BIOACCUMULATION OF CADMIUM, LEAD AND ZINC IN AGRICULTURE BASED INSECT FOOD CHAINS

Abida Butt[1]*, Qurat-ul-Ain[1], Kanwal Rehman[1], Muhammad Xaaceph Khan[1] and Thomas Hesselberg[2]

[1] Department of Zoology, University of the Punjab, Lahore, Pakistan

[2] Department of Zoology, University of Oxford, United Kingdom

*** Corresponding author: abdajawed@yahoo.com**

Abstract

Globally, the metals concentration in soil is increasing due to different anthropogenic and geogenic factors. These metals are taken up by plants and further transferred in the food chain through different routes. The present study was designed to assess the transfer and bioaccumulation of the heavy metals, cadmium (Cd), lead (Pb) and zinc (Zn), in food chains from soil to berseem plants (*Triofolium alexandrinum*), to insect herbivores (the grasshopper *Ailopus thalassinus* and the aphid *Sitobion avenae*) and to an insect carnivore (the ladybird beetle *Coccinella septempunctata*). The soil of studied berseem fields were slightly alkaline, silty loam in texture and moderate in organic matter. In soil, the concentration of Zn and Pb were under permissible level while Cd was above the permissible level. The accumulation of metals in *T. alexandrinum* were found in the order Zn>Cd>Pb. Grasshoppers showed higher accumulation of Pb than of Cd and Zn. In the soil-berseem-aphid-beetle food chain,metals enrichment was recorded. However, aphids did not show bioaccumulation for Cd. Metals accumulation in beetles showed that translocation of Zn, Cd and Pb was taking place in the third trophic level. Our study highlights the mobility of metals in insect food chains and showed that insect feeding style greatly influenced the bioaccumulation. However, different metals showed variable bioaccumulation rates depending on their toxicity and retention.

Key words: metals, food chain, agro-ecosystem, fodder crop, aphids, grasshoppers, beetles.

INTRODUCTION

Heavy metals naturally enter ecosystems through volcanic eruption, atmospheric deposition, erosion and weathering of rocks (Szyczewski et al. 2009). Anthropogenic activities like transportation, mining, smelting, military operations, industrialization, utilization of fertilizers and pesticides in agricultural crops further enhance metals concentrations (Mohammed et al. 2011). Metals remain in the environment for decades even after the removal of their point sources (Gall et al. 2015) and are frequently reported to have negative impact on a range of organisms. Concerns on accumulation and transfer of metals at different levels of food chains is increasing due to the rapid growth of industrialisation and agricultural intensification, especially in the developing world. The transfer and bioaccumulation rate of metals in different ecosystems depends on how differential properties of metals, how plant and animal species response to the metals, and the structure of food the chain. Uptake of metals by invertebrate taxa is related to their degree of direct contact with polluted substrates (e.g., soil-dwelling invertebrates) and their dietary habits. Heikens et al. (2001) studied Cd, Cu, Pb, and Zn accumulation in invertebrates and found thatthe accumulation levels of different invertebrates taxa varied with a factor between 2 and 12. Metal concentrations were high in Isopoda, intermediate in Lumbricidae, and low in Coleoptera. These results suggest that due to their habitat, diets, and physiological responses, certain invertebrate orders and families may be more likely to accumulate one metal over another.

Soil plays an important role as a metals reservoir for the atmosphere, hydrosphere and biota and it provides a medium for cycling of metals in nature (Cao et al. 2010). The transfer of metals from soil to plant depends upon its availability and mobility in soil. Metal cations bind to negatively charged organic compounds and clay particles. These metals ions, when unbounded in the soil solution, become available for uptake by plants (Neilson and Rajakaruna 2015). Soil pH also influences the mobility of metals. At alkaline pH, metal ions are immobilized by forming phosphates and carbonates. At acidic pH, H^+ ions compete at metals binding sites which cause increase in free metal ions that become available to plants (Sandrin and Hoffman 2007). Zinc (Zn), Cadmium (Cd), Chromium (Cr) and lead (Pb) are absorbed readily from soil at acidic pH (Blaylock and Huang 2000; Sandrin and Hoffman 2007). The values of electrical conductivity (EC) is also used to determine the concentration of salts in solution. High salinity promotes uptake of metals from soil by increasing the equilibrium dissociation constant (K_d) value of soil (Stevens et al. 2003). A soil with relatively low organic matter has a greater risk of metal contamination. Organic matter influences cation exchange capacity (CEC), buffering capacity and retention capacity of metals in soil by forming stable metal chelates. CEC is thus a direct indicator of organic matter in soil. Therefore, metals in mineral soil are more mobile and bioavailable compared to organic soil (Balasoiu et al. 2001).

Plants form the foundation of most food chains. Among various metals in soil, some, such as copper (Cu), iron (Fe), manganese (Mn), nickle (Ni) and zinc (Zn), have biological significance for plants while others including aluminum (Al), aresenic (As), cadium (Cd), lead (Pb) and mercury (Hg), are toxic even at low concentration (Arnon and Stout 1939). The electrochemical gradient of metal ions between the plasma membrane of root cells and soil contents cause their uptake in plants (Blaylock and Huang 2000). Plants avoid metals at their roots by binding them to organic acid or storing them in vacuoles, so that they cannot interfere with physiological activities (Hossain et al. 2012; Gall and Rajakaruna 2013). Most of the absorbed metals are stored in the roots and some are transferred to other parts by different mechanisms (Peralta-Videa et al. 2009). Some plants are hyper-accumulators and tolerate metal accumulation in aerial parts (Unterbrunner et al. 2007; Gall and Rajakaruna 2013)

In most terrestrial ecosystems, insects play a major role in providing protein rich food for different animal groups. They have variable feeding styles and can occupy various positions in the food chain (Labandeira 1997). Insect exposure to metals occur inadvertently through ingestion of contaminated plants / debris. They may also absorb metals through their exoskeleton (Heikens et al. 2001; Hobbelen et al. 2006). Those insects that ingest metals can further transfer it to their predators along the food chain (Zhang et al. 2009; Green et al. 2010). However, in response to metals toxicity, some insects have behavioural and physiological adaptations that limits the bio-accumulation of metal. It was, for example, observed that when the locust (*Schistocerca gregaria*) and the scale insect (*Saissetia* *neglecta*) initially taste a metal rich plant, they show aversion and a reduced ingestion rate (Behmer et al. 2005; Mathews et al. 2009). Some species of Hymenoptera and Lepidoptera excrete metals in their faeces thus limiting their accumulation in higher trophic levels (Lindqvist 1992). However, some species of Orthoptera and Coleoptera accumulate metals in their tissues including in the hepatopancrease, malpighian tubules, mid gut, ovarioles and mandibles (Warchałowska-Śliwa et al. 2005; Grzes 2010; Migula et al. 2011).

Plants also accumulate metals in their floral organs which have negative effect on their reproduction either through low pollen germination, decreased production of ovules and seeds or low viability of the seeds. Metal accumulation in floral parts of the plants also influence Plant – Insect interaction by decreasing the visitation rate and foraging time of honey bees and other herbivores (Meindl and Ashman 2015; Xun et al. 2017).

