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Bioaccumulation of Heavy Metals in Earthworms Found in Industrial Areas of Sirohi District of Southwestern Rajasthan

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Abstract
Earthworms are widely used as bioindicators of soil contamination, yet information on metal bioaccumulation in southwestern Rajasthan's industrial belt, India, remains scarce. This study quantified cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) concentrations in soil and earthworm tissue, and determined their bioaccumulation factors (BAFs) at four industrial sites in the Pindwara and Abu Road tehsils of Sirohi district, Rajasthan. Three sites comprised cement and mineral-processing units, while the fourth was a mixed industrial estate. We collected soil and adult earthworms in the pre-monsoon, monsoon, and post-monsoon seasons for two consecutive years (January 2024 to December 2025). Earthworms were identified to species level, and metals in acid-digested soil and tissue were measured using an atomic absorption spectrophotometer. Cadmium was quantifiable in soil only in the pre-monsoon season (1.50-5.40 mg/kg), when earthworm tissue contained 13.50-42.66 mg/kg, giving bioaccumulation factors of 7.9-9.0. Lead showed low transfer (bioaccumulation factor 0.04-0.49) despite higher soil concentrations (21.5-31.7 mg/kg in the pre-monsoon season). Arsenic showed a season-dependent pattern, with factors slightly above 1 in the pre-monsoon season (1.24-1.40) and below 1 (0.6-0.8) thereafter. Mercury was below the limit of quantification in all samples. The order of bioaccumulation was cadmium > arsenic > lead. The results indicate that earthworms efficiently accumulate cadmium from the industrially contaminated soils of the study region, suggesting their potential as bioindicators for monitoring soil cadmium pollution. In contrast, earthworms appeared to be less effective indicators of lead, arsenic, and mercury contamination at these sites.

Keywords: Bioaccumulation factor, cadmium, lead, mercury, arsenic, earthworms, industrial soil contamination

Introduction

Heavy metals are naturally occurring metallic elements with a comparatively high atomic number (above 20) and a density at least five times that of water (greater than 5 g/cm³), however, the term is variously defined and is often used interchangeably with trace elements or potentially toxic elements [1,2,5]. From a biological standpoint, they fall into two groups: essential metals such as iron, zinc, copper and manganese, which are required in trace amounts but become toxic beyond a threshold and non-essential metals such as cadmium (Cd), lead (Pb) and mercury (Hg), which have no known beneficial function and are toxic even at very low concentrations [1]. Arsenic (As) is strictly a metalloid but it is conventionally grouped with heavy metals in soil pollution studies because of its similar toxicity, persistence and accumulation behaviour [3]. Hg, Pb, Cd and As are the four elements identified by the Agency for Toxic Substances and Disease Registry (ATSDR) as highly toxic to animals, plants and humans [15,24,25] and they induce oxidative stress, organelle damage, genetic mutations and histone alterations [39,43].

Soils receive heavy metals from natural processes such as pedogenesis, volcanic eruptions and forest fires but anthropogenic inputs including mining, smelting, industrial emissions, transport, fertilizers, sewage sludge and wastewater irrigation are the major cause of contamination [38,41]. Metals are not degraded in soil. Only a small fraction is removed by leaching, plant uptake or erosion and the remainder is retained in the soil profile for decades [27]. The sources and behaviour of the four metals differ. Cd and Pb enter soil mainly through smelting, battery and pigment manufacture, electroplating and industrial dust and although they bind to organic matter and clay, they remain bioavailable for decades [3]. Hg is released mainly from chlor-alkali plants and coal combustion and its bioavailability depends strongly on speciation with methylmercury being far more readily accumulated by biota than inorganic salts [9]. The solubility and bioavailability of As, which originates from mining residues, pesticides and smelting are governed by soil pH, calcium carbonate content and iron and manganese oxides [30].

Rapid and often unplanned industrialization has raised soil metal concentrations well above natural background levels across India. Elevated levels of Cr, V, As, Cd, Zn and Cu have been reported from the Patancheru industrial area, Andhra Pradesh [11]; of Pb, Cr, Cu, Zn, Sr and V from the Pali industrial area of Rajasthan, where untreated textile and chemical effluent is discharged [17]; of Cr from the tannery belt of Jajmau and Unnao, Uttar Pradesh [12]; and of several toxic elements from the Balanagar industrial area, Hyderabad [23] and the Peenya industrial area, Bengaluru [30]. In Rohtak, Haryana, Cd was the metal of greatest ecological risk, with a mean of 7.54 mg/kg and a contamination factor of 37.68, increasing towards industrial zones [29]. Soil, water and crop contamination have also been documented in Sri Ganganagar, Rajasthan [33], central India [6] and the Buddha Nullah drain catchment in Ludhiana, Punjab [4]. These

surveys, however, measure total metal concentrations in soil, which do not indicate the fraction actually available to soil organisms.

Earthworms are widely regarded as useful bioindicators of soil contamination [7,13]. They ingest large quantities of soil and absorb metals through both the permeable body wall and the gut, so tissue concentrations tend to reflect the bioavailable fraction of metals in the surrounding soil [44]. Metals are sequestered in the chloragogenous tissue, which makes earthworms reliable indicators of soil contamination [36]. Accumulation is commonly expressed as the bioaccumulation factor (BAF), the ratio of the metal concentration in earthworm tissue to that in soil.

Field studies show that BAFs differ markedly among metals, species and sites. In forest soils, bioconcentration followed the order Cd > Hg > Pb and the endogeic *Octolasion cyaneum* accumulated more Hg and Cd than the epigeic *Lumbricus rubellus* [8]. Near the Kosovo smelter, the BAF order for two earthworm species was Cd >> Zn >> Cu > As = Pb = Sb [27]. In metal- polluted soils of Hunan Province, China, BAFs were highest for Cd (10.6–18.8) and below 1 for Pb (0.56–0.95) [40]. In Shaoguan, China, earthworms near mining and smelting zones carried Pb, Cd and As burdens up to 242.8, 138.1 and 14.5 times those of reference sites, with Cd BAFs reaching 24.40 [41]. Kokhia et al. (2022) also found that accumulation depends on species and body size [16], whereas in Punjab, Pakistan, Cd-polluted soils did not produce strong Cd or Pb bioaccumulation [32]. Internal regulation can further decouple tissue burden from soil concentration [26] and the ecological group of the earthworm influences metal mobility and exposure [34]. Taken together, these studies show that earthworm bioaccumulation cannot be extrapolated from soil concentrations alone and must be established locally.

Sirohi district in southwestern Rajasthan is a key producer of cement, marble, wollastonite and cement-grade limestone, with industrial clusters concentrated in the Pindwara and Abu Road tehsils. These semi-arid, calcareous soils differ from the soils studied elsewhere and, to our knowledge; no study has examined metal bioaccumulation in earthworms from this region or from industrial soils of Rajasthan. The present study was therefore undertaken to (i) determine the concentrations of Cd, Pb, Hg and As in soil and earthworm tissue at four industrial sites in the Pindwara and Abu Road tehsils over two consecutive years, across the pre-monsoon, monsoon and post-monsoon seasons; (ii) identify the earthworm species present; (iii) calculate the BAF of each metal and compare it among metals, sites and seasons; and (iv) assess the suitability of earthworms as bioindicators of soil metal contamination in the industrial belt of Sirohi district.

Material and methods

Study area

Sirohi district occupies the southwestern part of Rajasthan, between 24°20′–25°17′ N and 72°16′–73°10′ E, with an area of 5,136 km² (Portal, Govt. of Rajasthan). Pali district lies to its north-east, Udaipur to the east, Jalore to the west and Banaskantha district of Gujarat to the south and the district is divided into five tehsils: Pindwara, Abu Road, Reodar, Sirohi and Sheoganj [18]. The terrain is a mixture of plains and hills; the district includes the hill station of Mount Abu and is flanked by the Aravalli ranges, which are rich in minerals. Sirohi is a major producer of cement, export grade marble, wollastonite, cement grade limestone and granite (Portal, Govt. of Rajasthan).

The district lies on the eastern edge of the semi-arid zone of Rajasthan, and its climate ranges from dry to partly subhumid. The topography is undulating in the west and hilly in the north, centre and east, with the Abu-Sirohi hill ranges dividing the district into two parts. Groundwater is scarce: Pindwara and Abu Road blocks are classed as ‘critical’ (development close to 100%) and the Reodar, Sheoganj and Sirohi blocks as ‘over-exploited’.

Soils in the west of the district are sandy desert soils and those in the northwest are hard and rocky, while sandy loam predominates in the study areas. These soils reflect the Aravalli geological substrate and have been modified by decades of industrial and agricultural activity, which makes them a complex matrix for heavy metal dynamics.

Four industrial areas in the Pindwara and Abu Road tehsils were selected on the basis of industrial intensity (Fig. 1). Industry in the district is concentrated in Pindwara tehsil and in the Rajasthan State Industrial Development and Investment Corporation (RIICO) industrial area at Abu Road, where manufacturing and processing units can release heavy metals into the surrounding soil. Sampling was done in surrounding areas of the industrial premises, in all three seasons of two successive years. The four sites were as follows.

Site 1: Wolkem India Limited, Pindwara

Situated at 24.79° N, 73.03° E. A mineral-processing enterprise produces wollastonite, ground calcium carbonate (GCC) and wet ground calcium carbonate (WGCC) slurry.

Site 2: Ultratech Nathdwara Cement Limited, Pindwara

Situated at 24.81° N, 73.09° E. A cement plant manufactures Ordinary Portland Cement (OPC) and Portland Pozzolana Cement (PPC) for the construction sector of northern India.

Site 3: JK Lakshmi Cement Limited, Pindwara

Situated at 24.79° N, 73.04° E. A cement plant produces clinker, Portland cement, Pozzolana cement, concrete mix and ready-mixed concrete.

Site 4: RIICO Industrial Area, Abu Road

Situated at 24.45° N, 72.75° E. The area hosts marble and granite processing, miniature cement plants, HDPE bags, synthetic yarn, mineral grinding, ABS resin, steel, electronics and electrical goods and insulators. Large-scale units produce synthetic yarn and high-tension insulators; medium-scale units produce cement, marble and granite tiles and slabs, HCl, ERW tubes and HDPE pipes and fittings.

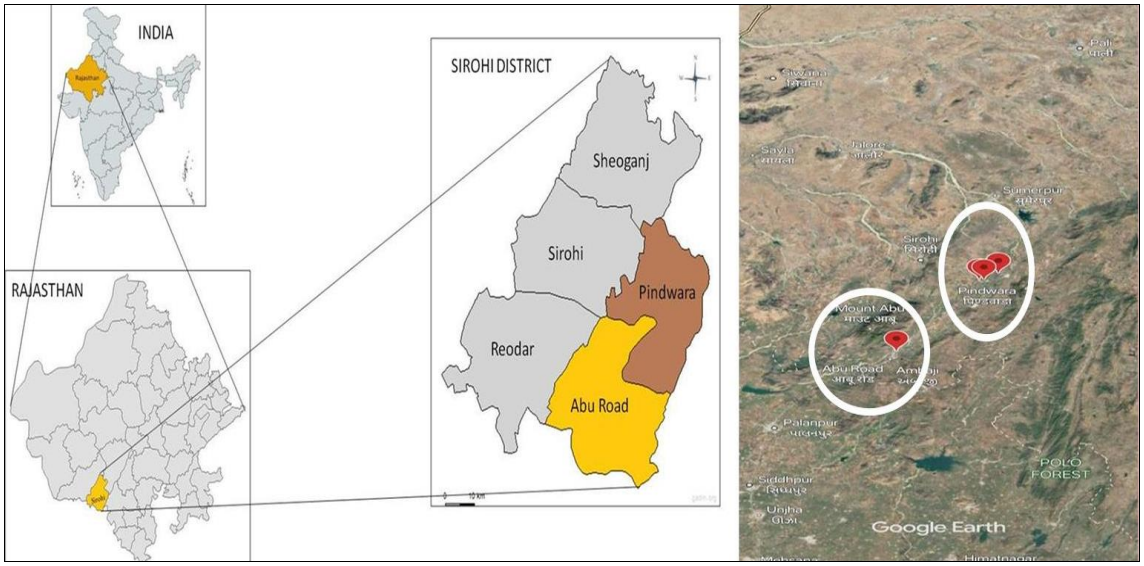


Fig 1: Location of the study area. Insets show Rajasthan within India (top left) and Sirohi district within Rajasthan (bottom left). The central map shows the five tehsils of Sirohi district, with the study tehsils, Pindwara (brown) and Abu Road (yellow), highlighted. The satellite image (right) marks the sampling sites near Pindwara and Abu Road (circled).

Sample collection and preservation

Sampling was carried out from January 2024 to December 2025. Three seasons were selected for the study: pre-monsoon (March–June), monsoon (July–October), and post-monsoon (November–February). Soil samples and adult earthworms were collected randomly from each site during every season.

Topsoil samples were collected from a depth of 5–20 cm in triplicate at each site, air-dried, and stored in appropriately labeled polythene bags prior to laboratory analysis.

Earthworms were collected by digging and hand-sorting from habitats near the industrial areas. Soil samples were excavated from marked 1 m^2 quadrats to a depth of at least 15 cm (30 cm where endogeic species were targeted), and the worms were sorted by hand.

To prevent mortality, collected worms were placed in labeled carry bags containing moist soil and kept cool and shaded until they were processed. For taxonomic work, worms were rinsed with tap water to remove adhering soil particles, narcotized in 30% ethanol until they stopped moving, and fixed in 70% ethanol (ethanol-to-worm ratio >4:1). The fixative was changed after 24–36 h for long-term preservation. Worms used for metal analysis were processed as described below.

Identification of earthworm species

Species were identified from adult specimens because the sexual characters essential for classification are developed only in adults. Body length was measured with a linear scale, preclitellar diameter with a Vernier caliper (130 mm, 0.05 mm graduation) and the dry mass of each individual was determined.

The external characters examined were the shape of the prostomium and clitellum, the segmental position of the clitellum, the presence of penial and copulatory setae, the number of setae on body segments and in pre- and post-clitellar segments, the segmental position of the first dorsal pore and genital markings (spermathecal, prostatic, male and female pores and genital tumescences). Internal characters were the number and position of seminal vesicles, testes, ovaries, spermathecae, prostate glands and nephridia, the position of the gizzard, typhlosole and oesophageal glands and the presence or absence of dorsal and subneural blood vessels.

Identification followed Stephenson (1923), Gates (1972) and Julka (1988) [10,14,35]. All species were confirmed by Dr. Chandra Kanta Mandal, Emeritus Scientist, Zoological Survey of India, Kolkata, and voucher specimens are deposited in the Department of Zoology, Government P.G. College, Sirohi, Rajasthan.

Metal analysis and calculation of the bioaccumulation factor

Soil: Total (aqua regia extractable) metals were determined in 1 g of soil digested with 12 mL of aqua regia (HCl: HNO_3, 3:1) in a closed microwave vessel (after a 15 min open pre-reaction to release CO_2; 1600 W, 10 min ramp to 160 °C, 15 min hold). After cooling to room temperature, the digest was diluted to a final volume of 25 mL with ultrapure water.

Earthworm tissue: Earthworms were washed with distilled water and kept individually in Petri dishes lined with Whatman No. 1 filter paper moistened with a few drops of water at 14°C for 7 days, changing the paper periodically to allow complete voiding of gut contents. They were then killed by freezing, oven-dried at 70°C for 48 h, cooled in desiccator and weighed. After grinding, 0.5 g of each sample was pre-digested for 12 h with 8 mL concentrated HNO_3 and 2 mL concentrated HCl and then gradually heated to 180°C for 2 h in a closed Teflon vessel in a microwave oven. After cooling to room temperature, the digest was diluted to a final volume of 25 mL with ultrapure water.

Metal (Cd and Pb) concentrations in the soil and tissue digests (FCO:1985 method) were determined, following suitable dilution, by atomic absorption spectrometry (ElementAS AAS4139A, ECIL) and for Hg and As (FL/SOP/12-25 based on EPA 7000 B-2007 & Agilent Technology Method 2008) by cold-vapour/hydride-generation AAS. Concentrations were calculated as:

The limits of quantification (LOQ) were 0.1 mg/kg (Cd, Hg), 0.2 mg/kg (Pb) and 0.3 mg/kg (As) in soil and the same in tissue: 0.1 mg/kg (Cd, Hg) and 0.2 mg/kg (Pb) and 0.3 mg/kg (As). Values below the LOQ are reported as BLQ

and no BAF was calculated when either the soil or the tissue value was BLQ.

$$\text{The concentration of heavy metals (mg/kg)} = \frac{\text{Reading from AAS} \times \text{Volume of digest}}{\text{Weight of sample}}$$

The bioaccumulation factor (BAF) was calculated for each metal, site and season as the ratio of the metal concentration in earthworm tissue to that in soil [38]:

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{C substance in the organism}}{\text{C substance in the soil}}$$

Where C is the concentration of the metal, expressed in the same units of mass per unit mass in both matrices.

Statistical analysis

Both physicochemical properties and concentrations of heavy metals in soil and earthworm tissue of all four study sites were compared using paired t-tests and all data were analyzed using SPSS (version 29.0.2) for Windows. To increase statistical power and perform site-wise overall comparisons, heavy metal concentration data (Cd, Pb and As) across all three seasons were pooled for each sampling site (yielding n=12, df=11). A two tailed paired t-test was conducted at a significance level of $\alpha = 0.1$.

Results

Earthworm species

Four earthworm species, representing three families, were recorded across the study sites: *Lampito mauritii* (Megascolecidae), *Octochaetona surensis* and *Octochaetona beatrix* (Octochaetidae), and *Dichogaster bolaui* (Benhamiidae). *L. mauritii* and *O. beatrix* were recorded at Sites 1 and 2, whereas only *L. mauritii* was observed at Site 3. At Site 4, *L. mauritii* occurred together with *O. surensis* and *D. bolaui*.

Table 1: Occurrence and diversity of earthworm species around industrial areas of Sirohi district of Rajasthan

S. No.	Family	Species	Site 1	Site 2	Site 3	Site 4
1.	Megascolecidae	*Lampito mauritii*	+	+	+	+
2.	Octochaetidae	*Octochaetona surensis*	-	-	-	+
		Octochaetona beatrix	+	+	-	-
3.	Benhamiidae	*Dichogaster bolaui*	-	-	-	+
Total	3	4	2	2	1	3

Heavy metal concentrations in soil

Table 2: Concentrations of Cd, Pb, Hg and As (mg/kg) in soil at the four sites in the pre-monsoon, monsoon and post-monsoon seasons. BLQ, below the limit of quantification.

Heavy metal	Season	Site 1	Site 2	Site 3	Site 4
Cd	Pre-monsoon	5.4	3.5	3.25	1.5
	Monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
	Post-monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
Pb	Pre-monsoon	31	31.7	30	21.5
	Monsoon	9.79	< 0.2 (BLQ)	7.85	< 0.2 (BLQ)
	Post-monsoon	< 0.2 (BLQ)	< 0.2 (BLQ)	1.73	0.43
Hg	Pre-monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
	Monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
	Post-monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
As	Pre-monsoon	0.34	2.69	< 0.3 (BLQ)	2.02
	Monsoon	7.85	2.07	0.63	0.68
	Post-monsoon	2.36	1.13	3.36	2.5

Soil Cd was detected only during the pre-monsoon season, at concentrations from 1.50 mg/kg (Site 4) to 5.40 mg/kg (Site 1), while it remained below the limit of quantification (BLQ, <0.1 mg/kg) at every site in the monsoon and post-monsoon seasons. The sharp seasonal drop suggests that cadmium (Cd) present in the soil during the dry pre-monsoon period is effectively mobilized and then diluted or leached out once the monsoon rainfall begins. This finding is consistent with the established increase in cadmium solubility and mobility under higher soil moisture and changing redox conditions.

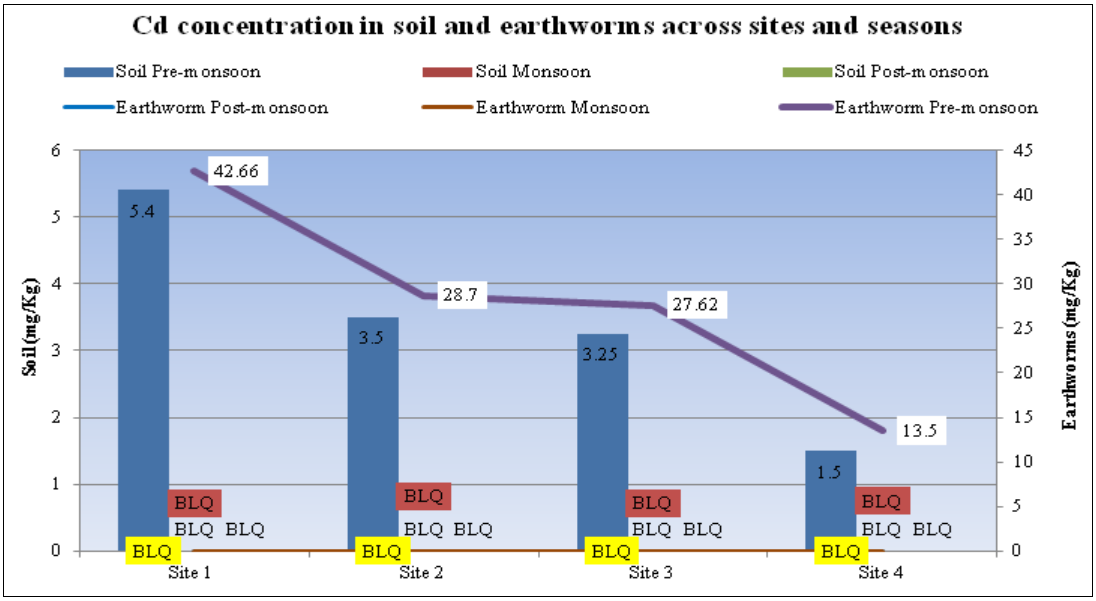
Lead (Pb) showed a comparable seasonal pattern, with the highest concentrations recorded in the pre-monsoon season (21.5–31.7 mg/kg across sites), falling sharply in the monsoon (BLQ to 9.79 mg/kg) and reaching near-negligible or BLQ levels in the post-monsoon season (BLQ to 1.73 mg/kg). The consistent site ranking Site 2 > Site 1 > Site 3 > Site 4 during the pre-monsoon season suggests a common anthropogenic source (e.g., traffic, industrial or agricultural inputs) affecting these sites to differing degrees, with Site 4 being the least impacted. Mercury (Hg) remained below the limit of quantification (<0.1 mg/kg) at all sites in all three seasons, indicating that Hg contamination was either absent or present only at trace levels not detectable by the analytical method used.

Unlike Cd and Pb, arsenic (As), did not show a simple pre-monsoon-dominant pattern. Its concentrations varied between seasons and sites, with the highest value recorded at Site 1 during the monsoon (7.85 mg/kg), while Site 3 was BLQ (<0.3 mg/kg) in the pre-monsoon but rose to 3.36 mg/kg in the post-monsoon. This more irregular seasonal behaviour suggests that As mobility in soil is governed less by a simple washout or dilution effect and more by shifts in redox potential and pH associated with soil saturation during and after the monsoon, which can alter As speciation and solubility.

Heavy metal concentrations in earthworm tissue

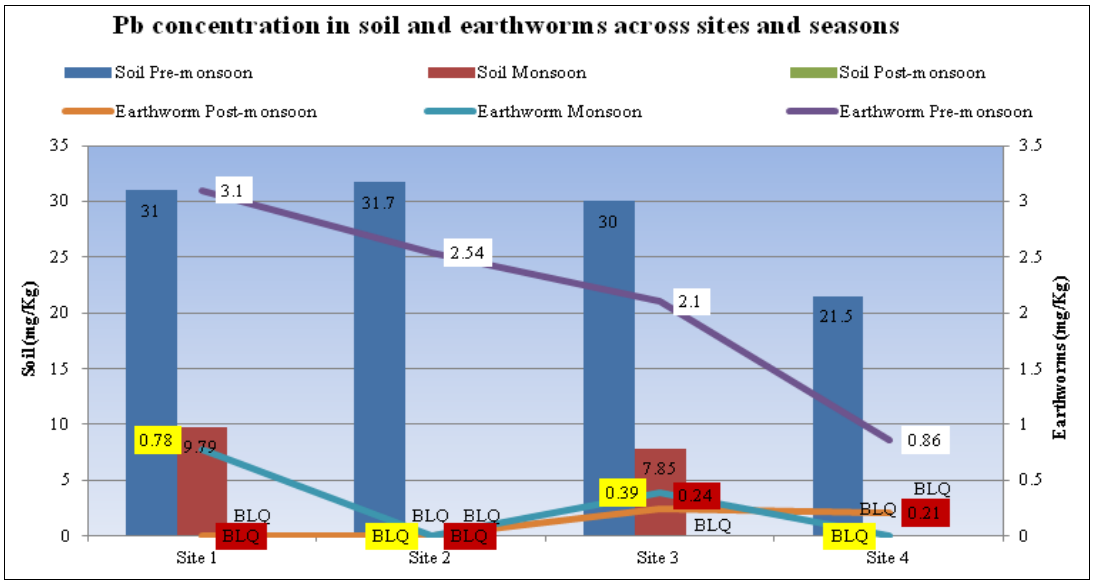
Table 3: Concentrations of Cd, Pb, Hg and as (mg/kg) in earthworm tissue at the four sites in the pre-monsoon, monsoon and post-monsoon seasons. BLQ, below the limit of quantification.

Heavy metal	Season	Site 1	Site 2	Site 3	Site 4
Cd	Pre-monsoon	42.66	28.7	27.62	13.5
	Monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
	Post-monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
Pb	Pre-monsoon	3.1	2.54	2.1	0.86
	Monsoon	0.78	< 0.2 (BLQ)	0.39	< 0.2 (BLQ)
	Post-monsoon	< 0.2 (BLQ)	< 0.2 (BLQ)	0.24	0.21
Hg	Pre-monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
	Monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
	Post-monsoon	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)	< 0.1 (BLQ)
As	Pre-monsoon	0.42	3.5	< 0.3 (BLQ)	2.83
	Monsoon	4.71	1.66	0.44	0.48
	Post-monsoon	1.65	0.9	2.69	1.5



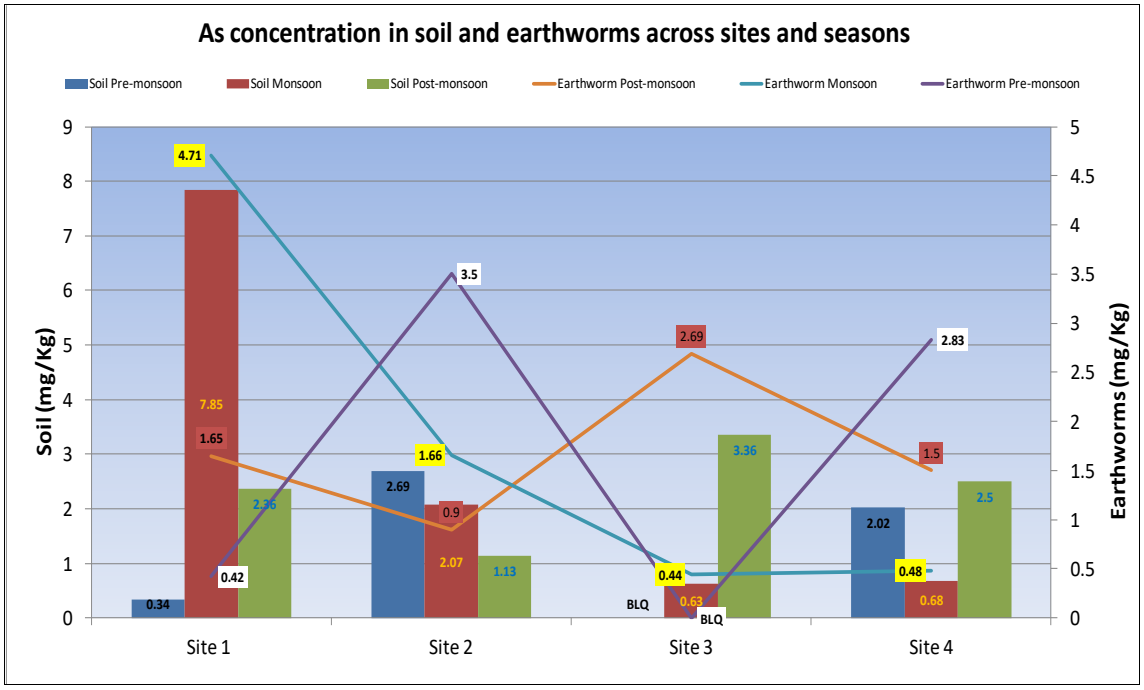
*BLQ= below limit of quantification

Fig 2: Comparative concentration of cadmium (Cd) in soil and earthworm tissue across the four study sites during the pre-monsoon, monsoon and post-monsoon seasons



*BLQ= below limit of quantification

Fig 3: Comparative concentration of lead (Pb) in soil and earthworm tissue across the four study sites during the pre-monsoon, monsoon and post-monsoon seasons



*BLQ= below limit of quantification

Fig 4: Comparative concentration of arsenic (As) in soil and earthworm tissue across the four study sites during the pre-monsoon, monsoon and post-monsoon seasons

Cadmium (Cd) concentrations in earthworm tissues followed a spatial pattern similar to that of soil Cd, although at substantially higher levels. During the pre-monsoon season, tissue Cd concentrations ranged from 13.50 mg/kg at Site 4 to 42.66 mg/kg at Site 1, whereas Cd was below the limit of quantification (BLQ) in both the monsoon and post-monsoon seasons. The mean tissue Cd concentration was approximately 7–9 times higher than the corresponding soil concentration, indicating pronounced bioaccumulation of Cd by earthworms during periods when Cd was detectable in the soil (Tab. 3; Fig. 2).

Pb concentrations in earthworm tissues were substantially lower than those in soil across all seasons, ranging from 0.86 to 3.10 mg/kg during the pre-monsoon period and declining to below the limit of quantification (BLQ) or trace levels after the monsoon. This pattern suggests relatively limited transfer of Pb from soil to earthworm tissue compared with Cd (Tab. 3; Fig. 3).

Hg remained BLQ in earthworm tissue in all seasons and sites, consistent with its absence in soil (Tab. 3).

Earthworm tissue concentrations generally followed the pattern observed in soil, with the highest value again recorded at Site 1 during the monsoon season (4.71 mg/kg). At most other site, season combinations, tissue concentrations were comparable to or somewhat lower than the corresponding soil concentrations (Tab. 3; Fig. 4)

Statistical comparison of heavy metal concentrations

Differences in heavy metal concentrations between the four sampling sites were tested evaluated separately for soil and earthworm tissue using two-tailed paired t-tests (significance level $\alpha = 0.1$). For each pairwise site comparison, paired observations consisted of the concentrations of Cd, Pb, Hg, and As measured across the three seasons (pre-monsoon, monsoon, and post-monsoon), yielding $n = 12$ paired observations ($df = 11$). For all metals, concentrations below the limit of quantification (LOQ) were substituted with half of the respective LOQ.

In soil, the paired t-test showed no significant difference in metal concentrations between most pairs of sites (Site 1 vs Site 2: t = 1.344, p = 0.206; Site 1 vs Site 3: t = 1.229, p = 0.245; Site 2 vs Site 3: t = -0.598, p = 0.562; Site 2 vs Site 4: t = 1.205, p = 0.253; Site 3 vs Site 4: t = 1.651, p = 0.127) (Table 4). A significant difference was observed between Site 1 and Site 4 (t = 2.035, p = 0.067), where Site 1 had higher concentrations (mean 4.76 mg/kg) than Site 4 (mean 2.41 mg/kg).

Table 4: Paired t-test (two-tailed) for heavy metal concentrations in soil between sampling sites

	Comparison	t (df = 11)	p-value	Significance (α = 0.1)
Pair 1	Site 1 vs Site 2	1.344	0.206	ns
Pair 2	Site 1 vs Site 3	1.229	0.245	ns
Pair 3	Site 1 vs Site 4	2.035	0.067	*
Pair 4	Site 2 vs Site 3	-0.598	0.562	ns
Pair 5	Site 2 vs Site 4	1.205	0.253	ns
Pair 6	Site 3 vs Site 4	1.651	0.127	ns

Note: * significant at p < 0.1; ns = not significant. All BLQ values were taken as LOQ/2.

In earthworm tissue, no significant difference was found between any pair of sites (Site 1 vs Site 2: t = 1.095, p = 0.297; Site 1 vs Site 3: t = 1.312, p = 0.216; Site 1 vs Site 4: t = 1.174, p = 0.265; Site 2 vs Site 3: t = 0.958, p = 0.359; Site 2 vs Site 4: t = 1.214, p = 0.250; Site 3 vs Site 4: t = 0.975, p = 0.351) (Tab. 5). Thus, the spatial difference observed in soil between Site 1 and Site 4 was not reflected in metal concentrations in earthworm tissue.

Table 5: Paired t-test (two-tailed) for heavy metal concentrations in earthworm tissue between sampling sites

	Comparison	t (df = 11)	p-value	Significance (α = 0.1)
Pair 1	Site 1 vs Site 2	1.095	0.297	ns
Pair 2	Site 1 vs Site 3	1.312	0.216	ns
Pair 3	Site 1 vs Site 4	1.174	0.265	ns
Pair 4	Site 2 vs Site 3	0.958	0.359	ns
Pair 5	Site 2 vs Site 4	1.214	0.250	ns
Pair 6	Site 3 vs Site 4	0.975	0.351	ns

Note: ns = non-significant. All BLQ values were taken as LOQ/2.

Soil and earthworm tissue concentrations were also compared at each site (Table 6). No significant difference was found at Site 1 (t = 0.063, p = 0.951), Site 2 (t = 0.081, p = 0.937), Site 3 (t = 0.315, p = 0.758) or Site 4 (t = 0.342, p = 0.739), nor when all sites were pooled (t = 0.365, p = 0.717). Overall, the metal concentrations in earthworm tissue were statistically comparable to those in soil.

Table 6: Paired t-test (two-tailed) between soil and earthworm tissue heavy metal concentrations at each site

Site	Soil mean (mg/kg)	Earthworm mean (mg/kg)	t (df = 11)	p-value	Significance (α = 0.1)
Site 1	4.76	4.47	0.063	0.951	ns
Site 2	3.46	3.14	0.081	0.937	ns
Site 3	3.93	2.82	0.315	0.758	ns
Site 4	2.41	1.64	0.342	0.739	ns
All sites pooled	3.64	3.02	0.365	0.717	ns

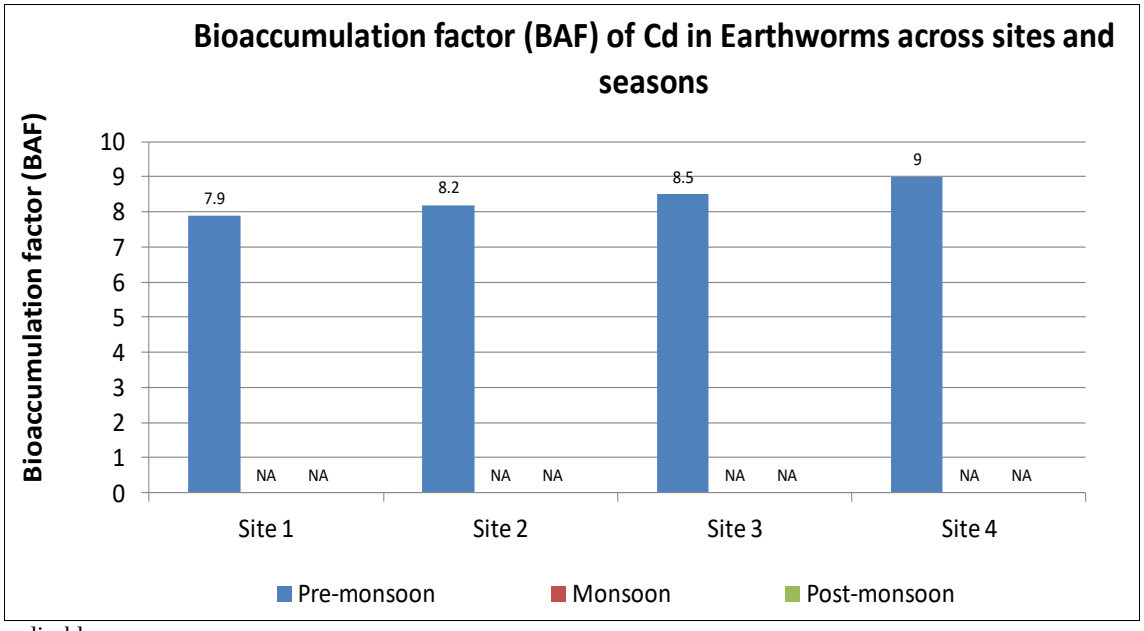
Note: ns = not significant

Bioaccumulation factors

A BAF>1 indicates that the organism accumulates the metal to a concentration higher than that in the surrounding soil, identifying it as a bioaccumulator. Conversely, a BAF<1 suggests limited uptake or partial exclusion of the metal relative to its soil concentration. BAF values were calculated only for those season–metal combinations in which both soil and earthworm concentrations exceeded the limit of quantification.

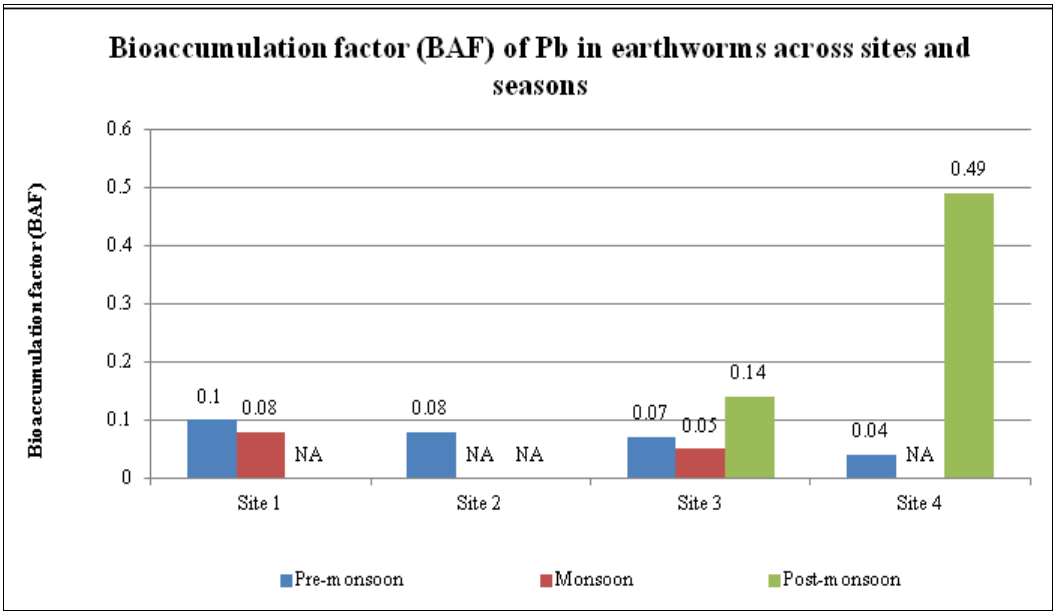
Table 3: Bioaccumulation factors (BAF, tissue/soil) of Cd, Pb, Hg and As at the four sites in the pre-monsoon, monsoon and post-monsoon seasons. A dash indicates that BAF could not be calculated because soil or tissue concentration was below the LOQ.

Heavy metal	Season	Site 1	Site 2	Site 3	Site 4
Cd	Pre-monsoon	7.9	8.2	8.5	9
	Monsoon	-	-	-	-
	Post-monsoon	-	-	-	-
Pb	Pre-monsoon	0.1	0.08	0.07	0.04
	Monsoon	0.08	-	0.05	-
	Post-monsoon	-	-	0.14	0.49
Hg	Pre-monsoon	-	-	-	-
	Monsoon	-	-	-	-
	Post-monsoon	-	-	-	-
As	Pre-monsoon	1.24	1.3	-	1.4
	Monsoon	0.6	0.8	0.7	0.7
	Post-monsoon	0.7	0.8	0.8	0.6



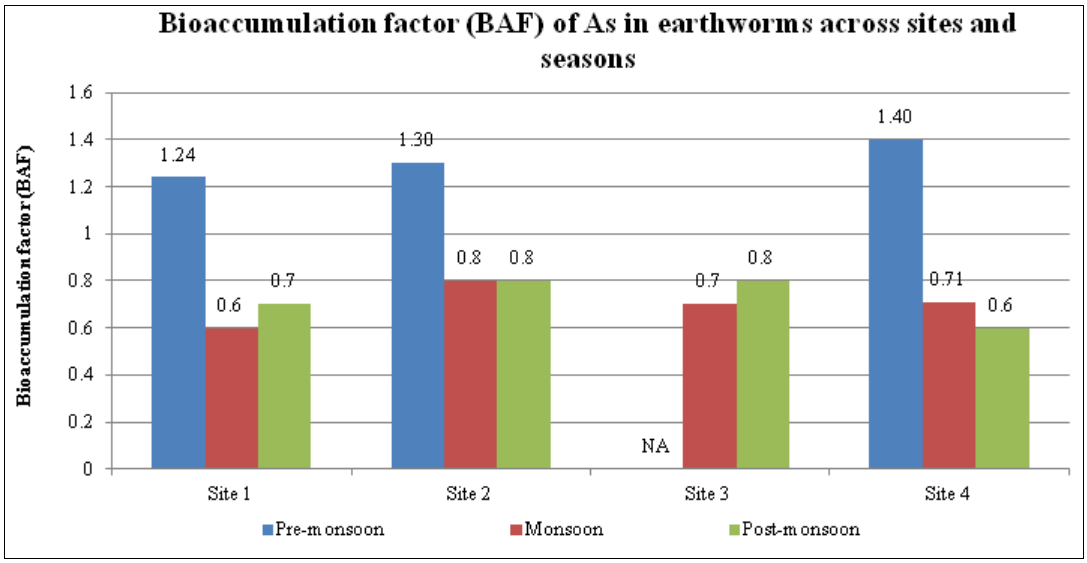
*NA= not applicable

Fig 5: Bioaccumulation factor (BAF) of cadmium (Cd) in earthworms across four sampling sites (Site 1–Site 4) during pre-monsoon, monsoon and post-monsoon seasons



*NA= not applicable

Fig 6: Bioaccumulation factor (BAF) of lead (Pb) in earthworms across four sampling sites (Site 1–Site 4) during pre-monsoon, monsoon and post-monsoon seasons



*NA= not applicable

Fig 7: Bioaccumulation factor (BAF) of arsenic (As) in earthworms across four sampling sites (Site 1–Site 4) during pre-monsoon, monsoon and post-monsoon seasons

Of all the metals analyzed, cadmium showed the highest bioaccumulation factor (BAF) values, ranging from 7.9 at Site 1 to 9.0 at Site 4 during the pre-monsoon season, the only season in which Cd was quantifiable. These values, far above 1, confirm that earthworms act as strong Cd accumulators. Notably, the BAF increased from Site 1 to Site 4 even though the absolute soil and tissue Cd concentrations decreased over the same sites, indicating that earthworms accumulate proportionally more Cd relative to ambient soil levels at the less contaminated sites, a pattern consistent with non-linear, concentration-dependent uptake kinetics rather than simple passive partitioning (Tab. 7; Fig. 5).

Lead showed consistently low BAF values (0.04–0.49) across all sites and the seasons in which it could be calculated, confirming that Pb is largely excluded or poorly assimilated by earthworms relative to its soil concentration, despite Pb being present in soil at higher absolute concentrations than Cd during the pre-monsoon season (Tab. 7; Fig. 6).

No mercury BAF could be calculated for any site or season as both soil and earthworm Hg concentrations remained below the limit of quantification throughout the study (Table 7).

Arsenic showed an intermediate and season-dependent pattern. In the pre-monsoon season, BAF values were slightly above 1 (1.24–1.40), indicating weak bioaccumulation, whereas in the monsoon and post-monsoon seasons, BAF values fell below 1 (0.6–0.8) at all sites, indicating a shift toward exclusion once soil moisture changed following the onset of rainfall (Tab. 7; Fig. 7).

Discussion

Earthworm diversity

A total of four earthworm species were recorded across the study sites in Sirohi district: *Lampito mauritii* was found at

all sites, *Octochaetona beatrix* at Sites 1 and 2 and *Octochaetona surensis* and *Dichogaster bolaui* only at Site 4. Of these, *L. mauritii*, *O. beatrix* and *D. bolaui* had previously been reported from Sirohi district by Kumar et al. (2017; 2021a; 2021b) [19,20,21], whereas *O. surensis* represents a new record for the region.

Cadmium bioaccumulation

Cadmium recorded by far the highest bioaccumulation factor of the four metals studied, ranging from 7.9 at Site 1 to 9.0 at Site 4 during the pre-monsoon season, the only season in which soil Cd was quantifiable at all four sites. These values are somewhat lower than, but of the same broad order of magnitude as, the Cd BAF of 10.6–18.8 reported across four earthworm species in the metal-polluted soils of Hunan province by [39] and the overall rank order recorded in the present study, Cd >> As > Pb reproduces the general bioaccumulation hierarchy Cd >> Zn >> Cu > As = Pb = Sb reported for Kosovo's mining-impacted soils by [27]. This consistency across three independent studies conducted in three different countries and industrial contexts (Indian cement/mineral processing, Chinese metal mining and Kosovan mining) lends considerable weight to the conclusion that Cd's disproportionately efficient bioaccumulation by earthworms, relative to Pb, As and other trace metals, is a genuinely general biological phenomenon rather than an artifact of any one study's particular soil chemistry or earthworm species composition. The most likely physiological basis for this pattern, as discussed in the broader ecotoxicological literature, lies in Cd's strong affinity for binding to metallothionein like proteins within the chloragogenous tissue that lines the earthworm's digestive tract and coelomic cavity. This tissue functions as a dedicated metal sequestration and detoxification organ and Cd appears to be bound and retained by it considerably more efficiently than Pb, which is instead more often sequestered in less soluble, less bioavailable mineral or carbonate associated forms within soil itself before it ever reaches the earthworm gut.

