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Bioaccumulation and Biotransportation of Heavy Metals Along a Water-Soil-Plant-Firefly (Coleoptera: Lampyridae: Luciolinae) Food Chain: A Case Study from the Southern Gangetic Plains, West Bengal, India

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ABSTRACT

Cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), zinc (Zn) were estimated along a water-soil-plant-firefly food chain for understanding bioaccumulation and biotransformation at the study site, i.e., areas surrounding a surface water body exposed to multidirectional anthropogenic usage (bathing, cleaning, washing) and periodic exposures of domestic sewerages and runoffs from nearby agricultural areas. In the food chain components, the trend of accumulation followed Fe > Zn > Cu > Pb > Cr > Cd. Chromium (Cr) and lead (Pb) levels were higher in sediment (80 ppm and 34.4 ppm respectively). Lower N/P ratio (0.08) together with high pH (7.8± 1.2) in water indicated the eutrophic conditions. Dissolved metals (<1.0 ppm) were lower at the sediment, from soil-water interface some fractions entered the macrophytes and through trophic cascades got bio-accumulated in the study insects, *Sclerotia aquatilis* (Coleoptera: Lampyridae: Luciolinae). Essential heavy metals, Fe, Zn and Cu levels were higher both in fireflies and their associated plants. Higher levels Pb and Cd (0.027 and 0.047 ppm respectively) may result from mixing of agriculture runoffs and road side leachates. Excessive Cr and Pb were marked in *Pistia stratiotes*, 15.1 and 13.4 ppm, followed by *Croton bonplandianum* 3.27 and 2.28 ppm, and *Alternanthera philoxeroides* 2.96 and 4.55 ppm, respectively. However, *Alternanthera philoxeroides* represented the rich source of Zn (649 ppm). Prolonged courtship activities of males in aquatic and macrophytic environment, led to asymmetric deposition of essential heavy metals Fe, Zn and Cu (higher levels in males 458, 149.7, 16.6 ppm, than those in females 208, 121, 13.8 ppm) in fireflies. Higher level of Pb in fireflies (8.88 in males and 5.98 ppm in females) may be sourced by direct or indirect utilisation of *Pista stratiotes* (13.4 ppm), which acted as an efficient nursery ground for larval instars of *Sclerotia aquatilis* and provided vital resource for the completion of their life cycle. This research highlights the utility of fireflies as potential indicator of heavy metal pollution.

INTRODUCTION

Fireflies (Coleoptera: Lampyridae: Luciolinae) could be considered as an entomospecies rich with the aesthetic impacts as well as ecological contributions, consists of around 2,200 species worldwide. In India, presently there are 35 species of Luciolinae fireflies (of those, two species are newly reported to science, *Triangulara frontoflava*, and *Medeopteryx bengalensis*) under 11 different genera (Ballantyne et al. 2019, Ghosh et al. 2021, 2023a, d, 2024 b) have been reported to exist. The sustainable growth, development, and survival of fireflies in their natural habitat gets influenced by various environmental factors like soil and water qualities (Takeda et al. 2006). On the other hand, they are reported to show sensitivity to the fluctuation of environmental parameters (Hazmi & Sagaff 2017). However, in recent era, like many other biotic organisms, fireflies are globally exposed to multifarious hazardous factors associated with anthropogenic activities resulting in habitat loss and degradation, climate change, poor water quality, encroachment of invasive species, uncontrolled exposure of agrochemicals, and light pollution (Fallon et al. 2021, Ghosh et al. 2024a).

Intense agricultural and industrial activities in modern time cause the release and deposition of excessive levels of heavy metals in immediate environmental components of air, water, and soil/sediments. Some of the heavy metals (e.g., Cu, Fe, and Zn) play vital biochemical and physiological functions in plants and animals, and therefore are considered essential in trace amounts. However, when present in excess amount, these exert toxic influence to living organisms, and triggers dangerous effects to human health through their bio transfer and bio transportation via the food chain. Few other metals (e.g., Cd and Pb) however are not reported to provide any essential function, rather those are toxic even at minute concentrations. Continuous interactions among different structural components of soil and water ecosystems harbouring an array of biotic components lead these toxic heavy metals to gain higher mobility and ultimately facilitate their entry to the local food chain. Heavy metals impose their threatening effects on all biotic and abiotic components of ecosystems through bioavailability, biomagnification, biotransformation, and bioaccumulation. The long retention times of toxic substances in the ecosystems, the transport of minute amounts through the food chain amplify the concentration of these metals leading to deterioration of the environment. As a result, the abundance, richness, and diversity of biotic entities get altered. Also, the community attributes and ecosystem functionalities get shifted which even may lead to the local extinction of sensitive species (Soliman et al. 2017).