In the present study, accumulation of Zn, Cd and Pb in the food chains of berseem - aphid, grasshopper - ladybird beetle was estimated in agricultural ecosystems in Pakistan. *Trifolium alexandrinum* (Berseem) is a cold season crop and is used as forage for dairy animals. Its forage is better than grasses due to its high mineral and protein contents (Laghari et al. 2000). It has potential for uptaking metals such as Cr, Cu, Cd, Co and Pb from soil thus exposing other components of the food chain, including humans, to metals (Bhatti et al. 2016). Berseem crop is attacked by a variety of pests that include foliage chewing grasshoppers and phloem feeder aphids. The grasshopper *A. thalassinus*, an important pest of berseem crop in Pakistan, is among the dominant members of the *Aiolopus* genus and have a polyvoltine breeding cycle (Soomro et al. 2015). Aphids are also major pest species on different crops including berseem (Crawford et al. 1995). The aphid *Sitobion avenae* is an abundant pest on berseem crop and is known to take up metals including Zn and Cd from wheat plants (Green et al. 2003). Finally, the ladybird beetle *Coccinella septempunctata* is an important biological control agent that voraciously feed on aphids (Ostman et al. 2003). Its larvae consume a variety of prey including small insects such as aphids and thrips (Ferrer et al. 2008). Zn and Cd accumulation was recorded in *C. septempunctata*, in a system amended with sewage sludge (Green et al. 2003). The primary objective of this study was to assess the bioaccumulation of metals (Zn, Cd and Pb) in *T. alexandrinum*, and their transfer to other species in plant-insect food chains.

MATERIAL AND METHOD

STUDY AREA:

Five agricultural sites were selected from the province of Punjab, Pakistan (Fig. 1, Table 1). Three sampling sites were located in district Lahore [Jallo (Site I), University of the Punjab (Site II), Raiwind (Site III)]; one in Kasur [Pattoki, (Site IV)] and one in Khushab [Noshera, Soon valley (Site V)] (Table 1). District Lahore and Kasur are located in the eastern central part of Punjab with an area of 4,000 km^2 and 1,800 km^2, respectively (Farooqi et al. 1999). In Lahore and Kasur, temperature ranges from 6 to 40 °C and average annual rainfall 607 and 334 mm, respectively. Soon valley is a hilly area covering 10,500 km^2 at the heart of the salt range, with an altitude between 400-1000m above sea level. The temperature of the valley ranges from -3 °C to 42 °C and it receives 350 to 500 mm of rain annually.

SAMPLING

At each site, five fields (each approximately 2 hectares) of berseem were selected randomly and soil, plants and insects samples were collected from March through April, 2016. From each field, two soil samples were taken (0-15cm depth) using a stainless steel auger (giving a total sample size of 50), and placed in a sealed polyethylene bag and taken to the laboratory for further analysis. At each field, three barseem plants were randomly selected (total N = 75) and their shoots and leaves were harvested using a pair of scissors. Approximately, 700 Aphids (*Sitobion avenae*), 10 adult grasshoppers (*Aiolopus thalassinus*) and 25 larvae (4th instar) of a ladybird beetle (*Coccinella septempunctata*) were also collected from each field by hand picking and sweep netting. Insects collected from each field were pooled and considered as a single sample (a total of N = 25 for each species). Plant and insect samples were placed in separate glass vials, brought to the laboratory, washed carefully with double distilled water, identified to species level and stored at -10^{o}C.

PHYSICOCHEMICAL ANALYSIS OF SOIL

Soil pH and electrical conductivity (EC) was measured in a suspension of soil and distilled water (1g : 2.5ml) using a pH meter (JESNCO 6173) and EC meter (HANNA HI 9811-5), respectively. Soil's physical characteristic was determined using a bouyoucos hydrometer (ASTM 152-H soil hydrometer). Soil organic matter was assessed using the wet oxidation method (Walkley and Black 1934). Cation exchange capacity (CEC) was determined by the methylene blue (filter paper spot test) titration method (Cokca and Birand 1993).

METAL EXTRACTION

Heavy metals were extracted from soil and plants by using the aqua-regia method (Khan et al. 2013). Insects were digested by using nitric acid (Green et al. 2005). Polarized Zeeman atomic absorption spectrophotometer (HITACHI Z-5000) was used to measure the concentration of Cd, Zn and Pb in soil, plant and insect extracts. The standard solutions for each metal were prepared by diluting their subsequent certified standard solutions from 0-20 ppm (Sigma-Aldrich).

BIOACCUMULATION FACTOR

The bioaccumulation factor (BAF) is a measure of the metal enrichment at each trophic level. BAF was calculated by dividing the metal concentration at the second or third trophic level with the metal concentration at the trophic level immediately below (Ghosh and Singh 2005; Bitterli et al. 2010).

STATISTICAL ANALYSIS:

The Kolmogorov–Smirnov test revealed that all the data used in the tests was normal distributed. The Pearson's correlation coefficient was calculated to assess the association between physico-chemical properties and metals content of soil. Analysis of Variance (ANOVA) and Tukey's tests were performed to check the differences in physico-chemical properties of soil and metals concentrations of soil, plant and insect samples collected from different sites. Correlation was also performed to assess metal concentration relationship within food chain compartments. A general linear model (GLM) with Bonferroni correction was used to examine the BAF at each level of the food chains. The effect of site, metal type and their interaction on BAF was calculated. All analyses were performed using Minitab 17 (Minitab Inc. 2010).

RESULTS

The soil at all study sites was similar and slightly alkaline (7.41± 0.95) in nature ($F_{4,20}$=0.32, P=0.86). However, soil EC varied ($F_{4,20}$=48.97, P=0.001) in different study areas. The highest EC value was observed at site I (450 ± 44.78) while the lowest was observed at site III (283 ± 35.23**)**. At all sites, the observed soil texture was silty loam except at site III which was sandy loam. In all study areas, clay content ranged from 4.1% to 15.3%, sand from 17.0% to 69.5% and silt from 15.1% to 68.8%. SOM ranged from 0.56% to 1.71%. The minimum value of SOM was recorded at site V (0.561 ± 0.001) while the maximum was recorded at site I (1.713 ± 0.009). CEC ranged from 10.27 meq/100g to 14.72 meq/100g and did not differ between sites ($F_{4.20}$=2.57, P=0.103) (Table 2). The quantity of sand and silt showed a strong negative correlation with each other (r^2=-0.975, P=0.005). A significant negative correlation was also observed between SOM and pH values (r^2=-0.986, P=0.002). The concentrations of Zn and Pb was lower while Cd was higher than the permissible level at all study sites. However, the quantity (mg/Kg of soil) of Pb was highest followed by Zn and Cd. The concentration of Pb and Zn showed no significant correlation with any studied physicochemical properties of the soil. However, Cd showed positive correlation with quantity of sand and negative with quantity of silt in the soil (Table 3).

In soil, the Zn concentration differed significantly between study sites ($F_{4,20}$=6.74, P=0.007). The maximum Zn concentration was recorded from site IV (**2.63±0.23** mg/kg**)** and the minimum from site I (**0.72±0.06** mg/kg**)**. Nevertheless, the Zn concentration at all sites was below the maximum permissible limit (1100 mg/kg) set by (USEPA 2002). The Zn concentration in plants did not differ between sites ($F_{4,20}$=1.190, P=0.374) and no significant correlation was found between the Zn concentration in plants and the total Zn concentration in the soil (r^2=0.128, P = 0.837). However, the Zn concentration in grasshoppers was significantly different at different sites ($F_{4,20}$=3.510, P=0.049), while no significant differences were found in aphids ($F_{4,20}$=2.63, P=0.098). The Zn concentration in ladybird beetle larvae that feed on aphids also differed significantly at different sites ($F_{4,20}$=16.61, P=0.001) (Table 4). No significant correlations were found between the Zn concentration in grasshoppers and plants (r^2=0.016, P=0.980), in aphids and plants (r^2=-0.859, P=0.062) or between beetle larvae and aphids (r^2=-0.454, P=0.442).

In soil, the Cd concentration did not differ between study sites ($F_{4,20}$=0.81, P=0.54) and all sites were above the maximum permissible level (0.480 mg/kg) set by (USEPA 2002). Similarly, the Cd concentration in plants was similar across all sites ($F_{4,20}$=3.05, P=0.07) and no significant correlation was found between the Cd concentration in plants and the total Cd concentration in soil (r^2=0.599, P = 0.286). The Cd concentration in grasshoppers ($F_{4,20=}$1.730, P=0.219) and aphids ($F_{4,20}$=2.600, P=0.10) did not differ between study sites. However, the Cd concentration in beetle larvae was different between study sites ($F_{4,20}$=4.580, P=0.023) (Table 5). No significant correlations were found between the Cd concentration in grasshoppers and plants (r^2=0.589, P=0.296), between aphids and plants (r^2=0.498, P=0.394) and between beetle larvae and aphids (r^2=0.744, P=0.150).

In soil, the Pb concentration depended on study site ($F_{4.20}$=5.160, P=0.016). The soil's maximum Pb was recorded at site IV (4.65±1.56 mg/kg) and the minimum at site II (1.54±0.96 mg/kg**)**, but was below the maximum permissible level (200 mg/kg) set by (USEPA 2002) at all sites. The Pb concentration in plants ($F_{4,20}$=0.640, P=0.644) and aphids ($F_{4,20}$=2.960, P=0.074) did not differ between sites. No significant correlations were found between the Pb concentration in plants and soil (r^2=0.374, P = 0.535) or between aphids and plants (r^2=-0.694, P=0.194). A significant difference between sites was observed in the Pb concentration of grasshoppers ($F_{4,20}$=11.790, P=0.001) and beetles larvae ($F_{4,20}$=11.590, P=0.001) (Table 6). However, no significant relationship was recorded between the Pb concentration of grasshoppers and plants (r^2=0.528, P=0.360) or between beetle larvae and aphids (r^2=-0.003, P=0.996).

BIOACCUMULATION FACTOR (BAF)

In the soil-plant subsystem, BAF values varied significantly for the metals (GLM; $F_{2,38}$=957.46, P=0.0001) (Fig. 2a). Zn (10.46±3.08) was the most accumulated metal, followed by Cd (3.74±0.54) and Pb (2.26±0.42). BAF value also varied for different sites (GLM; $F_{2,38}$=154.10, P=0.0001) and there was a significant interaction between site and metals (GLM; $F_{8,38}$=173.37, P=0.0001). Highest bioaccumulation of metals was recorded in plants of site I and Site V, followed by Site II, III and IV. In the plant-herbivore insect food chain, grasshoppers had a higher level of metals bioaccumulation than aphids (GLM; $F_{1,91}$=210.68, P=0.0001). In grasshoppers, BAF varies for metals (GLM; $F_{2,38}$=126.41, P=0.0001) and the highest BAF value was recorded for Pb (14.64±3.42) followed by Zn (10.21±1.56) and Cd (6.39±0.97) (Fig. 2b). In aphids, the BAF values varies for metals (GLM; $F_{2,47}$=16.51, P=0.0001) and recorded only for Zn (1.21±0.27) and Pb (1.40± 0.41) as Cd did not show accumulation in aphids as the average BAF was below 1 (0.97±0.23) (Fig. 2c). At the third trophic level, the ladybird beetle larvae showed metals bioaccumulation greater than aphids. The BAF values differed significantly for metals (GLM; $F_{2,38}$=52.69, P=0.0001). The highest average BAF value was recorded for Zn (3.35±0.90), the least for Cd (1.22±0.24) with intermediate values for Pb (2.94 ±1.31) (Fig. 2d).

DISCUSSION

Heavy metals in soil have the potential to accumulate in plants. Many studies have been conducted to assess its genetics, physiological and evolutionary significance (Pollard et al. 2002; Boyd 2004, 2007). Plants form the foundation of most food chains through which metals can transfer to the second and third trophic level (Zhang et al. 2009; Green et al. 2010; Dar et al. 2015; Bhatti et al. 2016). Plants absorb essential and non-essential elements through their roots either by diffusion or by selective uptake of the ions from the soil. Many serpentine outcrops accumulate high quantity of the metals due to high enrichment of the soil (Rajakaruna 2009). Heavy metal accumulation in plants depends on species, physiology and genetics (Peralta-Videa et al. 2009). In the present study, berseem plants showed a maximum accumulation for Zn followed by Cd and Pb. Zn is an essential micronutrient and plant roots readily absorb it from soil and translocate it to other parts of the plant (Green et al. 2010). Cd and Pb are nonessential elements for plants. The uptake of Cd in plants is through the same system that is involved in the transport of Zn. Cd is a chemical analogue of Zn and plants may not differentiate between the two ions (Chaney et al. 2004). Most of the absorbed Pb binds to the root surface and is not translocated to other parts of the plants (Blaylock and Huang 2000). Unbound Pb enter the plants and move through Calcium channels and accumulates in stems and leaves of different plants (Huang and Cunningham 1996; López et al. 2007). In the present study, the concentration of heavy metals in berseem plants was higher than in soil and above the permissible level. This highlights the metals accumulation ability of berseem plants and its potential use in phytoremediation of heavy metals (Ali et al. 2012; Bhat 2013).

The insects studied had different concentrations and bioaccumulation rates of heavy metals. Metal accumulation in insects depends on a range of factors including their age, sex, physiology and genetics (Lindqvist 1992; Sterenborg et al. 2003). Zn, Cd and Pb accumulation in aphids was lower than in grasshoppers and this difference likely reflects the feeding behaviour of the two insects. Aphids are phloem suckers and take up metals only in ionic form (War et al. 2012), while chewing insects such as grasshoppers ingest whole plant material (Price et al. 1974). The availability of metals in phloem is restricted because phloem consists of living cells that bind heavy metals (Greger 1999). In the present study, grasshoppers showed high BAF for Pb. It was reported that *Aiolopus thalassinus* can enrich Pb in almost all parts of its body, but particularly in the reproductive organs, gut and abdominal wings (Schmidt and Ibrahim 1994). In this study, the concentration of Zn and Cd in grasshoppers was also higher than the background level found in berseem plants. Grasshoppers may accumulate Zn due to its requirement for metabolic processes and may accumulate Cd due to its chemical similarities to Zn (Roth-Holzapfel 1990). The studied aphid (*Sitobion avenae)* showed a Cd BAF value below 1. This may be due to selective aversion of the aphids towards metal uptake, as high concentration of Cd causes disruption of normal development, survival and reproduction (Gao et al. 2012). The ladybird beetle *Coccinella septempunctata* is an important predator in agro-ecosystems. Metal accumulation in beetles indicates translocation of these metals to the third trophic level (Green et al. 2010). This was found in our study, where the BAFs for Zn, Cd and Pb were higher in beetle larvae compared to aphids. *C. septempunctata* is a generalist predator and it feeds on variety of herbivores, detritivores and other invertebrates, which may result in excessive accumulation of heavy metals (Hunter et al. 1987; Ferrer et al. 2008).

In this study, Zn and Pb concentrations in soil varied between different study areas, but were always under the permissible level. However, the Cd concentration did not differ between study sites and its average value was three times higher than the permissible level. This may be caused by the continuous and excessive use of phosphate fertilizers in agroecosystems (Mar and Okazaki 2012). In Pakistan, phosphate rocks are used to manufacture phosphate fertilizer, which are reported to contain a number of different heavy metals including Cd, As and Pb (Sabiha-Javied et al. 2009). Studies in different areas of Pakistan have reported high concentration of Zn, Cd and Pb in the soil (Muhammad et al. 2011; Perveen et al. 2012; Malik et al. 2010).

The mobility and availability of heavy metals from soil to plants depend on many biological and physicochemical properties of the soil including pH, organic matter, cation exchange capacity, soil texture and soil biota (Peralta-Videa et al. 2009). The soil in all study areas was slightly alkaline in nature and contained moderate levels of organic matter. SOM could influence metals mobility and availability in soil by adsorption or forming stable metal-humic chelates (Shuman 1999). However, high pH causes an increase in negative charges both in soil inorganic content and in organic matter. The repulsive forces between the negative charges result in breakdown of SOM to DOM (dissolved organic matter) (You et al. 1999). Soil texture affects the physical and chemical properties of soil including nutrients retention, water availability, infiltration, aeration, microbial activity and also the availability and mobility of heavy metals (Rizwan et al. 2016; Naidu et al. 1997). The soil texture was silt or sandy loam in all study sites. However, the invasion of non-indigenous material (for example from roads) can cause a change in local soil characteristic including soil texture (Greenberg et al. 1997). Sandy soil is more at risk of heavy metal contamination than clay soil, because sandy texture supports the free ionic form of heavy metals (Naidu et al. 1997). The high concentration of Cd at site III may be due to high sand content. The soil pH, EC, SOM and CEC are known to influence the bioavailability of heavy metals in soil (Olaniran et al. 2013; Violante et al. 2010). However, in the present study no significant correlations were found between soil physical properties (pH, EC, SOM, CEC) and Zn, Cd and Pb concentrations in soil. Similar results were also reported by other researchers (Lucho-Constantino et al. 2005; Bhatti et al. 2016).

The present study indicates the mobility of heavy metals in insect food chains in a tropical agri-ecological ecosystem. Zn is an essential micronutrient and organisms have developed different mechanisms to take it up and utilise it. However, some non-essential heavy metals such as Cd and Pb also accumulate in a food chain resulting in deleterious effects. Different biotic and abiotic factors affect the mobility of these metals in food chains. It is therefore suggested that in order to study bioaccumulation of metals in food chains, all external and internal factors must be taken into account for obtaining optimal results. Our study also assess indirectly the metal exposure and accumulation in higher trophic levels. Studies have reported that birds and mammals also accumulate different metals in their bodies (Wijnhoven et al. 2007; Zhuang et al.2009). The level of accumulation varies between species, type of organ and the capacity of the organism to excrete it from its body. In the present study, we found that berseem plants are good accumulator of different metals. In different parts of the world, berseem plants are used as fodder for many domestic animals. Similarly many birds have a high risk of exposure to these metals through feeding on contaminated seeds and insects. This may cause a rapid transfer of metals to local populations of animals and humans.

REFERENCES

Ali, H., Naseer, M., & Sajad, M. A. (2012). Phytoremediation of heavy metals by *Trifolium alexandrinum*. *International Journal of Environmental Sciences, 2*(3), 1459, doi:10.6088/ijes.00202030031.

Arnon, D. I., & Stout, P. R. (1939). The Essentiality of Certain Elements in Minute Quantity for Plants with Special Reference to Copper. *Plant Physiology, 14*(2), 371-375, http://www.jstor.org/stable/4257358.

Balasoiu, C. F., Zagury, G. J., & Deschenes, L. (2001). Partitioning and speciation of chromium, copper, and arsenic in CCA-contaminated soils: influence of soil composition. *Science of Total Environment, 280*(1-3), 239-255, https://doi.org/10.1016/S0048-9697(01)00833-6.

Behmer, S. T., Lloyd, C. M., Raubenheimer, D., Stewart-Clark, J., Knight, J., Leighton, R. S., et al. (2005). Metal hyperaccumulation in plants: mechanisms of defence against insect herbivores. *Functional Ecology, 19*(1), 55-66, doi: 10.1111/j.0269-8463.2005.00943.x.

Bhat, S. (2013). Phytoremidation properties and CLA content of Berseem (*Trifolium alexandrinum*). *Asian Journal Of Microbiology, Biotechnology And Environmental Sciences, 15*(3), 573-577, https://www.researchgate.net/profile/Shanky_Bhat/publication/258228887_Phytoremediation_and_CLA_content_in_Berseem/links/00b495277ab1ba73ee000000.pdf

Bhatti, S. S., Sambyal, V., & Nagpal, A. K. (2016). Heavy metals bioaccumulation in Berseem (*Trifolium alexandrinum*) cultivated in areas under intensive agriculture, Punjab, India. *Springerplus*, *5*(1), 173, doi:10.1186/s40064-016-1777-5.

Bitterli, C., Banuelos, G. S., & Schulin, R. (2010). Use of transfer factors to characterize uptake of selenium by plants. *Journal of Geochemical Exploration*, *107*(2), 206-216, doi:10.1016/j.gexplo.2010.09.009.

Blaylock, M. J., & Huang, J. W. (2000). Phytoextraction of metals. In Raskin, I. and BD Ensley (Eds.), *Phytoremediation of toxic metals: using plants to clean up the environment* (pp. 53–69). Toronto: John Wiley and Sons.

Boyd, R. S. (2004). Ecology of metal hyperaccumulation. *New Phytologist, 162*(3), 563-567.

Boyd, R. S. (2007). The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. *Plant and Soil, 293*(1-2), 153-176.

Cao, H. B., Chen, J. J., Zhang, J., Zhang, H., Qiao, L., & Men, Y. (2010). Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China. *Journal of Environmental Sciences, 22*(11), 1792-1799, doi:10.1016/S1001-0742(09)60321-1.

Chaney, R. L., Reeves, P. G., Ryan, J. A., Simmons, R. W., Welch, R. M., & Angle, J. S. (2004). An improved understanding of soil Cd risk to humans and low cost methods to phytoextract Cd from contaminated soils to prevent soil Cd risks. *Biometals, 17*(5), 549-553, https://doi.org/10.1023/B:BIOM.0000045737.85738.cf

Cokca, E., & Birand, A. (1993). Determination of cation exchange capacity of clayey soils by the methylene blue test. *Geotecnical Testing Journal*, 6(4), 518-524, https://doi.org/10.1520/GTJ10291J.

Crawford, L. A., Hodkinson, I. D., & Lepp, N. W. (1995). The Effects of Elevated Host-Plant Cadmium and Copper on the Performance of the Aphid Aphis-Fabae (Homoptera, Aphididae). *Journal of Applied Ecology*, *32*(3), 528-535, doi: 10.2307/2404650.

Dar, M. I., Khan, F. A., Green, I. D., & Naikoo, M. I. (2015). The transfer and fate of Pb from sewage sludge amended soil in a multi-trophic food chain: a comparison with the labile elements Cd and Zn. *Environmental Science And Pollution Research, 22*(20), 16133-16142, doi:10.1007/s11356-015-4836-5.

Farooqi, I. S., Jebb, S. A., Langmack, G., Lawrence, E., Cheetham, C. H., Prentice, A. M., et al. (1999). Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *The New England Journal Of Medicine, 341*(12), 879-884, doi:10.1056/NEJM199909163411204.

Ferrer, A., Dixon, A. F. G., & Hemptinne, J. L. (2008). Prey preference of ladybird larvae and its impact on larval mortality, some life-history traits of adults and female fitness. *Bulletin of Insectology, 61*(1), 5-10.

