Speciation of Nickel, Lead and Cadmium under Different Soil Land Uses of Lesser Himalayas

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Speciation of heavy metals in soils were analysed under different land uses of LesserHimalayas. The soil samples were subjected to Tessier sequential extraction with some modifications. Results indicated abundance of residual fraction indicating lithogenic origin. Cadmium was not detected in water soluble, exchangeable, carbonate and organicfractions while Pb was present in all fractions except water soluble. Assuming mobility and bioavailability, metals are related to their solubility and geochemical forms and decreased in order of extraction: Cd (79%) > Ni (52%) > Pb (45%). Cd was more mobile and bioavailable,so potential risk on food security and human health. pH had significant positive correlation with oxide -Ni (r = 0.367) and carbonate -Pb (r = 0.366). Organic - Ni was positively (r = 0.431) while oxide - Ni was negatively correlated (r = -3.90) with OC. Calcium carbonate had significant correlation with carbonate -Pb (r = 0.526).

Key words: *Bioavailability, chemical speciation, correlation, heavy metals, land uses*

Introduction

Soil is an essential natural resource for support of human life; but with time, its degradation has been constantly increasing due to the deposition of pollutants (Maldonado *et al.*, 2008). The background concentration of metals in virgin soil depends primarily on the bedrock type from which the soil parent material wasderived (Donahue *et al.*, 1983). Among the pollutant sources that cause the contamination of soils, heavy metal contamination is of great environmental concerns because of toxicity, persistent and nondegradable conditions in the environment (Yuan *et al.*, 2004; Nwuche and Ugoji, 2008; Mohiuddin *et al.*, 2010). Some of the elements that are potentially toxic for the plants and animals are accumulated in the food chain (Li *et al.*, 2008). Considering the existence of heavy metals resulted from industrial wastes, automobile emissions,mining activity, added through use of organic and fertilizer impurities (Alloway and Jackson, 1991), pesticides (Yang *et al.,* 2002), irrigation in agriculture (Lombi *et al.,* 2006), agricultural practice (Mantovi *et al.,* 2003) and several other industrial processes, it is necessary to address the effects of these contaminants. Their

uptake and accumulation in plants have been known to results in negative effects on plant growth. Inhibition of plant growth could be caused by the effect of heavy metals on different plant processes i.e. photosynthesis (Moya *et al.*, 1993); respiration (Keck, 1978); carbohydrate metabolism (Greger ang Lindberg, 1986) and water relations (Becerril *et al.*, 1988).

The impact of these metals in soils is their possible transfer into water or plants, which is defined by the term of bioavailability. This concern takes into account a variety of elements referred to in the scientific literature as toxic metals, among them Ni is a heavy metal which is essential for the growth and development of living organisms, but in excess can be toxic (Pakula and Kalembasa, 2009).Nickel is essential only for some plant species. It forms the active metallocenter of the enzyme urease (Gerendas *et al.*, 1999). It's toxic at high concentrations as it inhibits a large number of plant enzymes, such as those of nitrogen metabolism and sulphate assimilation (Lukowski and Wiater, 2009). Cadmium and lead are considered as the most important environmental pollutants in agricultural soils because of the potential harmful effects they may have on food quality and health of soil (Onweremadu and Duruigbo, 2007; Yobouet *et al.*, 2010). Lead is ranked as the number one priority hazardous substance by the Agency for Toxic Substances and Disease Registry (ATSDR) and the EPA (Hoilett, 2006). Cadmium is known as more mobile and soluble than many other metals in soils, but Pb is well known to be relatively immobile and unavailable for plant uptake. The mean concentrations of Cd and Pb in soils ranged from 0.37 mg kg^{-1}to 0.78 mg kg^{-1}and 10 to 40 mg kg^{-1} (Kabata-Pendias, 2001). Most soil Pb is thought to be bound to the solid phases and is, therefore, insoluble. The major concern about Pb is its relatively long residence time in the soil, due to a low solubility and a high affinity for adsorption (Badawy *et al.,* 2002).

Metal in soils can be divided into two fractions (Rachou and Sauvé, 2008): (i) inert fraction, assumed as the non-toxic fraction, and (ii) the labile fraction, assumed to be potentially toxic. Information about these forms helps the better understanding of these metal dynamics. Since total contents in soils provide, in most cases, limited information on the mobility and bioavailability of heavy metals and can be misleading when assessing environmental effects due to a potential overestimation of exposure risk. Sequential fractionation or speciation is a frequently used approach to evaluate metal distribution into different chemical forms present in a solid phase. It is defined as the identification and quantification of the different, defined species, forms or phases in which an element occurs and is essentially a function of the mineralogy and chemistry of the soil sample examined (Tack and Verloo, 1995).

Many studies have been carried out on speciation of heavy metals in different parts of this country. Since little work on speciation of heavy metals in soils in lesser Himalayas has been reported, it is expected that the results from this study would form a baseline data for future heavy metal pollution status of soils in the area under study.

2. Materials and Methods

2.1. Study Area

The study area lies approximately between longitude $33^0 30'$ and $34^0 30'$ N latitude and $74^0 10'$ and $75^0 03'$ E longitude. The area lies in the North-western corner of India, occupies the depression formed by the bifurcation of the Great Himalayan Range whose south-western arm is known as the Pir Panjal Range and the north-eastern arm as the main Himalayan Range.

2.2. Sampling and Analysis

Soil samples (n=44) were collected from five land uses (cereals, apple, saffron, vegetables, forests) of Lesser Himalayas soils (Fig. 1). The composite or representative samples got from each of the land uses are designated as samples from one land use. All the samples were air dried and ground to pass through a 2mm sieve. Soil pH values were determined using glass electrodes in a soil**:** water ratio of 1:2.5 (Jackson, 1973). The particle size distribution of soils was analyzed by the International pipette method (Piper, 1966), organic carbon by the Walkley and Black's (1934)rapid titration method, cation exchange capacity (CEC) was measured using ammonium acetate (pH 7) (Rhoades, 1982) and calcium carbonate ($CaCO_3$)by the method of Puri (1930).

Sequential ExtractionTechnique

The procedure of Tessier *et al*. (1979) with some modifications was used to separate the heavy metals into six operationally defined geochemical fractions (F1 to F6). One gram of the soil was placed in a 50ml polypropylene centrifuge tube and subjected to the following extraction processes:

Water-soluble fraction (F1): Soil extracted with 10ml of deionized water for one hour at 20°C.

Exchangeable fraction (F2): Residue from F1 extracted with 10ml of $1molL^{-1}$ $MgCl_2$, pH 7 for 1 hour.

Carbonate-bound fraction (F3): Residue from F2 extracted with 10ml 1molL^{-1} NaOAc pH 5 for 5 hours.

Fe-Mn oxide-bound fraction (F4): Residue from F3 extracted with 10ml 0.04molL^{-1} NH$_2$OH.HCl in 25% (v/v) HOAc at 900°Cin a water bath for five hours with occasional agitation.

Organic-bound fraction (F5): From F4 residue extracted with 7.5 ml of 30% (v/v) hydrogen peroxide, which has been adjusted to pH 2 with HNO$_3$. The mixture was heated to 85°C in a water bath for five hours with occasional agitation and allowed to cool down. Then 2.5 ml of 3.2 M ammonium acetate in 20% (v/v) nitric acid was added, shaken for half hour and centrifuged.

 Residual fraction (F6): Residue from F5 is digested using a HF/HCl/HNO$_3$ (hydrofluoric/aqua regia) digestion procedure.

All the solid phases from F1 to F6 were washed with 10ml of deionized water before further extraction. The supernatant washes were collected from the previous fraction. After each extraction, the supernatant was separated by centrifugation at 10,000 rpm for 10 minutes and analysis was carried out with Atomic absorption spectrophotometer.

