



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 the industrial belt of south-western Rajasthan, India, is scarce. This study determined cadmium, lead, mercury and arsenic in soil and earthworm tissue and calculated bioaccumulation factors at four industrial sites in the Pindwara and Abu Road tehsils of Sirohi district, three of them cement and mineral processing units and one a mixed industry estate. Soil and adult earthworms were collected 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 by atomic absorption spectroscopy. 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 0.04-0.10) 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 (1.3-1.6) 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. These results indicate that earthworms accumulate cadmium efficiently from industrial soils of the region and may serve as a biomonitoring tool for soil cadmium contamination, whereas they were less informative for lead, arsenic and mercury 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 (greater than 20) and a density at least five times that of water (greater than 5 g/cm³), although the term is variously defined and is often used interchangeably with trace elements or potentially toxic elements [1,2,5]. Biologically, 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 technically a metalloid but it is conventionally grouped with heavy metals in soil pollution studies because of its comparable 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, 21, 22], they induce oxidative stress, organelle damage, genetic mutations and histone alterations [36, 40].

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 [35, 38]. 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 [24]. 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, 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 [28].

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 [15] and the Peenya industrial area, Bengaluru [27]. 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 [26]. Soil, water and crop contamination has also been documented in Sri Ganganagar, Rajasthan [30], 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 [41]. Metals are sequestered in the chloragogenous tissue which makes earthworms reliable indicators of soil contamination [33]. 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 $Cd \gg Zn \gg Cu > As = Pb = Sb$ [23]. 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) [37]. 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 [38]. 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 [29]. Internal regulation can further decouple tissue burden from soil concentration [23] and the ecological group of the earthworm influences metal mobility and exposure [31]. 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.

Materials and methods

Study area

Sirohi district lies in the southwestern part of Rajasthan between $24^{\circ}20' - 25^{\circ}17' N$ and $72^{\circ}16' - 73^{\circ}10' E$ and covers 5,136 km² (Portal, Govt. of Rajasthan). It is bordered by Pali district to the north-east, Udaipur to the east, Jalore to the west and Banaskantha district of Gujarat to the south and comprises 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 margin 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 north-west are hard and rocky, 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

Located at $24.79^{\circ} N, 73.03^{\circ} E$. A mineral-processing enterprise producing wollastonite, ground calcium carbonate (GCC) and wet ground calcium carbonate (WGCC) slurry.

Site 2: Ultratech Nathdwara Cement Limited, Pindwara

Located at $24.81^{\circ} N, 73.09^{\circ} E$. A cement plant manufacturing Ordinary Portland Cement (OPC) and Portland Pozzolana Cement (PPC) for the construction sector of northern India.

Site 3: JK Lakshmi Cement Limited, Pindwara

Located at $24.79^{\circ} N, 73.04^{\circ} E$. A cement plant producing clinker, Portland cement, Pozzolana cement, concrete mix and ready-mixed concrete.

Site 4: RIICO Industrial Area, Abu Road

Located at $24.45^{\circ} N, 72.75^{\circ} E$. 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.