Gall, J. E., Boyd, R. S., & Rajakaruna, N. (2015). Transfer of heavy metals through terrestrial food webs: a review. *Environmental Monitoring and Assessment, 187*(4), 201, doi: 10.1007/S10661-015-4436-3.

Gall, J. E., & Rajakaruna, N. (2013). The physiology, functional genomics, and applied ecology of heavy metal-tolerant Brassicaceae. In *M. Lang* (Ed.), *Brassicaceae: characterization, functional genomics and health benefits* (*pp.* 121-148). New York: Nova Science Publisher.

Gao, H. H., Zhao, H. Y., Du, C., Deng, M. M., Du, E. X., Hu, Z. Q., et al. (2012). Life table evaluation of survival and reproduction of the aphid, *Sitobion avenae*, exposed to cadmium. *Journal Of Insect Science, 12*(1), 44, doi:10.1673/031.012.4401.

Ghosh, M., & Singh, S. (2005). A review on phytoremediation of heavy metals and utilization of it's by products. *Asian Jounal On Energy And Environment, 6*(4), 18.

Green, I. D., Diaz, A., & Tibbett, M. (2010). Factors affecting the concentration in seven-spotted ladybirds (*Coccinella septempunctata L.*) of Cd and Zn transferred through the food chain. *Environmental Pollution, 158*(1), 135-141, doi:10.1016/j.envpol.2009.07.032.

Green, I. D., Merrington, G., & Tibbett, M. (2003). Transfer of cadmium and zinc from sewage sludge amended soil through a plant-aphid system to newly emerged adult ladybirds (*Coccinella septempunctata*). *Agriculture Ecosystems & Environment, 99*(1-3), 171-178, doi:10.1016/S0167-8809(03)00147-6.

Green, I. D., Tibbett, M., & Diaz, A. (2005). Effects of aphid infestation on Cd and Zn concentration in wheat. *Agriculture Ecosystems & Environment, 109*(1-2), 175-178, doi:10.1016/j.agee.2005.02.014.

Greenberg, C. H., Crownover, S. H., & Gordon, D. R. (1997). Roadside soils: A corridor for invasion of xeric scrub by nonindigenous plants. *Natural Areas Journal, 17*(2), 99-109.

Greger, M. (1999). Metal availability and bioconcentration in plants. In *Heavy metal stress in plants* (pp. 1-27). Berlin: Springer, https://doi.org/10.1007/978-3-662-07745-0_1.

Grzes, I. M. (2010). Zinc and cadmium regulation efficiency in three ant species originating from a metal pollution gradient. *Bulletin of Environmental Contamination and Toxicology, 84*(1), 61-65, doi:10.1007/s00128-009-9893-3.

Heikens, A., Peijnenburg, W. J., & Hendriks, A. J. (2001). Bioaccumulation of heavy metals in terrestrial invertebrates. *Environmental Pollution, 113*(3), 385-393, https://doi.org/10.1016/S0269-7491(00)00179-2.

Hobbelen, P. H., Koolhaas, J. E., & van Gestel, C. A. (2006). Bioaccumulation of heavy metals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils. *Environmental Pollution, 144*(2), 639-646, doi:10.1016/j.envpol.2006.01.019.

Hossain, M. A., Piyatida, P., da Silva, J. A. T., & Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany, 2012,* http://dx.doi.org/10.1155/2012/872875.

Huang, J., & Cunningham, S. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New phytologist*, *134*(1), 75-84, https://doi.org/10.1111/j.1469-8137.1996.tb01147.x

Hunter, B. A., Johnson, M. S., & Thompson, D. J. (1987). Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. II. Invertebrates. *Journal of Applied Ecology*, 587-599, doi: 10.2307/2403895.

Khan, K., Lu, Y. L., Khan, H., Ishtiaq, M., Khan, S., Waqas, M., et al. (2013). Heavy metals in agricultural soils and crops and their health risks in Swat District, northern Pakistan. *Food and Chemical Toxicology, 58*, 449-458, doi:10.1016/j.fct.2013.05.014.

Labandeira, C. C. (1997). Insect mouthparts: Ascertaining the paleobiology of insect feeding strategies. *Annual Review of Ecology and Systematics, 28*(1), 153-193, doi:DOI 10.1146/annurev.ecolsys.28.1.153.

Laghari, H. H., Channa, A. D., Solangi, A. A., & Soomro, S. A. (2000). Comparative digestibility of different cuts of berseem (*Trifolium alexandrinum*) in sheep. *Pakistan Journal of Biological Sciences (Pakistan)*, 3, 1938-1939, http://agris.fao.org/agris-search/search.do?recordID=PK2000000622.

Lindqvist, L. (1992). Accumulation of cadmium, copper, and zinc in five species of phytophagous insects. *Environmental entomology*, *21*(1), 160-163, https://doi.org/10.1093/ee/21.1.160.

López, M. L., Peralta-Videa, J. R., Parsons, J. G., Benitez, T., & Gardea-Torresdey, J. L. (2007). Gibberellic acid, kinetin, and the mixture indole–3-acetic acid–kinetin assisted with EDTA-induced lead hyperaccumulation in alfalfa plants. *Environmental science & technology, 41*(23), 8165-8170, **doi:** 10.1021/es0714080

Lucho-Constantino, C. A., Alvarez-Suarez, M., Beltran-Hernandez, R. I., Prieto-Garcia, F., & Poggi-Varaldo, H. M. (2005). A multivariate analysis of the accumulation and fractionation of major and trace elements in agricultural soils in Hidalgo State, Mexico irrigated with raw wastewater. *Environmental International*, *31*(3), 313-323, doi:10.1016/j.envint.2004.08.002.

Malik, R. N., Husain, S. Z., & Nazir, I. (2010). Heavy Metal Contamination and Accumulation in Soil and Wild Plant Species from Industrial Area of Islamabad, Pakistan. *Pakistan Journal of Botany, 42*(1), 291-301, http://www.pakbs.org/pjbot/PDFs/42(1)/PJB42(1)291.pdf.

Mar, S. S., & Okazaki, M. (2012). Investigation of Cd contents in several phosphate rocks used for the production of fertilizer. *Microchemical Journal*, *104*, 17-21, doi:10.1016/j.microc.2012.03.020.

Mathews, S., Ma, L. Q., Rathinasabapathi, B., & Stamps, R. H. (2009). Arsenic reduced scale-insect infestation on arsenic hyperaccumulator *Pteris vittata L*. *Environmental and Experimental Botany, 65*(2-3), 282-286, doi:10.1016/j.envexpbot.2008.09.010.

Meindl, G. A., & Ashman, T. L. (2015). Effects of floral metal accumulation on floral visitor communities: Introducing the elemental filter hypothesis. *American Journal of Botany*, *102*(3), 379-389.

Migula, P., Przybyłowicz, W. J., Nakonieczny, M., Augustyniak, M., Tarnawska, M., & Mesjasz-Przybyłowicz, J. (2011). Micro-PIXE studies of Ni-elimination strategies in representatives of two families of beetles feeding on Ni-hyperaccumulating plant *Berkheya coddii*. *X-Ray Spectrometry*, *40*(3), 194-197, doi: 10.1002/xrs.1310.

Mohammed, A. S., Kapri, A., & Goel, R. (2011). Heavy metal pollution: source, impact, and remedies. In Khan M., Zaidi A., Goel R., Musarrat J. (Eds), *Biomanagement of metal-contaminated soils* (pp. 1-28). Dordrecht: Springer, https://doi.org/10.1007/978-94-007-1914-9_1.

Muhammad, S., Shah, M. T., & Khan, S. (2011). Heavy metal concentrations in soil and wild plants growing around Pb-Zn sulfide terrain in the Kohistan region, northern Pakistan. *Microchemical Journal, 99*(1), 67-75, doi:10.1016/j.microc.2011.03.012.