A further, subtler pattern emerged from the site-wise comparison of Cd BAF: BAF increased steadily from Site 1 (7.9) through to Site 4 (9.0), even though absolute soil and tissue Cd concentrations declined over the same site sequence (Site 1 having the highest soil and tissue Cd, Site 4 the lowest). In other words, earthworms at the least contaminated site accumulated a proportionally greater fraction of the bioavailable soil Cd into their tissues than those at the most contaminated site, despite being exposed to a smaller absolute Cd load. This is not consistent with simple linear, concentration-independent partitioning between soil and tissue, but is consistent with the non-linear, concentration-dependent uptake kinetics described by [41], who observed that earthworm internal Cd exposure does not scale proportionally with soil Cd across a wide contamination gradient, likely reflecting a combination of saturable uptake transporters at the gut epithelium and an increasingly effective detoxification/excretion response as internal Cd burden rises. Practically, this finding cautions against using BAF alone, without reference to the absolute tissue concentration, as a proxy for total ecological risk. A higher BAF at the less contaminated Site 4 does not imply greater risk to the earthworm population there than the lower BAF but far higher absolute tissue burden recorded at Site 1.

Lead bioaccumulation

Lead showed consistently and substantially lower BAF values than Cd at every site, ranging from just 0.04 at Site 4 to 0.10 at Site 1 during the pre-monsoon season (0.05–0.08 in the monsoon and 0.14–0.49 in the post-monsoon season, where quantifiable), indicating that earthworms at these sites overwhelmingly exclude rather than accumulate soil Pb. This is directionally consistent with the sub unity Pb BAF of 0.56–0.95 reported by [39]. The pre-monsoon values recorded in the present study are markedly lower still by roughly an order of magnitude. Two complementary explanations likely account for this difference. First, the soils of all four sites in the present study are consistently and strongly alkaline (pH 7.78–8.90), a condition well established in the literature [22] to promote tight Pb immobilization via carbonate precipitation and oxide/hydroxide sorption, likely rendering a smaller bioavailable Pb fraction here than in the presumably less uniformly alkaline soils studied by [40]. Second, despite the very low organic carbon levels recorded at all four sites, what little organic matter is present, together with the abundant mineral carbonate surfaces characteristic of cement and limestone associated soil, may still provide sufficient Pb binding capacity to keep the bioavailable fraction low, particularly given Pb's well documented strong affinity for both organic and carbonate mineral surfaces relative to its affinity for biological uptake pathways. Taken together, these results suggest that whatever ecological risk the elevated soil Pb concentrations documented in the present study may pose through other exposure pathways (for instance to burrowing vertebrates or through direct soil ingestion by other organisms), the risk of Pb entering the soil food web specifically via earthworm tissue bioaccumulation appears to be low at these four sites.

Mercury bioaccumulation

Mercury was not detected in earthworm tissue at any site or during any season throughout the two-year study period. This finding corresponded closely with the near-complete absence of mercury from the soil (Tab. 2), precluding calculation of a meaningful bioaccumulation factor (BAF) for this metal at the study sites. This finding is consistent with the general observation of [9] that Hg BAF in earthworms remains characteristically low in the absence of either a substantial soil Hg source or extensive anecic burrowing activity capable of vertically transporting Hg from deeper, historically contaminated soil layers up into the surface horizons that were sampled in the present study; neither condition applies at the present four sites, where no historical Hg source has been identified and where, even though *L. mauritii* is present as an anecic species with the burrowing capacity that could in principle mobilize deep Hg, there is no deep Hg reservoir for it to access. The absence of any detectable Hg bioaccumulation is, like the absence of soil Hg itself, a reassuring finding and further reinforces the conclusion reached above that Hg does not presently constitute a meaningful contamination or bioaccumulation concern at these four industrial sites, whatever priority it might warrant at other industry types not represented in this study.

Arsenic bioaccumulation

Arsenic occupied an intermediate position between Cd and Pb in terms of bioaccumulation efficiency and showed a

clear seasonal pattern in its BAF that mirrors the seasonal pattern already documented for soil As itself in the soil data (Tab. 2). Pre-monsoon BAF values ranged from approximately 1.24 to 1.4 (that is, above unity, indicating net accumulation) at three of the four sites (As was BLQ in soil and tissue at Site 3), while monsoon and post-monsoon BAF values fell to approximately 0.6–0.8 (below unity, indicating net exclusion) at all four sites. This seasonal switch from net accumulation to net exclusion mirrors the shift in soil As bioavailability with changing redox and moisture conditions proposed by [31] and reinforces the interpretation already advanced above that As mobility and bioavailability in these soils are governed principally by seasonal shifts in As speciation and soil redox state rather than by total soil As load, which itself did not follow a straightforward pre-monsoon-dominant seasonal pattern in the way that Cd and Pb did.

Site and soil tissue comparisons

Paired t-tests (two-tailed, $\alpha = 0.1$) showed no significant difference in heavy metal concentrations in earthworm tissue between any pair of sites ($p = 0.216–0.359$; Table 5), so the Site 1 versus Site 4 contrast seen in soil was not reflected in earthworm tissue. Soil and tissue concentrations also did not differ significantly at any individual site ($p = 0.739–0.951$) or when all sites were pooled ($p = 0.717$; Table 6). This statistical similarity should not be read as evidence that all metals transfer equally from soil to earthworms. This is likely to arise because the strongly accumulated Cd (BAF 7.9–9.0) and the poorly accumulated Pb (BAF 0.04–0.49) offset each other when the metals are pooled, which is why the metal-specific BAF values remain the more informative measure of bioaccumulation.

Comparative BAF hierarchy and its ecological significance

Drawing together the four metal specific discussions above, the overall bioaccumulation hierarchy established by this study, Cd (BAF 7.9–9.0) >> As (BAF 0.70-1.40, season dependent) > Pb (BAF 0.04–0.49) > Hg (not detected), is both internally consistent across sites and externally consistent with the wider international literature reviewed in the introduction and cited throughout this section. The practical significance of this hierarchy, for environmental monitoring and risk assessment purposes is considerable. It demonstrates that, of the four metals prioritized for this study on the basis of their general toxicological importance, only cadmium poses a meaningful risk of entering the soil food web via earthworm bioaccumulation at ecologically significant concentrations at these four sites. This does not mean that Pb, Hg and As are without any environmental significance here; Pb in particular remains present in soil at concentrations (21.5–31.7 mg/kg pre-monsoon) that may still be relevant to other exposure pathways not assessed in this study, such as direct soil ingestion by grazing animals or humans, but it does mean that earthworm tissue burden specifically, as a biomonitoring endpoint, is disproportionately informative for cadmium and comparatively uninformative for the other three metals at these sites. This distinction has a direct practical payoff; it justifies the use of earthworm Cd body burden as a targeted, low-cost, field-deployable biomonitoring tool at these and comparable semi-arid industrial sites in Rajasthan, while cautioning against relying on the same tissue sampling approach as a general-purpose indicator for the full suite of priority heavy metals.