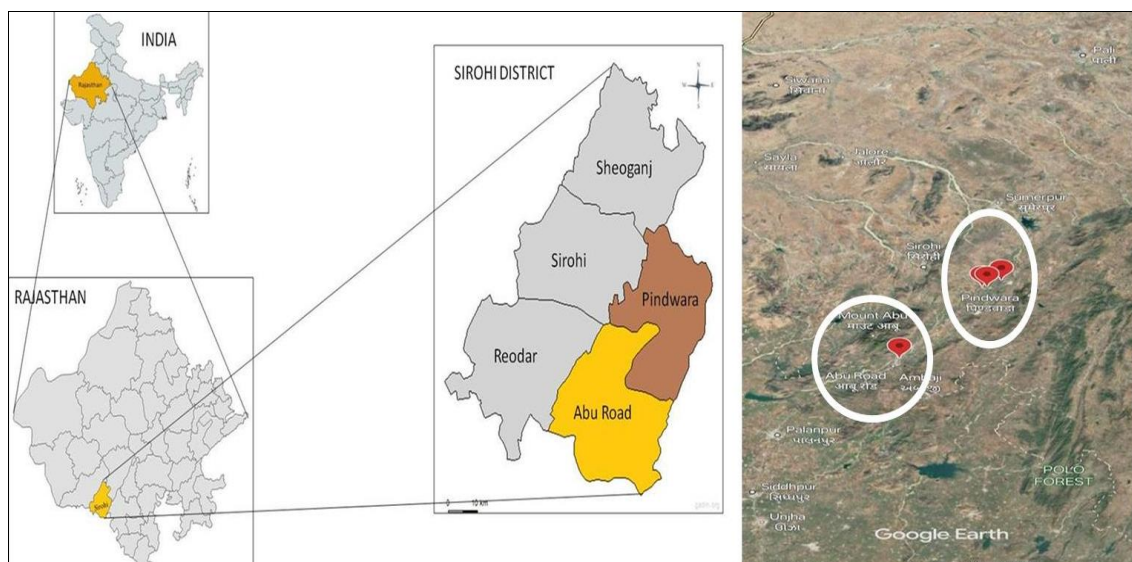


Fig 1: Location of the study area. Insets show Rajasthan within India (top left) and Sirohi district within Rajasthan (bottom left). The central map shows the five tehsils of Sirohi district, with the study tehsils, Pindwara (brown) and Abu Road (yellow), highlighted. The satellite image (right) marks 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 defined: pre-monsoon (March–June), monsoon (July–October) and post-monsoon (November–February). Soil and adult earthworms were collected at random at each site in every season.

Topsoil (5–20 cm depth) was collected in triplicate at each site, air dried and stored in labelled polythene bags until analysis.

Earthworms were collected by digging and hand sorting from habitats near the industrial areas. Soil was excavated from marked 1 m³ plots to a depth of at least 15 cm (30 cm where endogeic species were targeted) and the worms were removed by hand.

Collected worms were placed in labeled plastic bags containing moist soil and kept cool and out of direct sunlight until processing, to avoid mortality. For taxonomy, worms were rinsed with tap water to remove adhering soil, narcotized in 30% ethanol until motionless and fixed in 70% ethanol (ethanol to worm ratio >4:1). The fixative was replaced 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.

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) [10,14,32]. 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 1–2 g of soil digested with 12 ml of aqua regia (HCl:HNO₃, 3:1) in a closed microwave vessel at 1600 W (100% power) for 10 min, until the temperature reached 160 °C.

Earthworm tissue: Earthworms were washed with distilled water and held individually in Petri dishes lined with Whatman No. 1 filter paper moistened with a few drops of water at 14°C for 7 days, with the paper changed periodically to allow complete voiding of gut contents. They were then killed by freezing, oven dried at 70°C for 48 h, cooled in a desiccator and weighed. The dried worms were ground and 100 mg of each sample was pre-digested with 4 ml of concentrated HNO₃ and 2 ml of concentrated HCl for 12 h, then heated progressively to 150°C for 2 h in a closed Teflon vessel in a microwave oven. After cooling to ambient temperature, the digest was made up to 50 ml with ultrapure water.

Metal concentrations in the soil and tissue digests were measured, after appropriate dilution, by atomic absorption spectrometry (ElementAS AAS4139A, ECIL). 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 0.1 mg/kg (Cd, Hg) and 1.0 mg/kg (Pb, As) in tissue. Values below the LOQ are reported as BLQ and no BAF was calculated when either the soil or the tissue value was BLQ.

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 [35]:

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

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

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

Results

Earthworm species

Total four earthworm species from three families were identified at all sites: *Lampito mauritii* (Megascolecidae), *Octochaetona surensis* and *Octochaetona beatrix* (Octochaetidae) and *Dichogaster bolau* (Benhamiidae).

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	<i>Lampito mauritii</i>	+	+	+	+
2.	Acanthodrilidae	<i>Octochaetona surensis</i>	-	-	-	+
		<i>Octochaetona beatrix</i>	+	+	-	-
3.	Benhamiidae	<i>Dichogaster bolau</i>	-	-	-	+
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	0.14	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 quantifiable only in the pre-monsoon season, when it ranged from 1.50 mg/kg (Site 4) to 5.40 mg/kg (Site 1) (Tab. 1), declining in the order Site 1 > Site 2 > Site 3 > Site 4. At all sites it was below the LOQ (<0.1 mg/kg) in the monsoon and post-monsoon seasons.

