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POTENTIAL OF MACRO-INVERTEBRATES IN BIOACCUMULATION OF HEAVY METALS – A REVIEW

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Abstract – Benthic macro-invertebrates are the most appropriate and ideal organisms for bio-indicators of biodegradable organic pollution in surface water bodies. Apart from this specific characteristic many researchers have found that some of the orders (Molluscs, Annelides, Coleoptera, Polychaeta and Oligochaeta) of macroinvertebrates can also accumulate heavy metals. This review highlights the case studies of different groups of macro-invertebrates for heavy metal bioaccumulation, which help to understand the fact that macro invertebrates can serve as potential bioindicators of heavy metal pollution.

INTRODUCTION

Heavy metal pollution is a major global concern due to its non-degradable nature in the environment. It creates soil as well as water pollution that gets accumulated into aquatic organisms. This accumulation further travels into higher organisms through the food chain and causes harmful health effects to humans and animals (Cheng, 2003). Benthic macro-invertebrates are very useful bio-indicators of organic pollution. According to their sensitivity towards organic pollution, the taxonomic orders have been categorized into pollution sensitive, moderately tolerant, and highly tolerant groups. Pollution sensitive group includes Ephemeroptera, Trichoptera and Plecoptera orders that can survive only in the waterbody where dissolved oxygen is high and biodegradable organic pollutants are less (Ghosh and Biswas, 2017).

Contrary to this, a highly tolerant group includes few families of Oligochaeta and Diptera orders. They make several adaptations in their body, due to which they can survive even in significantly less/nil dissolved oxygen conditions and very high amounts of organic pollutants. Adaptations such as siphon in the Syrphidae family, the red pigment in Chironomidae worms, and cuticle layers in Naididae make them tolerant in highly polluted environments (Porst *et al.*, 2012). The distribution of benthic macroinvertebrates was widely studied as biomonitors and the rivers were bio-mapped in European countries based on the presence/absence of these macro-invertebrates.

Many have developed bio-alarm systems based on the characteristics of benthic macroinvertebrates to monitor sudden changes in water bodies due to pollution. The heavy metals are available in sediment, or water becomes available for the benthic macroinvertebrates. These invertebrates accumulate them into their different body parts such as tissues, organs, etc. Bettles belonging to Carabidae family have been found to segregate heavy metals and convert them into less active forms through different detoxification mechanisms (Hopkin, 1989). They might have a system to accumulate metals in the hindgut wall (Butovsky, 2011). The concentrations of heavy metals accumulation depend on the contamination in the environment and vary with the organisms. Heavy metals such as Zn, Cu might be required for their metabolism in significantly less amount, but they are bound to uptake other heavy metals into their bodies, such as cadmium, arsenic, lead, etc., due to their high concentrations in the surrounding environment (Table 1). However, it has been studied that in living organisms, due to the internally metabolically available heavy metals, all vital or non-vital metals become lethal to the organism despite their concentration (Rainbow, 2007). These macroinvertebrates can serve as bioindicators to enumerate the bioavailable amount of pollutants in water bodies. (Zulkifli *et al.*, 2012). Research studies have shown that molluscs can store high concentrations of heavy metals and survive in adverse environments (Usero *et al.*, 1997; Ananthan *et al.*, 2006; Amirad *et al.*, 1986; Joiris and Azokwu, 1999; Kesavan *et al.*, 2013). The reason may be that molluscs feed on phytoplankton and bottom sediments, containing high levels of pollutants (Luoma and Jenne, 1976). The prime reason of heavy metal exposure may be the diet (Poteat and Buchwalter, 2014) instead of direct aqueous exposure (Xie *et al.*, 2010). A correlation pattern has been observed between levels of Rare Earth Elements (REE) and the density of macro-invertebrates category belonging to collector-gatherers which defines that predatory organisms are more efficient in accumulating REEs than non-predatory invertebrates and therefore, collectors-gatherers can be useful organisms to trace elements in freshwater ecosystems.

However, there is a vast gap in the literature; some successful attempts have been made to understand the bioaccumulation of heavy metals in macro-invertebrates (Table 2). Thus, the present review highlights such research studies, which will help explore the use of macroinvertebrates in heavy metal bioaccumulation and their use as Bio-indicators of heavy metal pollution in water bodies.