Naidu, R., Kookana, R. S., Sumner, M. E., Harter, R. D., & Tiller, K. G. (1997). Cadmium sorption and transport in variable charge soils: A review. *Journal of Environmental Quality, 26*(3), 602-617, doi:10.2134/jeq1997.00472425002600030004x.

Neilson, S., & Rajakaruna, N. (2015). Phytoremediation of agricultural soils: using plants to clean metal-contaminated arable land. In: Ansari A., Gill S., Gill R., Lanza G., Newman L. (Eds), *Phytoremediation* (pp. 159-168). Cham: Springer.

Olaniran, A. O., Balgobind, A., & Pillay, B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International Journal Of Molecular Sciences, 14*(5), 10197-10228, doi:10.3390/ijms140510197.

Ostman, O., Ekbom, B., & Bengtsson, J. (2003). Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecological Economics, 45*(1), 149-158, doi:10.1016/S0921-8009(03)00007-7.

Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J. (2009). The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. *The International Jorurnal Of Biochemistry And Cell Biology, 41*(8-9), 1665-1677, doi:10.1016/j.biocel.2009.03.005.

Perveen, S., Samad, A., Nazif, W., & Shah, S. (2012). Impact of Sewage Water on Vegetables Quality with Respect to Heavy Metals in Peshawar Pakistan. *Pakistan Journal of Botany, 44*(6), 1923-1931, https://www.pakbs.org/pjbot/PDFs/44(6)/15.pdf.

Pollard, A. J., Powell, K. D., Harper, F. A., & Smith, J. A. C. (2002). The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences, 21*(6), 539-566.

Price, P. W., Rathcke, B. J., & Gentry, D. A. (1974). Lead in terrestrial arthropods: evidence for biological concentration. *Environmental entomology, 3*(3), 370-372, https://www.pakbs.org/pjbot/PDFs/44(6)/15.pdf.

Rajakaruna, N. (2009). Serpentinophiles from california and across the world gather in maine to highlight recent research on soil-biota relations of serpentine outcrops. *Fremontia, 37*(1), 21-24.

Rizwan, M., Siddique, M. T., Ahmed, H., Iqbal, M., & Ziad, T. (2016). Spatial variability of selected physico-chemical properties and macronutrients in the shale and sandstone derived soils. *Soil & Environment, 35*(1), 12-21.

Roth-Holzapfel, M. (1990). Multi-element analysis of invertebrate animals in a forest ecosystem (*Picea abies L.*). In Lieth, H., Markert, B. (Eds.), *Element concentration cadasters in ecosystems: methods of assessment and evaluation* (281-295). Weinheim: VCH Publishers.

Sabiha-Javied, Mehmood, T., Chaudhry, M. M., Tufail, M., & Irfan, N. (2009). Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. *Microchemical Journal, 91*(1), 94-99, doi:10.1016/j.microc.2008.08.009.

Sandrin, T. R., & Hoffman, D. R. (2007). Bioremediation of organic and metal co-contaminated environments: effects of metal toxicity, speciation, and bioavailability on biodegradation. *Environmental Bioremediation Technologies, 1*, 1-34, doi: 10.1007/978-3-540-34793-4_1.

Schmidt, G. H., & Ibrahim, N. M. M. (1994). Heavy-Metal Content (Hg2+, Cd2+, Pb2+) in Various Body Parts - Its Impact on Cholinesterase Activity and Binding Glycoproteins in the Grasshopper *Aiolopus thalassinus* Adults. *Ecotoxicology and Environmental Safety, 29*(2), 148-164, doi: 10.1016/0147-6513(94)90016-7.

Shuman, L. M. (1999). Organic waste amendments effect on zinc fractions of two soils. *Journal of Environmental Quality, 28*(5), 1442-1447, doi:10.2134/jeq1999.00472425002800050008x.

Soomro, F., Sultana, R., Wagan, M. S., Abbasi, A. R., & Solongi, B. K. (2015). Studies on the immature stages of *Aiolopus thalassinus thalassinus* (Fabricius)(Oedipodinae: Acrididae: Orthoptera). *Sindh University Research Journal-SURJ (Science Series)*, 47(2), 267–274.

Sterenborg, I., Vork, N. A., Verkade, S. K., van Gestel, C. A. M., & van Straalen, N. M. (2003). Dietary zinc reduces uptake but not metallothionein binding and elimination of cadmium in the springtail, *Orchesella cincta*. *Environmental Toxicology and Chemistry, 22*(5), 1167-1171, doi: 10.1002/etc.5620220528.

Stevens, D. P., McLaughlin, M. J., & Heinrich, T. (2003). Determining toxicity of lead and zinc runoff in soils: salinity effects on metal partitioning and on phytotoxicity. *Environmental Toxicology and Chemistry, 22*(12), 3017-3024, doi: 10.1897/02-290.

Szyczewski, P., Siepak, J., Niedzielski, P., & Sobczynski, T. (2009). Research on Heavy Metals in Poland. *Polish Journal of Environmental Studies, 18*(5), 755-768, http://www.6csnfn.pjoes.com/pdf/18.5/755-768.pdf.

Unterbrunner, R., Puschenreiter, M., Sommer, P., Wieshammer, G., Tlustos, P., Zupan, M., et al. (2007). Heavy metal accumulation in trees growing on contaminated sites in Central Europe. *Environmental Pollution, 148*(1), 107-114, doi:10.1016/j.envpol.2006.10.035.

USEPA (2002). Supplemental guidance for developing soil screening levels for superfund sites. Office of Solid Waste and Emergency Response, Washington, D.C. http://www.epa.gov/superfund/health/conmedia/soil/index.htm

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., & Pigna, M. (2010). Mobility and Bioavailability of Heavy Metals and Metalloids in Soil Environments. *Journal of Soil Science and Plant Nutrition, 10*(3), 268-292, doi:10.4067/S0718-95162010000100005.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science, 37*(1), 29-38.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., et al. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signal and Behavior, 7*(10), 1306-1320, doi:10.4161/psb.21663.

Warchałowska-Śliwa, E., Niklińska, M., Görlich, A., Michailova, P., & Pyza, E. (2005). Heavy metal accumulation, heat shock protein expression and cytogenetic changes in *Tetrix tenuicornis (L.)* (Tetrigidae, Orthoptera) from polluted areas. *Environmental Pollution, 133*(2), 373-381, https://doi.org/10.1016/j.envpol.2004.05.013.

Wijnhoven, S., Leuven, R., van der Velde, G., Jungheim, G., Koelemij, E., De Vries, F. T., et al. (2007). Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: importance of species-and location-specific characteristics. *Archives of Environmental Contamination and Toxicology, 52*(4), 603-613.

WHO (1996). permissible limits of heavy metals in soil and plants (Geneva: World Health Organization). switzerland.

Xun, E., Zhang, Y., Zhao, J., & Guo, J. (2017). Translocation of heavy metals from soils into floral organs and rewards of Cucurbita pepo: Implications for plant reproductive fitness. *Ecotoxicology and environmental safety, 145*, 235-243.

You, S. J., Yin, Y. J., & Allen, H. E. (1999). Partitioning of organic matter in soils: effects of pH and water/soil ratio. *Science of the Total Environment, 227*(2-3), 155-160, doi: 10.1016/S0048-9697(99)00024-8.

