	53.33	4.76	1.78	25.80
	BCF		0.28	0.31	0.64	0.94	0.14	**1.05**	0.15
	TF		0.41	0.63	0.59	0.71	**1.50**	0.56	0.44
	TC		0.12	0.20	**0.38**	0.67	**0.21**	0.59	0.07
Helichrysum foetidium (L.) Mönch (Asteraceae)	Shoot	4	14.91	9.36	17.27	39.35	11.16	1.34	10.27
	Root	1.6	22.92	22.06	44.94	58.36	45.60	4.59	13.78
	BCF		0.27	0.26	0.76	**1.03**	**1.33**	**2.72**	0.08
	TF		0.65	0.42	0.38	0.67	0.24	0.29	0.74
	TC		0.18	0.11	**0.29**	0.69	**0.32**	0.79	0.06
2. Eastern Waste Dump		**DM**	**Cu**	**Ni**	**Co**	**Zn**	**Pb**	**Cd**	**Cr**
Trifolium acaule Steud. Ex A. Rich. (Leguminosae –	Shoot	146.3	11.84	97.40	24.83	32.92	1.85	2.98	38.93
	Root	98.8	2.97	104.40	23.32	12.95	7.44	2.23	14.99
	BCF		0.05	**1.02**	0.39	0.17	**1.38**	0.99	0.09

Papilionoideae)	TF		**3.98**	0.93	**1.06**	**2.54**	0.25	**1.33**	**2.60**
	TC		0.21	0.95	**0.42**	0.42	**0.34**	1.32	**0.23**
Vernonia myriiantha Hook. f. (Asteraceae)	Shoot	78.1	30.47	24.63	20.85	54.19	1.22	2.96	17.11
	Root	39.9	41.73	31.04	28.20	45.37	6.79	3.15	34.78
	BCF		0.75	0.30	0.48	0.58	**1.26**	**1.40**	0.21
	TF		0.73	0.79	0.74	**1.19**	0.18	0.94	0.49
	TC		0.54	0.24	**0.35**	0.70	**0.23**	1.31	0.10
Vernonia myriiantha Hook. f.	Twig	101.5	35.05	25.99	24.39	52.04	5.01	2.27	16.69
	TC		0.63	0.25	**0.41**	0.67	**0.93**	1.01	0.10
Panicum maximum Jacq. (Poaceae)	Shoot	227.5	17.39	29.26	18.22	32.98	6.05	1.45	16.85
	Root	37.3	39.97	114.41	30.65	37.76	25.64	5.77	157.21
	BCF		0.71	**1.12**	0.52	0.49	**4.75**	**2.56**	0.94
	TF		0.44	0.26	0.59	0.87	0.24	0.25	0.11
	TC		0.31	0.29	**0.31**	0.42	**1.12**	0.64	0.10
Laggera pterodonta (DC) Oliv. (Asteraceae)	Shoot	124.7	37.20	60.00	27.60	36.67	6.30	2.30	47.20
	Root	58.6	29.30	39.60	20.20	30.91	6.90	2.10	31.20
	BCF		0.52	0.25	0.39	0.15	**3.41**	**9.86**	0.13
	TF		**1.27**	**1.51**	**1.37**	**1.19**	0.91	**1.09**	**1.51**
	TC		0.66	0.59	**0.47**	0.47	**1.17**	1.01	**0.28**
Hyparrhenia filipendula (Hochst.) Stapf. (Poaceae)	Shoot	214.4	19.50	48.90	29.11	39.06	11.60	3.69	49.49
	Root	127.5	22.40	85.63	41.09	41.87	12.29	4.95	57.32
	BCF		0.40	0.84	0.69	0.54	**2.28**	**2.19**	0.34
	TF		0.87	0.57	0.71	0.93	0.94	0.75	0.86
	TC		0.35	0.48	**0.49**	0.50	**2.15**	1.64	**0.30**
Solanum indicum L. (Solanaceae)	Shoot	135.6	25.07	27.90	21.82	32.35	6.18	2.71	16.95
	Root	23.5	17.54	22.74	22.04	28.52	8.23	2.65	15.93
	BCF		0.31	0.22	0.37	0.37	**1.53**	**1.18**	0.10
	TF		**1.43**	**1.23**	0.99	**1.13**	0.75	**1.02**	**1.07**
	TC		0.45	0.27	**0.37**	0.42	**1.15**	1.20	0.10
Tagetes minuta L. (Asteraceae)	Shoot	125.3	23.31	34.47	6.87	53.24	8.53	2.20	5.16
	Root	28.8	29.11	31.26	12.47	39.03	24.96	3.04	22.81
	BCF		0.52	0.31	0.21	0.50	**4.63**	**1.35**	0.14
	TF		0.80	**1.10**	0.55	**1.36**	0.34	0.72	0.23
	TC		0.42	0.34	**0.12**	0.68	**1.58**	0.97	0.03
Datura stramonium L. (Solanaceae)	Shoot	228.4	44.86	43.64	8.08	109.78	10.54	2.83	9.91
	Root	25	23.79	28.19	7.60	148.23	23.75	2.65	8.00
	BCF		0.42	0.28	0.13	**1.91**	**4.40**	**1.18**	0.05
	TF		**1.89**	**1.55**	**1.06**	0.74	0.44	**1.06**	**1.24**
	TC		0.80	0.43	**0.14**	1.41	**1.95**	1.25	0.06
Helichrysum odoratissimum (L.) Less. (Asteraceae)	Shoot	126.9	33.29	23.70	15.38	60.6	16.57	2.96	17.06
	Root	16.6	31.00	16.09	16.02	42.18	8.88	2.67	14.10
	BCF		0.55	0.16	0.27	0.54	**1.65**	**1.19**	0.08
	TF		**1.07**	**1.47**	0.96	**1.44**	**1.87**	**1.11**	**1.21**
	TC		0.59	0.23	**0.26**	0.78	**3.07**	1.31	0.10
Persicaria senegalensis (Meisn.) Sojak. (Polygonaceae)	Shoot	46.7	40.57	65.18	15.94	53.67	21.80	2.20	24.89
	Root	3.7	40.07	28.54	24.08	54.28	72.41	3.57	1.78
	BCF		0.72	0.28	0.41	0.70	**13.43**	1.58	0.01
	TF		**1.01**	**2.28**	0.66	0.99	0.30	0.61	**14.01**
	TC		0.72	0.64	**0.27**	0.69	**4.04**	0.97	**0.15**
Croton macrostachyus Hochst. ex Del	Twig	99.6	32.58	35.86	14.73	39.57	14.28	1.49	7.47
	TC		0.58	0.35	**0.25**	0.51	**2.65**	0.66	0.04
3. Southern Waste Dump		**DM**	**Cu**	**Ni**	**Co**	**Zn**	**Pb**	**Cd**	**Cr**
Trifolium acaule Steud. Ex A. Rich. (Leguminosae – Papilionoideae)	Shoot	44.7	16.01	52.51	12.35	38.12	6.16	1.76	4.41
	Root	14.1	14.32	47.60	11.14	25.6	20.94	1.77	7.39
	BCF		0.19	0.25	0.20	0.49	0.25	0.84	0.05
	TF		**1.12**	**1.10**	**1.11**	**1.49**	0.29	0.99	0.60

	TC		0.21	0.28	**0.22**	0.74	0.07	0.83	0.03
Vernonia myriiantha Hook. f. (Asteraceae)	Shoot	37.1	23.47	55.96	15.85	55.5	15.63	1.79	2.29
	Root	9.7	28.21	45.39	18.76	41.42	30.7	1.68	6.63
	BCF		0.37	0.24	0.34	0.80	0.37	0.80	0.04
	TF		0.83	**1.23**	0.85	**1.34**	0.51	**1.06**	0.35
	TC		0.30	0.30	**0.29**	1.07	**0.19**	0.85	0.02
Solanum indicum L. (Solanaceae)	Shoot	14.4	56.39	65.30	13.32	90.51	10.89	1.71	12.19
	Root	4.3	25.55	34.17	11.09	120.58	10.71	1.58	8.58
	BCF		0.33	0.18	0.20	**2.33**	0.13	0.75	0.06
	TF		**2.21**	**1.91**	**1.20**	0.75	**1.02**	**1.09**	**1.42**
	TC		0.73	0.35	**0.24**	1.75	**0.13**	0.81	0.08
Hyparrhenia filipendula (Hochst.) Stapf. (Poaceae)	Shoot	142.4	18.94	17.32	9.24	64.49	49.64	1.79	17.92
	Root	122.4	36.54	104.18	30.39	91.31	52.12	1.64	48.06
	BCF		0.47	0.55	0.55	**1.76**	0.63	0.78	0.32
	TF		0.52	0.17	0.30	0.71	0.95	**1.09**	0.37
	TC		0.25	0.09	**0.17**	1.24	**0.60**	0.85	**0.12**
[a]*Vetiveria zizaniodes* (Poaceae)	Shoot	185.9	10.97	16.20	9.36	28.1	19.79	1.81	12.09
	Root	178.3	37.63	36.59	15.01	116.98	9.62	1.70	31.09
	BCF		0.49	0.19	0.27	**2.26**	0.12	0.80	0.21
	TF		0.29	0.44	0.62	0.24	**2.06**	**1.06**	0.39
	TC		0.14	0.09	**0.17**	0.54	**0.24**	0.86	0.08
Tagetes minuta L. (Asteraceae)	Shoot	47.9	16.96	53.81	11.78	115.63	8.07	1.68	30.14
	Root	11.7	11.99	17.08	8.10	35.15	5.59	1.82	9.15
	BCF		0.16	0.09	0.15	0.68	0.07	0.86	0.06
	TF		**1.41**	**3.15**	**1.46**	**3.29**	**1.44**	0.92	**3.29**
	TC		0.22	0.29	**0.21**	2.23	0.10	0.80	**0.20**
[a]*Grevillea robusta* A. Cunn. Ex R.Br (Proteaceae)	Twig	59	15.08	58.62	11.04	57.43	14.78	1.04	12.51
	TC		0.20	0.31	**0.20**	1.11	**0.18**	0.49	0.08
[a]*Schinus molle* L. (Anacardiaceae)	Twig	110.8	31.32	29.63	6.46	43.10	53.18	1.59	13.27
	TC		0.41	0.16	**0.12**	0.83	**0.64**	0.75	0.09
Grewia bicolor (Tiliaceae)	Twig	95.8	20.03	49.85	13.35	47.04	6.32	1.21	18.11
	TC		0.26	0.26	**0.24**	0.91	0.08	0.57	**0.12**
[a]*Jacaranda mimosifolia* D. Don (Bignoniaceae)	Twig	168.5	19.59	23.36	9.36	43.72	7.83	1.48	13.08
	TC		0.25	0.12	**0.17**	0.84	0.09	0.70	0.09
Croton macrostachyus Hochst. ex Del (Euphorbiaceae)	Twig	140.3	23.40	48.63	10.22	37.53	8.26	1.71	16.48
	TC		0.30	0.26	**0.18**	0.72	0.10	0.81	**0.11**
Ricinus communis L. (Euphorbiaceae)	Twig	36.4	22.92	44.26	11.61	56.90	5.35	1.74	18.22
	TC		0.30	0.23	**0.21**	1.10	0.06	0.83	**0.12**
[a]*Melia azadrach* L. (Meliaceae)	Twig	60.8	13.17	12.96	9.63	60.46	12.55	1.74	10.89
	TC		0.17	0.07	**0.17**	1.17	**0.15**	0.83	0.07

[a]***Represents those species deliberately planted on Southern Dump Site by the environmental safety department of the Midroc gold company.***
Those values in bold represent BCF or TF > 1 or TC > 0.1

The minimum (0.01 mg kg^{-1}) and the maximum (5.77 mg kg^{-1}) Cd levels were observed in roots of *Cyperus sp.* (TD) and *P. maximum* (ED), in that order. Although such Cd levels fairly exceed the normal range in plant materials (0.05–0.2 mg kg^{-1}: Pendias and Pendias, 2001), nearly all plants had slightly lower Cd levels than the toxic (5–30 mg kg^{-1}) range reported by Pendias and Pendias (2001). Conversely, *H. filipendula* (ED) accommodated the highest shoot Cd (3.69 mg kg^{-1}). On average, the

Cd contents of roots were relatively higher than the corresponding levels in the shoots (Table 3.20), though the differences were not statistically significant ($p > 0.05$). In general, *T. acaule*, *P. maximum*, *H. filipendula*, *S. indicum*, *D. stramonium*, *Helichrysum odoratissiumum* (ED); and *V. zizanioides* (SWD) extracted palpable amounts of Cd (EE: 330–790 µg; 420–1421 µg plant^{-1}) from their respective soils.

Cr concentrations of the plants ranged from 1.78–160.50 mg kg^{-1}, with the highest value observed for the root of *A. conyzoides* from TD^{a} (Table 3.20). In addition, the roots of *Cyperus sp*., *H. filipendula* (TD^{a}); and *P. maximum* (ED) accommodated substantial amounts of Cr (103–157 mg kg^{-1}). Conversely, *Bothriocline schimperi* (53.30 mg kg^{-1}), *O. urticifolium* (52.73 mg kg^{-1}), *Helinus integrifolius* (52.18 mg kg^{-1}), TD^{a}; *H. filipendula* (49.49 mg kg^{-1}) and *L. pterodonta* (47.20 mg kg^{-1} from ED contained considerable amounts of Cr in their shoots. Although the phytotoxic levels of Cr in tops of plants could vary from 1–100 mg kg^{-1} (Pendias and Pendias, 2001), usually narrower range of 5–30 mg kg^{-1} has been indicated as phytotoxic/excessive Cr levels (Ross, 1994; Pendias and Pendias, 2001). Many plants accommodated higher Cr levels in their shoots and / or roots than the latter common phytotoxic range (Table 3.20). Consistent with the reported trend (Pendias and Pendias, 2001), the roots of the plants exhibited comparatively higher mean root Cr levels than the same in the shoots (Table 3.20). Nevertheless, only plants from TD^{a} had significant variations ($p < 0.05$) between their mean shoot and root Cr contents. While the biomass was considered, *O. urticifolium*, *Bothriocline schimperi* (TD^{a}); *T. acaule*, *P. maximum*, *L. pterodonta*, and *H. filipendula* (ED) took considerable amounts of Cr (EE: 2915.40–10610.25 µg; 6729.50–17918.43 µg plant^{-1}) out of the respective mine spoils. It is notable that *H. filipendula* not only extracted the highest amount of Cr from ED, but also performed well on TD^{a} and SWD (Table 3.20).

