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Distribution of Geochemical fractions of Zn, Fe, Cu and Mn under different landuses of Temperate Himalayas

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Abstract

Research interests have moved from total element composition of the soil sample towards speciation analysis to separate and to identify specific or binding forms of metals and allows assessing the availability and mobility of metals in order to understand their chemical behavior. Soils were collected from the five agricultural landuses (cereals, apple orchards, vegetables, saffron, pastures and forests) of Temperate Himalayas. A sequential extraction procedure was used to fractionate heavy metals in six geochemical fractions i.e., water-soluble, exchangeable, carbonate bound, iron and manganese oxide bound, organic bound and residual fractions. Results indicated that the chemical partitioning trend and per cent distribution in each geochemical fraction varied under each land use system due to varied soil properties and agricultural practices. Water soluble, exchangeable and organic bound fractions of heavy metals were highest in pasture and forest soils while as carbonate bound fraction was maximum in saffron soils. In the five land uses studied, the average potential mobility of Zn, Fe and Mn was highest in forest and pasture soils while as Cu mobility was found highest in cereal soils. The results suggest that there is no serious contamination hazard with the Zn, Fe, and Mn studied, considering the geochemical phases in which they were associated but the Cu with relatively high mobility factors coupled with its comparatively high concentration in the exchangeable fraction in cereal soils indicates high mobility, liability and bioavailability for cereals and may pose a threat of toxicity in cereal crops. Influence of soil properties on different fractions of heavy metals was evaluated by simple correlation and multiple regression analysis. Multiple regression indicated that pH and organic carbon of soils correlate greatly with available (water soluble and exchangeable) and organic bound fractions. Calcium carbonate content correlated significantly with carbonate fraction of Zn and Cu while as CEC contributed greatly to water soluble Mn. The results of this study indicated that the sequential extraction procedure, in conjunction with multiple regression analysis, may be useful for understanding the influence of landuses on the distribution of heavy metals in soils and the soil properties that greatly influence the different fractions.

Keywords: Geochemical fractions, Heavy metals, Landuses, Temperate Himalayas

1. Introduction

Metal species identification is used by researchers studying soil fertility, water quality, soil genesis and geomorphology, environmental quality, soil ecology, and soil remediation. The ubiquity of metals combined with the complexity of soils makes the study of metals one of the most important disciplines of soil chemistry (Walna *et al.* 2010). Heavy metals occur naturally in rocks, soils, sediments and water but their anthropogenic components have increased significantly since the Industrial Revolution. This increase has caused serious environmental problems that have affected the food chain and consequently the health of organisms, including humans (Forstner, 1990). High levels of metals are usually found in superficial soil and vegetation of areas affected by anthropogenic activities such as mining, agriculture, metal industries and traffic emissions (Maiz *et al.* 2000). Metals from anthropogenic sources tend to be more mobile than pedogenic or lithogenic ones (Chlopecka *et al.* 1996).

Some elements play a double role—they are essential for leaving organisms in a limited concentration and above it they become toxic. The toxicity of metals depends on their chemical forms and in such cases the speciation analysis should be used (Maria Cioroi 2009). Therefore speciation analysis is very important to separate and to identify specific or binding forms of metals and allows assessing the availability and mobility of metals in order to understand their chemical behavior. Cu, Zn, Fe and Mn are nutrients required by plants in trace

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quantities for normal growth and metabolism of plants but if their concentration exceeded the required amount, it may have toxic effects on the plants (Hansch and Mendel 2009).

Zinc, iron, copper and manganese are essential micronutrients for almost all living organisms including plants. Zinc is present in the structure of cell wall and in its deficit, the phosphorus transfer from the superficial root cells to xylem is not controlled and the phosphorus level in the air-borne parts of plants increases (Farshid Aref 2010). It influences the quality and yield of crops (Chidanandappa et al. 2008) and plays an important role in auxin metabolism, preferential accumulation of chlorophyll, protein synthesis and starch metabolism. It is a necessary cofactor in more than 300 enzymes such as dehydrogenases, adolase, isomerase, proteinases, peptidases, phosphohydrolases, carbonic-anhydrase and superoxide dismutase and numerous transcription factors (Haug et al. 2008).

Copper acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Marschner 1995; Raven et al. 1999). Iron serves as an integral cofactor for numerous proteins. In plants, Fe is essential for chlorophyll biosynthesis and the synthesis of heme and its deficiency significantly mars plant growth and development (Bashir 2010). Similarly, Mn is required as a cofactor or activator for enzymes belonging to different functional groups which perform various roles. Mn deficiency causes interveinal chlorosis and a reduction in the percentage of fructans and structural carbohydrates, resulting in lax leaves (Ishimaro 2012).

Simple selection of methods for the extraction of heavy metals is not sufficient for conceptualization of their availability, sources or interconnection with a phase of a soil (Roa et al. 2008; Sharma et al. 2008). Many authors suggested the employment of a sequential extraction procedure for investigation of heavy metals in the environment, sediment of natural origin or in substrates secondarily enriched with heavy metals (*e.g.*, agriculture, industry, road traffic) (Tessier 1979; Sanchez Martinet et al. 2007). These methods eliminate the disadvantages of individual extraction procedures and provide information on the total and available content of selected heavy metals in soils, the strength of the bonding and their relationship with specific compounds in the solid phase of soil. Many single or sequential extraction procedures mainly based on the Tessier procedure or different versions thereof, have been applied to soils and sediments to fractionate metals by using different extractants or reagents to obtain more useful information about the bioavailability and mobility of metals (Milivojevic et al. 2005; Polic 1991; Petrovic et al. 2010).