Human and ecological risk implications

Beyond their direct ecological significance for the earthworm populations of these four sites, the bioaccumulation findings summarized above carry implications for the wider soil food web that extend past the scope of the present study's direct measurements. Earthworms are a key prey for a range of insectivorous birds, small mammals and reptiles and are also directly ingested by free-ranging livestock and poultry common in and around the industrial areas surveyed. Where earthworm Cd body burden reaches 13.5–42.7 mg/kg as recorded here, any predator or grazing animal consuming a substantial proportion of its diet as earthworms from these specific sites would be exposed to a materially concentrated Cd source relative to the surrounding soil, by the same seven-to nine-fold factor documented in Cd section above. This trophic transfer pathway was not directly measured in the present study; no predator tissue was sampled and should accordingly be treated as a probable risk pathway flagged by the present findings rather than a risk that has been directly quantified. It is, however, precisely the kind of pathway that the future biomarker and trophic transfer studies should address. By contrast, the very low Pb BAF and non detectable Hg documented in Pb and Hg sections above suggest that the earthworm-mediated trophic transfer pathway is of comparatively little concern for these two metals at the present sites, even though both may still warrant attention via other, non-earthworm-mediated exposure routes such as direct soil or dust ingestion, airborne particulate exposure or dietary uptake through plant material grown in or near the affected soils, none of which fall within the scope of the present earthworm-focused study.

Taken together, the four objectives of the present study converge on a single, internally consistent picture of these four industrial sites in Sirohi district. The soils surveyed are consistently alkaline, non-saline, organic carbon-poor, and carry a heavy metal burden that is highly metal-specific: a seasonally mobile, pre-monsoon-dominant Cd and Pb signature, a complete absence of Hg and a redox-sensitive rather than source-driven As signature. The earthworm community inhabiting these soils comprises a small, semi-arid-adapted species pool dominated by the widely tolerant anecic species *Lampito mauritii*, with community structure – density, richness and evenness alike- shaped far more strongly by soil pH and moisture than by total heavy metal load and following a sharply monsoon-synchronized seasonal cycle that is remarkably consistent across the two years studied. Within this community, bioaccumulation of the four studied metals is highly selective: cadmium is accumulated efficiently and consistently, at levels seven to nine-fold higher in earthworm tissue than in the surrounding soil, while lead is almost entirely excluded and mercury is undetectable throughout. Arsenic occupies an intermediate, season-switching position that reflects the same redox sensitivity already evident in the soil phase data.

The single most actionable conclusion to emerge from this integrated picture is that earthworm cadmium body burden functions at these sites as a genuinely field-deployable biomonitoring indicator; it is analytically straightforward to measure; it responds to soil Cd in a consistent and

interpretable manner across all four sites and both years of the study; and it aggregates a metal that is otherwise difficult to monitor reliably in soil alone because of its sharp seasonal disappearance from the quantifiable soil pool after the pre-monsoon period. By contrast, the same tissue sampling approach would be a poor choice as a general-purpose indicator for Pb, Hg, or As at these specific sites, each of which would require a different monitoring strategy: direct soil sampling timed to the pre-monsoon season for Pb, no dedicated monitoring at all for Hg on present evidence, and season-resolved soil (rather than tissue) sampling, informed by redox and moisture status for As.

It is also worth stressing the methodological robustness underlying these conclusions. The findings summarized above are not based on a single snapshot survey but on a two-year, three-season, four-site sampling programme, with statistical significance formally tested via paired-samples t-tests for every physicochemical parameter and every possible site pair combination and via Pearson's correlation for the relationship between each parameters and earthworm abundance. The high degree of reproducibility observed between the two years of the study, evident most clearly in the near-identical seasonal abundance cycles documented in the abundance data but also apparent in the consistency of site rankings for Cd and Pb across both years, provides confidence that the patterns discussed throughout this paper reflect stable, recurring features of these four industrial sites rather than artifacts of any single year's particular weather or operational conditions. This temporal consistency is itself an important, if easily overlooked, finding; it indicates that a single well-designed monitoring cycle, appropriately timed to span the pre-monsoon to monsoon transition identified throughout this paper as ecologically pivotal, is likely to be sufficient to characterize the broad contamination and bioaccumulation status of a given industrial site of this type without necessarily requiring multi-year replication for routine regulatory or management purposes, even though the two-year design adopted in the present study was valuable for establishing that baseline confidence in the first instance.

Conclusion

Earthworm diversity: Four earthworm species belonging to three families (*Lampito mauritii*, *Octochaetona surensis*, *Octochaetona beatrix*, *Dichogaster bolaui*) were identified across the study sites. *Lampito mauritii* was the only ubiquitous, site-tolerant species. The RIICO Industrial Area (Site 4) supported the richest and most even earthworm community ($H' = 0.99$), where *Octochaetona surensis* was found for the first time, while JK Lakshmi Cement Ltd. (Site 3) supported only a single species, indicating that habitat heterogeneity strongly shapes earthworm community structure across these industrial landscapes.

Heavy metal bioaccumulation: Cadmium was present in soil only during the pre-monsoon season; lead was highest in the pre-monsoon season and declined to BLQ in the monsoon and post-monsoon seasons; mercury remained below the limit of quantification throughout, and arsenic showed a more variable, redox-dependent seasonal pattern. Among the four metals, cadmium exhibited the highest bioaccumulation factor (BAF), ranging from 7.9 to 9.0 across all sites, indicating that it poses the greatest ecological concern. In contrast, lead was largely excluded by earthworms, with BAF values of only 0.04–0.49, whereas arsenic showed an intermediate and season-dependent pattern of bioaccumulation. Paired t-tests ($\alpha = 0.1$) showed no significant differences in heavy metal concentrations between sites in earthworm tissue or between soil and earthworm tissue at any site, while in soil only Site 1 and Site 4 differed ($p = 0.067$). Earthworms therefore act as effective bioindicators of soil cadmium contamination in the cement- and mineral-processing-dominated industrial belt of Sirohi district.

Significant findings

Cadmium was detected in soil only during the pre-monsoon season (1.50–5.40 mg/kg) and fell below the limit of quantification in the monsoon and post-monsoon seasons, whereas lead was highest in the pre-monsoon season (21.5–31.7 mg/kg) and declined sharply thereafter, remaining quantifiable only at Sites 1 and 3 in the monsoon (7.85–9.79 mg/kg) and at Sites 3 and 4 in the post-monsoon season (0.43–1.73 mg/kg).

Mercury remained below the limit of quantification in both soil and earthworm tissue at every site and season throughout the study, indicating no measurable Hg contamination from any of the four industrial sources.

Cadmium concentration in earthworm tissue (13.50–42.66 mg/kg during the pre-monsoon) was approximately 7-9 folds higher than the corresponding soil concentration, indicating evident bioaccumulation of this metal.

Cadmium recorded the highest Bioaccumulation Factor of all four metals studied (BAF 7.9–9.0), confirming earthworms as strong Cd bioaccumulators; lead recorded the lowest BAF (0.04–0.49), indicating near-total exclusion; and arsenic showed an intermediate, season-dependent BAF, exceeding 1 (1.24-1.4) only in the pre-monsoon season and falling below 1 thereafter (0.6–0.8).

Paired t-tests (two-tailed, $\alpha = 0.1$) on Cd, Pb, and As concentrations showed no significant difference between most pairs of sites in soil (the exception being Site 1 versus Site 4, $p = 0.067$) or between any pair of sites in earthworm tissue ($p > 0.2$), and soil and earthworm tissue concentrations did not differ significantly at any site ($p > 0.7$).

Limitations and future work

This study has several limitations. No uncontaminated reference site was sampled, so industrial enrichment relative to local background could not be assessed. Aqua-regia digestion gives pseudo-total soil metal and not the bioavailable fraction, and the LOQs were relatively high for Hg and for tissue Pb and As. Only four sites were studied, and results are descriptive. Future work should include reference sites, species specific BAFs, sequential extraction or speciation of soil Cd and As, lower detection limits for Hg, biomarker responses in earthworms and measurements of trophic transfer to predators.

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