When samples of the same species collected from the different sites were compared, relatively higher EE and total plant levels were observed for those from ED than other sites (Table 3.20). Likewise, the mean biomass of plants from ED as well their mean EE values for Cu, Ni, Co Cd and Cr were significantly higher ($p < 0.05$) than the same for plants from the other two mine spoils. Moreover, the mean soil–shoot transfer (TC) values for Cu, Ni, and Cd of plants from ED were significantly higher ($p < 0.05$) than the mean TC values from TD^{a} and SWD. By all appearances, factors that

contributed to higher biomass accretion as well higher soil–shoot transfer of heavy metals in plants from ED could have enhanced the efficiency of the plants to extract heavy metals from their substrate. As is indicted in (Brady and Weil, 1996), these factors could entail increased nutrient cycling due to relatively important proportion of active pool of organic matter, soil pH (5.67) fairly close to the optimum for satisfactory release of nutrients (5.5–6.5), symbiotic association with mycorrhizae for improved nutrient and water acquisition as well as protection from excessive uptake of toxic metals. Conversely, available phosphorus on ED and SWD (Table 3.20) might have ameliorated, to certain extent, the otherwise higher release and uptake of heavy metals by plants expected at their acidic pHs (5.67 and 3.49, correspondingly). Moreover, the highest (Ca + Mg)/K ratio (30.52) of ED which is within the favorable range (25–40) of the relative proportion of cations, could have certain contribution to the nutrient demands of the plants. However, these surmises need to be substantiated with further study on nutrient dynamics and heavy metal–nutrient interactions vis–à–vis the bio–physico–chemical nature of the mine spoils.

On the other hand, significant positive associations (R^2 = 27–47 %, $p < 0.01$) were observed between the shoot levels of Cu with Co, Zn, and Cr; Co with Cr; and Zn with Cd. Correspondingly, significant positive relationship (R^2 = 37–60 %, $p < 0.01$) between the TF values of Cu, Ni, Co and Zn were observed. Besides, the TC values for Cu and Pb as well as Cr and Co were highly correlated (R^2 = 29–39 %, $p < 0.01$). In similar studies, the shoot Cu and Zn levels had weak correlation (Yanqun *et al.,* 2004) while strong positive correspondence was reported for their TF values (Yoon *et al.,* 2006). In contrast, the TC values for Pb–Cu were uncorrelated (Yanqun *et al.,* 2004). Conversely, strong positive associations (R^2 = 40–77 %, $p < 0.01$) between Cu, Ni, Co and Zn for the root levels as well as BCF values were observed in all plants under the present study. As Yoon et al. (2006) suggested, positive relationships of BCFs and TFs among heavy metals might indicate similarity in effectiveness of the plants in their uptake and translocation the heavy metals. Although it is difficult to draw firm conclusion from literature, Cu–Ni and Ni–Zn synergistic interactions have been reported (Pendias and Pendias, 2001). By all appearances, plant nutrient-heavy metal interactions and various heavy metal–heavy metal interactions occurring outside and / or inside the plants as well as plant specific interactions might modify the nature of interactions.

3.4.4 Potential candidate plants from mine spoils with considerable extraction efficiency and total plant accumulations of heavy metals

Yoon et al. (2006) pointed out that BCF and TF values of various plants can be used to compare their capacity for heavy metal uptake and translocation to the shoots, in that order. The highest BCF and TF values found were 13.39 for Pb in *P. senegalensis* and 178 for Cd from *Cyperus sp*. (Table 3.20). Moreover, *L. alata* (1.65–20.29, TD[a]), *Dodonaea angustifolia* (1.17–2.06, TD[a]), *H. odoratissiumum* (1.1–1.87, TD[a]), *Triumfetta tomentosa* (1.23–1.98, TD[a]), *T. minuta* (1.44-3.29, SWD), *L. pterodonta* (1.1–1.51, ED), *S. indicum* (1.02–1.43, ED; 1.02–2.21, SWD), *O. urticifolium* (1.43–2.31, TD[a]), *D. stramonium* (1.06–1.89, ED) and *T. acuale* (1.06–3.98, ED) showed TF > 1 for five or more heavy metals. However, for a species to qualify as a candidate plant for phytoextraction, both BCF and TF values should be greater than one (Yoon *et al.,* 2006). Among the species studied, only *E. ramosissimum* (Cd), *C. stricta* (Zn), *C. ambrosioides* (Zn and Cd), *A. conyzoides* (Pb) (TD[a]); *S. indicum* (Zn, TD[a]; and Cd, ED); *D. stramonium* (Cd), *H. odoratissiumum* (Pb and Cd) (ED) met the above criteria. Nevertheless, the concentrations of heavy metals in the shoot and / or corresponding shoot biomass production possibly have a bearing on the species choice. Accordingly, *H. filipendula*, *D. stramonium*, *O. urticifolium*, *L. pterodonta*, *T. acaule*, and *P. maximum* exhibited remarkable capacity to extract different heavy metals from mine spoils with considerable EE (213.87–25073 µg where Zn > Ni > Cr > Cu > Pb > Co >>> Cd) and substantial amount of total plant levels (285.62–28779.50 µg plant^{-1} with Zn > Ni > Cr > Pb > Co > Cu >>> Cd). It should be noted that the relative position of heavy metals within the above (general) ranges for EE and total plant levels pivoted on species concerned, the nature of mine spoil and the heavy metal considered. In addition, a given species (e.g., *H. filipendula*) could be a candidate for phytoextraction and phytostabilization in conditions like ED, but it might be an appropriate species for phytostabilization of soils with similar properties to TD[a] and SWD.

Apart from this, *T. minuta*, *H. odoratissiumum*, *S. indicum*, *Schoenoplectus corymbosus*, *P. senegalensis*, *H. integrifolius*, *V. myriantha*, *Bothriocline schimperi*, *A. conyzoides*, *Sporobolus pyramidalis* and *V. zizanioides* presented palpable EE

(57.82–7690.14 µg) and / or had important amounts of total plant levels (104.01–26081.32 µg plant^{-1}) with the high values for two or more heavy metals. Among this category, *B. schimperi*, *A. conyzoides*, *S. pyramidalis* and *V. zizanioides* could be used for phytostabilization while the others may be suitable for the both phytoextraction and phytostabilization.

Of all the species screened, *E. ramosissimum* (Cd), *C. stricta* (Zn), *C. ambrosioides* (Zn and Cd), *A. conyzoides* (Pb) (TD[a]); *S. indicum* (Zn, TD[a]; and Cd, ED); *D. stramonium* (Cd), *H. odoratissiumum* (Pb and Cd) (ED) with both BCFs and TFs above one or TC above one (TC = TF × BCF > 1) may have the potential utility for phytoextraction of the heavy metals indicated. Conversely, heavy metal tolerant plants with high BCF and correspondingly low TF can be used for phytostabilization of contaminated soils, together with a vegetative cover (Yoon *et al.,* 2006). Accordingly, A. *conyzoides* (Cu, Ni, Co, Zn and Cd), *Cyperus sp* (Cu, Ni, Co, and Zn), *H. foetidum* (Zn, Pb, and Cd), *C. stricta* (Ni and Cd), and *P. maximum* (Ni, Pb and Cd), *P. senegalensis* (Pb and Cd), *Phragmites sp.* (Co and Cd), *E. ramosissimum* (Zn) with BCF > 1 could be important. In essence, for effective phytoextraction as well as phytostabilization, the heavy metal levels of the substrates, the corresponding levels in the shoots and roots and / or the shoot as well as root biomass production of the above plants are of paramount importance.

4. CONCLUSIONS

- Laboratory assessment of soil samples collected from five uncontaminated soils (Vertisol, Fluvisol, Solonetz, Andosol, and Nitosol) showed that their total levels of heavy metals and metalloids (barring Zn in Vertisol, Andosol, and Nitosol) were lower than the corresponding mean values for world soils or common values for soils. It was also found that the soil types as well as soil variables (clay, organic carbon, pH, and texture) influenced the levels as well as depth–wise distribution of heavy metals/metalloids in uncontaminated soils. Seen in this light, the total contents as well as the distributions of heavy metals/metalloids in these soils are considered the product of their respective parent rocks/materials and the pedogenic processes entailed. Assessment of the natural abundance of heavy metals/metalloids is central for evaluation and formulation of the feasible counter measures for soil pollution as well as for monitoring the impacts of human activities on the quality of soil chemicals of similar soils.