Soil Pb also peaked in the pre-monsoon season (21.5–31.7 mg/kg; Site 2 > Site 1 > Site 3 > Site 4), fell in the monsoon season (BLQ–9.79 mg/kg)

and was BLQ–1.73 mg/kg in the post-monsoon season.

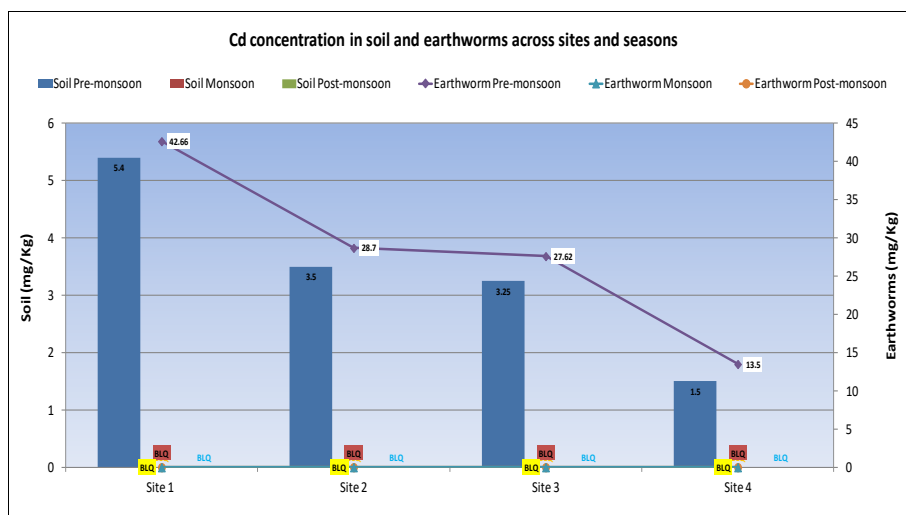
Soil Hg was below the LOQ (<0.1 mg/kg) at all sites in all seasons.

Soil As did not follow this pattern. Concentrations varied among sites and seasons (BLQ–7.85 mg/kg), the maximum occurring at Site 1 in the monsoon season (7.85 mg/kg). At Site 3, As was BLQ (<0.3 mg/kg) in the pre-monsoon season but reached 3.36 mg/kg in the post-monsoon season.

Heavy metal concentrations in earthworm tissue

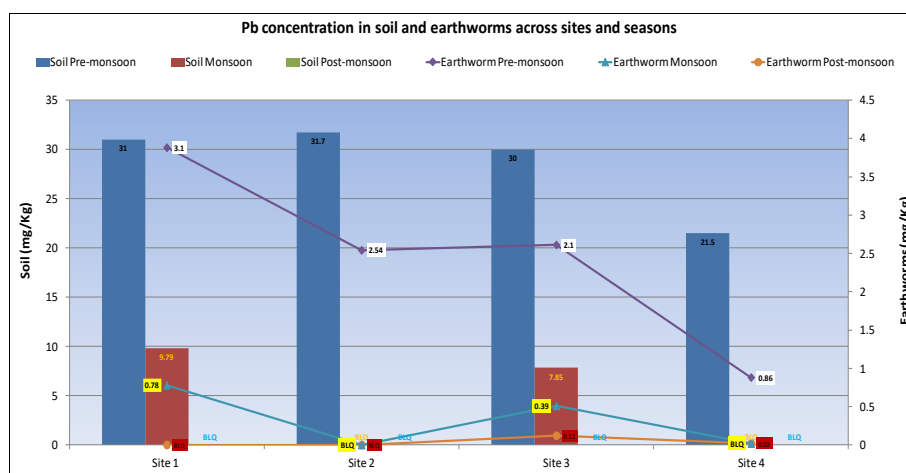
Table 3: Concentrations of Cd, Pb, Hg and as (mg/kg) in earthworm tissue at the four sites in the pre-monsoon, monsoon and post-monsoon seasons. BLQ, below the limit of quantification.