Results and Discussion

Soil Analysis

Various physicochemical properties of the soils of Lesser Himalayas used in the study are shown in Table 1. The results showed that majority of the soils were slightly acidic to neutral reaction with good amount of organic matter content. The clay percentage of soils varies from 21.9-40.6%. The pH ranged from 4.5 to 7.7 with a mean value of 6.4. The pH was minimum in forest (5.5) followed by apple (6.2), vegetable (6.6), cereal (6.7) and saffron (6.9) soils (Table 1). The pH influences oxidation reactions, solubility and ionic forms of several metallic constituents. Soil organic matter was variable, ranging between 0.9 to 5.2% with a mean value of 2.2%. The percentage of organic carbon found in the forest soils (3.8%) was more likely to have higher values than other land uses. The cation exchange capacity varied between 8.1 and 23.1 cmol$_c$ kg^{-1} with an average value of 13.6 cmol$_c$ kg^{-1}, highest in forest (15.6 cmol$_c$ kg^{-1}) and lowest in saffron cultivated soils (10.2 cmol$_c$ kg^{-1}). Results of present study show that calcium carbonate content lies between 0.0 to 4.8% with a mean of 0.2%.

Speciation of nickel, cadmium and lead

Chemical fractions of Ni, Cd and Pb are presented in Table 2, 3 and 4 and percentages of their 'total' are given in Table 5, 6 and 7.

Water soluble fraction:

Water soluble fraction of microelements consists of non-adsorbed ions and can be extracted by water. This fraction is relatively labile and thus, may be potentially bioavailable (Kumar *et al.*, 2011), as soil solution is a naturally dynamic medium for the transportation of metals from soil to plant (Rahmani *et al.*, 2012) where exchange, adsorption, and complexation reactions take place (Harter and Naidu, 2001). It is first to be brought in process of fractionation. This fraction is usually negligible, except in areas where evaporites are present.

The speciation results indicate that the average value of water soluble Ni was 0.60 mg kg^{-1} (Table 2). Notably, the average percentage of the Ni in this fraction was as lowest as 1.26% (Table 5). Fractionation results of Shober (2007) showed that the proportion of Ni that is potentially bioavailable is quite small. Osakwe (2012) reported low levels of Ni (0.03-0.13 mg kg^{-1}) in water soluble fraction, while relatively higher values have been found by Aydinalp. (2009) in the Vertisols of Serbia. Among different land uses, soils with vegetable cultivation had highest percentage (2.27%) of water soluble Ni over other land uses. Lead as well as Cd were below detection limit (0.00 mg kg^{-1})(Table 3, 4) on atomic absorption spectrophotometer (detection limit were 0.2 mg L^{-1}) in all the soils analyzed.

Dissolved or Exchangeable fraction:

This fraction of microelements includes weakly adsorbed metals retained on the solid surface by relatively weak electrostatic interactions. The metals in the exchangeable fraction held by electrostatic adsorption represent the most mobile and readily available for biological uptake in the environment, thus this fraction can be regarded as a pollution indicator (Zakir, 2008). Metals corresponding to the exchangeable fraction usually represent a small portion of the total metal content. Thus, this fraction generally accounts for less than 2% of the total metal present in the soil (Emmerson *et al.*, 2000).

The concentration of metals in this phase indicates the environmental impact (Kumar *et al.*, 2011). In the Lesser Himalayas soils the Ni and Pb associated with exchangeable fraction had the average values of 3.77 and 1.88 mg kg^{-1} (Table 2, 3) and contributed 7.96 and 4.37% (Table 5, 6) to the total respectively. These results are in line with finding of Yobouet *et al.* (2010) who reported

less than 5% of Pb in exchangeable fraction. Data given in Table 5 and 6 indicate that the Ni and Pb associated with exchangeable fraction were highest in saffron (10.66% and 7.13% respectively). Cadmium was not detected in any soil sample from any land use of Lesser Himalayas.

Carbonate fraction (Acid soluble):

Carbonates are an important host of heavy metals (Maskall and Thornton, 1998). Carbonate bound or acid soluble fraction contains the metals which are precipitated or co-precipitated with carbonate. The carbonate form is a loosely bound phase and liable to change with environmental conditions. This phase is susceptible to changes in pH, being generally targeted by use of a mild acid (Filgueiras *et al*., 2002).

From the current study it is revealed that the mean value of Ni in carbonate bound fraction (Table 2, 5) is 4.10 mg kg^{-1} (8.68%). The values are slightly more than that found by Olubunmi (2010) who found 1.34-3.28 mg kg^{-1} of Ni in carbonate form in soils of Agbabu. The results also revealed that thepercentage of Niassociated with carbonate bound fraction was highest in forest soils (10.55%) and lowest in saffron soils (6.88%).

Carbonate bound fraction (Table 3) of Pb (8.97 mg kg^{-1}) constitutes the second highest fraction (20.50%) (Table 6) and is more likely due to the reason that Pb is most likely controlled by a mixture of Pb minerals, comprising Pb carbonates (Teutsch *et al.,* 2001). Different land uses had different percentages of Pb associated with carbonates (Table 5), highest in cereal soils (22.81%) and lowest in forest soils (18.97%).Wang *et al.* (2010) found that approximately 10-25% of Pb was associated with carbonate minerals in soils of northeast China. Further support to the results comes from Adaikpoh (2011) and Osakwe (2012). However, Cd in carbonate bound fraction was below detection limit (Table 4). Similar result was reported by Chen*et al.* (2009) who found carbonate bound Cd below detection limit in Beijing soils of China.

Fe-Mn oxide fraction (Reducible):

In comparison with carbonate minerals, Fe-Mn oxide minerals have relatively large area and surface site density (Forstner and Wittmann, 1981). The Fe-Mn oxide, the reducible phase of the soil under oxidizing conditions is a significant sink for the heavy metals. Nickel concentration in this pool (Table 2) varied from 3.27 to 17.06 mg kg^{-1} averaging 9.20mg kg^{-1}. It is second most concentrated fraction (19.38%) after the residual fraction. The distribution of Ni in different land uses (Table 5) on the basis of average concentrations shows maximum percentage in forests (8.60%) and least in apple orchards (4.05%). This may be due to the ability of Fe^{2+} and Fe^{3+} species

to scavenge metals from soil solution that would normally not precipitate (Zakir., 2008). This fraction was most dominant in vegetable soils (83.59%) and lowest in pasture soils (75.43%). Som and Joshi (2002) quantified the degree of substitution in goethite on a laterite, finding Ni:Fe ratios ranging from about 1:50 to 1:26. Singh *et al*. (2000) found that up to 6% of Ni can substitute for iron in the hematite structure. This fraction could be considered relatively stable, but could change with variations in redox conditions of the soil (Horsfall and Spiff, 2005).

The fractionation results from the present study found only small amount Pb (2.56 mg kg^{-1}, 5.77% of total) associated with this fraction (Table 3). Minkina *et al.* (2008) found 1.8 mg kg^{-1} of Pb in oxide bound fraction in chernozem soils while Olubunmi (2010) examined that the oxide bound Pb varied from 0.00-1.35 mg kg^{-1} with an average value of 0.88 mg kg^{-1} in Agbabu soils. Cadmium was chiefly concentrated in the oxide bound fraction (79.34%) having average values of 0.44 mg kg^{-1}(Table 4).The findings are supported by the fact that hydrous oxides of manganese and iron furnish the principal control on the fixation of cadmium in soils. Hydrous oxides of manganese and iron are extracted together, the well-known 'sinks' in the surface environment for heavy metals. Scavenging by these secondary oxides, present as coatings on mineral surfaces or as fine discrete particles, can occur by any or a combination of mechanisms such as coprecipitation, adsorption, surface complex formation, ion exchange and penetration of the lattice. This fraction could be considered relatively stable, but could change with variations in redox conditions of the soil (Horsfall and Spiff, 2005).