- The data acquired from Vertisol and Fluvisol under wastewater irrigation at Akaki, conversely, revealed that the soils accommodated higher levels of heavy metals and metalloids than uncontaminated Vertisol and Fluvisol did. Selfsame study also brought into prominence that the average levels of most heavy metals and metalloids in the soils exceeded the corresponding mean levels plus two standard deviations (known as pollution criteria) of the uncontaminated Vertisol and Fluvisol. Such comparison of the heavy metal/metalloid levels could impart an important clue about the contribution of anthropogenic inputs of heavy metals to the soil environment. Apart from certain variations attributed to local pedological and/or geological consequences, wastewater irrigation may contribute to the accumulation of most of the elements. Moreover, it was observed that most of these elements exhibited considerable associations with the non–residual (the soluble) fractions and thus, may depict their potential mobility or bioavailability as well as the impending threat of heavy metal buildup on a given environment.

- The study made known that most of the heavy metals and metalloids in contaminated Vertisol and Fluvisol were highly phytoavailable and hence accumulated in the roots as well as remarkably translocated to the shoot. The plant

levels of most heavy metals/metalloids were not only higher than the corresponding levels in similar plants, but also were comparable to those plants grown in soils with polymetallic pollution. Overall, in the greenhouse experiment, grasses and legumes depicted a general inclination to accumulate most heavy metals/metalloids in their roots and shoots, in that order. Conversely, Clover, Oat and Alfalfa on Vertisol, Rhodes on Fluvisol and Setaria on both soils appeared to be effective in the soil–shoot transfer of heavy metals/metalloids under the field conditions. In general, heavy metal/metalloid sequestration in the greenhouse averaged over the soil types and plant parts decreased in the order of Setaria > Desmodium > Rhodes > Alfalfa > Oat while the same averaged over the soil types under the field condition had the sequence of Clover > Setaria/Rhodes > Alfalfa/Rhodes. Largely, Vertisol promoted root–shoot and soil–shoot transfer of most of the heavy metals/metalloids, whereas Fluvisol appeared to encourage root accumulation (soil–root). The exchangeable, carbonate, reducible, and oxidizable fractions as well as pH and CEC were important soil variables in explaining the variations in the plant heavy metal/metalloid levels. Accordingly, the uptake, distribution, accumulations of heavy metals/metalloids in the roots and shoots of the plants reflect the potential environmental and health hazard attributable to the use of fodder grasses and legumes, and cultivation of vegetables on contaminated Vertisol and Fluvisol as well as on similar soils. Consequently, for the study area as well as similar sites, the plant factors (species, and plant part), soil factors (soil type, heavy metal/metalloid associated with the different geochemical phases of the soil, pH, and CEC), and their interactions could govern heavy metal uptake, translocation, sequestration and ultimately, transfer through the food chain.

- The result of the study showed that the mine spoils were unsuitable substrates for plant growth: elevated levels of heavy metals, poor nutrient status, slightly to strongly acidic pH, and low CEC. Notwithstanding, all plants effectively tolerated these edaphic stressors of the mine spoils, and most of the plants screened exhibited important potential to accommodate heavy metals in their above– and belowground parts and / or produced reasonably high biomass. Among the species studied, *E. ramosissimum* (Cd), *C. stricta* (Zn), *C. ambrosioides* (Zn and Cd), *A. conyzoides* (Pb) (TD[a]); *S. indicum* (Zn, TD[a]; and Cd, ED); *D. stramonium* (Cd), *H. odoratissiumum* (Pb and Cd) (ED) had both BCF and TF > 1 and thus could

qualify as candidate plants for phytoextraction. Nonetheless, the concentrations of heavy metals in the shoot and / or corresponding shoot biomass production possibly have an impact on the species choice. Accordingly, *H. filipendula*, *D. stramonium*, *O. urticifolium*, *L. pterodonta*, *T. acaule*, and *P. maximum* exhibited remarkable capacity to extract different heavy metals from mine spoils with considerable EE (213.87–25073 µg where Zn > Ni > Cr > Cu > Pb > Co >>> Cd) and substantial amount of total plant levels (285.62–28779.50 µg plant^{-1} with Zn > Ni > Cr > Pb > Co > Cu >>> Cd). Conversely, heavy metal tolerant plants with high BCF and correspondingly low TF can be used for phytostabilization of contaminated soils. Accordingly, A. *conyzoides* (Cu, Ni, Co, Zn and Cd), *Cyperus sp* (Cu, Ni, Co, and Zn), *H. foetidum* (Zn, Pb, and Cd), *C. stricta* (Ni and Cd), and *P. maximum* (Ni, Pb and Cd), *P. senegalensis* (Pb and Cd), *Phragmites sp.* (Co and Cd), *E. ramosissimum* (Zn) with BCF > 1 could be important. In essence, for effective phytoextraction as well as phytostabilization, the heavy metal levels of the substrates, the corresponding levels in the shoots and roots and / or the shoot as well as root biomass production of the above plants are of paramount importance. Long plant–growing season, increased soil temperature, absence of funds allocated for prohibitively costly traditional soil remediation methods, heavy metal/metalloid polluted sites, and the prevailing biodiversity resources make phytoremediation more appealing to Ethiopia and other developing countries in the tropics.

5. RECOMMENDATIONS

The study on heavy metals/metalloids of uncontaminated soils portrayed the general feature about the distribution and status of these elements vis–à–vis the main soil characteristics of each soil type. Since the occurrence of heavy metals/metalloids in a given ecosystem is governed by their sources (which could be natural and/ or anthropogenic), identifying the contribution of natural sources is mandatory in any conceivable risk assessment strategy of a given development activity on the receiving environment. However, all–inclusive environmental management of soils affected by heavy metals/metalloids can best be instituted if the natural background concentration of trace metals/metalloids of different soils under various landuses (forest, grassland, agricultural, etc.) is known. It is thus plain that establishing further research in the area of background levels of heavy metals/metalloids in a given soil type across variety of environmental settings and/ or for different soils in a specific region would prop up the long term management of environmental and mineral resources with the requisite, comprehensive, and reliable data.

The study put on view that the contaminated soils had high amount of most of heavy metals/metalloids and the same were demonstrated to have potential mobility/availability. Apparently, for the same type of waste water application, the chemical form and nature of heavy metals/metalloid as well as important soil factors (e.g., the chemical, physical, biological and mineralogical characters of the soil) could influence the status, distribution among the different soil fractions and eventually their mobility/availability from the non–residual fractions of the study soils. Further study, therefore, is deemed requisite on the identification of the chemical species of heavy metals/metalloids of the wastewater, temporal trends of the composition as well as the levels of these chemical forms in the wastewater, and mineralogical constituent of the study soils.

The data on the uptake and distribution of heavy metals/metalloids in the plants imparted about the potential environmental and health hazard that could be caused by direct uptake of contaminants from soil, or indirectly through eating contaminated plant and/or animal tissue, etc. However, comprehensive understanding of the same issue necessitates acquisition of further information on distribution of heavy metals

within similar forage plants as well as the vegetables grown on the same soils using direct application of the wastewater under controlled and field experiments. Depending on the type of the plant, heavy metal/metalloid and plant part consumed, lower yield/biomass due to reduced nutrient availability could result in relatively higher level of a given contaminant per unit dry matter and may thus, pose health risk to humans and their stock in low input agriculture. In view of that, future studies pertaining to the impact of heavy metals/metalloids on soil biota would furnish important information on their accumulation (biomagnification through soil food chain/web) and potential ecological consequences (e.g., effect on soil biological processes: N mineralization, litter decomposition, Nitrogen fixation and enzyme activities, etc.).

