© Kamla-Raj 2019 J Hum Ecol, 68(1-3): 195-202 (2019)
PRINT: ISSN 0970-9274 ONLINE: ISSN 2456-6608 DOI: 10.31901/24566608.2019/68.1-3.3181

Bioaccumulation of Heavy Metals from Mining Effluents in the Tissues of Fish *Puntius narayani*

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KEYWORDS Aquatic Pollution. Biomagnification. Fish. Human Health. Risk Assessment

ABSTRACT Unregulated mining activities not only cause air pollution but also affects the water quality and contributes to the release of heavy metals in the aquatic ecosystem. This poses a major health problem for aquatic organisms. The present study involved heavy metal evaluation, of eight water bodies in the vicinity of mines and study of bioaccumulation of these metals in the tissues of the fish *Puntius narayani*. Result of this study shows interesting findings on the risk posed to humans on account of preference of specific heavy metal bioaccumulation in the tissues of fishes. Liver showed a strong preference to accumulate Ni and Cu, Gills showed accumulation preference for Zn, Cu, Ni and Fe, while Muscle tissue showed affinity to accumulate Cu, Zn, and Ni. Given the findings of this study, it is suggested that the Environmental Impact Assessment should include bioaccumulation studies before the issue/renewal of mining licenses.

INTRODUCTION

Unregulated mining leads to the release of harmful substances into the environment and is responsible for having devastating effects inflicting a wide variety of air, water, and soil pollution. Mining effluents get entry into the freshwater systems through seepage from tailing and waste rock impoundment and can have a significant impact in imposing various stresses onto the organisms, thereby disrupting the aquatic food chain. The pollution of air, water and land on account of mining is reported by many researchers (Tchounwou et al. 2012; Fashola et al. 2016). Mining effluents deteriorate the water quality by altering its physicochemical parameters. Studies have reported genotoxicity in the fishes exposed to heavy metals released in the water bodies via mining effluents (Fernandes 2018). Heavy metals occur in natural water bodies at levels below their toxic thresholds. However, due to their non-degradable nature, such sub-lethal concentrations may still pose the risks of damage via uptake and subsequent bioaccumulation in organisms which cannot effectively metabolize and excrete the absorbed metals. Bioaccumulants have a long term effect on the aquatic organisms as they can adversely affect the food chain ultimately impacting human health at large (Arif et al. 2015). Results of many field studies of metal accumulation in fish living in polluted waters show that considerable amounts of metals may be deposited in fish tissues without causing mortality (Akan et al. 2012). Among animal species, fishes are the ones occupying the place from where they cannot escape the detrimental effects of this heavy-metal pollution (Tchounwou et al. 2012). The high concentration of metals in the aquatic ecosystem, bioaccumulate in the aquatic organisms, damage tissues and interfere with the normal growth and metabolism (Fashola et al. 2016).These heavy metals can be passed on to humans through the food chain. Presence of trace heavy metals in diet can cause serious health problems and can affect the excretory organs, nervous system and act as a potent carcinogen if ingested over a long period (Igwegbe et al. 2014). Genotoxic and bioaccumulation studies, therefore, play a major role in assessing the extent of pollution of the river system.

Objective

The present study was undertaken to assess bioaccumulation of metals in different tissues of the fishes in water bodies contaminated with

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mining effluents and to know the bioaccumulation preference for specific tissues by metals.

MATERIAL AND METHODS

To assess bioaccumulation of metals from mining effluents in different tissues of the fishes, four water bodies in mining areas of Kalay, Ponda, Codli and Rivona in south Goa, were identified using GPS / Google maps. The main constituents of the ore extracted from these areas included Fe ore, Mn, basalt, and laterite. Based on the location of the influx of effluents from the nearby prominent mines, eight sampling sites were selected for the study. Five litres of water samples and live fishes were collected from these sites. The heavy metal analysis was done by atomic absorption spectrophotometer included detection of Fe, Mn, Zn, Cu, Cd and Ni. Tissues such as gills, liver, and muscles of *Puntius narayani* were also evaluated for the determination of metal concentrations. These tissue samples were dried, powdered, digested according to the standard procedure of Sreedevi et al. (1992) and analyzed using Atomic Absorption Spectrophotometer. The standardized procedures used for heavy metal detection in the tissues was as per IS 3025 Part 53: 2003, SOP/ITG/AAS/INST-01. The concentration of heavy metals was expressed as mg/ltr for water and mg/gm body weight for fish tissue. Bioaccumulation factor (BAF) was then calculated to see the accumulation of these metals in three different body tissues namely Liver, Muscle and Gills by using the mean metal concentrations in fish and the corresponding metal concentrations for water (Bu-Olayan et al. 2008). The formula for calculating bioaccumulation factor:

$$BAF = \frac{\text{Concentration of metal in body tissue}}{\text{Concentration of metal in the environment (water)}}$$

The data was tabulated and analyzed statistically for significance by Fisher's exact test using graphpad.com.

RESULTS

The present study showed interesting findings on the risk posed to humans on account of heavy metal pollution in the water bodies. The bioaccumulation studies indicated that the metals have a specific preference of being deposited in specific tissues of the fishes. Predominant metals in the water samples were Mn, Fe, and Zn. An important finding of the present study is that though the metal concentration in the water was within permissible limits, it showed a high rate of bioaccumulation in the body tissues of the fish *Puntius narayani*. Bioaccumulation studies showed a specific pattern of metal preference for a specific tissue of the fish.

1) Metal Concentration Determination

The metals detected in the water samples and fish tissues were Fe, Cu, Zn, Mn, Cd and Ni.

a) