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Heavy Metal Pollution and Biota-to-soil Accumulation Factor (BSAF) of in Situ Earthworms in Western Zone of Punjab, India

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Abstract: Earthworms are a key species in the terrestrial ecosystem as their responses to contaminated soil reflects the health of ecosystem functioning. The present study was undertaken to ascertain the effect of heavy metal pollution on in situ earthworm communities of Punjab, India. The presence of heavy metals in both soil and earthworm samples collected from all three districts of western zone of Punjab (Fazilka, Ferozepur, Bathinda). The content of heavy metal in soil samples and in earthworms collected from different locations followed the trend: Mn>Zn>Cu>Cr>Ni>Pb>As>Cd and Zn>Mn>Cu>As>Cr>Ni>Pb>Cd respectively. The heavy metal contents were higher in earthworms in comparison to soil samples. The oxidative stress in earthworms was highly significant and positive correlation for enzyme glutathione reductase with cadmium and chromium while negative correlation was observed of glutathione S transferase and glutathione peroxidise with chromium and manganese and copper, respectively. The biota-to-soil accumulation factor (BSAF) followed the trend: Cd>As>Zn>Cu>Pb>Ni>Cr>Mn. The current study is a step forward in the direction of in-situ study of earthworms and to understand the complex relations of various factors that together operate as determinants of bioaccumulation as opposed to the widely prevalent and controlled laboratory studies.

Keywords: Heavy metals, Earthworms, Stress enzyme, Soil, BSAF, Western Zone of Punjab

Soil is a dynamic and vital ecosystem that forms the basis of life on this planet. The soil organisms like earthworms play an important role in the functioning of soil ecosystems (Spurgeon et al., 2003). In this ecosystem earthworms have a special place due to their complex role in the various stages of soil formation as well as production of vermicast and maintaining the dynamics of the soil ecosystem. Although most other soil organisms are protected by the thick cuticle outside their bodies however earthworms are susceptible to soil chemicals as these hazardous substances are ingested and absorbed in their bodies which leads to bioaccumulation which is then passed on to other organisms in the food chain. This bioaccumulation in earthworms may have significant effects on the animal and on other higher level organisms as well (Reinecke and Reinecke 2007).

The rapid industrialization has added new stressors in the ecosystem in the form of pesticides and heavy metals which are the major environmental pollutants released from activities like mining, combustion of fossil fuels disposal of wastewater and sewage sludge on land. The metals like zinc, arsenic, cadmium, chromium etc are harmful to both the environment and the organisms even in minute concentrations (Aulakh et al., 2022, Friis et al., 2004, Mahmood 2020, Yadav et al., 2023). Earthworms are indispensable organisms especially for the study of the degree and effects of various pollutants in the soil primarily due to their edaphic habitat which keeps them in intimate contact with both solid and aquatic phases of soil. The heavy metal contents are accumulated by earthworms inside their tissues during feeding in addition to the uptake of heavy metals by earthworms they also return a portion of these pollutants back to the soil when put into vermicast. Higher concentrations of these pollutants are a serious threat to the survival of these species in the soil ecosystem. When exposed to these pollutants the natural reaction of the body is to release various anti-oxidant defence systems that an organism employs to protect and deactivate the radical toxicity from the exposure to substances. The traces of the metal elements have been implicated with the oxidative stress at cellular level via (Lijun et al., 2005) several enzymes including superoxide dismutase, catalase, and glutathione peroxidase (GPX). The reactive oxygen species (ROS) affects the lipids, proteins, carbohydrates and nucleic acids including the superoxide radical (O^{2}-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). These biochemical responses can be early warning signs of environmental pollution and its severity. These metal pollutants accumulate over time resulting in bioaccumulation, studied using the bioaccumulation factor as a measure of quantification of metal bioaccumulation into biota (Melake et al., 2023). Despite the crucial role of earthworms within the soil ecosystem, there is scarcity of studies with a specific focus on the oxidative stress and antioxidant defences in relation to the natural availability of environmental pollutants. The present investigation is an attempt to bridge the gap by studying the effects of heavy metals and their relations to the organism's biochemical defence system through the release of certain enzymes under the in-situ and dynamic conditions of the natural habitat.

MATERIAL AND METHODS

Collection and preparation of sample: The present study was conducted in the Department of Zoology, Punjab Agricultural University, Ludhiana to check the heavy metal residues in western regions of Punjab. The samples of soil and earthworms were collected from the rice fields of three districts in Western zone of Punjab: Fazilka (A), Ferozepur (B), and Bathinda (C). Two villages namely Mansa and Choharian Wali from Fazilka, Pindi and Jiwan Arian from Ferozepur, Pirkot and Chauka from Bathinda were selected for soil sampling. The soil samples up to the depth of 30cm were collected. The soil aggregates were disintegrated while they were still moist in the laboratory and then air dried before sieving. Then these samples were sieved through 2 mm mesh sieve before they were placed in plastic bags for further testing. Earthworms collected from all the three sites were first rinsed with distilled water and placed in petri dishes with Whatman No. 1 filter paper and a few drops of distilled water were put to maintain them. The earthworms were placed alive on moist filter paper with no food source for 24 hours to allow soil to be egested from their gut. The worms were killed by freezing and then oven dried (48 h at 70-80°C) to constant weight. The dry earthworms were processed for heavy metal detection (Bade et al., 2012) by using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

Antioxidant activity in earthworms: After washing the earthworms in distilled water, they were homogenised in 0.02M phosphate buffer (pH 7.5) and centrifuged at 3000 rpm for ten minutes. The homogenate supernatant was used for further enzyme analysis. The enzymes were analysed using standard methods: which include glutathione peroxidase (Hafeman et al., 1974), superoxide (Marklund and Marklund 1974), catalase (Aebi (1983); glutathione reductase (Carlberg and Mannervik 1985) and glutathione-S-transferase (Habig et al., 1974).

Biota-to-Soil accumulation factor : The BSAF is calculated (Cortet et al., 1999).

BSAF = Metal content in biota (earthworm tissue) / Total metal content in soil.

The BSAF was calculated for As, Mn, Zn, Cd, Cr, Ni, Pb and Cu.

RESULTS AND DISCUSSION

The higher contents of heavy metals (As, Mn, Zn, Cd, Cr, Ni, Pb and Cu) were in tissues of earthworm as compared to soil samples from all the three selected sites (Table 1). The metal concentrations in the soil samples collected indicated the trend: Mn> Zn> Cu> Cr> Ni>Pb>As>Cd whereas the content of heavy metal in earthworm species collected from different locations show the trend: Zn>Mn> Cu> As>Cr> Ni>Pb>Cd. This difference in the presence of various heavy metals in soils and their presence as observed in the earthworm tissues are due to the differences in the very nature of these metals. The high value of heavy metals may be attributable to chemical fertilisers which are employed in agricultural activities such as insecticides (Krishna and Govil 2004). The average concentrations of as in the earthworm from all the study locations were in decreasing trend maximum in site B (38.25) followed by site A and C. The concentrations of As in soil was maximum in site B (6.62) followed by A and C. The average concentrations of Cd in earthworms from all the study locations followed the trend, site A(3.4) >site B (3.25) > site C (2.75) while the average concentrations of Cd in soil from all the study locations followed the trend, site B(0.5) >site A (0.44) >site C(0.31). The average concentrations of Ni in earthworms was

Table 1. Heavy metal content in soils and earthworms collected from western zone of Punjab (ppm)

Location	A-Fazilka		B-Ferozepur		C-Bathinda	
Metal	Soil	Earthworm	Soil	Earthworm	Soil	Earthworm
Arsenic	4.68±0.45	21.5±3.59	6.62±1.86	38.25±2.67	4.12±0.78	15.50±2.38
Cadmium	0.44±0.12	3.40±0.58	0.50±0.14	3.25±0.58	0.31±0.08	2.75±0.48
Nickel	17.37±2.74	12.33±1.15	18.94±3.82	18.50±2.45	13.31±2.26	10.75±1.73
Manganese	247.18±40.53	117.5±6.60	295.56±59.74	238.50±35.86	165.21±28.11	127.50±24.72
Zinc	52.37±9.61	254.25±20.51	79.81±19.18	336.50±63.87	67.68±9.41	284.5±17.91
Lead	10.25±1.33	10.50±1.89	11.42±2.39	13.00±1.91	5.68±0.87	7.50±0.58
Chromium	18.56±4.66	10.50±1.91	20.12±2.03	18.50±2.50	15.18±2.86	9.50±1.73
Copper	19.22±2.99	36.85±6.44	26.51±8.28	49.57±4.511	10.27±1.40	26.20±1.61

Values are mean ±S.E

maximum in site B (18.5) followed A and C. The average concentrations of was maximum (18.94) in site B followed closely by site A (17.37). The average concentrations of Mn in earthworms was maximum in site B (238.5) quite low in C (127.5) and A (117.5) The Mn in soil in site B was maximum (295.56) followed by A (247.18) and C (165.21). The average concentrations of Zn in earthworms was maximum at site B(336.5) and was low in C (284.5) and A (254.25). The average concentrations of Zn in soil was maximum in site B (79.81) followed by C (67.68) and A (52.37). The average concentrations of Pb in earthworms from all the study locations followed the trend: B (13) >A (10.5) >C(7.5) while the average concentrations of Pb in soil from all the study locations followed the same trend being maximum in B (11.42) followed by A and C. The average concentrations of Cr in earthworms was high in B (18.5) >A (10.5) >C (9.5) while the average concentrations of Cr in soil from all the study locations followed same the trend, B (20.12) >A (18.56) > C (15.18). The average concentrations of Cu in earthworms was maximum in B (49.57) followed by A (36.85) and C(26.20). The average concentrations of Cu in soil from all the study locations was maximum in site B (26.51) and minimum in C (10.27). Except for Mn, Ni and Cr, the concentration of heavy metals Pb, Cu, As and Zn was higher in earthworms in soil samples collected from different districts.

Analysis of the presence of various enzymes revealed that highest catalase activity in earthworm was at site B (50.32 nmolH_2O_2 consumed/min/mg of protein) followed by C and A (Table 2). The catalase activity in earthworms positive trend for most of the heavy metals. This can be attributed to the increased substrate concentration as a result of heavy metal exposure (Liu *et al* 2011). The present results corroborate with the findings that catalase has a trend of increase and decrease as per the duration of exposure to toxic metals in *E. fetida* (Yadav et al., 2022, Lin et al., 2010). Superoxide dismutase is a metalloenzyme that acts as an antioxidant, highest SOD activity in earthworm was at site B

Table 2. Activity of antioxidative enzymes in earthworms collected from different districts

Oxidative enzymes	A-Fazilka	B-Ferozepur	C-Bathinda
Catalase (nmolH_2O_2consumed/min/mg of protein)	34.33±0.61 [a]	50.32±1.20 [c]	40.24±0.88 [b]
SOD (U /mg of protein)	32.54±0.64 [a]	41.23±0.96 [b]	33.43±0.38 [a]
Glutathione peroxidase (U/mg of protein)	37.87±0.56 [a]	61.76±0.55 [c]	54.22±0.75 [b]
Glutathione S-transferase (nmol/min/mg of protein)	135.33±0.70 [a]	150.44±0.43 [c]	140.44±0.57 [b]
Glutathione reductase (μmol of NADPH conjugate/min/mg of protein)	10.54±0.45 [b]	6.43±0.71 [a]	9.65±0.46 [b]

Values are mean ±S.E
Values with different superscript (a, b and c) are significantly different ($p<0.05$)

Table 3. Correlation coefficient of heavy metal content in earthworms with antioxidant activity

Enzymes	Locations	Heavy metals							
		As	Cd	Pb	Zn	Cr	Ni	Mn	Cu
Catalase	A	+0.76	+0.18	-0.86	-0.59	-0.75	-0.75	-0.92	-0.71
	B	+0.87	+0.69	+0.75	+0.94	-0.14	+0.34	+0.24	+0.65
	C	+0.24	+0.19	-0.03	+0.28	-0.06	+0.01	+0.10	-0.69
Glutathione	A	-0.51	+0.59	+0.11	-0.43	+0.59	-0.01	+0.19	-0.41
Reductase	B	-0.19	+0.91	+0.26	-0.33	+0.95	+0.71	+0.78	+0.05
	C	+0.02	-0.02	-0.13	-0.26	-0.13	-0.12	-0.23	+0.80
SOD	A	-0.45	+0.99	-0.63	-0.87	+0.48	+0.11	-0.005	-0.55
	B	-0.15	-0.10	+0.60	-0.10	+0.43	+0.55	+0.50	+0.83
	C	-0.83	-0.38	+0.56	-0.08	+0.41	+0.40	+0.40	0.27
GPX	A	-0.54	+0.67	+0.03	-0.51	+0.62	+0.01	+0.19	-0.44
	B	-0.16	+0.72	-0.58	-0.03	-0.99	-0.92	-0.95	-0.30
	C	-0.78	-0.05	+0.84	+0.47	+0.28	+0.74	+0.83	-0.42
GST	A	+0.39	-0.51	-0.11	+0.42	-0.47	+0.14	-0.08	0.47
	B	-0.04	+0.81	-0.45	+0.09	-0.99	-0.85	-0.90	-0.18
	C	-0.53	-0.71	+0.09	-0.35	+0.84	-0.10	+0.04	-0.93