This study pioneered the screening of a number of native plants grown on waste dumps and soil affected by tailings material and it was found that the potential utility most of the plants could be exploited for phytoremediation of soils with polymetallic pollution (mine–degraded landscapes, industrial–waste–polluted soils, etc,). Notwithstanding, to optimize the phytoremediation potential of these plants, further investigations should be directed towards means of improving their uptake and/or translocation of heavy metals/metalloids with parallel increase in their biomasses (using chemical or organic fertilizers, irrigation and chelates) under controlled as well as field conditions. Since different heavy metal/metalloid contaminated soils differ in their nature and the types of local environmental conditions under which an array of contaminants prevail, phytoremediation of each one soil requires selection of plant species suitable for particular condition from those already available/recognized. Albeit the growing worldwide acceptance of phytoremediation as a low–tech, potentially cheap *in situ* treatment of contaminants that renders visually unobtrusive sites and thus an attractive alternative over the traditional physicochemical remediation, no single species (saving the present study) has hitherto been reported as a potential candidate for phytoremediation purpose in Ethiopia. Consequently, further geobotanical surveys and plant screening are of the essence for identification of additional species suitable for the phytoremediation of affected soils under different sets of heavy metal/metalloid–soil type–local environment conditions.

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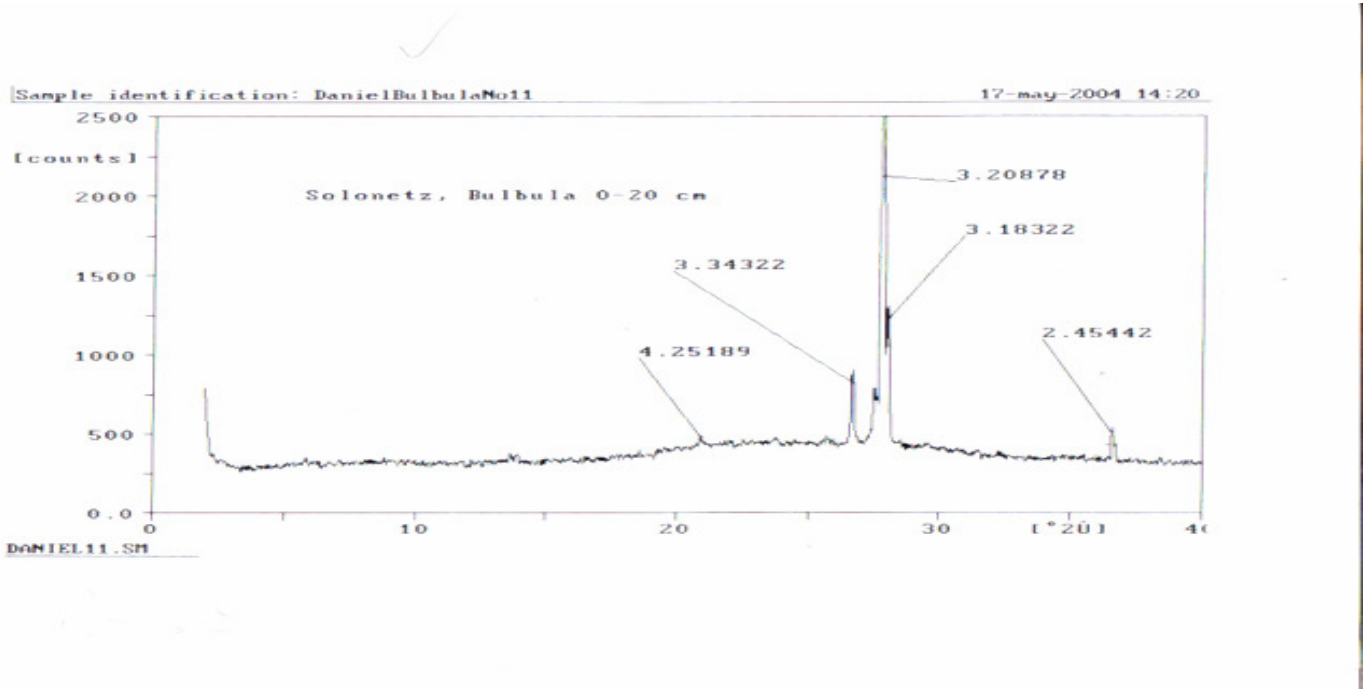
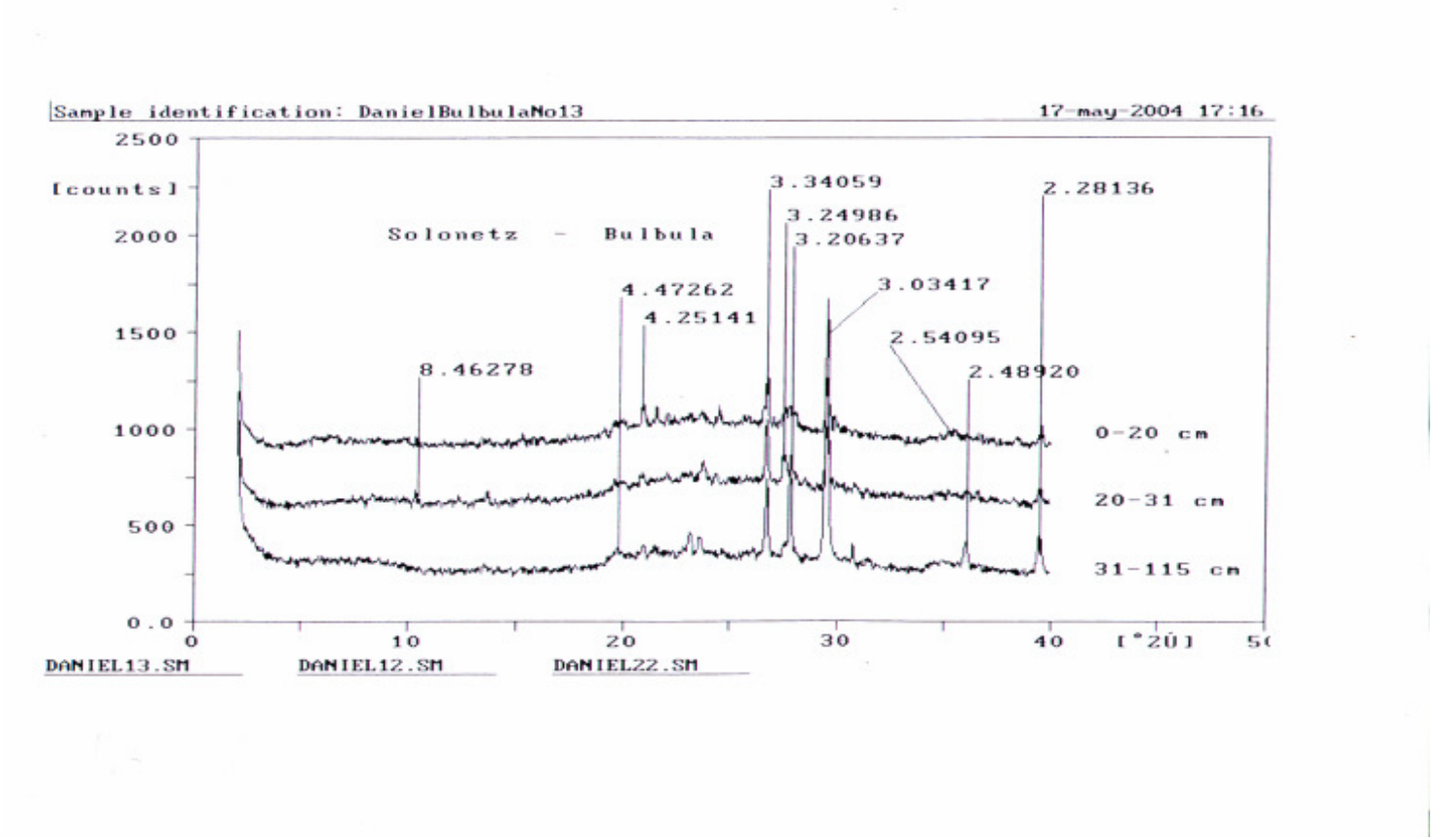
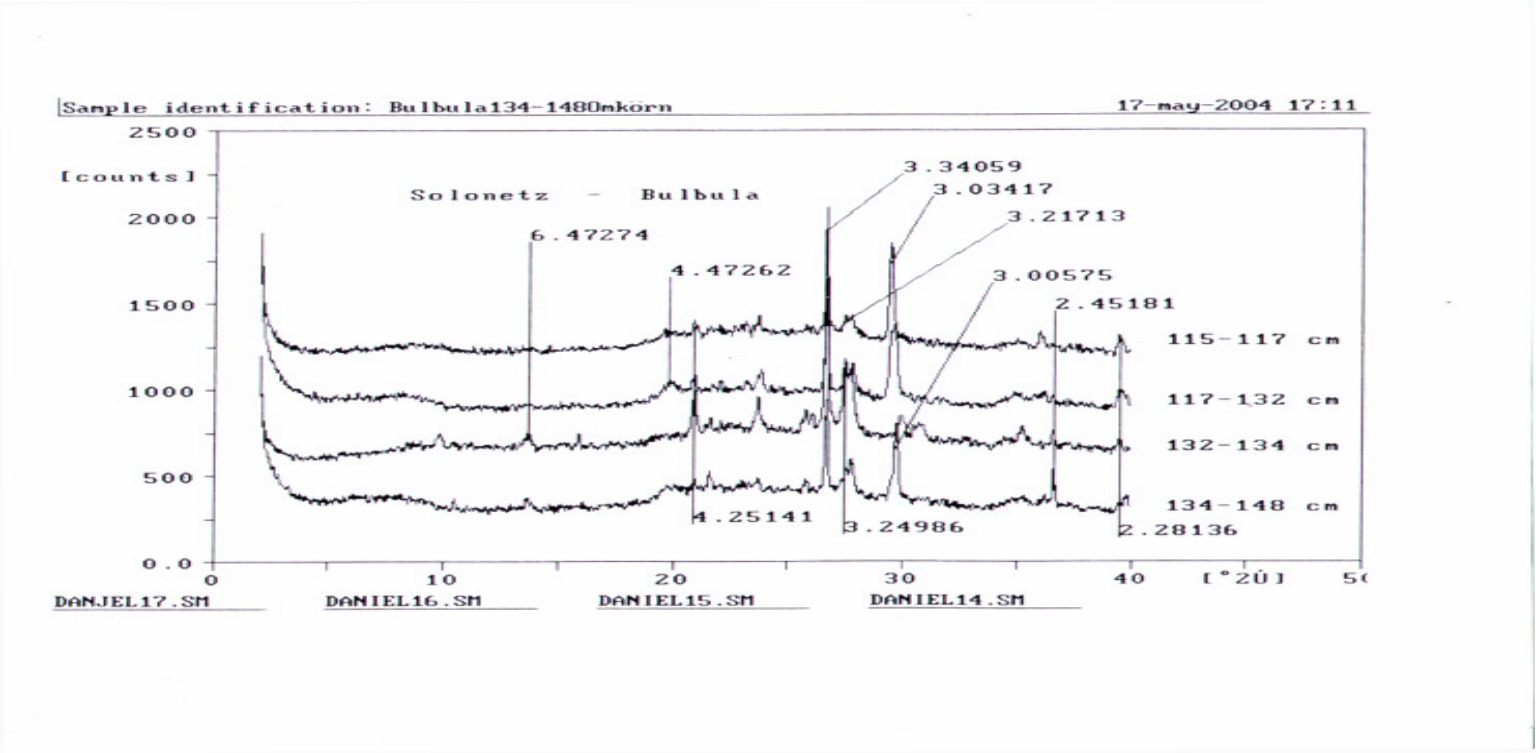
7. APPENDICES