Organic fraction (Oxidizable):

Organic matter plays an important role in the distribution and dispersion of metals by mechanisms of chelating and cation exchange. This includes metals associated through complexation or bioaccumulation process with various kinds of organic materials such as living organisms, detritus or coatings on mineral particles (Tokalioglu *et al.,* 2002). Carboxyl, phenolic, hydroxyl and carbonyl functional groups are assumed to be primarily responsible for metal binding.

The concentrations of heavy metals found in the organic fraction (Table 5, 6, 7) are 15.05% (Ni), 13.99% (Pb) and 0.00% (Cd). The relatively higher values of organic Ni may be attributed toits organophilic nature (Cunningham *et al*., 1975). It is pointed out from the Table 5 and 6 that the highest and lowest percentage of organic bound Ni was noticed in vegetable soils (20.27%) and cereal soils (9.65%) and Pb in apple orchards (21.71%) and saffron soils (10.90%), respectively.

Residual fraction:

The residual fraction is a major carrier of metals in most environmental systems and can be taken as a guide to the degree of non-availability of metals (Horsfall and Ma, 2005). As a result of weathering, a fraction of the trace constituent content is gradually transferred to forms accessible to plants (Hlavay *et al.*, 2004). The smaller the percentages of the metal present in this fraction, the greater the pollution of the area. In all the samples analyzed the both the Ni and Pb (47.65%, 55.35%) were found to be mostly associated with the residual fraction (Table 5, 6) and is well supported by Yobouet *et al.* (2010) who concluded that the residual Pb is the major form (53%) in the entire fractions. The low content of Cd (Table 7) in residual soil fraction (20.66%)may pose a threat to the environment.Gondek (2006)reported below detection limit of the residual Cd in soils of Poland. Osakwe (2012) reported that the residual Cd ranged from 0.53-2.80 mg kg^{-1}.

So the distribution patterns of the studied metals in various fractions (Table 5, 6, 7) were found to be as follows:-

Nickel:residual >oxide bound>organic bound>carbonate bound> exchangeable > water soluble

Lead: residual >carbonate bound> organic bound>oxide bound> exchangeable

Cadmium: reducible > residual

Availability of heavy metals

The environmental impact of the five-speciation fractions of the heavy metals depends upon case of remobilization (Kumar *et al.*, 2011). Tessier *et al.* (1979) defined a model for heavy metal association with geochemical fractions. In this model, metals found in ion-exchangeable, carbonate, reducible fraction and organic phases are those considered to be readily available to water column and the biota operationally described as bioavailable metals, while those metals in the residual phase is considered as the non-available metal.

It is evident from Figure 2 that the percentage potential availability of Cd, Ni and Pb were 79%, 52% and 45%, respectively. Figure 2 also shows that most fraction of Cd was found in the potentially available form while as Pb was mostly concentrated in non-available form and Ni shows almost an equal percentage of available and non available fraction (Figure 3, 4 and 5). This potential availability of the heavy metals indicates that metals have contamination risk in Lesser Himalayas environment.

Correlation studies

Simple correlation studies between microelements such as nickel, cadmium and lead were made with some physico-chemical properties as shown in Table 8, 9 and 10.

Relationship of nickel (Ni) fractions with soil properties

The water soluble, exchangeable and carbonate bound Ni(Table 8) exhibited non-significant correlation with all the studied soil properties (pH, Organic carbon, cation exchange capacity and clay content).

There was a positive and significant correlation of organic boundNi with organic carbon, while as other soil properties (pH, cation exchange capacity, calcium carbonate and clay content) showed non-significant correlation with organic boundNi. The oxide boundNi had a positive significant correlation with pH (r = 0.367[*]) and was significantly and negatively correlated with organic carbon (r = -0.39[**]). The present results confirm the findings of Ping *et al.* (2011). Significant and positive correlation of organic boundNi with organic carbon is supported by the results of Pakula and Kalembasa (2009).

Relationship of lead (Pb) fractions with soil properties

The relationship of exchangeable Pb with pH, organic carbon, calcium carbonate, cation exchange capacity and clay content (Table 9) are in accordance with the findings of Finzgar *et al.* (2007) and Ping *et al.* (2011). Carbonate bound Pb observed a positive significant correlations with pH and calcium carbonate content (r = 0.366[*] and 0.526[**]) and non-significant relationships with organic carbon,cation exchange capacity and clay content, thus revealing that the carbonate bound Pbin the soils under investigation was largely influenced by pH and calcium carbonate content and not by organic carbon,cation exchange capacity and clay content. Similar results were also stated by Li and Thornton (2001), Davies *et al. (*2003) and Ashraf *et al.* (2012).

Lead associated with the oxide, organic and residual fractions showed non-significant correlations with all the soil properties (pH, organic carbon, calcium carbonate, cation exchange capacity and clay content).These results are in consonance with those reported by Fernandez *et al.* (2004), Finzgar *et al.* (2007) and Laurent and Pierre (2010).

Relationship of cadmium (Cd) fractions with soil properties

Oxide bound Cdshowed positive correlations with organic carbon,calcium carbonate, cation exchange capacity and negative coefficient of correlations with organic carbon and clay content (Table

10). Yet the correlations were not significant.These results support the observations of Fernandez *et al.* (2004) and Ping *et al.* (2011).

The positive correlations of residual fraction of Cd with organic carbon andcation exchange capacity are in agreement with the results of Ping *et al.* (2011) and the negative correlations with pH, calcium carbonateand clay content are in accordance with the observations of Kashem *et al.* (2007).

Conclusion

Geochemical forms of microelements affect their solubility which directly influences their bioavailability in soils and sediments. Sequential extraction was used to fractionate Ni, Pband Cd from five land uses of Lesser Himalayas into six operationally defined pools: water soluble, exchangeable, carbonate, oxide, organic and residual. Such assessment assumes that metal bioavailability decreases with each successive extraction step. The residual phase was most abundant for all the analyzed metals except Cd which was abundant in oxide bound fraction in soils examined. Among the non-residual fractions the major portions of Ni were associated with reducible followed by organic and carbonate, Pb with carbonate bound followed by organic and oxide, Cd with oxide followed by residual fractions. The results also indicate that the soil samples collected from five land uses contain varying amounts of metal in each fraction. The mobility and bioavailability of the five metals declined in the following order: Cd > Ni > and Pb. Among the analyzed soil properties pH, organic carbon and calcium carbonate content were the principal factors governing distribution of microelements in various chemical pools. The study will help in monitoring levels of heavy elements (Ni, Cd and Pb) in soils of Lesser Himalayas.

References

Adaikpoh, E. O. 2011. Metal fractionation in soil profiles in Umutu Oil Field, northwest Niger delta Nigeria. *Int. J. Chem.***3**,22-36.

Alloway, B. J., and Jackson, A. P. 1991. The behavior of heavy metals in sewage sludge amended soils. *Sci. Total Environ.* **100**, 151-176.

Ashraf, M. A., Maah, M. J., and Yuso, I. 2012. Chemical speciation and potential mobility of heavy metals in the soil of former tin mining catchment. *J. Scien. World* **10**, 1-11.

Aydinalp, C. 2009. Concentration and speciation of Cu, Ni, Pb and Zn in cultivated and uncultivated soils. *Bulg. J. Agric. Sci.* **15**,129-134.

Badawy, S. H., Helal, M. I. D., Chaudri, A. M., Lawlor, K., and McGrath, S. P. 2002. Soil solid-phase controls lead activity in soil solution. *J. Environ. Qual.* **31**, 162-167.

Becerril, J. M., Gonzlez-Murua, C., Munoz-Rueda, A., and De Felipe, M. R. 1988. The effects of cadmium and Lucerne. *Plant Physiol.Biochem*. **26,** 257-363.