Methodology for analysis of bioaccumulation studies in macro-invertebrates

The common methodology was adapted in all the available literatures with modifications according to the type of organisms. The water/soil/sediments and macro-invertebrates samples from the contaminated sites were collected. The macrobenthos samples are washed thoroughly in double distilled water to remove the debris or soil particles, further, fixed in 4% formaldehyde to prevent decomposition of organisms. For heavy metal analysis, macrobenthos samples (shells of molluscs and soft tissues in case of other macrobenthos) are first dried in oven at a temperature of 60%C. Dried/powdered tissues (0.1 g) were after wards acid digested with 2 mL of a mixture of nitric andperchloric acid (ratio 9:1) (Julshmamn *et al.*, 1982). Digested samples are filtered through Whatman filter paper no.41. The metal concentrations were determined using AAS (Dar *et al.*, 2018).

The heavy metal concentration in surrounding water/sediment and organisms' body are compared to understand the Bioaccumulation Factor (BAF) according to Klavins *et al.* (1998) (Equations 1 and2):

$BAF_{Wv} = M_{benthos} / M_{wat}$ (1)

$BAFSv = M_{benthos} / M_{sed}$ (2)

where $M_{benthos}$ is metal concentration in benthos; M_{wat} is metal in the water and M_{sed} is metal in sediment.

Case studies

Latif *et al.* (2011), examined the suitability of oligochaete *Eiseniella tetraedra* as a bioindicator of cadmium (Cd) and lead (Pb) contamination in Iran. The concentration of heavy metals was analysed in soil and earthworm tissues from two locations; Polur Falls and the riverside area of Abali. Pb and Cd concentrations in Earthworms collected from Abali were found to be higher than the earthworms collected from Polur (Abali=199.92±21.58 ppm; Pb: Polur=38.02±4.44 ppm; Abali=16.77±2.92 ppm; Cd: Polur=7.35±0.97 ppm). The accumulation of Pb and Cd in earthworms from Abali was 5 and 2 times higher, respectively than from Polur. Also, it was observed that Pb and Cd at both stations were higher in earthworms than the surrounding soils. Hence, they recommended the use of *E. tetraedra* as a bioindicator of heavy metals contaminated locations (Latif *et al.*, 2011).

Dange and Manoj (2015) reported that the polychaete worms were used for bioaccumulation from Purna river estuary, South Gujarat. Heavy metals Copper, Nickel, Zinc and Cadmium concentrations were determined in sediment and several tissues of polychaetes. It was observed that the body wall and gut are the major body parts in Nereididae family of polychaete used for bioaccumulation of copper. In the case of Mud crab (*Rhithropanopeus harrisii*), heavy metals Copper, Nickel and Zinc were accumulated in different body parts such as chelipeds, walking legs, carapace etc. except Cadmium, Cu and Zn are essential elements for crabs as they are required for different biological functions but in limited concentrations. (Dange and Manoj, 2015).

Carabidae beetles make the major part of soil bioindicators. It has been found that beetles have gut as the exceptionally important body part which allows uptake, transmission, accumulation and excretion of metals (Agnieszka *et al.*, 2016) while midgut restricts the toxic materials inside the body while eating (Rawi, 2011). Nasr and Khater (2020) examined their use as an indicator of heavy metal pollution in soil. The heavy metal (As, Hg, Al, Cd, Se, and Pb) concentrations were determined in soil and midgut of *C. Chlorostictum* beetle. Also, physicochemical properties of the sampling site were also studied. The data reflected that As was found to be the most accumulated metal (11.858 μg/ g^{-1}) in the midgut of insects collected from polluted site in comparison to reference site this may be due to the higher concentration of arsenic in the zone. Concentrations of other metals found in the insect midgut were in the order as Al>Pb>Cd>Hg>Se. Transmission Electron Microscopic (TEM) results showed that heavy metals caused ultrastructural changes in the midgut epithelial layer of the ground beetles, collected from control and contaminated sites. Apart from structural changes correlation studies of heavy metals concentrations among soil and insects midgut were also done among the which reflected highest correlation for As and lowest observed was for Al (Nasr *et al.,* 2020)

Hydrophilidae is one of the widely distributed families of order Coleoptera, it haswell developed taxonomy. Since, Hydrophilidae larvae need various environmental surroundings, they can be suitable as heavy metal bioindicators (Fikáèek *et al.,* 2010).

The potential of Hydrophilidae was explored by Aydoðan *et al.* (2017) they assessed the heavy metal concentrations in in surrounding environment, fish tissues and bettle family Hydrophilidae (Coleoptera: Hydrophilidae) concentrations in two study areas in Erzurum, Turkey (Aºkale and Dumlu sites). Total 11 metals (Cr, Mn, Ni, Al, Fe, As, Cd, Cu, Zn, Pb, Hg) were analysed in fish tissues were analysed, and in insects (*Laccobius (Dimorphol accobius) syriacus, Laccobius (D.) imulatrix, Laccobius (D.) bipunctatus, Enochrus (Lumetus) bicolor, Enochrus (L.) fuscipennis, Enochrus (L.) halophilus*, and *Helochares lividoides* of hydrophilidae family, 14 elements (Cr, Ti, Mn, V, Co, Cu, Fe, Ni, Se, Zn, As, Pb, Br, Sr). They concluded that insects were affected by heavy metal contamination in the surrounding area and could accumulate higher concentrations of metals in comparison to fishes (Aydoðan *et al.*, 2017).