Water quality in any aquatic ecosystem depends on soil and sediment characteristics. Common sources of exposure heavy metals in aqueous environment include agricultural run-offs, untreated municipal and industrial sewage disposal, modern farming, vehicular exhaustion etc. The soil sediment level acts as the major sink for heavy metals which get deposited by industrial emissions, disposal of high concentration metal wastes, leaded gasoline and paints, overuse of agrochemicals, wastewater irrigation, coal combustion residues, spillage of petrochemicals (Sainz et al. 2004).

Heavy metals impose physiological effects on insects at various levels like changes in protein content in haemolymph, inhibition of enzymatic activities, changes in nucleic acid ratio and alteration of rate of cellular metabolism. All these alterations ultimately get manifested through acute and chronic effects like inhibition of growth, decline in the rate of fecundity and irregularities of natural courses of development. Insects may be exposed to heavy metals either by direct contact through dissolved elements in aquatic systems or contaminated soils or from airborne pollution or atmospheric deposition. Indirectly insects procure heavy metals by consuming plants or other organisms in the local food chain those assimilate nutrition from plant materials and then have sequestered those metals (Vickerman & Trumble 2003).

Fireflies often maintain a biphasic life history strategy (egg, larva, pupa develop and settles in aquatic ecosystem, whereas, adults thrive in semiaquatic and terrestrial ecosystems). Besides, they have shown characteristics diet switching events during their journey from larval (predatory) to adult stage (either nonfeeding or nectarivore or plant sap eater) (Wahida et al. 2018). Larvae of the present study specimen is exclusively aquatic in nature and they maintain carnivorous diet, particularly preying upon the gastropod molluscs (Ghosh et al. 2023c). Their molluscan preys inhabit soil substratum layer and surfaces of macrophytes surrounding aquatic bodies (Fu et al. 2006). In different phases of their life cycle, the fireflies are dependent on different associated plants or display plants (macro phytic vegetation, herbs, shrubs, and trees) (Jaikla et al. 2020). So, there is a chance that fireflies could be exposed to heavy metals from various environmental sources.

Despite being a tiny creature, fireflies have potentially significant impacts on local ecosystem and community of people as well as they can act as pollinators, natural biocontrol agents (as their larvae consume the gastropod species which impose harms to local crops), nutrient recyclers, and ecotourism objects (Ghosh et al. 2023a, c). Particularly, the aquatic species of fireflies undergo a biphasic lifecycle and show dependence on macrophytes (as sites for courtship, mating, breeding, oviposition, perching, resting, larval foraging) and gastropod snails inhabiting aquatic and semi aquatic habitats adjacent to waterbodies. Maintenance of integrity and hygiene of aquatic and terrestrial ecological setups in vicinity of firefly habitats are to be maintained for their sustainable existence. Thus, qualitative, and quantitative monitoring of exposure of heavy metals firefly populations, their habitats and other related food chain components on periodic basis become vital. The findings of these systematic monitoring helps assessing the possible adverse effects of heavy metal contamination on soil, water, and food chain components related to fireflies and pave way for their potential activity as bioindicators against heavy metal pollution (Takeda et al. 2006).

The present research aims to provide baseline information on the sensitivity of fireflies towards the heavy metal exposure in their natural habitats. Besides, certain geochemical investigations related to the aqueous phase and the sediment base influencing the distribution of fireflies has been conducted in the present study site.

In respect of these, present research stands as the pilot study attempting to

- estimate the levels of six heavy metals, Cadmium, Chromium, Copper, Iron, Lead, Zinc, from soil and aquatic samples, from selected macrophytes where the insects commonly observed to reside and develop to maturity and ultimately from the males and females of adult firefly species, *Sclerotia aquatilis* (*Scl. aquatilis*) with the aim of introspecting the trend of bioaccumulation or biomagnification of these heavy metals in the course of locally existing food chain
- analyse certain soil and water qualities as an indicator of habitat quality of local populations of Luciolinae species
- document the baseline idea about the effects imposed by heavy metals on insects

MATERIALS AND METHODS

Study site

The study was conducted in a particular semi-urban ecozone near Diamond Harbour, Kulpi, (22° 04' 44" N, 88° 14' 42" E), with average elevation of 6 metres from the sea level, situated at South 24 Parganas, West Bengal. The water and sediment soil were collected from a surface waterbody in the study site. Mostly the water was stagnant and used for domestic purposes. No direct agricultural or industrial activities were found to be involved, rather the multidirectional anthropogenic usage like cleaning, washing, bathing etc. were common and it was periodically exposed to domestic sewerage as well as agricultural runoffs from nearby agricultural areas and occasional cattle bathing.