The extent to which each fraction of micronutrients is present and the transformations in equilibrium between various fractions is influenced by soil properties such as pH, cation exchange capacity, texture and soil organic matter. Generally, the total micronutrients increases with increasing cation exchange capacity (CEC) and the clay and silt content (Sharma et al. 2008). Complexation of micronutrients by soil organic matter may result in increased plant availability, and microbial exudates can supply additional for plant routes (MacKowiak et al. 2001). Likewise, exchangeable forms adsorbed onto inorganic sites and diethylenetriaminepentaacetic acid (DTPA) extractable increase with increasing soil organic matter but decrease with higher soil pH and calcium carbonate content (Kabata-Pendias and Pendias 2001; Jakovljevic et al. 2005).

Since no fractionation studies on heavy metals under different landuses in soils in this part of the country has been yet reported, it is expected that the results from this study would form a baseline data for future heavy metal status, mobility, availability and distribution in soils of the area under study. This research is also aimed at determining effect of land use on mobility and bioavailability of soil Zn, Cu, Fe and Mn.

Materials and Methods

Study area

The study area (Figure 1) 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. At the northern limit of the valley is the Qazi-Nag Range. In between the complexly folded and faulted mountain ramparts lies the bowl shaped Kashmir Valley between the parallels of 33°30' and 34°30' N latitude and 74°10' and 75°03' E longitude. The average height above mean sea level is 1850 meters. The climate of the valley is temperate to Mediterranean type characterized by mild summers and chilling winters. Due to latitudinal differences, there is a wide variation in climatic conditions in different parts experiencing a typical temperate climate in high altitudes with snowfall and severe cold in the winter and mild temperate at low altitudes. Annual rainfall of the valley recorded is about 75 cm. December has 80 percent humidity which is the highest and May has 71 percent which is the lowest.

Soil sampling

Forty four geo-referenced sites (Figure 1) were selected in soils of Temperate Himalayas from surface horizons of five land use systems (cereals, apple, vegetables, saffron and forests). The samples were collected using soil auger to avoid any contamination. The samples were air dried, ground, and passed through 2 mm sieve before analysis. Soil reaction (pH) of the sample was measured in 1:2.5 soil: water suspension with a digital glass electrode pH meter (Jackson 1973). Cation exchange capacity (CEC) of soils was done according to the procedure given by Rhoades (1982). Calcium carbonate (CaCO₃) was determined by the method of Puri (1930) and Walkley and Black's rapid titration method was used for determination of organic carbon (OC). The mechanical analysis of the soil samples was done by following the International Pipette method as described by Piper (1966). The International Society of Soil Science textural triangle was used for determining the textural class.

Quantification of chemical fractions of heavy metals

The procedure of Tessier et al. (1979) with some modifications was used to separate the soil heavy metals into six operationally defined geochemical fractions; water soluble (Ws.), exchangeable (Exch.), bound to carbonates (Carb.), bound to Fe-Mn oxides (Fe/Mn ox.), bound to organic matter (Orga.) and residual (Resi.) fraction. The extraction was carried out using 1.0 g of soil and involved following steps:

Water soluble (F1): Samples of soil (1.0 g) were extracted at 20 °C for 1 hour with 10.0 ml of distilled water.

Exchangeable (F2): The residue of F1 was extracted at room temperature for 1 hour with 10.0 ml of magnesium chloride solution (1.0 M MgCl₂) at pH 7.