Chen, Z., Ye, Z., Li, Q., Qiao, J., Tian, Q., and Liu, X. 2009. Heavy metal contents and chemical speciations in sewage-irrigated soils from the eastern suburb of Beijing, China.*J. of Food, Agric. and Environ.* **7**, 3-4.

Cunningham, J. D., Keeney, D. R., and Rayan, J. A. 1975. Phytotoxity and uptake of metals added to soils as inorganic salts or in sewage sludge*. J. Environ. Qual*. **4**, 460-462.

Davies, N. A., Hodson, M. E., and Black, S. 2003. Is the OECD acute worm toxicity test environmentally relevant? the effect of mineral form on calculated lead toxicity. *Environ. Pollut.* **121**, 49-54.

Donahue, R. L., Miller, R. W., and Shickluna, J. C. 1983. Soils and introduction to soils and plant growth. Fifth edition. Prentice Hall, Inc., Englewood Cliffs, New Jersey1-45

Emmerson, R. H. C., Birkett, J. W. M., and Scrimshawand and Lester, J. N. 2000. The importance of chemical speciation in environmental processes. *Sci. Total Environ.* **254**, 75-79.

Fernandeza, E., Jimeneza, R., Lallenab, and Aguilar, A. M. J. 2004. Evaluation of the BCR sequential extraction procedure applied for two unpolluted Spanish soils. *Environ. Pollut.* **131**, 355-364.

Filgueiras, V., Lavilla, I., and Bendicho, C. 2002. Chemical sequential extraction for metal partitioning in environmental solid samples. *J. Environ. Monit.***4**, 823-857.

Finzgar, N., Tlustos, P., and Lestan, D. 2007. Relationship of soil properties to fractionation, bioavailability and mobility of lead and zinc in soil.*Plant and Soil Environ.***53**, 225-238.

Forstner, U., and Wittmann, G. T. W. 1981. *Metal Pollution in the Aquatic Environment.* New York, Springer-Verlag.

Gerendas, J., Polacco, J. C., Freyermuth, S. K., and Sattelmacher, B. 1999**.** Significance of nickel for plant growth and metabolism. *J. Plant Nutr. and Soil Sci.***162**, 241-245.

Gondek, K. 2006. Contents of various forms of cadmium, copper, lead and chromium in soil after application of untreated and composted tannery sewage sludge. *Plant and Soil Environ.* **52**, 199-210.

Guerra, P., Ahumada, I., and Carrasco, A. 2007. Effect of biosolid incorporation to Mollisol soils on Cr, Cu, Ni, Pb, and Zn fractionation and relationship with their bioavailability. *Chemosphere* **77**, 739-800.

Greger, M., and Lindberg, S. 1986. Effects of Cd^{2+} and EDTA on young sugarbeets (Beta vulgaris). Cd^{2+} uptake and sugar accumulation. *Physiol. Plant.* **66,** 69-74.

Harter, R., and Naidu, R. 2001. An assessment of environmental and solution parameter impact on trace-metal sorption by soils. *Soil Sci. Soc. Am. J.* **65**, 597-612.

Hlavay, J., Prohaska, T., Weisz, M., Wenzel, W. W., and Stingeder, G. J. 2004. Determination of trace elements bound tosoils and sediment fractions.*Environmentalist* **26**,123-128.

Hoilett, N. 2006. Microbial properties as affected by in situ phosphate treatment in lead contaminated soils. *M.Sc. dissertation* submitted to University of Missouri, Columbia MO.

Horsfall, M., and Spiff, A. 2005. Speciation and bioavailability of heavy metals in sediment of Diobu River, Port Harcourt, Nigeria. *Europ. J. Sci. and Res.***6**,20-36.

Jackson, M. L. 1973. *Soil Chemical Analysis*. Prentice Hall of India Private Limited, New Delhi.

Kabata-Pendias, A. 2001. *Trace Elements in Soils and Plants.* 3rd edition, CRC Press, Boca Raton FL, USA.

Kashem, M. A., Singh, R., Kondo, Imamul-Huq, M., and Kawai, S. 2007. Comparison of extractability of Cd, Cu, Pb and Zn with sequential extraction in contaminated and non-contaminated soils. *Int. J. Environ. Sci. Tech.* **4**, 169-176.

Keck, R.W. 1978. Cadmium alteration of root physiology and potassium ion fluxes. *PlantPhysiol.* **62,** 94-96.

Khurana, M. P. S., and Bansal, R. L. 2008. Impact of sewage irrigation on speciation of nickel in soils and its accumulation in crops of industrial towns of Punjab. *J. Environ. Bio.***29**, 793-798.

Kumar, M., and Babel, A. L. 2011.Available micronutrient status and their relationship with soilproperties of Jhunjhunu Tehsil, District Jhunjhunu, Rajasthan. *Indian J. Agril. Sci.* **3**, 20 - 31.

Laurent, M., and Pierre, T. J. 2010. Multivariate statistical analysis of trace elements in soil on spoil heap, south east Congo-Brazzaville. *African J. basic and appl. Sci.***2**, 81-88.

Li, B., Wei, M., Alin Shen, Xu, J., Zhang, H., and Hao, F.2008.Changes of yields, soil properties and micronutrients as affected by seventeen years of fertilization treatments. *J. of Food, Agric. and Environ.* **7**, 408-413.

Li, X., and Thornton, I. 2001. Chemical partitioning of trace and major elements in soils contaminated by mining and smelting activities. *Appl. Geoche.* **16**, 1693-1706.

Lombi, E., Nolan, A. L., and McLaughlin, M. J. 2006. Short-term natural attenuation of copper in soils, effects of time, temperature and soil characteristics. *Environ. Toxicol. and Chemis.* **25**, 652-658.

Lukowski, A., and Wiater, J.The Influence of Mineral Fertilization on HeavyMetal Fraction Contents in Soil. Part II, Copper and Nickel.2009*. Polish J. of Environ. Stud.* **18,**645-650.

Maldonado, V. M. H., Rubio., Arias, O., Quintana, R., Saucedo, R. A., Gutierrez, M., Ortega, J. A., and Nevarez, G. V. 2008. Heavy Metal Content in Soils under Different Waste water Irrigation Patterns in Chihuahua., Mexico. *Int. J. Environ. Res. Public Health* **5,** 441-449.

Mantovi, P. G., Bonazzi, E., Maestri and Marmiroh, N. 2003. Accumulation of copper and zinc from liquid manure in agricultural soils and crop plants. *Plant Soil* **250**, 249-257.

Maskall, J. E., and Thornton I. 1998. Chemical partitioning of heavy metals in soils, clays and rocks at historical lead smelting sites. *Water Air and Soil Pollut.* **108**, 391-409.

Minkina, T. M., Motuzova, G. V., Nazarenko, O. G., Kryshchenko, V. S., and Mandzhieva, S. S. 2008. Combined approach for fractioning metal compounds in soils. ***Eurasian Soil Sci.*** **11**, 1324-1333.

Mohiuddin, K. M., Zakir, H. M., Otomo, K., Sharmin, S., and Shikazono, N. 2010. Geochemical distribution of trace metal pollutants in water and sediments of downstream of an urban river. *Int. J. Environ. Sci. Tech.* **7,** 17-28.

Moya, J. L., Ros, R., and Picazo, I. 1993. Influence of Cd and Ni on growth, net photosynthesis and carbohydrate distribution in rice plants. *Photos. Res.* **36,** 75-80.

Nwuche, C. O., Ugoji, E. O. 2008. Effects of heavy metal pollution on the soil microbial activity. *Int. J. Environ. Sci. Tech.* **5,** 409-414.

Olubunmi, F. E. 2010. Speciation of heavy metals in soil of bitumen deposit impacted area of Western Nigeria. *European J. Scif. Res.* **47**, 265-277.