Duration of study
Collection of soil, water, plants (leaves from selected display plants), and insects (both larvae and adult males and females) were conducted during the premonsoon season, along the months March to June in the year 2018, characterised with higher temperature (average temperature 31°C) and occasionally accompanied by thunder-storms and rain (average annual precipitation recorded to be around 190 mm; source: https://tcktcktck.org/).
Specimens of study
The firefly species *Sclerotia aquatilis* (*Scl. aquatilis*) belonging to the order Coleoptera, family Lampyridae and subfamily Luciolinae, was selected for heavy metal estimation. It had been represented as one of the abundant species of fireflies in the locality with exclusively aquatic larval phase (Ghosh et al. 2023a).
Collection of water, soil, plants, and insect specimens
Water samples were collected from four different places covering the side and middle sector of the selected water body from the study site. These were made composite by thorough mixing and were subjected to analysis of different parameters and estimation of heavy metals.
Soil samples were taken from sediment layers from edges of the water body by hand operated grab sampler. Four sub-samples were collected from different locations and were mixed for forming a composite soil sample. After sorting and removing the pebbles, stems and organic debris by hand picking, the soil specimens were preserved in plastic bags, oven dried and mixed for composite sample, and finally analysis of the components was performed by the methods following standardized literature (Hazmi & Sagaff 2017). All the environmental factors (Table 1) were estimated with triplicate analysis. The peripheral parts of the waterbody lie under the shade of the canopy of display trees surrounding the pond. Leaves were collected from different **associated plants (APs) and display plants (DPs)** including macrophytes (M), herbs (H), shrubs (S) and trees (T) with higher canopy, namely, *Croton bonplandianum* (H), *Pistia stratiotes* (H), *Cephalandra indica* (H), *Alternanthera philoxeroides* (H, M), *Enhydra fluctuans* (H, M), *Hibiscus rosa sinensis* (S), *Acacia auriculiformes* (T), *Streblus asper* (T). In these plants the insects were commonly observed to reside and develop to maturity. The collected leaves were shattered with a stainless crusher and stored in dry forms in sealed bags before further analysis (Wang et al. 2014).
As the **insect specimens**, the adult males, and females of firefly species, *Scl. aquatilis* were collected by hand picking from soil and plant surfaces.
Six heavy metals, Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Zinc (Zn) were measured from soil and aquatic levels, from leave specimens of selected APs and DPs and from adult males and females of *Scl. aquatilis*.
Assessment of soil and water parameters
Several water (pH, temperature, alkalinity, hardness, TDS, Chloride, Phosphate, Phosphorus, Nitrate, Nitrogen: Phosphorus ratio) and soil parameters (pH, conductivity, sand: silt: clay composition) were estimated as a reflector of overall habitat quality.
Methods for estimating levels of heavy metals (Cr, Cd, Pb, Fe, Zn, Ni, and Cu) in aquatic bodies, soil substratum, macrophytes and males and females of aquatic firefly species, *Sclerotia aquatilis*
The collected surface water sample was filtered through a 0.45-μm filter at the time of collection and then the liquid phase was acidified with nitric acid. During analysis the sample was heated with acid and substantially reduced in volume. The digestate was again filtered and diluted to volume and was prepared for heavy metal analysis. The collected soil sample were oven- dried, ground into finer particles, passed through 2 mm sieve. The plant and insect samples were digested in duplicate with concentrated Nitric acid and Hydrogen peroxide (MacFarlane et al. 2003) at 110 °C for 12h in an electrical oven. On cooling, the samples were redigested with hydrogen peroxide (30%) at 110 °C for 30 minutes. After the removal of remaining Nitric acid by heating, the samples were centrifuged for 2

minutes at 1500 rpm and then subjected to dilution by distilled water and were stored at 4 °C until analysis. These samples were then subjected to digestion and heavy metal analysis (USEPA 2005). Heavy metals from these samples were estimated by Atomic Absorption Spectrophotometer (AAS, model: Agilent Technology 200 Series AA).

Statistical analyses

During heavy metal analysis, following the normality test (Shapiro Wilk test), the sex best differences in fireflies were statistically analysed by one sample t test to detect that whether there was any significant difference of heavy metal contents between males and females. Kruskal Wallis test helped to find out that whether any significant difference exists among heavy metal contents in different associated plants of the study insect. Microsoft Excel, Microsoft® Excel® 2019 MSO16.0.14228.20200, 64-bit and PAST, version 4.03 software were utilised.

RESULTS

Heavy metals in different components of food chain at the study site

One sample t test had shown that there was no significant difference of heavy metal contents between males (t-= 1.42 at p= 0.22) and females (t= 1.64 at p= 0.16), thus highlighting a probability that individualistic physiological set up of males and females does not influence the property of heavy metal accumulation in the study species. Kruscal Wallis test helped to find out that no significant difference exists among heavy metal contents (H= 2.9 at p= 0.9) in different associated plants of the study insect.

Status of soil and water parameters of the study site

The prevailing environmental conditions with respect to soil and water parameters at the study site were documented (Table 1). It depicted that the water medium was alkaline in nature (pH 7.8± 1.2) possibly due to presence of appreciable amount of alkalinity (425± 24.2 mg/l) contributing substances (e.g., detergents). Total hardness (250± 43.5 mg/l) content was about half of that of alkalinity values implying the presence of significant amount of sodium and potassium in dissolved state either in the form of carbonate or bicarbonate. Lower hardness value also signified that dissolved calcium and magnesium remained in non-carbonate forms associated with chloride and sulphate anions. Hardness of water is a factor which modifies the effectiveness of the toxicity of heavy metals towards aquatic organisms. The bioavailability of Cd, Cu, Pb and Zn in freshwater typically decreases as the hardness increases. Toxicity of Pb, particularly to the early developmental stages of aquatic organisms like larvae of *Sclerotia aquatilis* is highly influenced by water quality parameters like pH, salinity, and hardness (Girgin et al. 2010). Dissolved available nutritional elements of nitrogen and phosphorous (N/P) were found to be present in extremely low ratio (0.08± 0.03 mg/l). Lower N/P ratio (0.08) together with high pH (7.8± 1.2) in water is an indicator of accelerated rate of eutrophication. Higher total dissolved solids (TDS) (732± 26.6 ppm) as well as chloride content (237± 11.8 mg/l) might be ascribed to the gradual evaporation of water during premonsoon season and by various anthropogenic activities or mixing of domestic sewage in the system.

The nature of soil was almost neutral (pH 7.1) (Table 1) with potentiality to release water soluble mineral components (salts of Na, K, Ca, Mg) into the overlying layer of water. Macrophytes imbibe the minerals and gain higher productivity resulting in increased rate of utilisation of those plant parts by study insects. The plants often play role as the associated plants (APs) and/ or display plants (DPs) (Ghosh et al. 2023b). Clay- silt- sand composition of soil sediment (sand: silt: clay= 38: 25: 37) with higher levels of minute particles with higher surface area facilitates the absorption of huge organic matter together with heavy metals. Soil sediment layer acts as major sink for heavy metals like Pb, Cr, Zn, Cd, Cu. Through microbial degradations, heavy metals find their ways to overlying water and ultimately gets bioavailable to the study insect via the macrophytic plants. Heavy metals in soil are reported to enhance the production of hsp 70, a stress indicator protein in insects which are exposed to those toxic metals (Zhou et al. 2008).

Table 1. Physicochemical parameters of water and soil at the study site.

Physicochemical parameters of water	Average ± SE	Physicochemical parameters of soil	Average ± SE
pH	7.8± 1.2	pH	7.1
Temperature (°C)	31± 2.4	Temperature (˚C)	28± 2.44
Total alkalinity (as $CaCO_3$) mg/ l	425± 24.2	Moisture content	25± 0.63
Total hardness (as $CaCO_3$) mg/ l	250± 43.5	Organic Carbon (%)	3± 0.66
TDS (ppm)	732± 26.6	Conductivity(ɥs)	31
Chloride (as Cl^-) (mg/ l)	237± 11.8	Sand (%)	38
Phosphate (as PO_4) (mg/ l)	9.6± 0.8	Silt (%)	25
Phosphorus (as P)	3.13± 0.5	Clay (%)	37
Nitrate (as NO_3-N) (mg/ l)	0.78± 0.33		
N/P ratio (mg/ l)	0.08± 0.03		

Table 2. Levels of occurrences of essential and nonessential metals in the aqueous medium and soil sediment at the study site in comparison with references to the baseline level of metals.

Heavy metals		Aqueous medium Concentration (ppm)	Sediment base amount (ɥg/gm)	Continental shale (ppm) (Turekian & Wedepohl 1961)	Continental crust (ppm) (Wedepohl 1995)	Clean sediments (ppm) (DR 1995)	Pre industrial reference level (ppm) (Hakanson 1980)
Non-essential metals	Cadmium (Cd)	0.047	1.5	0.30	0.20	1	1
	Chromium (Cr)	0.043	80	90	196	50	90

	Lead (Pb)	0.027	34.46	20	16	50	70
Essential metals	Copper (Cu)	0.044	20	45	70	35	50
	Zinc (Zn)	0.015	30.4	95	65	100	175
	Iron (Fe)	0.105	4.24	67.5%	42.2%	-	-

Heavy metal contents in the associated plants (APs) and display plants (DPs) and the study insect, *Scl. aquatilis*

The essential metals like Fe, Zn and Cu were found at quite higher levels in plants, as well as in males and females of fireflies (Tables 3 and 4). Bioaccumulation of metals has been noted to follow a cascading process with higher concentrations in organisms at higher trophic levels (Soliman et al. 2017). In present scenario, natural prey item of *Scl. aquatilis* larvae significantly consisted of gastropod molluscs (Ghosh et al. 2023c) which are known to possess high concentrations of heavy metals (Zhou et al. 2008, Zhang et al. 2021). Thus, they are capable of playing vital role in transferring heavy metals to fireflies.

In the present research, among the plants (Table 3), Cr and Pb were recorded considerably higher in *Pistia stratiotes* (15.1 and 13.4 ppm) followed by *Croton bonplandianum* (3.27 and 2.28 ppm) and *Alternanthera philoxeroides* (2.96 and 4.55 ppm). Hexavalent Cr (Cr VI) has shown a tendency of accumulation in plant roots of *Alternanthera philoxeroides* (Trumble & Jensen 2004). Natural level of occurrence of Cr in plants has been prescribed as 0.2 ppm only which has been exceeded in cases of all the plants. Hence excess values would have been responsible for drastic effects in these plants. On the other hand, Zn was higher about more than 5 times in *Alternanthera philoxeroides* (649 ppm) in comparison to the natural concentration as mentioned by Pais, 1997. While the other plants contained lesser amount of Zn than the natural level. Thus, *Alternanthera philoxeroides* could be considered as a potential source of this most vital metal Zn in this environment.

The limit values of Cd for aquatic life ranges between 0.00066 to 0.002 mg/l. Limit of Pb, permissible for aquatic organisms ranges between 0.0013 to 0.077 mg/l. The limit values of Cu fall between 0.0065 to 0.021mg/ l (USEPA 2005). Fe, though a micronutrient, may become toxic when present in excess dose (Soliman et al. 2017). In case of fireflies (Table 4), in males, the levels of all these heavy metals were found to be higher than those in females reflecting the prolonged courtship activities of males in aquatic and vegetation-based environments. Among the non- essential toxic metals only the Pb was found in quite higher levels in *Scl. aquatilis* individuals (5.98 ppm in female to 8.88 ppm in male). Such an enhanced level of Pb in insects indicates the uptake of resource materials either from aquatic components as well as from their floral partners (e.g., *Pista stratiotes*). Alternative sources of Pb uptake (e. g., from soil-based nutrients, larval predatory diet etc.) may also be present.

Table 3. Levels of heavy metals in associated plants (APs) of the firefly species
[Natural content (mg/ kg or ppm) of metal in plants Cd- Nil, Cr-0.2, Pb-Nil, Cu-14, Zn-100, and Fe-200 (Pais 1997)]

Levels	Parts of associated plants (APs)

of heavy metals (mg/ kg or ppm)		*Alternanthera philoxeroides*	*Cephalandra indica*	*Hibiscus rosa sinensis*	*Streblus asper*	*Croton bonplandianum*	*Enhydra fluctuans*	*Acacia auriculiformes*	*Pistia stratiotes*
Non-essential metals	Cadmium (Cd)	0.69	Nil	Nil	0.376	0.169	0.135	0.073	0.82
	Chromium (Cr)	2.96	0.717	1.79	1.07	3.27	0.73	0.493	15.1
	Lead (Pb)	4.55	1.53	3.41	2.95	2.28	3.27	2.92	13.4
Essential metals	Copper (Cu)	12.9	3.41	6.46	20.4	8.29	5.49	7.82	16.9
	Zinc (Zn)	649	27.7	22.4	32.6	35.5	25.1	29.4	79.8
	Iron (Fe)	1096	175	953	192	426	139	101.8	11945

Table 4. Levels of heavy metals in males and females of fireflies.