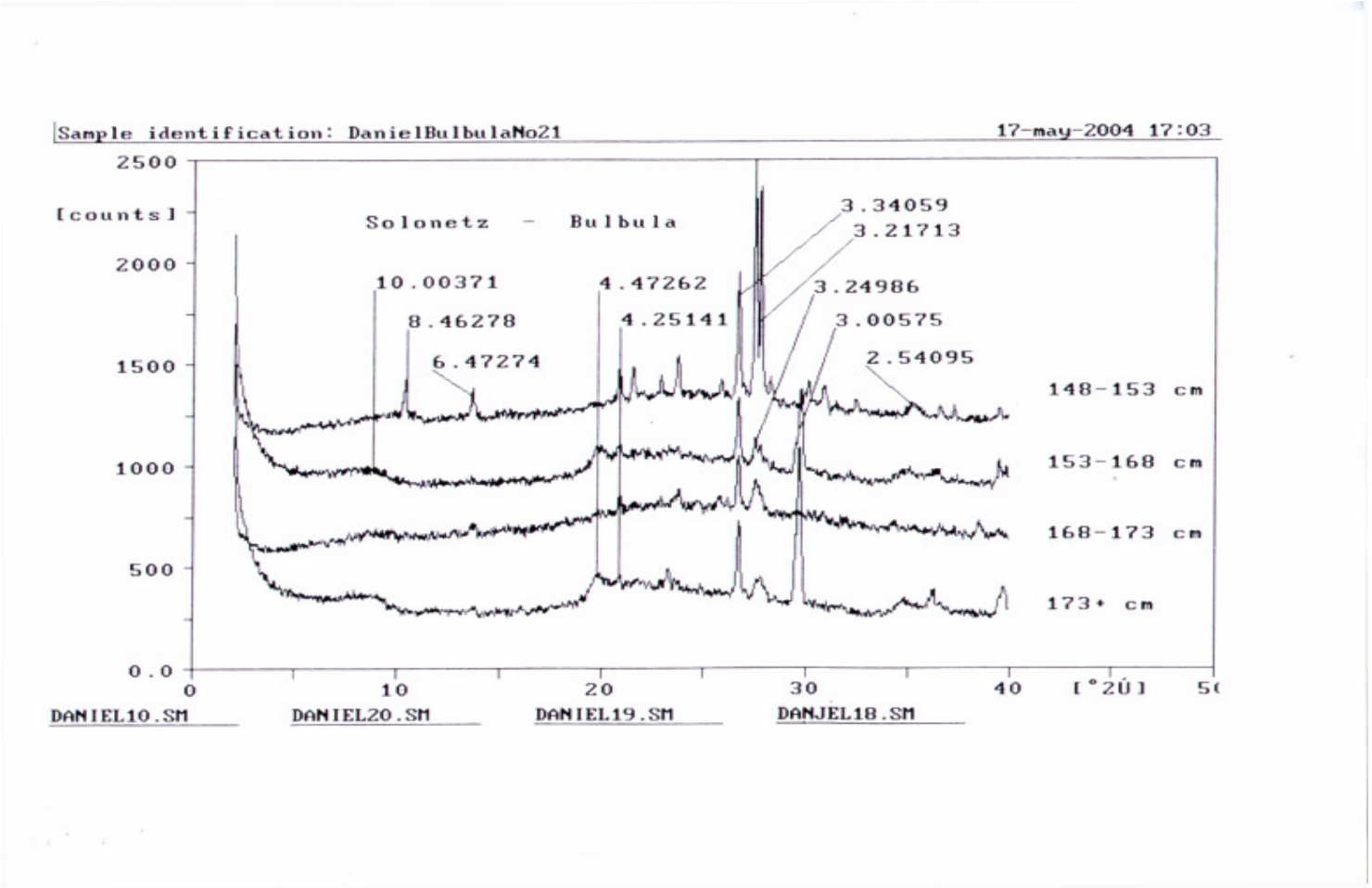
Appendix 1 X-ray graph for the crushed surficial layer (0-20 cm) of Solonetz (see for the same graph on Fig 11)