Onweremadu, E. U., and Duruigbo, C. I. 2007. Assessment of Cd concentration of crude oil pollution arable soils. *Int. J. Environ. Sci. Tech.* **4,** 409-412.

Osakwe, S. A. 2012. Chemical Partitioning of iron, cadmium, nickel and chromium in contaminated soils of south-eastern Nigeria. *Res. J. Chem. Sci.* **2**, 1-9.

Pakula, K., and Kalembasa, D. 2009. Distribution of Nickel fractions in forest luvisols in the south Podlasie lowland. *J. Elementol* **14,** 517- 525.

Ping, G. U. O., Zhong-lei, X. I. E., and Jun, L. I. 2011. Fractionation of Pb, Cd, Cu, Zn and Ni in urban soils of Changchun, China. *China acad. J.* **2**, 180-187.

Piper, C. S. 1966. *Soil and Plant Analysis*. Hans Publishers, Bombay, pp 164.

Puri, A. N. 1930. A new method of estimating total carbonates in soils. *Pusa Bulletin, No. 73,* Imperial Agriculture Research, New Delhi.

Rachou, J., and Sauve, S. 2008. Evaluation of affinity constants of Cu, Cd, Ca and H for active soil surfaces for a solid phase-controlled soil ligand model. *Environ. Chem.* **5,** 1065- 1071.

Rahmani, B., Tehrani, M. M., Khanmirzaei, A., and Shahbazi, K. 2012. Cadmium fractions and its uptake by the wheat plant in some calcareous soils of Iran. *Int. J. Agri. Res. and Rev.***2**, 461-466.

Rhoades, J. D. 1982. Cation exchange capacity.**In**, *Methods of Soil Analysis: Chemical and Microbiological Properties*. Part-II (Editors Page, A. L., Miller, R. H., and Keeney, D. R.). American Society of Agronomy and Soil Science Society of America, Madison, Wiscosin, USA.

Shober, A. L. 2007. Chemical fractionation of trace elements in biosolid-amended soils and correlation with trace elements in crop tissue. *Commun. in Soil Sci. and Plant Analy.* **38**, 1029-1046.

Singh, B., Sherman, D. M., Gilkes, R. J., Wells, M., and Mosselmans, J. F. W. 2000. Structural chemistry of Fe, Mn and Ni in synthetic hematite's as determined by extended x-ray absorption fine structure spectroscopy. *Clays and Clay Minerals***48**, 521-527.

Som, S. K., and Joshi, R. 2002. Chemical weathering of serpentinite and Ni enrichment in Fe oxide at Sukina area, Jajpur district, Orissa, India. *Economic Geol.* **97**, 165-172.

Tack, F. M. G., and Verloo, M. G. 1995. Chemical speciation and fractionation in soil and sediment heavy metal analysis, a review. *Int. J. Environ. Anal. Chem.* **59**, 225-238.

Tessier, A., Campbell, P. G. C and Bisson, M. 1979. Sequential extraction procedure for the speciation of particulate traces metals**.** *Anal. Chem.* **51**,844-851.

Teutsch, N., Yigal, E., Halicz, L., and Banin, A. 2001. Distribution of natural and anthropogenic lead in Mediterranean soils.*Geochimica et Cosmochimica Acta***65**, 285-2864.

Tokalioglu, S., Kartal, S., and Elc, L. 2002. The use of extractants in studies on trace metals in soils. *Analytical Chimica Acta***33**, 410-413.

Walkley, A. and Black, C.A. (1934) An examination of Egtareff method for determining soil organic matter and a proposed modification of chromic acid titration method. *Soil Sci.***37**: 29-31.

Wang, D. C., Cui, Y. S., Liu, X. M., Dong, Y. T., and Christie, P. 2010. Soil contamination and plant uptake of heavy metals at polluted sites in China. *J. Environ. Sci. and Health* **38**, 823-838.

Yang, J., Mosby, D. E., Casteel, S. W., and Blanchar, R. W. 2002. In vitro lead bioaccessibility and phosphate leaching as affected by surface application of phosphoric acid in lead-contaminated soil. *Environ. Contam. and Toxicol.***43**, 399-405.

Yobouet, Y. A., Adouby, K., Trokourey, A., and Yao, B.2010. Cadmium, copper, lead and zinc speciation in contaminated soils.*Int. J. Eng. Science and Tech.***2**,802-812

Yuan., C. J., Shi, B., He, J., Liu, L., and Jiang, G. 2004. Speciation of heavy metals in marine sediments from the East China sea by ICP-MS with sequential extraction. *Environ. Int.***30,** 769-783.

Zakir, H. M. 2008. Geochemical partitioning of trace metals, An evaluation of different fractionation methods and assessment of anthropogenic pollution in river sediments. *Ph.D. dissertation* submitted to Keio University of Yokohama, Japan, pp 223-8522
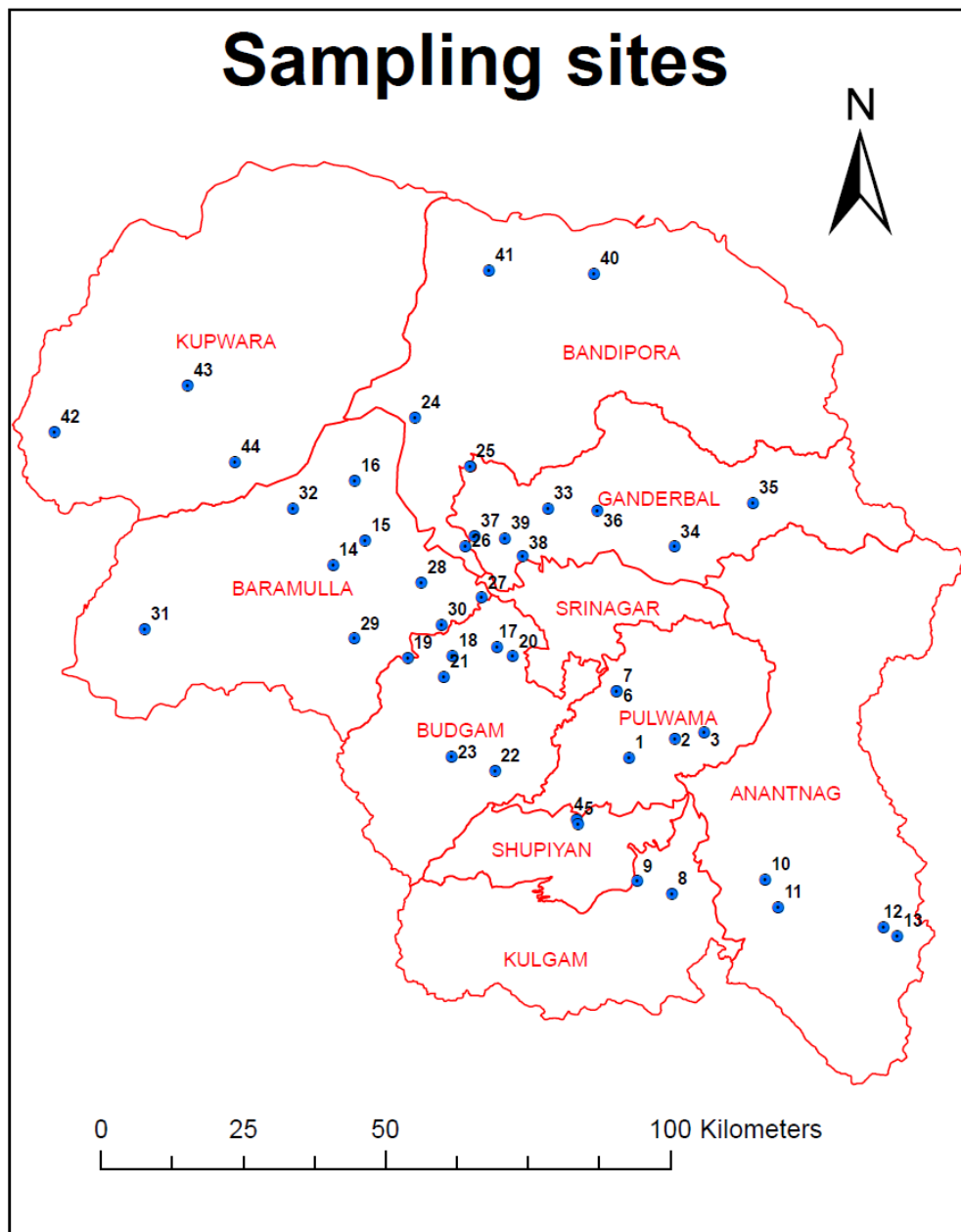
Figure 1. Description of geo-referenced sampling locations of Kashmir Himalayas