Heavy metals		**Levels of heavy metals (mg/ kg or ppm)**	
		Fireflies	
		Males	Females
Non-essential metals	Cadmium (Cd)	0	0
	Chromium (Cr)	0	0
	Lead (Pb)	8.88	5.98
Essential metals	Copper (Cu)	16.6	13.8
	Zinc (Zn)	149.7	121
	Iron (Fe)	458	208

Effects of heavy metals on the study insects

Heavy metals that play essential roles only in trace amounts include Cu, Fe and Zn. Those are essential as are involved in different biochemical and physiological functions in biotic organisms (Marschner 2012). However, if present in excess, these turn out to become toxic to living organisms through bio transfer and bioaccumulation via the food chain (Chaffai and Koyama 2011). Contrastingly, other metals (e.g., Cd, Cr and Pb) are not known to have any essential function, and are known to impart toxic effects even at low concentrations (Nordberg et al. 2014). Cd being chemically alike to Zn, the essential trace metal, often shows a tendency of getting accumulated in different parts of plants, like roots, shoots, pods, and flowers etc. (Hladun et al. 2015). Zn and Cd both show pro-oxidative nature (Kafel et al. 2014).

The study insects were significantly vulnerable to the potential risk factors imposed by heavy metals as the study was conducted during premonsoon, which is the period overlapping the prebreeding stage of the fireflies. In the present study site, agrochemicals were the major source of heavy metals. Metabolic disorders and oxidative stress have been recorded to be the most potential ones among the significant effects of heavy metal toxicity on physiology of plants and insects (Table 5). In insects' body heavy metals get deposited in alimentary canal, fat bosies, haemolymph, integument etc. Sometimes, chronic toxicity caused by prolonged exposure of heavy metals gets manifested in the altered patterns of foraging, locomotory and reproductive behaviours of insects (Mogren & Trumble 2010).

Results of Pearson correlation analyses between bioaccumulation contents of heavy metals in soil/water/ associated plants and those of firefly (males and females) have provided stronger evidence for bioaccumulation trends. The heavy metal contents of aqueous medium had shown significantly positive correlation ($p \leq 0.05$) with those of the macrophytes *Cassia* sp., *Barbostylis densa*, *Streblus asper*, *Croton bonplandianum*. Again, Bioaccumulation of heavy metals in insects (both in male and female individuals) had shown significantly positive correlation ($p \leq 0.05$) to those of macrophytes *Alternanthera philoxeroides*, *Cassia* sp., *Barbostylis densa*, *Streblus asper*, *Croton bonplandianum, Therpesea populnea*, *Acacia auriculiformes*, *Pistia stratiotes*.

Table 5. Significant effects of heavy metal toxicity in physiology of plants and insects.

Heavy metals		**Environmental sources**	**Significant physiological effects**	**References**
Non-essential metals	Cadmium (Cd)	Chemical fertilizers, sewage sludges, mining, and industrial wastes	interference in protein synthesis; alterations in signalling receptor activity; affects CO_2 fixation by inhibiting photosynthesis and transpiration; affects cellular metabolism by replacing Zn in many enzymatic reactions	Kafel et al. 2014, Hladun et al. 2015
	Chromium (Cr)	Industrial factories	increases oxidative stress; damages DNA, proteins, and membrane lipids disrupting cellular integrity and functions	Bravo et al. 2017
	Lead (Pb)	Agrochemicals, factory wastes, automobile, and air plane exhausts	Not very much toxic to plants but remain accumulated in leaves, shoots, roots of roadside plants; produces various deleterious effects on the hematopoietic, reproductive systems through increased oxidative stress	Hladun et al. 2015
Essenti al	Copper (Cu)	Industrial discharge	creates oxidative stress; damages reproductive tissues; with various sublethal effects	Hladun et al. 2015

	Iron (Fe)	Industrial discharge	generates an array of harmful free radicals; corrosive effect on the gastrointestinal tract and cellular damage; increases lipid peroxidation with resulting membrane damage to mitochondria, microsomes, and other cellular organelles; respiratory damage	Albretsen 2006
	Zinc (Zn)	Industrial discharge	Essential roles: enzyme activities, anti-oxidant activities, arrest apoptosis and maintains cellular integrity Toxic effects: disturbs cellular environment by increasing oxidative stress	Kafel et al. 2014

DISCUSSION

Overall scenario of levels of heavy metals at the study site

Various anthropogenic activities including agricultural runoff, roadside washery, domestic sewerage, automobile exhausts and aerial dust fall remained responsible for the exposure of aqueous and sediment layers of the selected waterbody in the study site to heavy metals. It had been reflected that plants are not being systematically utilized to nullify the effects of heavy metals by absorbing these, rather they act as potential sink for heavy metals which they store as they get exposed to these and in turn release the metals in food chain by furnishing bioavailability. This led to the chance of imposing varying levels of toxic effects to the insect of study manifested in their population structure and ecobiological attributes.