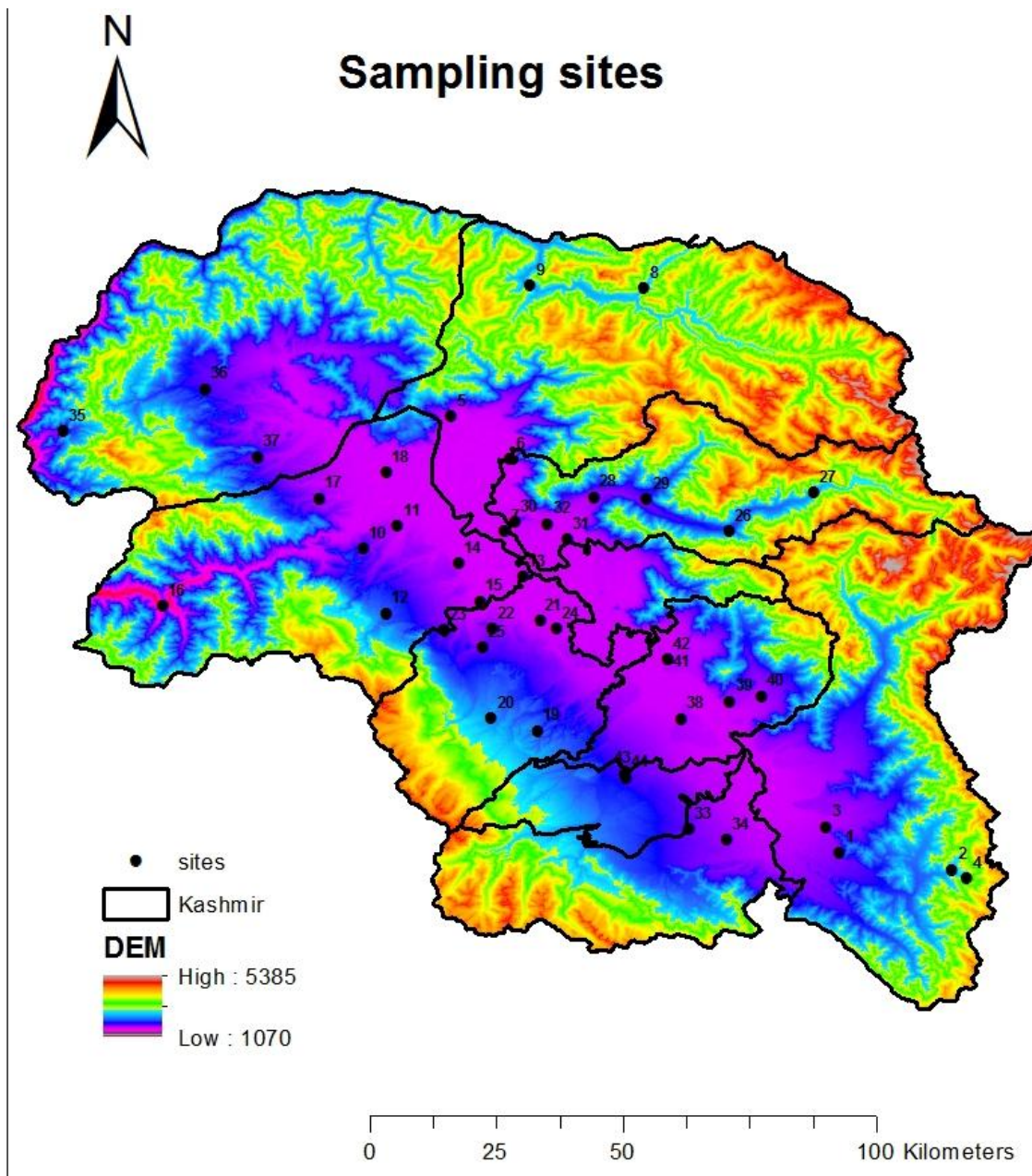


Fig 1: Description of geo-referenced sampling locations under different landuses in soils of Kashmir Himalayas

Carbonate bound (F3): The residue of F2 was extracted with 10.0 ml of 1.0 M sodium acetate/acetic acid buffer at pH 5.0 for 5 hours at room temperature.

Fe/Mn oxide bound (F4): The residue from F3 was extracted with 10.0 ml of 0.4 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 25% (v/v) acetic acid (pH 2.0) with agitation at 96 °C in a water bath for 5 hours.

Organic bound (F5): The residue from F4 was oxidized as follows: 7.5 ml of 30% (v/v) hydrogen peroxide, which has been adjusted to pH 2 with HNO_3 , was added to the residue. The mixture was heated to 85 °C in a water bath for 5 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 0.5 hour and centrifuged.

Residual bound (F6): Residue from fraction F5 was digested with a mixture of 5 ml concentrated HNO_3 (HNO_3 , 70% w/w), 10 ml of hydrofluoric acid (HF, 40% w/w) and 10 ml of perchloric acid (HClO_4 , 60% w/w). The cooled solution was transferred to a 50 ml volumetric flask and subsequently diluted to volume with deionized H_2O and stored for analysis.

Total: Total metal was calculated as a sum of all the fractions determined.

The supernatant from each extraction step was removed with a pipette and analyzed for total metal concentrations using acetylene flame Atomic Absorption Spectrophotometer (Hitachi AA-1275), fitted with deuterium lamp for background correction, following the procedure of Sakata (1983). When continuous agitation was required, the samples were shaken lengthwise on a mechanical shaker at 265 oscillations per minute with a stroke of 8cm.

Heating of the samples was done using Al block heater. Standards for all metals were prepared for each extraction step in the same matrix as then extracting reagent to minimize matrix effects.

Results and Discussion

Physico-chemical characteristics of soils under different land uses

The range and mean values of the physico-chemical properties of the soils 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 content in soils varied between 21.9 and 40.6%. The pH of the soils ranged from 4.50 to 7.70. Lowest pH was observed in forest (5.5) followed by apple (6.2), vegetable (6.6), cereal (6.7) and saffron (6.9) soils. The low pH noticed in some soils may be ascribed to leaching of soluble salts and higher content of organic matter that brings down pH by releasing organic acid. Based on soil test data, all the soil samples were found high in organic carbon ranging between 0.9 to 5.2% with a mean value of 2.2%, with most observed to be high in forests (3.8%) and lowest in saffron (1.3%) soils. The percentage of organic carbon found in the forest soils was more likely to have higher values than other land uses. In addition to the above properties, cation exchange capacity in surface soils ranged from 8.1 and 23.1 cmolc kg⁻¹, and the highest value was obtained in forest (15.6 cmolc kg⁻¹) and the lowest in saffron cultivated soils (10.2 cmolc kg⁻¹). This property is found to be an important predictor of metal retention, movement (Udom *et al.* 2004) and extractability (Rieuwerts *et al.* 2006) in soils. Wani *et al.* (2010) also reported a higher range of cation exchange capacity in some soils, relating it with their corresponding high levels of organic matter and clay. Results of present study show that calcium carbonate content lies between 0.0 to 4.8% with a mean of 0.2%. The overall low content of calcium carbonate in surface soils can be assigned to leaching down of calcium carbonate to sub-surface horizons. The findings are in agreement with the work of Bhat (2009) and Wani *et al.* (2010) who also reported low calcium carbonate content in surface soils of Kashmir Himalayas.

Effect of different landuses on geochemical fractions of heavy metals

From the sequential extraction of heavy metals, it is possible to determine the form of their location in the soil, allowing for a clear understanding of their potential mobility and accessibility for plants. Table 1 to 4 presented the results of the different chemical pools of metals and figure 2 to 5 showed percentages of their 'total' under different land use systems.

Water soluble fraction

Water soluble fraction 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 fractionation results indicated that the average value of water soluble Zn (Table 1) in cereals, apple, vegetables, saffron, forest and pastures was 0.41, 0.89, 0.49, 0.33, 3.25 and 1.08 mgkg⁻¹, respectively; water soluble Fe was 4.00, 9.01, 8.66, 9.11, 17.44 and 17.88 mgkg⁻¹, respectively (Table 2); water soluble Cu was 1.67, 1.88, 1.81, 1.59, 2.94 and 2.84 mgkg⁻¹ (Table 3), respectively and water soluble Mn was 0.35, 1.89, 1.68, 0.71, 2.22 and 1.92 mgkg⁻¹, respectively (Table 4). The above data clearly indicated that among the different landuses (Figure 2), forest and pasture soils contain relatively higher percent of water soluble fraction and may be attributed to lower pH and higher organic matter content in these soils (Businelli *et al.* 2009). Similar results were noticed by Zauyah

et al. (2004) and Kumar and Babel (2011). In addition to this, current data also revealed that except Fe water soluble fraction of other heavy metals were least among all fractions.

Dissolved or Exchangeable fraction:

This fraction of metals 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; Kumar and Babel 2011). 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).

In the soils of Temperate Himalayas, the landuses depicted great variation in this fraction but contributed very little to the total content. The content of exchangeable Zn, Fe, Cu and Mn was 1.36, 4.42, 1.85, 8.89 mgkg⁻¹ respectively in cereals; 1.77, 4.48, 2.44, 10.14 mgkg⁻¹ respectively in apple orchards; 1.42, 6.04, 3.10, 13.24 mgkg⁻¹ respectively in vegetable field soils; 0.94, 3.32, 1.69, 6.67 mgkg⁻¹ respectively in saffron cultivated soils; 2.49, 13.93, 2.95, 5.77 mgkg⁻¹ respectively in forest soils and 4.18, 4.12, 3.88 and 8.94 mgkg⁻¹ respectively in pasture soils. These values are in line with the findings of Razdan (1995), Aydinalp *et al.* (2009), Olubunmi (2010) and Ibrahim *et al.* (2011). With exception to Mn, the exchangeable fraction of Zn, Cu and Fe were highest in forests and pastures (Figure 2) comparing to the cultivated fields (i.e., cereals, apple, vegetables, saffron). These findings may be attributed to the high amount of organic carbon and low pH in forest and grassland soils. Lindsay and Norvell (1978) confirmed that the solubility of micronutrients in soil solution increased 100 fold for each unit decrease in pH. Organic matter on decomposition releases organic acids which chelates the micronutrients and keeps it in readily available form. As it has been proposed that plant uptake is largely from this pool (Shuman 1981), it will therefore be expected that it relies on the other pools, especially those with intermediate concentrations (carbonate bound and organic bound) to replenish it.

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).

The pattern of carbonate bound Zn and Cu didn't differ significantly with land uses, however, they exhibited an increasing trend from saffron to forest soils. The lowest percentage of Zn and Cu in this pool in forest soils can be ascribed to lowest amount of calcium carbonate in these soils. Usually the carbonate bound zinc and copper is seen only in soils with high pH and lime content as reported by (Rajakumar 1994). Notably, Mn and Fe fraction associated with carbonates presented the different results where the highest values were found in pasture and cereal soils (77.26 and 42.69 mgkg⁻¹) respectively and lowest in cereal and apple orchard soils (20.76 and 10.01 mgkg⁻¹) respectively (Table 5 and Figure 2). These findings are in contrast with the findings

of Ramos *et al.* (1994) who reported that calcium carbonate act as a strong absorbent for Fe and Mn metals and could complex as double salts like $\text{CaCO}_3 \cdot \text{MCO}_3$.

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 including micronutrients. As for as mobility and availability is concerned reducible iron and manganese oxide bound fractions are known to be more important for controlling the mobility and bioavailability of metals (Bhattacharyya *et al.* 2006). Metals associated with oxide minerals are likely to be released in reducing conditions. Reductive dissolution of the oxide minerals occurs at $E_h < \sim +250$ mV for manganese oxides and $+100$ mV for iron oxides. Relatively small changes in Rh toward reducing conditions would cause reduction of iron and manganese oxide species. This will cause dissolution of iron and manganese oxide minerals, thereby allowing release of associated metals (Ma and Roa 1997).

In the current study the oxide fraction of Zn was the second most concentrated fraction after the residual fraction while as oxide fraction of Mn was most concentrated fraction among all fractions and may be attributed to diffusion mechanism and stabilization of Zn and Mn oxides due to high stability constant of Zn and Mn oxides (Ma and Rao 1997). A good percentage of Zn and Mn in this fraction imply that they are especially easy to be absorbed and immobilized by oxyhydroxide particles of iron and manganese (Chen *et al.* 2009). The distribution of oxide bound Zn in different land uses (Table 1 and Figure 2) on the basis of average concentrations shows maximum amount in pasture (9.72 mgkg^{-1}) and least in saffron cultivated soils (4.41 mgkg^{-1}), whereas oxide bound Mn content was highest in saffron cultivated soils (173.40 mgkg^{-1}) and can be assigned to well drained condition in saffron fields as also confirmed in research by Moja (2007) who concludes that in well-drained soils at pH (above 6) much of the manganese exists as manganese oxides. Similar to the zinc, the oxide bound fraction of Fe and Cu was maximum in pastures (420.10 and 6.26 mgkg^{-1} , respectively) and might be due to enrichment of these pasture soils with Fe and Mn oxide minerals. Vega *et al.* (2007) confirmed that although organic matter is the main component affecting sorption of Cu, especially at neutral pH, oxides control the adsorption at lower pH. Sims (1986) reported that when soils are waterlogged for 2 or 3 days, the oxygen is lost from the soil and microorganisms use the chemically combined oxygen in manganese and iron oxides

for their respiratory needs. This process releases manganese from non-available (MnO_2) to available form (Mn^{2+}) and increases the pool of available manganese in soil.

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 bound fraction (Table 1 to 4) was highest in pasture soils which may be due to higher percentage of organic matter in these soils. The preference of heavy metals for organic matter is supported by the high stability constant of the metal complexes with organic matter (Gonzalez *et al.* 1994, Chlopecka *et al.* 1996, Ramos *et al.* 1999). Studies of Prasad *et al.* (1995) and Randhawa and Singh (1995) revealed that higher and lower content of organically bound Zn in alluvial soils of Punjab and old alluvial rice growing soils of Southern Bihar was due to the higher amount of organic matter in the former and lower amount in the later soils.

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 Spiff 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.