Zhang, Z. S., Lu, X. G., Wang, Q. C., & Zheng, D. M. (2009). Mercury, cadmium and lead biogeochemistry in the soil-plant-insect system in Huludao City. *Bulletin of Environmental Contamination and Toxicology, 83*(2), 255-259, doi:10.1007/s00128-009-9688-6.

Zhuang, P., Zou, H., & Shu, W. (2009). Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: Field study. *Journal of Environmental Sciences, 21*(6), 849-853.

Table 1. List of sampling areas with their districts and coordinates.

Sites	Site name	District	Coordinates
Site I	Jallo	Lahore	31.585083^{0}N, 74.503399^{0}E
Site II	University of the Punjab	Lahore	31.489034^{0}N, 74.293530^{0}E
Site III	Raiwind	Lahore	31.267061^{0}N, 74.223645^{0}E
Site IV	Pattoki	Kasur	31.046329^{0}N, 73.863838^{0}E
Site V	Noshera	Khushab	32.574148^{0}N,72.151258^{0}E

Table 2. Chemical properties of the soil (Mean ± standard deviation) from all study areas.

Chemical character	Site I	Site II	Site III	Site IV	Site V
pH	7.16 ± 0.91^{a}	7.20 ± 0.95^{a}	7.23 ± 0.96^{a}	7.21 ± 0.97^{a}	7.83 ± 0.99^{a}
EC (µs/cm)	450 ± 44.78^{a}	343 ± 40.33^{b}	283 ± 35.23^{c}	353 ± 41.76^{b}	316 ± 39.66^{bc}
Clay %	4.12 ± 0.02	12.10 ± 0.05	15.32 ± 0.02	5.67 ± 0.03	14.25 ± 0.05
Sand %	28.62 ± 0.01	22.21 ± 0.03	69.54 ± 0.04	44.10 ± 0.03	16.95 ± 0.01
Silt %	67.26 ± 0.02	65.69 ± 0.04	15.14 ± 0.01	51.23 ± 0.02	68.80 ± 0.05
SOM %	1.713 ± 0.009	1.577 ± 0.004	1.395 ± 0.006	1.519 ± 0.009	0.561 ± 0.001
CEC(meq/100g)	12.65 ± 2.06^{a}	14.72 ± 2.03^{a}	13.42 ± 2.09^{a}	11.36 ± 1.08^{a}	10.27 ± 2.08^{a}

Site I: Jallo, Site II: University of the Punjab, Site III: Raiwind, Site IV: Pattoki, Site V: Noshera, EC: electrical conductivity, SOM: Soil organic matter, CEC: cation exchange capacity. Comparison was carried out between site for each chemical character using ANOVA ($P < 0.05$) and Tukey's multiple range test.

Table 3. Pearson's correlation matrix of physicochemical properties and heavy metals contents of soil from studied area

	pH	EC	sand	silt	clay	SOM	CEC	Zn	Cd
EC	-0.378								
Sand	-0.449	-0.412							
silt	0.310	0.581	-0.975*						
clay	0.491	-0.853	0.155	-0.372					
SOM	-0.986*	0.515	0.311	-0.160	-0.584				
CEC	-0.703	0.023	0.217	-0.235	0.138	0.681			
Zn	-0.514	-0.348	0.356	-0.330	-0.019	0.433	0.417		
Cd	-0.241	-0.767	0.823*	-0.871*	0.431	0.082	0.225	0.686	
Pb	-0.084	-0.201	0.643	-0.536	-0.264	0.000	-0.507	0.263	0.520

Abbreviations: EC electrical conductivity, SOM Soil organic matter, CEC cation exchange capacity. Zn zinc, Cd cadmium, Pb lead, *correlation is significant at $P<0.05$.

Table 4. Concentration of Zn (mg/kg) in soil, berseem and insects food chain.

Samples/Species	Sites					Average
	I	II	III	IV	V	
soil	**0.72±0.06^{b}**	**2.41±0.88^{a}**	**1.85±0.98^{ab}**	**2.63±0.23^{a}**	**0.76±0.05^{b}**	**1.67±0.42**
T. alexandrinum	**12.51±0.95 ^{a}**	**12.56±3.66 ^{a}**	**9.62±3.32 ^{a}**	**15.62±2.03 ^{a}**	**14.12±5.78 ^{a}**	**12.88±0.99**
A. thalassinus	**82.32±13.09^{b}**	**188.45±66.74^{a}**	**120.34±29.56^{ab}**	**120.66±20.34^{ab}**	**130.36±18.67^{ab}**	**128.40±17.10**
***S.* avenae**	**15.25±1.05 ^{a}**	**15.62±4.56 ^{a}**	**21.25±8.56 ^{a}**	**12.54±3.23 ^{a}**	**8.75±3.78 ^{a}**	**14.68±2.05**
C. septempunctata	**46.09±3.09^{ab}**	**63.56±9.76^{a}**	**24.45±8.45^{c}**	**26.51±6.87^{bc}**	**56.43±7.34^{a}**	**43.43±7.85**

Zn permissible level in soil is 1100 mg/kg (USEPA 2002) and in plants is 0.60 mg/kg (WHO 1996). Site I: Jallo, Site II: university of the Punjab, Site III: Raiwind, Site IV: Pattoki, Site V: Noshera. Metal concentration is expressed as mean value and standard deviation. Comparison was carried out between the values of the site through the Tukey's multiple range test ($P < 0.05$).

Table 5. Concentration of Cd (mg/kg) in soil, berseem and insects food chain.

Sample/Species	Sites					Average
	I	II	III	IV	V	
Soil	1.04±0.02^{a}	1.56±0.98^{a}	2.16±1.03^{a}	1.83±0.93^{a}	1.33±0.78^{a}	1.58±0.21
T. alexandrinum	4.43±2.60^{a}	8.25±1.77^{a}	8.87±3.34^{a}	4.12±2.11^{a}	3.75±2.10^{a}	5.88±0.75
A. thalassinus	30.56±3.34^{a}	32.34±12.65^{a}	48.28±18.43^{a}	40.33±16.23^{a}	22.31±8.76^{a}	34.76±3.70
***S.* avenae**	6.66±0.92^{a}	7.51±4.34^{a}	5.99±3.01^{a}	1.25±0.92^{a}	5.55±2.12^{a}	5.39±0.81
C. septempunctata	7.89±1.90^{ab}	10.22±4.89^{a}	2.76±0.43^{b}	2.45±0.12^{b}	6.54±2.99^{ab}	5.97±1.50

Cd permissible level in soil is 0.48 mg/kg (USEPA 2002) and in plants is 0.02 mg/kg (WHO 1996). Site I: Jallo, Site II: University of the Punjab, Site III: Raiwind, Site IV: Pattoki, Site V: Noshera. Metal concentration is expressed as mean value and standard deviation. Comparison was carried out between the values of the site through the Tukey's multiple range test ($P < 0.05$).

Table 6. Concentration of Pb (mg/kg) in soil, berseem and insects food chain.