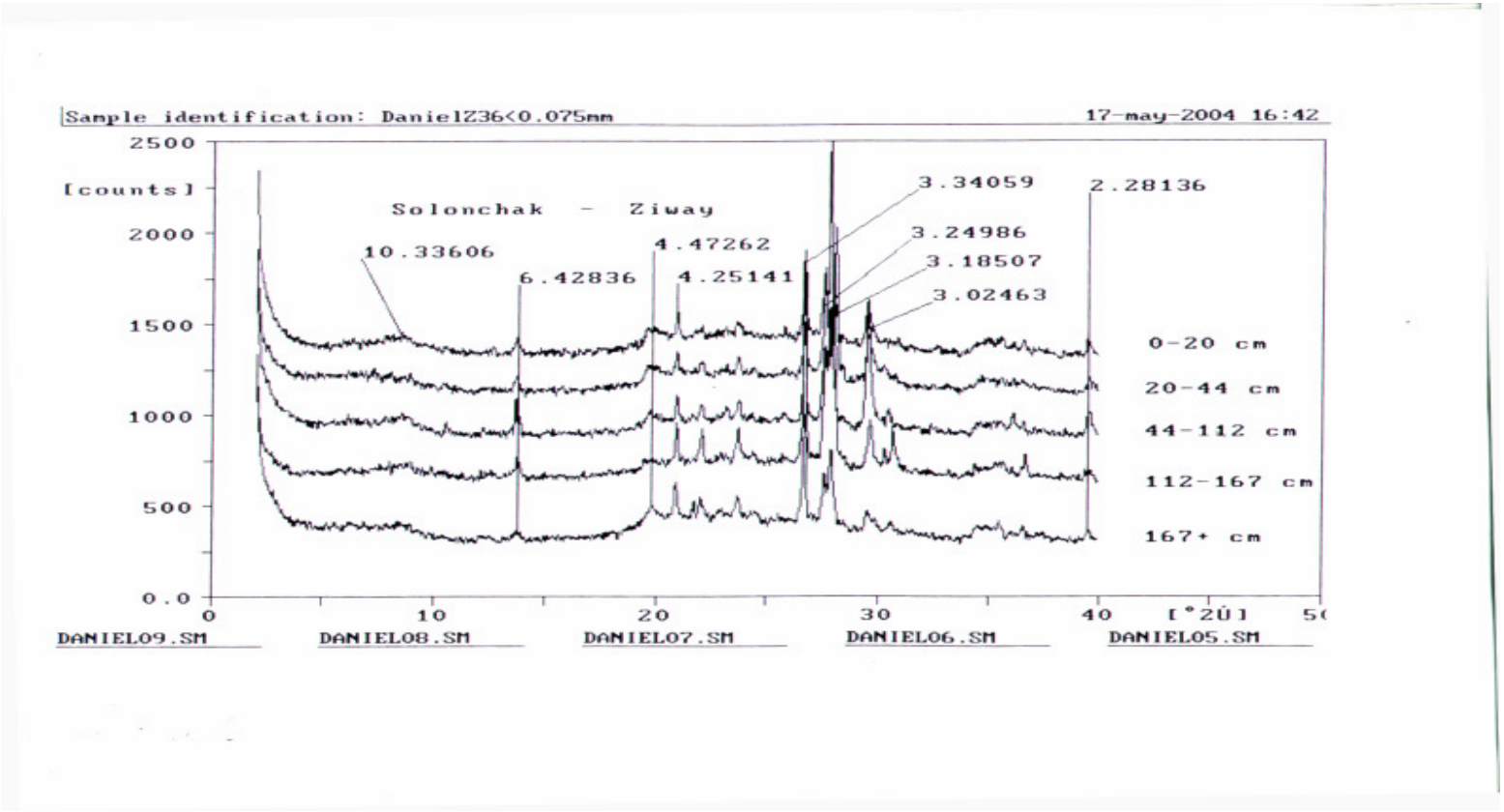
Appendix 2 X-ray graphs for samples collected from 0-20, 20-31, and 31-115 cm depth of Solonetz.



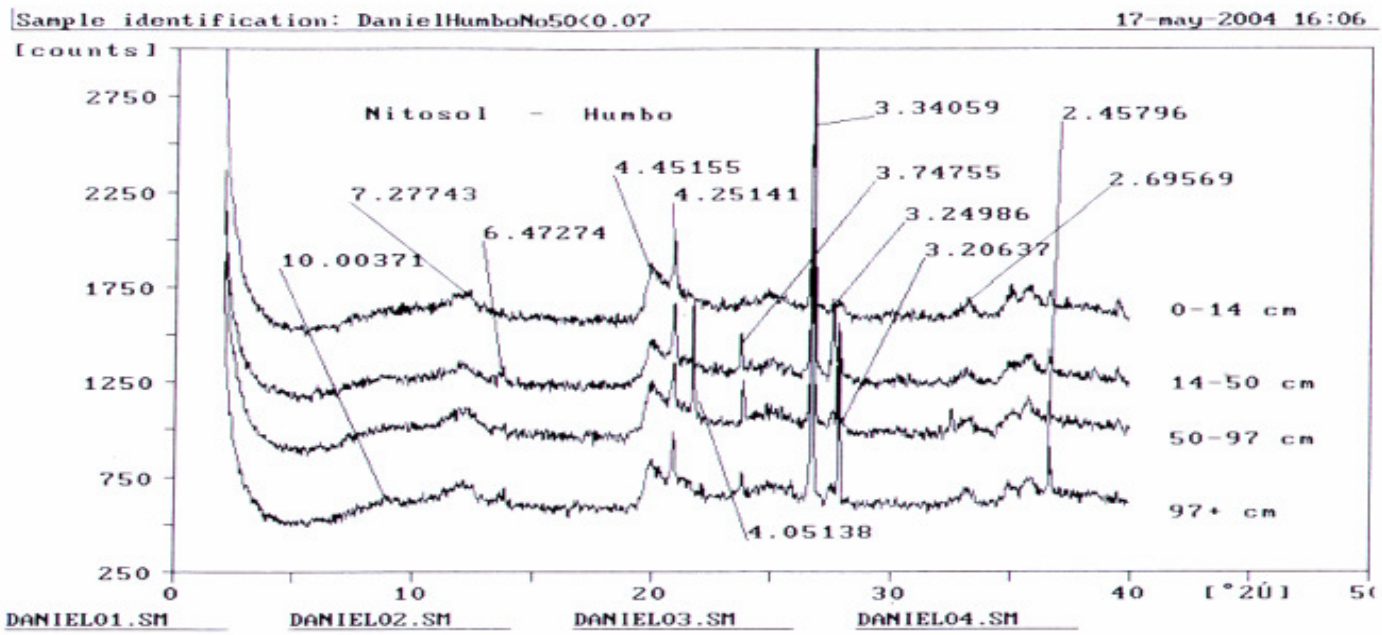
Appendix 3 X-ray graphs for samples collected from 115-117, 117-132, 132-134, and 134-148 cm depths of Solonetz.