From the data it is evident that residual fraction is the dominant fraction in all the soil samples analysed for heavy metals under different landuses (except Mn which was found most abundant in oxide fraction) and are well concurrence with the findings of Yobouet *et al.* (2010), Kumar and Babel (2011) and Olubunmi (2010), who concluded that the residual form of metals is the major form in the entire fractions. The highest percentage in residual fraction explains lithogenic origin of heavy metals (Xuelu *et al.* 2008) and may indicate that they had stronger capacity to associate with the crystalline structures of the minerals (Nemati *et al.* 2009). The current results indicated that large reserves of Zn and Mn in the form of residual fraction were in vegetable growing soils (69.76 and 170.56 mgkg^{-1} , respectively), iron in cereal soils (1535 mgkg^{-1}) and Cu in apple growing soils (41.55 mgkg^{-1}) of Temperate Himalayas (Table 1-4).

Table 1: Zinc concentrations in each of the Operationally Defined Geochemical Fractions of the soils (mgkg^{-1}) under different land uses

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
cereals	0.41	1.36	1.75	5.88	4.9	67.2
Apple	0.89	1.77	1.51	6.43	5.1	61.13
Vegetables	0.49	1.42	2.33	6.83	6.3	69.76
Saffron	0.33	0.94	2.81	4.41	4.93	61.78
Forest	3.25	2.49	0.53	6.66	7.86	61.8
Pasture	1.08	4.18	0.76	9.72	9.45	59.35

Table 2: Iron concentrations in each of the Operationally Defined Geochemical Fractions of the soils (mgkg^{-1}) under different land uses

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
cereals	4	4.42	20.76	383.61	348.3	1535
Apple	9.01	4.48	57.23	415.5	341.5	1195
Vegetables	8.66	6.04	42.4	375.1	433.9	1295

Saffron	9.11	3.32	28.49	306.7	233.8	1323
Forest	17.44	13.93	61.47	357.2	443.5	1234
Pasture	17.88	4.12	77.26	420.1	542.6	1335

Table 3: Copper concentrations in each of the Operationally Defined Geochemical Fractions of the soils (mgkg⁻¹) under different land uses

Landuse	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
cereals	1.67	1.85	1.9	2.23	5	19.19
Apple	1.88	2.44	1.84	5.5	5.64	41.55
Vegetables	1.81	3.1	1.91	4.17	6.3	37.29
Saffron	1.59	1.69	2.14	5.58	7.05	40.73
Forest	2.94	2.95	0.36	4.9	7.05	37.43
Pasture	2.84	3.88	1.72	6.26	7.21	39.9

Table 4: Manganese concentrations in each of the Operationally Defined Geochemical Fractions of the soils (mgkg⁻¹) under different land uses

Land use	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
cereals	0.35	8.89	15.96	127.94	17.79	170.56
Apple	1.89	10.14	10.01	113.03	37.28	63.03
Vegetables	1.68	13.24	15.7	115.52	34.63	106.69
Saffron	0.71	6.67	42.69	173.4	40.81	139.1
Forest	2.22	5.77	20.85	171.55	31.37	76.43
Pasture	1.92	8.94	16.82	142.9	46.4	63.85

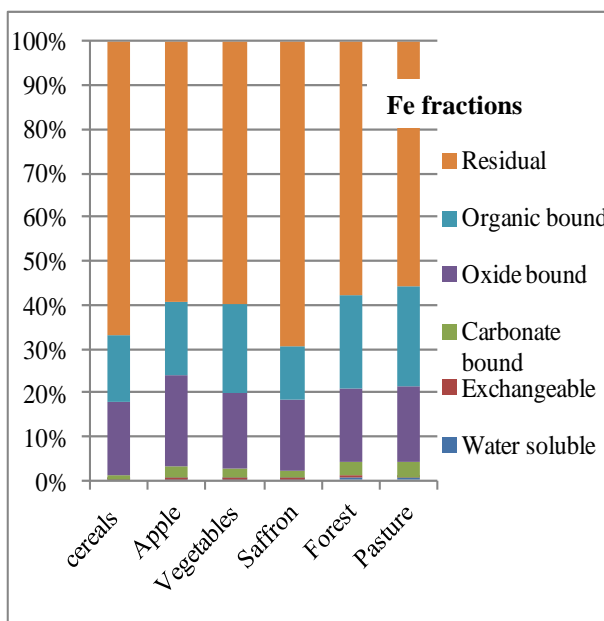
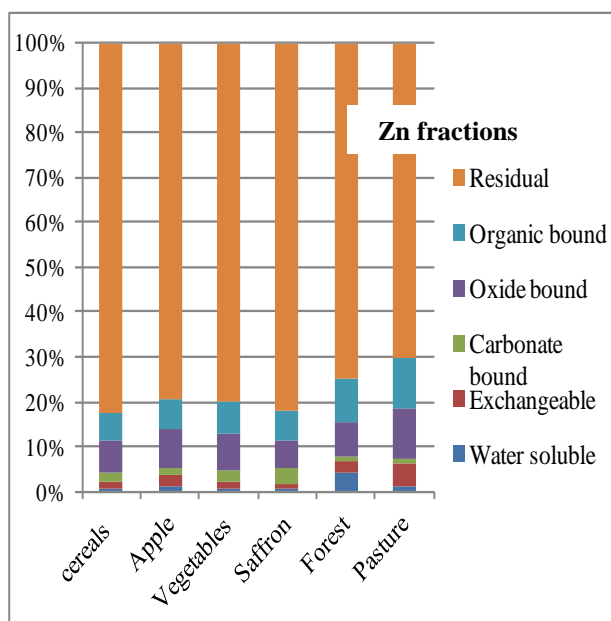
Effect of landuses on Mobility factor of heavy metals