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	< 1.0 (BLQ)	0.39	< 1.0 (BLQ)
	Post-monsoon	< 1.0 (BLQ)	< 1.0 (BLQ)	0.12	0.02
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	0.22	3.5	< 1.0 (BLQ)	2.83
	Monsoon	4.71	1.66	0.44	0.48
	Post-monsoon	1.65	0.9	2.69	1.5



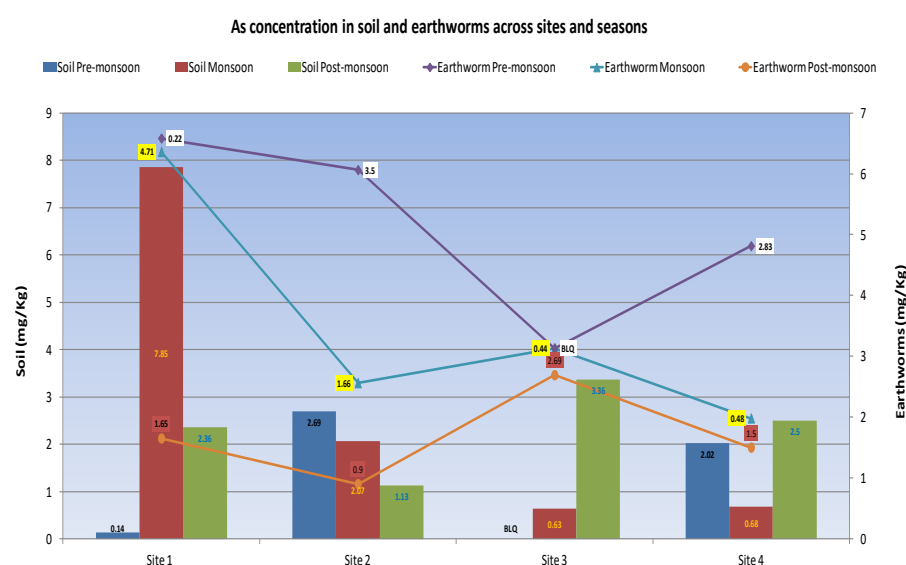
*BLQ= below limit of quantification

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

Tissue Cd was quantifiable only in the pre-monsoon season, when it ranged from 13.50 mg/kg (Site 4) to 42.66 mg/kg (Site 1), following the same site order as soil Cd; it was BLQ in the monsoon and post-monsoon seasons (Tab. 2, Fig. 2).

Tissue Pb was much lower than soil Pb in every season: 0.86–3.10 mg/kg in the pre-monsoon season and BLQ or trace values (≤ 0.78 mg/kg) thereafter (Tab. 2, Fig. 3).

Tissue Hg was BLQ at all sites in all seasons.

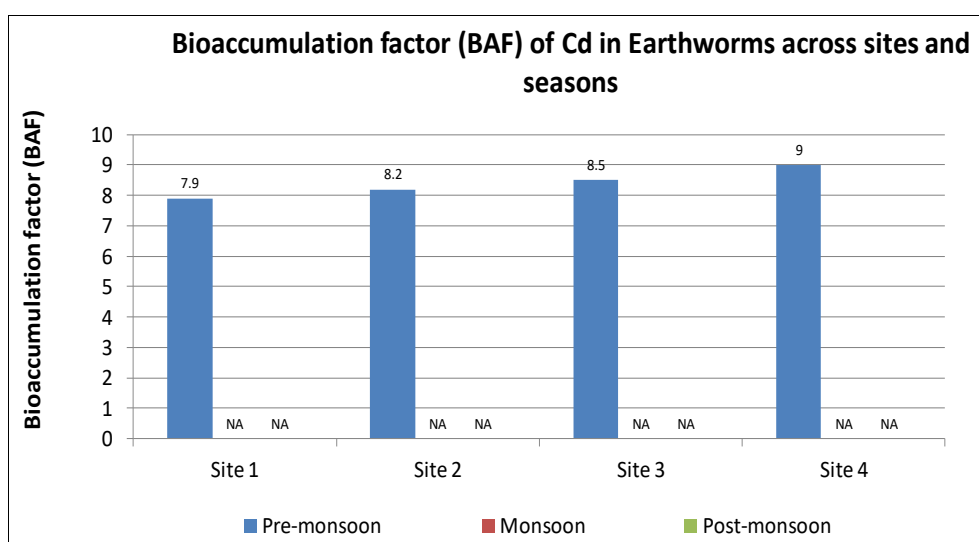
Tissue As broadly followed soil As (Fig. 4), with the maximum at Site 1 in the monsoon season (4.71 mg/kg). Tissue As exceeded soil As in the pre-monsoon season and was lower than soil As in the monsoon and post-monsoon seasons (Tab. 2, Fig. 4).