Table 1.Physico-chemical characteristics of soils under different land uses of Lesser Himalayas

Land use	Statistics	pH (1:2.5)	CaCO$_3$ (%)	OC (%)	CEC (cmol$_c$ kg^{-1})	Clay (%)
Cereals	Range	4.7-7.6	0.0-1.8	0.9-4.2	9.1-23.1	21.9-40.67
	Mean ± SD	6.7±0.7	0.2±0.5	1.9±0.9	15.1±3.0	30.5±5.19
Apple	Range	4.5-7.1	0.0-0.1	1.0-1.8	9.1-15.3	24.5-33.8
	Mean ± SD	6.2±0.1	0.03±0.05	1.5±0.3	12.4±2.43	29.5±3.6
Vegetables	Range	5.3-7.5	0.0-4.8	1.6-4.2	11.5-17.6	26.1-38.5
	Mean ± SD	6.6±0.9	0.9±2.0	2.3±0.9	14.5±2.4	32.6±4.2
Saffron	Range	6.2-7.7	0.0-0.2	1.2-1.3	8.1-12.3	25.7-26.6
	Mean ± SD	6.9±1.0	0.1±0.1	1.3±0.1	10.2±3.0	26.1±0.6
Forest	Range	5.2-5.8	0.0-0.0	2.1-5.2	13.2-18.5	23.2-31.9
	Mean ± SD	5.5±0.3	0.0±0.0	3.8±1.57	15.6±2.7	27.9±4.4
Over all Mean	Range	4.5-7.7	0.0-4.8	0.9-5.2	8.1-23.1	21.9-40.6
	Mean ± SD	6.4±0.6	0.2±0.4	2.2±1.0	13.6±2.2	29.3±2.5

0.0 Below detection limit

Table 2. Chemical fractionation of Ni (mg kg^{-1}) in five land uses of Lesser Himalayas

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual	Total
Cereals	0.00-2.23 (0.76±0.60)	2.52-6.34 (4.22±1.23)	0.72-7.84 (4.36±1.70)	3.27-17.06 (11.61±4.19)	0.36-12.60 (5.07±3.58)	13.30-44.75 (26.48±8.64)	36.78-75.58 (52.49±9.77)
Apple	0.00-1.56 (0.80±0.58)	1.32-5.06 (3.23±1.41)	1.78-6.48 (4.01±1.87)	6.94-13.18 (10.56±1.79)	3.87-9.59 (6.39±2.25)	12.65-26.80 (19.28±5.81)	35.90-52.39 (44.26±5.72)
Vegetables	0.24-2.03 (1.09±0.65)	1.84-4.34 (3.13±0.97)	3.18-5.24 (4.15±0.84)	4.14-11.16 (8.80±2.95)	8.85-10.53 (9.73±0.76)	16.15-37.50 (21.13±8.10)	37.83-68.13 (48.02±10.88)
Saffron	0.00-0.59 (0.29±0.41)	4.12-5.86 (4.99±1.23)	2.78-3.66 (3.22±0.62)	6.94-9.28 (8.11±1.65)	4.78-8.05 (6.42±2.31)	16.15-31.4 (23.78±10.78)	42.70-50.90 (46.80±5.79)
Forest	0.00-0.22 (0.07±0.13)	2.72-3.67 (3.30±0.51)	3.76-6.00 (4.78±1.13)	6.48-7.88 (6.94±0.80)	4.76-10.12 (7.78±2.74)	12.65-27.85 (22.40±8.46)	31.43-53.87 (45.29±12.12)
Average	0.00-2.23 (0.60±0.41%)	1.32-6.34 (3.77±0.81)	0.72-7.84 (4.10±0.57)	3.27-17.06 (9.20±1.88)	0.36-12.60 (7.08±1.77)	12.65-44.75 (22.61±2.72)	31.43-75.58 (47.37±3.20)

0.00 = Below detection limit

In parenthesis = (Mean±SD)

Table 3. Chemical fractionation of Pb (mg kg^{-1}) in five land uses of Lesser Himalayas

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual	Total
Cereals	0.00	0.00-4.38	4.72-14.79	0.62-6.75	0.23-14.23	5.20-43.50	14.43-60.88
	0.00	(1.66±1.37)	(8.72±2.47)	(2.50±1.73)	(4.27±3.56)	(21.09±12.33)	(38.23±12.21)
Apple	0.00	0.58-6.80	4.58-12.02	0.92-4.56	2.37-16.14	10.45-35.65	29.79-60.15
	0.00	(2.15±2.27)	(8.40±2.85)	(1.68±1.44)	(9.00±4.69)	(20.21±9.56)	(41.44±11.05)
Vegetables	0.00	0.72-3.96	4.72-16.16	0.92-5.78	3.85-8.85	18.00-41.00	36.98-59.46
	0.00	(1.81±1.18)	(9.81±4.67)	(2.31±1.93)	(6.46±2.01)	(28.38±8.32)	(48.77±8.84)
Saffron	0.00	2.72-3.42	8.36-9.16	0.92-3.36	0.32-9.07	23.20-25.55	35.52-50.56
	0.00	(3.07±0.49)	(8.76±0.57)	(2.14±1.73)	(4.69±6.19)	(24.38±1.66)	(43.04±10.63)
Forest	0.00	0.72-0.72	5.94-12.02	2.14-5.78	0.60-10.86	18.00-40.70	44.94-52.52
	0.00	(0.72±0.00)	(9.18±3.06)	(4.16±1.85)	(6.26±5.21)	(28.08±11.56)	(48.40±3.83)
Average	0.00	0.00-6.80	4.58-16.16	0.62-6.75	0.23-16.14	5.20-43.50	14.43-60.88
	0.00	(1.88±0.85)	(8.97±0.54)	(2.56±0.95)	(6.14±1.86)	(24.43±3.80)	(43.98±4.55

0.00 = Below detection limit

In parenthesis = (Mean±SD)

Table 4. Chemical fractionation of Cd (mg kg^{-1}) in five land uses of Lesser Himalayas

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual	Total
Cereals	0.00	0.00	0.00	0.00-0.82 (0.28±0.23)	0.00	0.00-0.23 (0.08±0.07)	0.00-0.98 (0.37±0.30)
Apple	0.00	0.00	0.00	0.00-0.54 (0.28±0.25)	0.00	0.00-0.19 (0.08±0.07)	0.00-0.72 (0.36±0.32)
Vegetables	0.00	0.00	0.00	0.00-0.82 (0.45±0.29)	0.00	0.00-0.16 (0.09±0.06)	0.00-0.98 (0.54±0.35)
Saffron	0.00	0.00	0.00	0.50-0.63 (0.56±0.09)	0.00	0.11-0.14 (0.13±0.02)	0.62-0.78 (0.70±0.11)
Forest	0.00	0.00	0.00	0.58-0.66 (0.61±0.04)	0.00	0.19-0.21 (0.20±0.01)	0.78-0.88 (0.81±0.05)
Average	0.00	0.00	0.00	0.00-0.82 (0.44±0.15)	0.00	0.00-0.23 (0.12±0.05)	0.00-0.98 (0.56±0.20)