Ratio of relatively metal bioavailable and mobile fractions to stable and less mobile fractions is defined as mobility factor. In any sequential extraction procedure the early fractions capture the most mobile and bioavailable fractions. On this basis high mobility factor (MF) values have been reported or interpreted as evidence of relatively high reactivity, high liability and high biological availability of elements in soil (Ahumuda et al. 1999; Narwal et al. 1999; Kabala and Singh 2001). The availability of the metals in the soil may be evaluated on the basis of absolute and relative contents of fractions weakly bound to soil components. In this study mobility factors were calculated according to (Salbu et al. 1998; Narwal et al. 1999; Kabala and Singh 2001) as:

$$\frac{F1 + F2 + F3}{F1 + F2 + F3 + F4 + F5 + F6}$$

- Where F1 = Water soluble fraction
- F2 = Exchangeable fraction
- F3 = Carbonate fractions
- F4 = Fe-Mn oxide fraction
- F5 = Organic matter fraction
- F6 = Residual fraction

The mobility factors are shown in figure 3. High values of mobility factor for metals are an evidence of relative high bio-availability. The relatively highest mobility factors of Zn, Fe and Mn was observed for pasture and forest soils and are quite in agreement with the high percentage of available fraction (i.e., water soluble and exchangeable fraction) recorded from the chemical fractionation results in these soils (Osakwe 2010). Exceptionally, mobility factor of Cu was found highest in cereal soils.



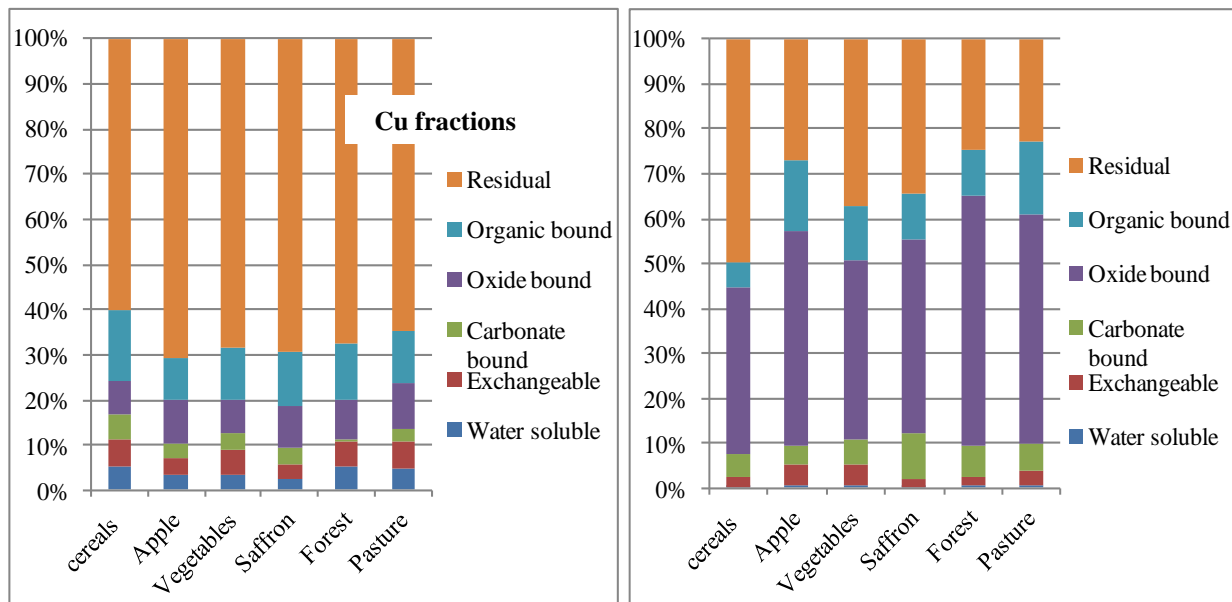


Fig 2: The relative distribution of Zn, Fe, Cu and Mn among six fractions under different landuses of Temperate Himalayas.

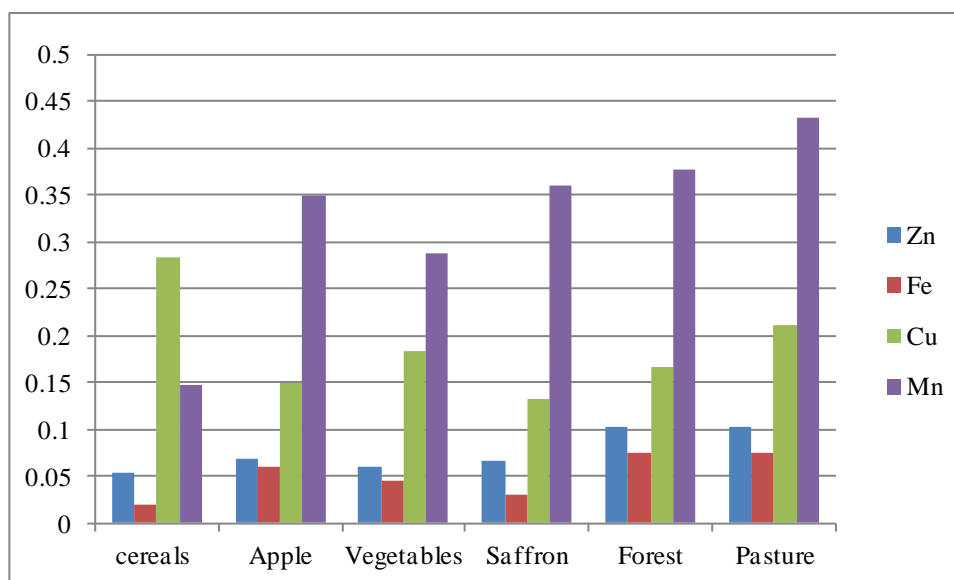


Fig 3: Mobility factors of the heavy metals under different landuses

Correlations between heavy metal fractions and soil properties

To determine the most important factors affecting the different fractions of heavy metals, a simple correlation and multiple regression procedure was carried out. The results are presented in table 5 to 8. The distribution of different fractions may vary significantly in response to changing soil properties. The optimum plant growth and crop yield depends on the bioavailability of metal present in the soil at a particular time which in turn is controlled by physico-chemical properties like soil texture, organic carbon, calcium carbonate, cation exchange capacity and pH (Bell and Dell 2008; Wijebandara *et al.* 2011).