Influence of abiotic parameters on heavy metal toxicity levels

Anthropogenic activities like bathing and washing using soap and detergents remain responsible for contributing extensive amount of dissolved phosphorous content relative to nitrogen leading towards higher level of alkalinity. Higher level of eutrophication promoted algal bloom and often the macrophytic growth found to offer shelter to the larvae of study insects.

In an eutrophicated waterbody, the excessive nutrient enrichment leads to algal bloom, which can absorb heavy metals from the water column and heavy metals get concentrated within the algal biomass. When the algae degenerate, the metals get released back into the aquatic system and become potentially more bioavailable. Algal blooms also cause fluctuations in diurnal pH levels influencing the solubility and bioavailability of heavy metals (Huang et al. 2023).

Sometimes previously bound heavy metals become available in water column as excess nutrients act as stimulating agents for the decomposition of organic matters in sediments (Jaiswal & Pandey 2020).

Phosphorus, one of the key nutrients in eutrophication, often complexes with heavy metals, and potentially increases their solubility and bioavailability (Wang et al. 2024).

Last but not the least, eutrophication leads to the alteration of microbial communities in the water column and sediment levels. It causes the transformation and bioavailability of heavy metals through reduction and oxidation of metallic components (Yao et al. 2022).

Higher alkaline condition in water promotes the precipitation of dissolved heavy metals on the underlying sediments. It results in lower content of heavy metal concentration in aquatic phase and higher content in the sediment layer, as evident from the study. A comparison of heavy metal levels from various sources suggested the presence of higher amount of Cd (1.5 ppm), Pb (34.46), and Cr (80 ppm) in sediment. In water (Table 2), higher levels of toxic metals Pb and Cd (0.027 and 0.047 ppm respectively, whereas in unpolluted freshwater the average Cd content is < 0.0001 to < 0.0006 ppm, as described by IPCS 1992) were found to be present resulting from the mixing of agricultural runoff and road side leachates.

Bioavailability of heavy metal contents in the study insects through food chain components (water, soil, APs, DPs, prey molluscs)

The distribution of the local populations of *Scl. aquatilis* is found to be relatively higher near aquatic sites in comparison to the nearby paddy fields or grassland or roadside vegetation patches. Thus, it could be emphasized that the species, *Scl aquatilis* preferred the habitat patches adjacent to aquatic sites for the availability of nutritional and other resources which support their development, maintenance, and survival. The presence of toxic heavy metals was observed in water, soil sediment, and the parts of macrophytes and other vegetations often playing role as APs and DPs (Tables 2, 3). Thus, probability exists that the study insects could get exposed to the risks of heavy metal contamination from their environmental resources. On the other hand, for the insects, there is scope of nourishment, maintenance, and above all, the nutritional enrichment by procuring heavy metals like Cu, Fe and Zinc from water, sediment layer, and plants (Soliman et al. 2017).

In general, the variation in metal levels followed the order of Fe > Zn > Cu > Pb > Cr > Cd in associated plants and males and females of fireflies indicating their respective preference of accumulation in the organisms. The plants often exhibit higher tolerance to heavy metals withstanding the hazardous effects of cellular disintegration, damage of respiratory and photosynthetic activities, growth inhibition etc. imposed by those metals (Zhou et al. 2008).

Scope of utilisation of insects as potential bioindicators against heavy metal pollution

In insects acute and chronic heavy metal toxicities are reported to result in growth inhibition, retarded course of development, reduced fecundity, larval mortality etc (Azam et al. 2015). Report of synergistic effect of combined exposure of the pesticide (e. g., Chlorpyriphos) and the heavy metal (e. g., Cd) has been reported to alter the metabolic activity levels by decreasing the mitochondrial potential and inducing the production of reactive oxygen species (Singh et al. 2017).