0.00 = Below detection limit

In parenthesis (Mean±SD)

Table 5. Percentage concentrations of Ni(% of 'total') in each of the operationally defined geochemical fractions

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
Cereals	1.45	8.04	8.30	22.11	9.65	50.45
Apple	1.81	7.29	9.05	23.85	14.44	43.56
Vegetables	2.27	6.52	8.64	18.31	20.27	43.99
Saffron	0.63	10.66	6.88	17.33	13.70	50.8
Forest	0.15	7.29	10.55	15.37	17.18	49.46
Average	1.26±0.86	7.96±1.60	8.68±1.32	19.38±3.50	15.05±3.97	47.65±3.58

± = SD

Table 6. Percentage concentrations of Pb (% of 'total') in each of the operationally defined geochemical fractions

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
Cereals	0.00	4.33	22.81	6.53	11.17	55.16
Apple	0.00	5.19	20.26	4.05	21.71	48.79
Vegetables	0.00	3.71	20.12	4.74	13.25	58.18
Saffron	0.00	7.13	20.36	4.97	10.90	56.64
Forest	0.00	1.49	18.97	8.60	12.93	58.02
Average	0.00	4.37±2.06	20.50±1.40	5.77±1.82	13.99±4.44	55.35±3.87

± = SD

Table 7. Percentage concentrations of Cd(% of 'total') in each of the operationally defined geochemical fractions

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
Cereals	0.00	0.00	0.00	77.83	0.00	22.17
Apple	0.00	0.00	0.00	78.65	0.00	21.35
Vegetables	0.00	0.00	0.00	83.59	0.00	16.41
Saffron	0.00	0.00	0.00	81.21	0.00	18.80
Forest	0.00	0.00	0.00	75.44	0.00	24.56
Average	0.00	0.00	0.00	79.34±3.14	0.00	20.66±3.14

± = SD

Table 8. Correlation coefficients between different fractions of nickel and soil properties (n = 44)

	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual	Total
pH	0.232	0.111	-0.009	0.367*	-0.21	-0.006	0.003
OC	-0.148	0.14	0.236	-0.390**	0.431**	0.128	0.163
$CaCO_3$	0.241	-0.006	0.216	0.116	0.01	-0.132	0.008
CEC	-0.007	0.105	0.146	-0.005	0.162	0.189	0.245
Clay	-0.001	0.136	0.001	-0.007	0.253	0.151	0.217

Table 9. Correlation coefficients between different fractions of lead and soil properties (n = 44)

	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual	Total
pH	-	0.251	0.366*	0.214	0.008	-0.008	0.107
OC	-	-0.161	-0.137	0.004	-0.01	-0.009	-0.009
$CaCO_3$	-	-0.107	0.526**	-0.004	-0.003	0.007	0.17
CEC	-	-0.008	0.001	0.003	-0.01	0.007	0.003
Clay	-	-0.003	0.106	-0.104	-0.189	0.007	0.006

*   Significant at the 0.05 level
** Significant at the 0.01 level

Table 10. Correlation coefficients between different fractions of cadmium and soil properties

	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual	Total
pH	-	-	-	-0.007	-	-0.143	-0.009
OC	-	-	-	0.228	-	0.27	0.244
CaCO$_3$	-	-	-	0.137	-	-0.001	0.109
CEC	-	-	-	0.005	-	0.004	0.005
Clay	-	-	-	-0.002	-	-0.009	-0.004

Figure 2. The available and non-available concentrations of heavy metals in soils
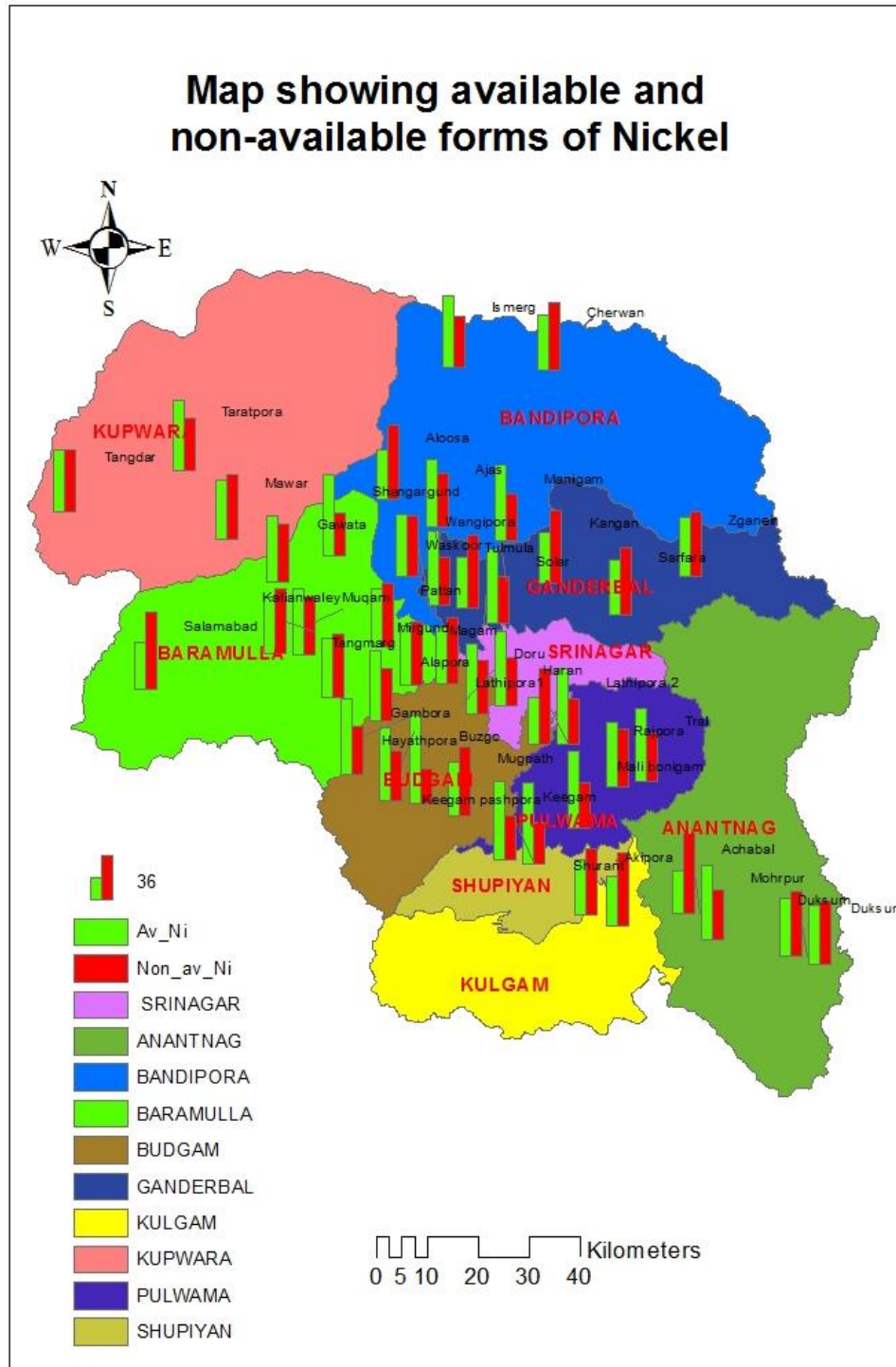
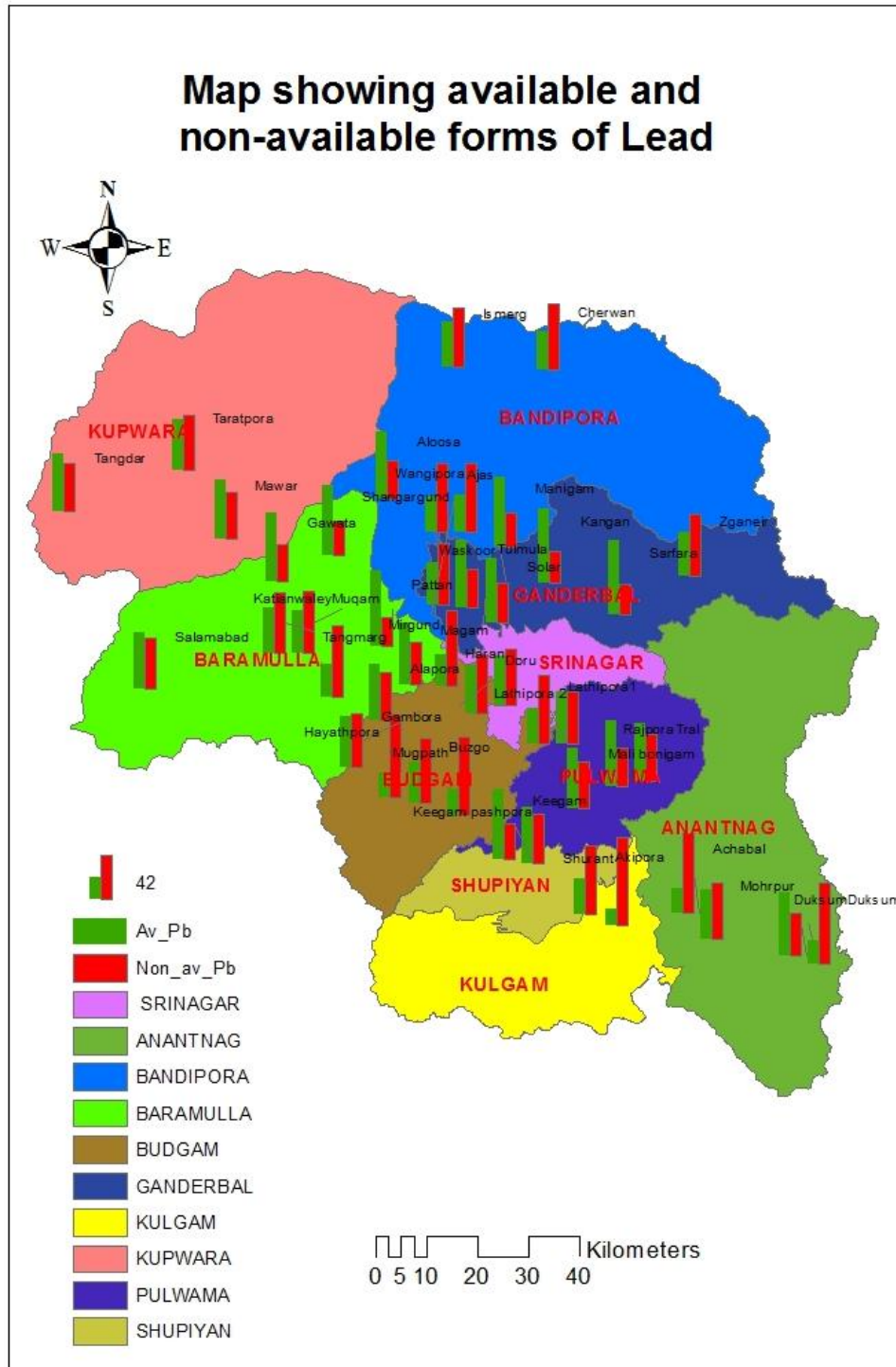**Figure 3.** The Location map showing available and non-available concentrations of Nickel in soils