Molluscshave a capacity to accumulate heavy metals differentially in the soft tissues and calcium carbonate shells of the bivalvesand thus, can be very good bio-indicators of heavy metal pollution (Szefer and Szefer, 1990). However, most studies suggest the majority of accumulation occurs in the soft tissues but based on some studies, shells offer more accurate information about heavy metals accumulation (Thorn *et al.,* 1995; Yasoshima and Takano, 2001; Huanxin *et al.,* 2000, Carriker *et al.,* 1982). Also, due to their long-life span (for example- *Margaritifera margaritifera* and *Velesunio ambiguus*, is upto100 & 30 years respectively), they can provide long-term impact of pollutants on aquatic bodies. (Carell *et al*., 1987; Walker, 1992)

Dar *et al.* (2018) studied various trends of heavy metals mineralisation in some species of bivalves and molluscs which were collected from the Timsah and Great Bitter. The capability of molluscs to bio-mineralize heavy metals into their shells was studied and depicted as Bio-mineralisation factor (BMF). At Timsah lake, *Cardium papyraceum* and *Paratapes undulatus* each showed highest BMFM ratios (0.95) of Ni. For Pb, highest BMFM ratio was found to be 0.92 in *Cardium papyraceum*. At the Great Bitter Lake, high Fe biomineralization was observed in *Modiolusa uriculatus* and the *Bulla ampulla* within their shells in comparison to the other studied. *Brachidontes pharaonis* accumulated highest Cd and Cu and similarly, *Fusinus sp.* accumulated highest concentrations of Ni and Zn. At the Timsah Lake, the accumulation of heavy metals was found in the following order Fe >Mn>Pb> Ni > Zn > Cu > Cd and at the Great Bitter Lake it was in following order Fe >Mn>Pb> Ni > Cd > Zn > Cu (Dar *et al.*, 2018).

Four bivalve molluscs *Anadara granosa*, *Meretrix meretrix* and *Pelecyora trigona* were collected from intertidal regions of the *Sunderban mangrove* wetland, north-eastern part of the Bay of Bengal and accumulation of heavy metals (Cu, Pb, Cd, Zn, and Hg) was studied by Sarkar *et al.* (2008). Contrary to many other research studies, shells have less accumulation capability than other soft body tissues specifically, gills and mantle. The reason for high accumulation might be the ion exchange mechanism of the mucous layer layering these tissues. The higher accumulation of heavy metals into these

Table 1. Major Organs for metal accumulation in Macroinvertebrates

S.No.	Macroinvertebrates	Major organs for metal accumulation
1.	Molluscs	Shell and soft body tissues (gill, visceral mass, mantle, podium, shell, adductor muscle)
2.	Insects (beetles)	Gut, mid gut
3.	Annelids	Gut
4.	Crustacea	Chelipeds, Walking legs, carapace

bivalves may create harmful health effects for humans as they are consumed as seafood. On the basis of the results, it was observed that heavy metal accumulation varies with the species and affected by many factors such as size, age, sex and metal speciation, salinity etc. Also, each species of marine macroinvertebrates adopt different mechanism for each metal. Maximum accumulation of Cu and Zn, 175.14 and 2382 μg/g dry weight respectively in visceral mass of *S. acuminata* (bigger) at Gangasagar and in gills of *A. granosa* (larger at Jharkhali), which might be due to the fact that these two are essentiallyrequiredin most of the metabolic processes of these bivalves (Greenfield *et al.,* 2003; Paez-Osuna *et al.,* 2002). *S. acuminata* could accumulate Pb 46.34 μg/g dry weight in the gill. The organ specificity trend against metal accumulation is observed in following sequence: gill> visceral mass> mentle> podium> shell> adductor muscle (Sarkar *et al.,* 2008).