Insects possess the ability to counteract the toxic effects of metals with the help of some intrinsic mechanisms, like the ability to form and excrete certain intercellular granular concretions which are nothing but the storage of the metal. Metallothionines provide protection against contaminating metals by binding with them. Enzymatic and non-enzymatic oxidative enzymes and organismal proteins help to eliminate excess metallic load from insects' bodies (Kafel et al. 2014).

Thus, by tolerating and withstanding the toxic load of heavy metals, fireflies can act as potential bioindicators against the pollution induced by heavy metal exposures (Takeda et al. 2006, Jaikla et al. 2020).

CONCLUSIONS

The present research remains significant as a pilot study for baseline data generation regarding the mode of bioaccumulation, biomagnification and biotolerance of heavy metals in a food chain consisting of fireflies, the insect whose lifecycle stages show biphasic distribution through aquatic and terrestrial habitats.

It highlights the explicit importance of fireflies as bioindicator in comparison to other known bioindicators (e.g., insects belonging to orders Diptera, Coleoptera, Hemiptera, Hymenoptera, molluscs etc.) as they possess basic criteria for monitoring the heavy metal pollution in environment. Those criteria include richness and diversity of species, ease of handling, ecological faithfulness, fragility to minute environmental changes, and efficient responses to environmental cues. The larvae being aquatic are highly sensitive to heavy metal contaminations in aquatic systems.

Moreover, future scopes are there to utilise this insect as potential indicator of heavy metal pollution, particularly for long-term monitoring or comparisons with less contaminated sites in several ecosystems like grassland, coastal, forest, plantation, etc distributed in rural, semiurban, and urban ecozones.

Bioaccumulation of heavy metals, viz., Cd, Cu, Pb, has been reported in many insects (larvae and adults of grasshoppers, locusts, termites, ants, honey bees, flies etc) including the beetles (Coleoptera). The present study has established that fireflies are not an exception in respect of heavy metal accumulation in their bodies. Scope remains to investigate the pattern of tissue specific deposition of different heavy metals in fireflies.

The gathered information leads to generation of several hypotheses:

First, the adaptation for biotolerance against heavy metals in fireflies may be dependent on environmental cues which can be evidenced by the fact of presence of higher concentrations of heavy metals like Cd, Zn and Pb in the intestinal tissues of beetles obtained from industrial areas in comparison to those from an area free of industrialisation encroachments.

Secondly, the plants which are capable of bio tolerating considerable levels of toxic heavy metals like Pb, Cd, Cr, and excess load of essential metals like Cu, Fe etc. often play vital role as phytomediators and pave way for sustainability of faunal species depending on them in a natural habitat exposed to hazards of heavy metals.

Thirdly, as primary consumers, firefly larvae play vital role in transferring heavy metals to higher trophic levels.

Fourthly, chronic toxicity induced by heavy metals may be reflected in the altered patterns of ethological manifestations related to the foraging, locomotory and reproductive behaviours of insects.

Fifthly, synergistic effects of pesticides and heavy metals often turns out to become dreadful for abiotic environment and living organisms.

Sixthly, the modes of sensitivity- resistance- tolerance towards heavy metals in insects get elicited in species specific manner.

Seventhly, the heavy metal concentration may influence the demographic attributes of firefly populations reflected in the abundance, resource utilization profile and ecosystem functionalities of their local populations.

From the overall findings of the present research, it can be stated that scope remains there to utilize these insects as potential bioindicator for the estimation of risk induced by heavy metal pollution and pave way for their future conservation.

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AUTHORS' CONTRIBUTION

Conceptualization (S.G., S.K.C.); Methodology (S.G.); collection of samples of soil, water, and plants and insect specimens (S. G.); writing manuscript (S.G.); heavy metal analyses (SG- from NABL accredited laboratory R. V. Briggs and Company Pvt. Ltd.), editing of manuscript (S.G., S.K.C.); Data analysis (S.G., S.K.C.); Supervision (S.K.C.).

DECLARATION

The authors declare that they have no conflict of interests.

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List of Tables

Table 1. Physicochemical parameters of water and soil at the study site
Table 2. Levels of occurrences of essential and nonessential metals in the aqueous medium and soil sediment at the study site in comparison with references to the baseline level of metals
Table 3. Levels of heavy metals in associated plants (APs) of the firefly species
[Natural content (mg/ kg or ppm) of metal in plants Cd- Nil, Cr-0.2, Pb-Nil, Cu-14, Zn-100, and Fe-200 (Pais 1997)]
Table 4. Levels of heavy metals in males and females of fireflies
Table 5. Significant effects of heavy metal toxicity in physiology of plants and insects