Figure 4. The Location map showing available and non-available concentrations of Lead in soils
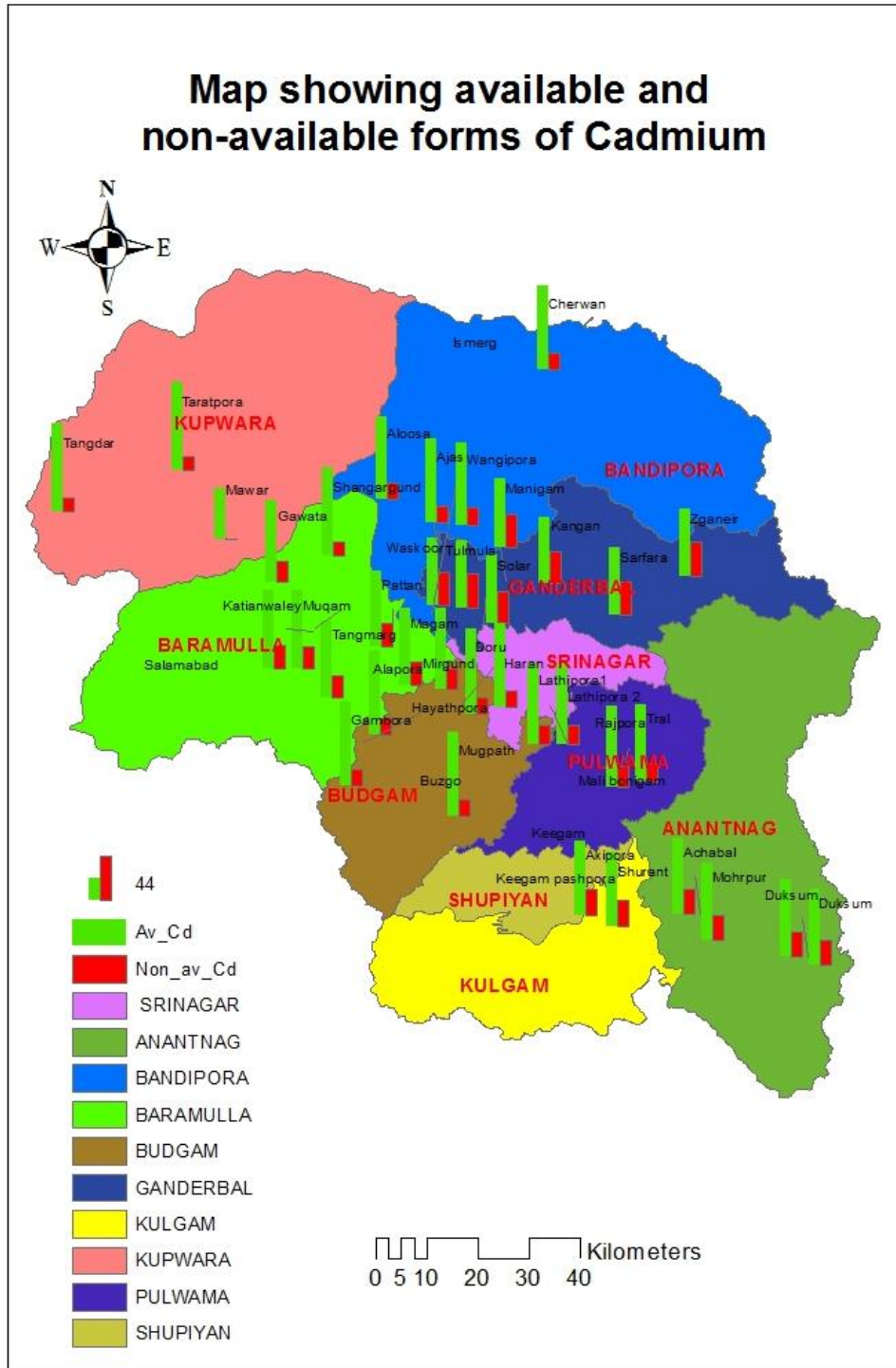
Figure 5. The Location map showing available and non-available concentrations of Cadmium in soils