Bio-sorption of heavy metals (Cd, Pb, Zn) using bivalve molluscs was carried out by Mahendra *et al.* (2020). Shells were dried and crushed, then, shell powder was used to understand the sorption capacity of bivalve shells for Pb^{2+}, Cd^{2+}, and Zn^{2+} was studied. Ion exchange mechanism Maximum concentration 489 mg/g for Pb 460 mg/g for Cd and 450 mg/g for Zn was obtained by seashell powder. The seashells are made up with $CaCO_3$ and present in calcite and aragonite forms (Vijayaraghavan *et al.,* 2011,Afroze and Sen, 2018). Bio-sorption process was found to be influenced by temperature and pH. Sorption process was found to decrease with increase in temperature due to the damage of active binding sites. The effective pH range for bio-sorbent was found to be in range from 6-8. The metal ions replace the calcium ions present of shell surface through ion exchange mechanism, indicating the sorption process of metal uptake rather than adsorption (Mahendra *et al.,* 2020). Shahzad *et al.* (2017), conducted a similar study on removing Pb (II) ions using shell biomass of cylindrical paper shell mussel (*Anodontoidesferussacianus*). Results indicated that the 200 mg/L of Pb (II) was removed through biosorption. The effect of some physico-chemical parameters such as pH, contact time, temperature and biosorbent concentration etc. on biosorption process of *A. ferussacianus* shell was evaluated. Ideal pH and temperature were found to be 6 and 30°C respectively. The occurrence of some functional groups (–OH, NH, C=O and S=O) on the surface of bivalve mollusks helps in surface binding mechanisms of metals (Liu *et al.,* 2009).

In addition to above mentioned studies, Bhalachandra *et al.(*2011) studied the heavy metal uptake/accumulation into soft body tissues of the freshwater bivalves species namely, *Parreysia cylindrica, Corbicula striatella, Parreysia corrugata, Lamellidens corrianus, Lamellidens marginalis and Indonaia caeruleus* in laboratory conditions. The

Table 2. Common heavy metals and macroinvertebrates used in the bioaccumulation studies

Order	Name of Macro-invertebrates	Heavy metals studied	References
Oligochaeta	*Eiseniella tetraedra*	Zn, Cu	Latif *et al.* (2011)
Polychaeta	*Nereididae*	Cd, Pb,	Dange and Manoj (2015)
Mollusca	*Brachidont espharaonis*	Cd, Cu	Dar *et al*. (2018)
	Cardium papyraceum, Paratapes undulates	Ni, Pb	
	Modiolus auriculatus, Bulla ampulla	Fe	
	Fusinus sp.	Ni, Zn	
	Sanguinolaria acuminata, Anadara granosa	Cu,Zn	Sarkar *et al*. (2008)
	Anodontoides ferussacianus	Pb	Liu *et al.* (2009)
	Lamellidens corrianus	As, Pb	Bhalchandra *et al.* (2011)
	Lamellidens marginalis	Cu, Hg	
	Parreysia cylindrica	Cd	
	Corbicula striatella	Zn	
	Indonaia caeruleus	Cu, Hg	
Insecta	*Calosoma chlorostictum*	As, Hg, Al, Cd, Se, and Pb	Nasr and Khater (2020)
	Laccobius (Dimorpholaccobius) syriacus, (*Laccobius (D.) imulatrix*), *Laccobius (D.) bipunctatus, Enochrus (Lumetus) bicolor, Enochrus (L.) fuscipennis, Enochrus (L.) halophilus,* and *Helocha reslividoides*	Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Br, Pb	Aydoðan *et al.* (2017)
Crustacea	*Rhithropano peusharrisii*	Cu, Ni,Zn	Dange and Manoj, (2015)

heavy metal concentrations used to acclimatize these bivalves were 0.1719 ppm for arsenic, 0.1411 ppm for cadmium, 0.033 ppm for copper, 1.50ppm for lead, 0.0443 ppm for mercury and 1.8589 ppm for zinc. The bivalves were exposed with heavy metals for up to 30 days in laboratory and accumulated concentration was analysed in bivalve tissues an dcontrol with a regular interval of 10, 20 and 30 days. Results indicated highest concentration of arsenic (37.9 µg) and lead (1235.4 µg) was accumulated by *Lamellidens corrianus*, *Lamellidens marginalis* could accumulate Cu upto 826.7 µg, Hg upto 5.87 µg, *Parreysia cylindrica* could accumulate Cd upto 182.62 µg and Zn upto 4139.2 mg was found in the per gram dry tissues of *Corbicula striatella*. Accordingly, bioconcentration factor (BCF) was also evaluated in same order as the heavy metal accumulation in bivalves. Based on the accumulation rate of different species, the *Corbicula striatella* can be used as an indicator organism for biomonitoring of Zn, *Lamellidens corrianus* can be used for As and Pb, *Parreysia cylindrica* for Cd and *Lamellidens marginalis* for Cu and Hg in freshwater ecosystem (Bhalchandra *et al*., 2011).

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