Bioaccumulation factors

BAFs could be calculated only for the metal–season combinations in which both soil and tissue concentrations were quantifiable (Tab. 3). A BAF >1 indicates that tissue concentrations exceed those in soil (accumulation), whereas a BAF <1 indicates limited uptake or partial exclusion.

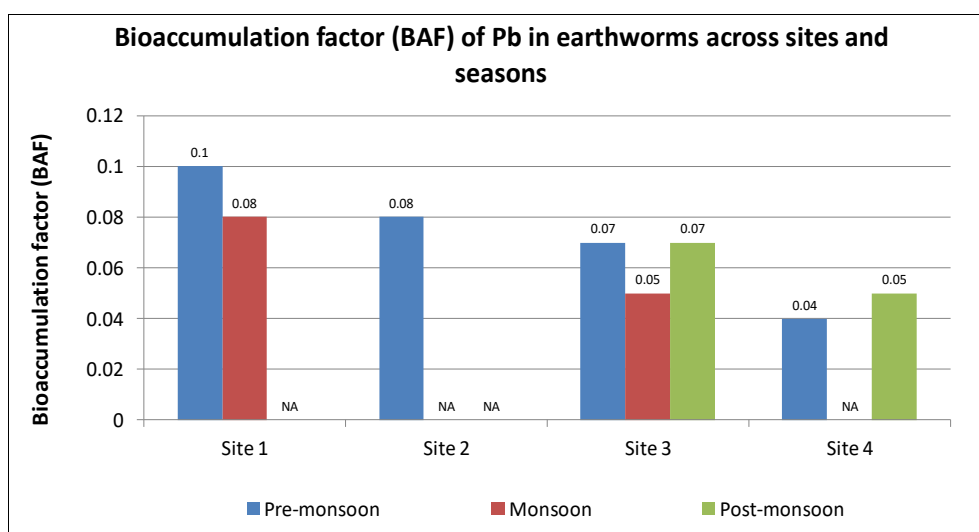
Table 3: Bioaccumulation factors (BAF, tissue/soil) of Cd, Pb, Hg and As at the four sites in the pre-monsoon, monsoon and post-monsoon seasons. A dash indicates that BAF could not be calculated because soil or tissue concentration was below the LOQ.

Bioaccumulation Factor (BAF)					
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	-	-	0.07	0.05
Hg	Pre-monsoon	-	-	-	-
	Monsoon	-	-	-	-
	Post-monsoon	-	-	-	-
As	Pre-monsoon	1.6	1.3	-	1.4
	Monsoon	0.6	0.8	0.7	0.7
	Post-monsoon	0.7	0.8	0.8	0.6



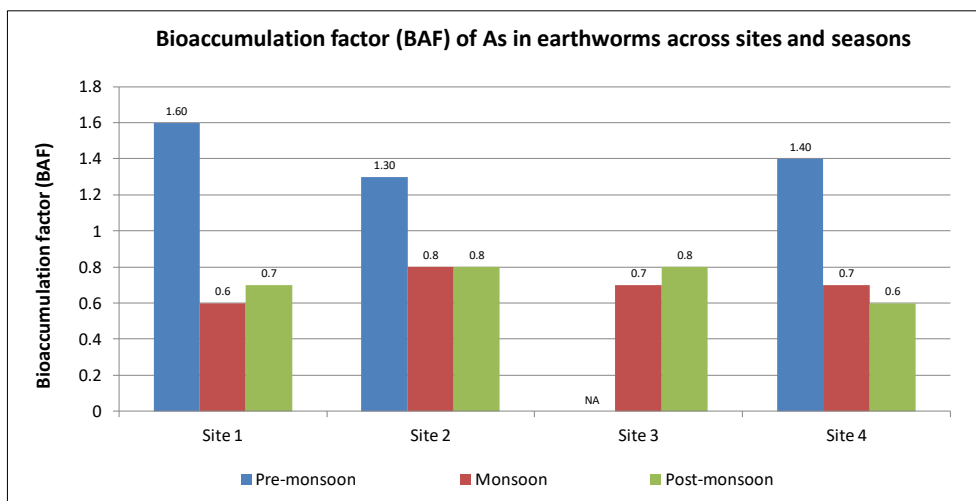
*NA= not applicable

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

Cd had the highest BAFs: 7.9, 8.2, 8.5 and 9.0 at Sites 1, 2, 3 and 4, respectively, in the pre-monsoon season, the only season in which BAF could be calculated (Fig. 5). BAF rose from Site 1 to Site 4 while soil and tissue Cd concentrations fell.

Pb BAFs were consistently low (0.04–0.10) at all sites and in all seasons in which they could be calculated (Fig. 6), although soil Pb exceeded soil Cd in the pre-monsoon season.

Hg BAF could not be calculated because soil and tissue Hg were BLQ throughout.

As BAFs were 1.3–1.6 at Sites 1, 2 and 4 in the pre-monsoon season (not calculable at Site 3, where soil As was BLQ) and 0.6–0.8 at all sites in the monsoon and post-monsoon seasons (Fig. 7). The overall order of BAF was therefore Cd > As > Pb, with Hg not evaluable.

Discussion

Cadmium bioaccumulation

Cd had by far the highest BAF of the four metals (7.9–9.0), showing that earthworms in the industrial belt of Sirohi strongly accumulate Cd from soil. These values are of the same order as, though lower than, the BAFs of 10.6–18.8 reported for earthworms from metal-polluted soils in Hunan Province, China [37] and the Cd BAFs of up to 24.4 recorded near mining and smelting zones in Shaoguan [38]. The ranking Cd > As > Pb also matches the strong preference for Cd over As and Pb reported for two earthworm species near a smelter in northern Kosovo [24] and the Cd > Pb ordering found in forest soils [8]. That similar rankings arise in different countries, soil types and contamination sources suggests that preferential Cd accumulation is a general property of earthworms rather than a site-specific effect. It is usually attributed to the high affinity of Cd for metallothionein-like proteins and its sequestration in the chloragogenous tissue, which serves as a metal storage and detoxification organ [33,41], whereas Pb is largely held in poorly soluble forms in soil. The pattern is not universal, however: Cd polluted soils in Punjab, Pakistan, did not produce strong Cd bioaccumulation [29] and accumulation varies with species and body size [16].

BAF increased from 7.9 at Site 1 to 9.0 at Site 4 while soil and tissue Cd declined over the same sequence, so worms at

the least contaminated site accumulated proportionally more Cd. A decline in BAF with rising soil concentration is typical of regulated uptake and internal detoxification [23] and is not consistent with simple, concentration independent partitioning between soil and tissue.

The BAF range is narrow, however in the absence of replicate variance it may lie within analytical uncertainty; differences in species composition among sites [16,31] could also contribute. The finding is therefore tentative. It does show that BAF should not be used alone as a measure of risk: Site 1 had the lowest BAF but the highest tissue Cd (42.66 mg/kg), and thus the greatest potential dietary exposure for predators.

A further feature was the disappearance of Cd from both soil and tissue after the pre-monsoon season. Several non-exclusive processes could explain this: dry-season deposition of dust and emissions from cement, mineral grinding and transport activities, followed by wash off, leaching and dilution once the rains begin. Because earthworms are active mainly when soils are moist, monsoon and post-monsoon samples may also consist of newly emerged individuals with little prior exposure, whereas pre-monsoon worms had accumulated Cd over a longer period. These explanations were not tested here. They also mean that BAF could be calculated only when soil Cd was above the LOQ, so seasonal comparisons of Cd BAF were not possible.

Lead bioaccumulation

Pb BAFs (0.04–0.10) were consistently low, indicating that earthworms largely exclude soil Pb even though soil Pb (21.5–31.7 mg/kg in the pre-monsoon season) exceeded soil Cd. Sub-unity Pb BAFs have been reported elsewhere (0.56–0.95) [37] but the values here are about an order of magnitude lower. A likely reason is that the soils of the sites are alkaline (pH 7.78–8.90) and calcareous, conditions that promote Pb immobilization through carbonate precipitation and sorption onto oxides and hydroxides [19] and so reduce the bioavailable fraction. Even the small amount of organic matter, together with abundant carbonate mineral surfaces typical of cement and limestone soils, may bind Pb strongly enough to keep it unavailable for uptake. The results therefore suggest that, whatever risk soil Pb poses through

other pathways, such as soil or dust ingestion, transfer via earthworm tissue is low at these sites.