Sample/Species	Sites					Average
	I	II	III	IV	V	
Soil	**2.67±0.67^{ab}**	**1.54±0.96^{b}**	**3.71±0.01^{ab}**	**4.65±1.56^{a}**	**2.82±0.43^{ab}**	**3.078±0.523**
T. alexandrinum	**4.25±0.87 ^{a}**	**5.75±3.22 ^{a}**	**7.25±2.99 ^{a}**	**6.62±2.12 ^{a}**	**7.75±3.56 ^{a}**	**6.024±0.680**
A. thalassinus	**22.57±3.76^{c}**	**120.34±32.55^{ab}**	**66.98±12.87^{bc}**	**155.35±44.76^{a}**	**110.45±10.89^{ab}**	**95.10±13.50**
***S.* avenae**	**12.53±3.78 ^{a}**	**8.75±2.87 ^{a}**	**5.76±3.00 ^{a}**	**4.37±2.45 ^{a}**	**8.64±3.56 ^{a}**	**8.01±1.00**
C. septempunctata	**32.51±4.87 ^{a}**	**7.34±3.87 ^{b}**	**13.76±4.65 ^{b}**	**34.87±12.56 ^{a}**	**7.92±3.87 ^{b}**	**19.28±6.00**

Pb permissible level in soil is 2000 mg/kg (USEPA 2002) and in plants is 2.00 mg/kg (WHO 1996). Site I: Jallo, Site II: University of the Punjab, Site III: Raiwind, Site IV: Pattoki, Site V: Noshera. Metal concentration is expressed as mean value and standard deviation. Comparison was carried out between the values of the site through the Tukey's multiple range test ($P < 0.05$).

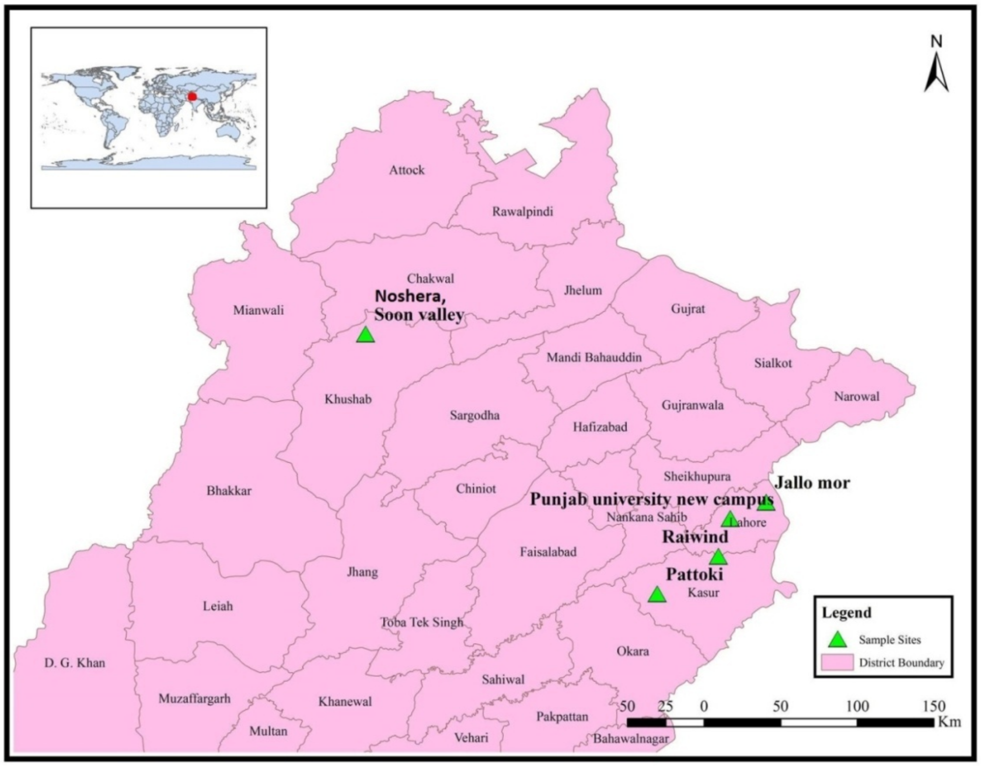

Figure 1. Geographical distribution of sampling sites.

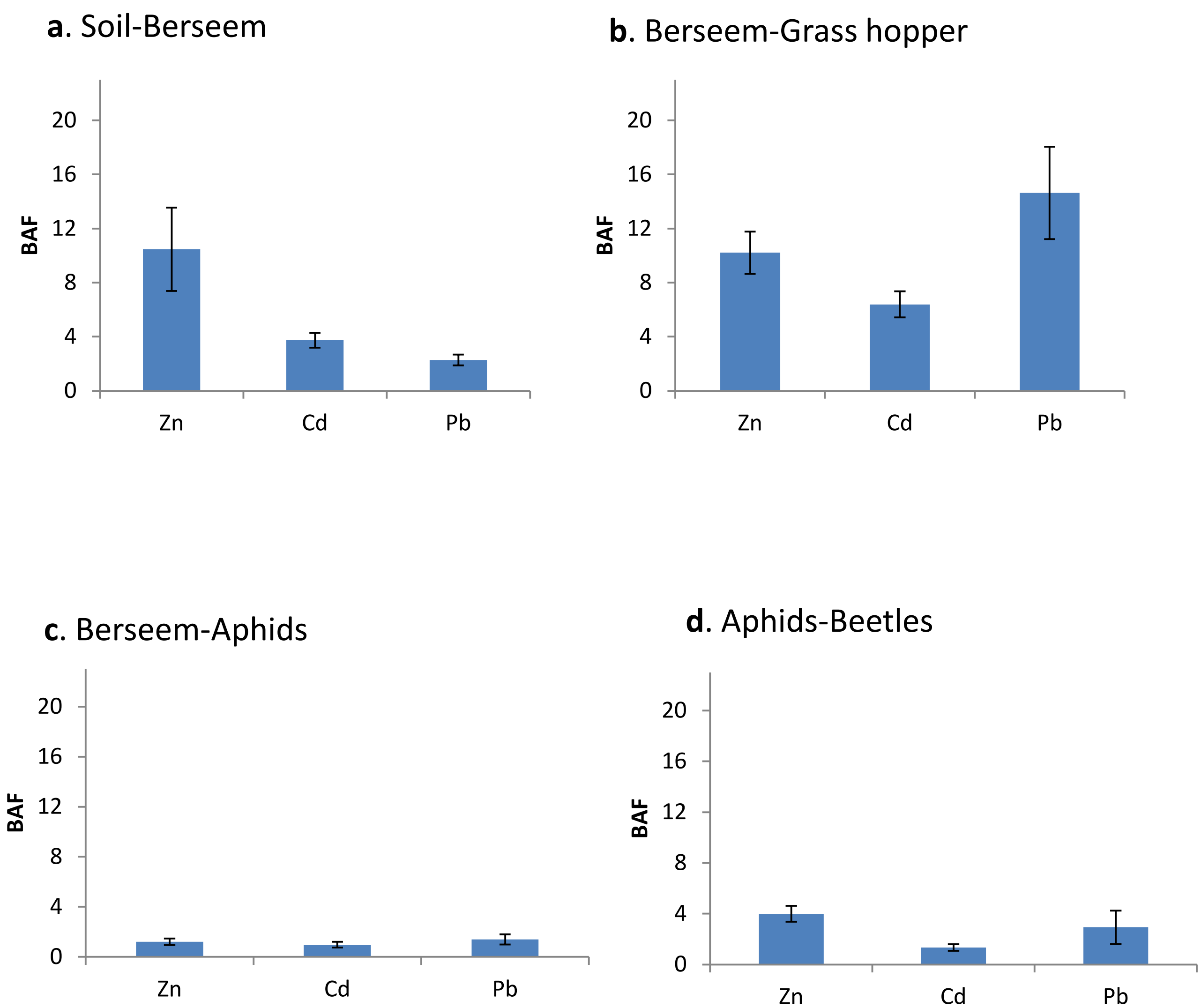

Figure 2. Bioaccumulation factor (BAF) of heavy metals for (a) plant *Triofolium alexandrinum*, (b) grasshopper *Ailopus thalassinus*, (c) aphid *Sitobion avenae*, and (d) *Coccinella septempunctata* in food chain