Water soluble metals (Zn, Fe, Cu and Mn) correlated significantly and negatively with pH ($r = -0.535^{**}$, -0.734^{**} , -0.58^{**} , -0.553^{**} , respectively) (Table 4). A negative and significant correlation with pH may be due to the reason that they are most soluble under acid conditions. For every unit increase in pH, solubility of cationic microelements may decrease from 100 fold for divalent (e.g., Mn, Cu, Zn) to 1000 fold for trivalent ions (e.g., Fe) (Rengel 2001) as the ionic forms of microelement cations are changed first to the

hydroxyl ions and finally to the insoluble hydroxides or oxides of the elements (Brady and Weil 2002) when the soil pH is increased. This relationship is also found by Sharma *et al.* (2003) and Jelic *et al.* (2011). A significant positive correlation ($r = 0.485^{**}$, 0.335^{*}) between water soluble Zn and Fe and organic carbon was observed. Organic matter present in soil provides sites for the sorption of microelement and this form of microelement comes into solution with time and will be available for crop plants. Similar observations were recorded by Obrado *et al.* (2007) and Adeboye (2011). Among the metals analysed only the water soluble Mn was correlated significantly with CEC (0.301^{*}) and is in consistent with many reports (Laurent and Pierre 2010; Ibrahim *et al.* 2011).

In order to determine the most important soil factors affecting different fractions of metals, the data were also put through a multiple regression. Each of the potential independent variables was examined for its ability to meet the criteria of entry into or remove from the regression equation. The probability of "F-to-enter" at each step was 0.05, and the maximum "F-to-remove" was 0.10. The consistently significant regression equations obtained for some fractions of

metals were listed in Table 9. Regression equations showed that 43.6, 64.1, 38.70 and 40.80 per cent variation in water soluble Zn, Fe, Cu and Mn respectively was due to the soil properties studied where the pH, organic carbon and CEC were main contributors.

A significant negative correlation was found between the exchangeable fractions with soil pH and positive with organic carbon indicating that increase in organic carbon content would increase the sorbed metal content of soil and decrease with an increase in pH. Similar results were reported in literature by Nazif *et al.* (2006) and Ibrahim *et al.* (2011). From this association it is inferred that micronutrient deficiency would be serious problem at higher soil pH and lower organic matter content. Standard regression coefficients (Table 9) for exchangeable Zn and Fe illustrated 36.7 and 48.1 per cent variability caused due to pH, organic carbon, CEC, CaCO₃ and clay respectively in soils of Kashmir Himalayas.

Important soil factors contributing to the carbonate bound fraction of heavy metals are pH and calcium carbonate indicating that calcium carbonate offers specific retention sites for metals on carbonates. Similar type of relationship was reported by Ashraf *et al.* (2012) and Jelic *et al.* (2011). From the data it is clear that significant amount of variation in carbonate bound Zn (66.2%) fraction is explained by studied soil properties whereas only a small but significant amount of variation in carbonate bound Fe and Cu (36.7 and 30.2%, respectively) is due to pH, organic carbon, calcium carbonate, cation exchange capacity and clay content.

The less pronounced but significant negative correlation coefficients ($r = -0.431^*$, 0.338^*) found between oxide bound fraction of Zn and Fe respectively showed natural reduction in oxide solubility and concentrations as pH increases.

Shiowatana *et al.* (2005) made similar observation when they analyzed soils in Thailand. They concluded that soil pH significantly determined the adsorption and sorption of metals and that its reduction increased the concentration of metal ions in the soil solution. Similar results were also derived by Mathur *et al.* (2006) and Jelic *et al.* (2011). Soil properties analysed in current research could only account 33.8% variation in oxide bound Zn.

The important fraction of metals i.e., organic bound fraction correlate significant positively with organic carbon but negatively with soil pH. The positive correlation between the metals content in the organic bound fraction and the pH value of the soil indicates that the content of metals in this fraction increase with increasing soil organic matter because organic matter by means of organic acids or other functional groups provides exchange sites for the adsorption of metals. Similar relationship was reported by Ping *et al.* (2011) and Ashraf *et al.* (2012). Current study also indicated significant variability in organic bound fraction of Zn, Fe and Cu (54.7, 39.0, 27.0%, respectively) explained by pH, organic carbon, calcium carbonate, cation exchange capacity and clay content. Positive and significant correlations were found between residual fraction of Zn with pH and CaCO₃ content of soils ($r = 0.609^{**}$, 0.387^{**}) and residual Fe with CEC (0.465^{**}). The positive correlation between Zn in residual fractions and soil pH indicates that base oxidation and environmental conditions cause Zn deposition but reducing conditions its hydrolysis. Similar conclusions have been made by Minhas and Chhibba (1999), Lestan *et al.* (2003), Jelic *et al.* (2011), Wijebandara *et al.* (2011), Ashraf *et al.* (2012) and Mekichirwa *et al.* (2012). Studied soil properties explain only 43.40 and 32.7% variation of residual Zn and Fe respectively.