Mercury bioaccumulation

Hg was below the LOQ (0.1 mg/kg) in soil and tissue at every site and season, so no BAF could be computed. This indicates that Hg inputs from the sampled industries are small, but low-level contamination below the LOQ cannot be excluded. Because bioavailability depends strongly on speciation, with methylmercury accumulated far more readily than inorganic Hg ^[9], a more sensitive technique such as cold vapour atomic absorption or fluorescence, ideally with speciation, would be needed to confirm that Hg is truly negligible.

Arsenic bioaccumulation

As was intermediate between Cd and Pb. BAFs were modestly above 1 (1.3–1.6) in the pre-monsoon season at the three sites where they could be calculated and fell to 0.6–0.8 at all sites in the monsoon and post-monsoon seasons, a shift from net accumulation to net exclusion that parallels the irregular seasonal behaviour of soil As. As solubility and bioavailability are governed by soil pH, carbonate content and Fe and Mn oxides ^[28], so seasonal changes in moisture and redox state, which alter as speciation, are a plausible cause; this was not measured here. The Site 1 pre-monsoon BAF (1.6) rests on very low soil (0.14 mg/kg) and tissue (0.22 mg/kg) values close to the LOQ and should be interpreted cautiously. Since the aqua-regia extract estimates total rather than bioavailable As, total soil As is a weak predictor of tissue As in these soils.

Implications for biomonitoring

The BAF hierarchy Cd (7.9–9.0) > As (≤ 1.6 , season-dependent) > Pb (0.04–0.10), with Hg not quantifiable, shows that of the four metals only Cd was concentrated by earthworms to a substantial degree at these sites. Earthworm Cd burden is therefore a sensitive and analytically practical biomonitoring endpoint: tissue Cd was roughly eight times higher than soil Cd, which makes low level soil contamination easier to detect, and pre-monsoon sampling appears to be the optimal window. The same approach is much less informative for the other metals. Pb is better monitored by direct soil sampling in the pre-monsoon season, As by season resolved soil sampling with redox and moisture data, and Hg would require more sensitive analytical methods. This does not make Pb, Hg and As unimportant in the region; Pb in particular is present in soil at concentrations that may matter for exposure pathways not assessed here.

Ecological risk implications

Earthworms are prey for insectivorous birds, small mammals and reptiles and are eaten by free-ranging poultry and livestock. With tissue Cd of 13.5–42.7 mg/kg in the pre-monsoon season, predators feeding largely on earthworms from these sites would encounter Cd at roughly 8-fold the concentration in the surrounding soil. Trophic transfer was not measured, so this is a potential pathway rather than a quantified risk. By contrast, the low Pb BAF and non-quantifiable Hg suggest limited earthworm-mediated transfer of these two metals, although they may still reach organisms through soil and dust ingestion, airborne

particulates or plants grown on the affected soils, none of which were studied.

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.

Conclusions

This two-year study measured Cd, Pb, Hg and As in soil and earthworm tissue at four industrial sites (three cement and mineral processing units and one mixed industrial estate) in Pindwara and Abu Road, Sirohi district and identified four earthworm species from three families with common species *L. mauritii* at all four sites. Cd and Pb were quantifiable in soil only in the pre-monsoon season (Cd 1.50–5.40 mg/kg; Pb 21.5–31.7 mg/kg), Hg was below the limit of quantification throughout, and as varied irregularly. Cd was strongly bioaccumulated (tissue 13.50–42.66 mg/kg; BAF 7.9–9.0), As showed a season-dependent intermediate BAF (1.3–1.6 in the pre-monsoon season, 0.6–0.8 thereafter) and Pb was largely excluded (BAF 0.04–0.10). Earthworms are thus effective bioindicators of soil Cd contamination in this semiarid industrial belt but not of Pb, As or Hg. Pre-monsoon sampling of earthworm Cd is recommended for routine surveillance, supported by soil monitoring for the other metals.

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