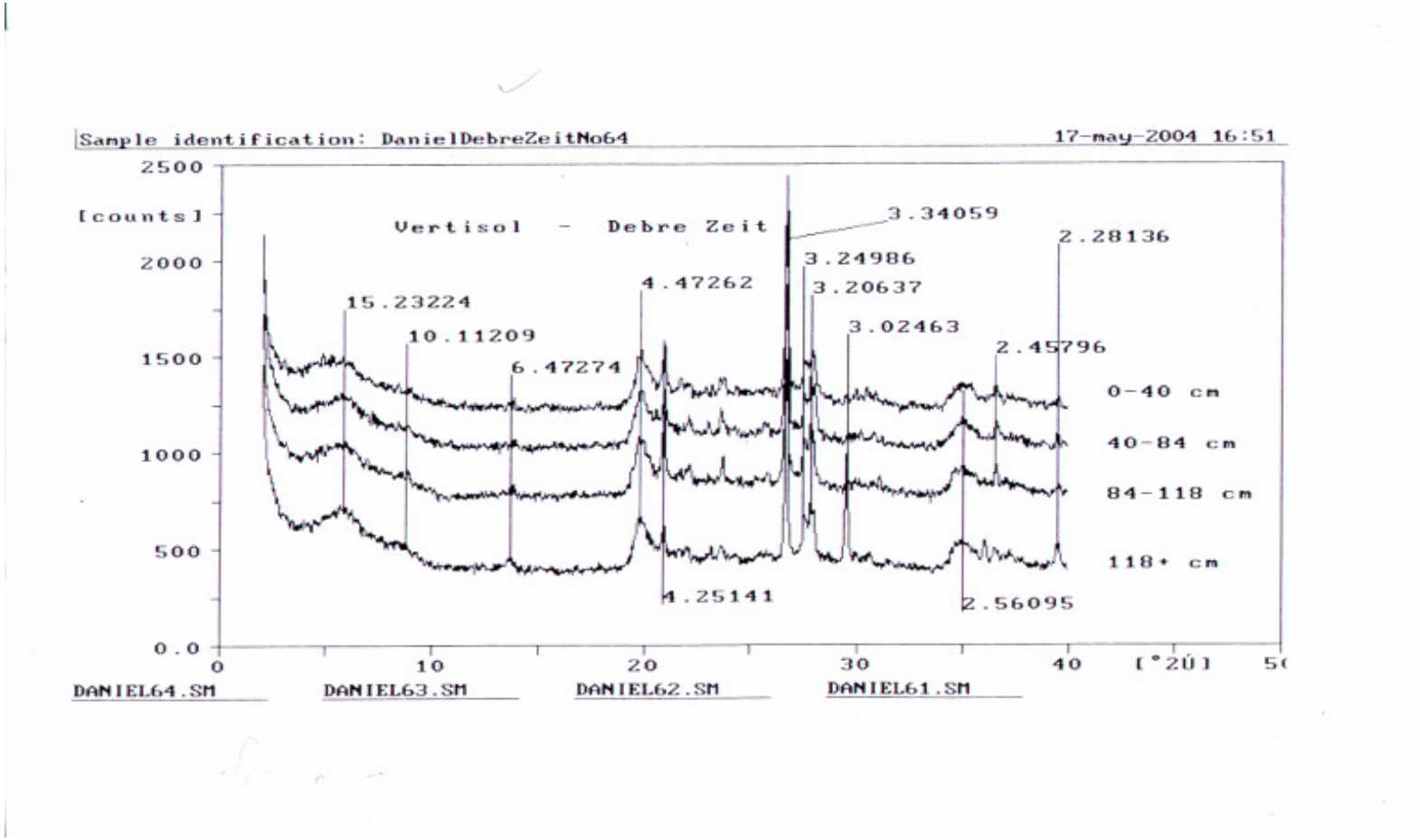
Appendix 4 X-ray graph for the underlying layers (148-153, 153-168, 168-173, > 173 cm) of Solonetz



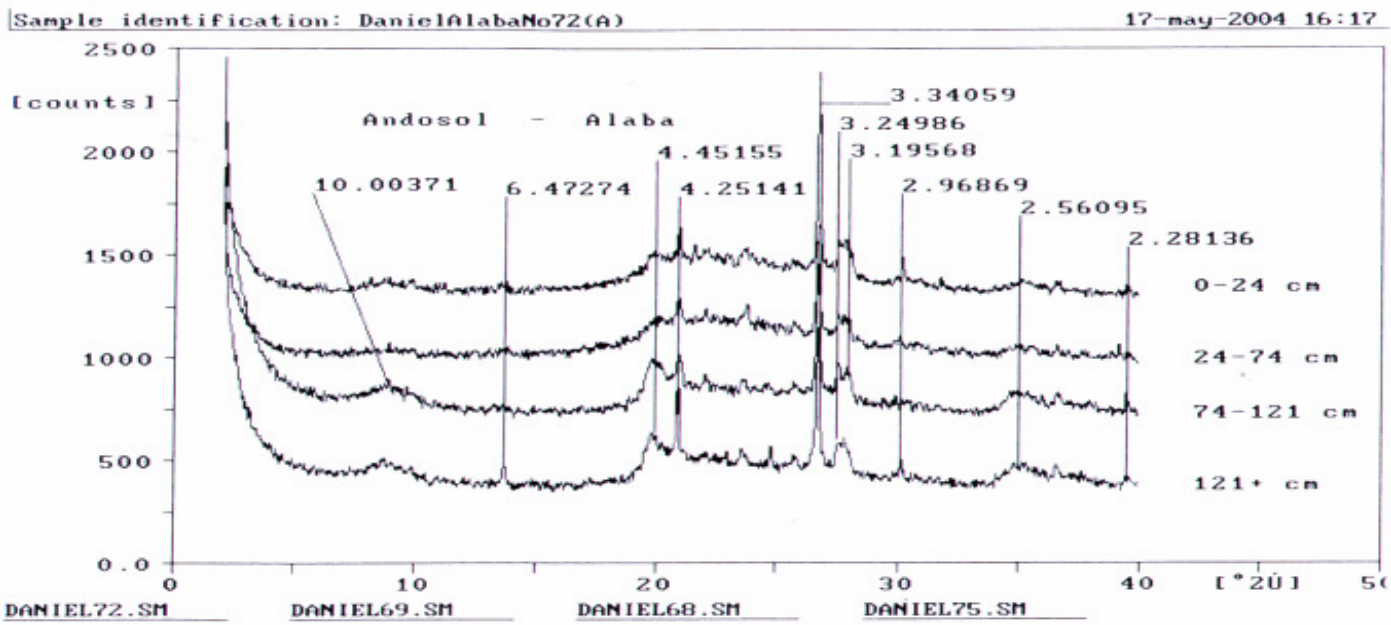
Appendix 5 X-ray diffractogram for soil samples from the different horizons of Fluvisol, Ziway.



Appendix 6 XRD graph for horizon samples collected from the four layers of Nitosol, Humbo.



Appendix 7 XRD for horizon samples collected from Vertisol, Debre Zeit.



Appendix 8 XRD for samples collected from four layers of Andosol, Alaba.

Declaration

I, the undersigned, declare that this thesis is based on my original work and that it has not been presented for a degree in any other university. All sources of materials have been duly acknowledged.

Daniel Fitamo Signature……

This thesis has been submitted for examination with my approval as supervisor of the thesis.

Dr. Seyoum Leta Signature……