Table 5: Correlation coefficients between different fractions of zinc and soil properties

Soil Properties	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
pH	-0.535**	-0.478**	0.519**	-0.431**	-0.373*	0.609**
OC	0.485**	0.424**	-0.180	0.214	0.679**	-0.159
CaCO ₃	-0.170	-0.254	0.759**	0.161	-0.257	0.387**
CEC	0.149	0.254	0.002	-0.008	0.292	0.195
Clay	-0.002	0.104	0.103	0.103	0.277	0.206

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

Table 6: Correlation coefficients between different fractions of iron and soil properties

Soil properties	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
pH	-0.734**	-0.591**	-0.574**	-0.338*	-0.392**	0.118
OC	0.335*	0.468**	0.213	-0.180	0.528**	0.006
CaCO ₃	-0.250	-0.239	-0.005	-0.294	-0.003	-0.201
CEC	-0.206	0.198	-0.110	-0.201	0.258	0.465**
Clay	-0.128	0.007	-0.004	-0.135	0.283	0.268

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

Table 7: Correlation coefficients between different fractions of copper and soil properties

Soil properties	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
pH	-0.580**	-0.210	0.221	-0.261	-0.270	-0.170
OC	0.230	0.322*	-0.301*	0.000	0.460**	0.000
CaCO ₃	-0.010	0.210	0.392**	0.200	0.010	0.200
CEC	-0.010	0.010	-0.280	-0.260	0.010	-0.190
Clay	-0.145	0.157	-0.002	-0.141	0.003	-0.006

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

Table 8: Correlation coefficients between different fractions of manganese and soil properties

Soil properties	Water soluble	Exchangeable	Carbonate bound	Oxide bound	Organic bound	Residual
pH	-0.553**	-0.207	0.291	0.006	-0.109	0.246
OC	0.132	0.154	0.006	0.006	-0.003	-0.123
CaCO ₃	-0.110	0.163	0.239	-0.006	-0.149	-0.007
CEC	-0.301*	0.006	-0.002	0.228	-0.008	0.240
Clay	-0.293	0.246	0.002	0.107	-0.001	0.159

*Correlation is significant at the 0.05 level

**Correlation is significant at the 0.01 level

Table 9: Regression models of chemical fractions of heavy metals with soil properties

Fractions	Regression equation	r ²
W S (Zn)	3.933-0.441(pH) + 0.356 (OC) + 0.022 (CaCO ₃) + 0.181(CEC)-0.233 (Clay)	0.436*
EX (Zn)	3.867-0.424(pH) + 0.184 (OC)-0.093 (CaCO ₃) + 0.291 (CEC)-0.108 (Clay)	0.367*
CB (Zn)	-1.262 + 0.280(pH)-0.072 (OC) + 0.663 (CaCO ₃) + 0.007 (CEC) + 0.005(Clay)	0.662*
OX (Zn)	14.060-0.478(pH) + 0.155(OC) + 0.295 (CaCO ₃)-0.293 (CEC)-0.273 (Clay)	0.338*
ORG (Zn)	4.130-0.121 (pH) + 0.665 (OC)-0.204 (CaCO ₃)-0.195 (CEC) + 0.197 (Clay)	0.547*
RES (Zn)	20.353 + 0.476 (pH)-0.123 (OC) + 0.214 (CaCO ₃) + 0.152 (CEC)-0.058 (Clay)	0.434*
WS (Fe)	39.369-0.589(pH) + 0.378 (OC)-0.045 (CaCO ₃)-0.387 (CEC) + 0.068(Clay)	0.641*
EX (Fe)	22.124-0.524 (pH) + 0.280 (OC)-0.043 (CaCO ₃) + 0.192 (CEC)-0.115 (Clay)	0.481*
CB (Fe)	194.435-0.564 (pH) + 0.156 (OC) + 0.130(CaCO ₃)-0.173 (CEC) + 0.065 (Clay)	0.367*
ORG (Fe)	463.983-0.337(pH) + 0.417 (OC) + 0.104 (CaCO ₃)-0.065 (CEC)-0.192 (Clay)	0.390*
RES (Fe)	715.707 + 0.052(pH)-0.278 (OC)-0.230 (CaCO ₃) + 0.667 (CEC)-0.077 (Clay)	0.327*
WS (Cu)	4.982-0.589 (pH) + 0.130 (OC) + 0.157 (CaCO ₃) + 0.095 (CEC)-0.224 (Clay)	0.387*
CB (Cu)	1.207 + 0.115(pH)-0.128 (OC) + 0.317 (CaCO ₃)-0.426 (CEC)-0.274 (Clay)	0.302*
ORG (Cu)	7.374-0.136 (pH) + 0.551 (OC) + 0.088 (CaCO ₃)-0.193 (CEC)-0.037 (Clay)	0.270*
WS (Mn)	7.206-0.481 (pH) + 0.217 (OC) + 0.080 (CaCO ₃)-0.244 (CEC)-0.164 (Clay)	0.408*

*Correlation is significant at the 0.05 level

Conclusion

The sequential fractional procedure was confirmed as suitable for accessing the content and availability of heavy metals in the Temperate Himalayas soils. Residual fraction is the major binding site for Zn, Fe and Cu, indicating that the major proportion of the metal is associated in the silicate mineral matrix. Fe-Mn oxide fraction is the important binding site for Mn. In the five land use soils studied, the distribution, availability and mobility of different metals varied depended upon different soil properties and agricultural practices. Unstable species, i.e. water soluble, exchangeable and bound to organic matter was highest in pasture and forest soils while as carbonate bound fraction was maximum in saffron soils. The average potential mobility of Zn, Fe and Mn estimated under all land uses was highest in forest and pasture soils while as Cu mobility was found highest in cereal soils. The results suggest that there is no serious contamination hazard with the Zn, Fe, and Mn studied, considering the geochemical phases in which were associated but the Cu with relatively high mobility factors coupled with its comparatively high concentration in the exchangeable fraction in cereal soils indicates high mobility, liability and bioavailability for cereals and may pose a threat of toxicity in cereal crops. Influence of soil properties on different fractions of heavy metals was evaluated by simple correlation and multiple regression analysis. The obtained results show a good correlation between pH and organic carbon of soils with available (water soluble and exchangeable) and organic bound fractions of metals. So an important aspect is to monitor from time to time the pH of soil and to adjust it until slightly acidic character. Thus the results from current study may indicate that an appropriate selection of land use are required for better crop production as well as environmental benefits, particularly in the areas of intensive cropping systems with higher external input use.

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