A-Fazilka, B-Ferozepur and C-Bathinda

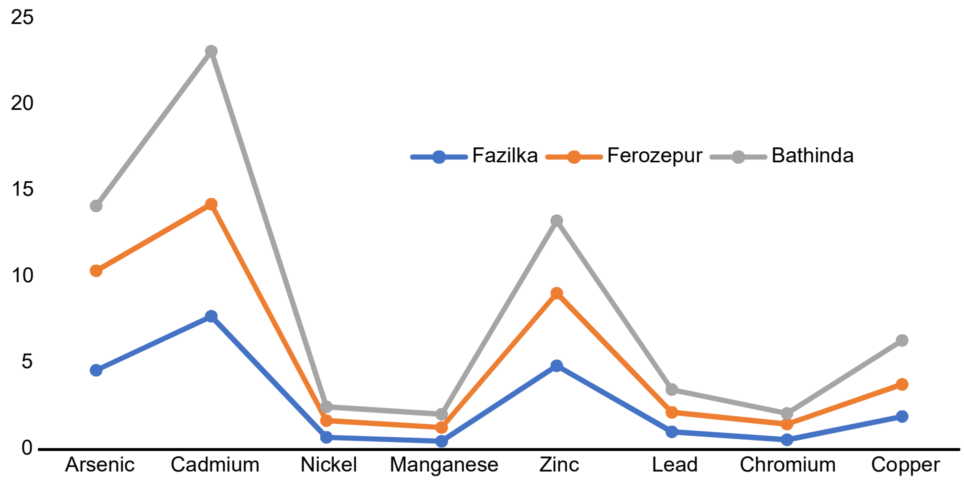

Fig. 1. Biota-to-soil accumulation factor of heavy metals in tissue of earthworm

(41.23 U /mg of protein) followed by site C and site A. The highest GPx activity in earthworm was at site B (61.76 nmol U/mg of protein) followed by site C and site A. GST, a cytosolic enzyme, is involved in the detoxification and biotransformation of a variety of electrophilic chemicals through glutathione consumption. The highest GST activity in earthworm was at site B (150.44nmol H_2O_2 consumed/min/mg of protein) followed by site C and site A. For the enzyme glutathione reductase, highest activity in earthworm was at site A (10.54μmolof NADPH conjugate/min/mg of protein) followed by site C and site B. The negative effect of Cd, As and Zn on the SOD levels can be due to the accumulation of superoxide radicals after prolonged exposure to these heavy metals (El-Demerdash et al., 2009). The activity of enzymes GST, GPx and GR were severely affected by the presence of heavy metals in soils, these findings are in agreement. Laszczyca et al. also (2004) observed that the gradient of heavy metal pollution leads to a gradual increase and subsequent decrease due to hormesis which is a mechanism of balance between the instantaneous production and degradation of specific proteins.

The correlation analysis of heavy metals in earthworm tissues and the activity of various enzymes of site A revealed a highly significant and high positive correlation for the enzyme glutathione reductase and cadmium. The enzyme glutathione reductase has significant high positive correlation for chromium while the enzyme GPX has significantly high negative correlation with chromium and manganese and for the enzyme GST there is a significantly high negative correlation for chromium in samples from site B. At site C, the enzyme GPX has significantly high negative correlation for copper. The Biota-to-soil accumulation factor (BSAF) for the present study follows the trend of Cd>As>Zn>Cu>Pb>Ni>Cr>Mn (Fig. 1) which are in agreement with Dai et al. (2004).

CONCLUSION

The study provides valuable insights into the heavy metal contamination among in situ earthworms in Western zone of Punjab. The Biota-to-soil accumulation factor (BSAF) revealed the trend: Cd>As>Zn>Cu>Pb>Ni>Cr>Mn. There is significant relationship of the presence of oxidative stress enzymes in earthworms with specific heavy metals such as glutathione reductase with cadmium while a significant inverse relation was found with glutathione S transferase with chromium and manganese as well as of glutathione peroxidase with copper. Thereby, the study of various oxidative stress enzymes provides a reliable measure of bioaccumulation of environmental pollutants in the earthworms as well as the severity and penetration of pollutants within the soil ecosystem.

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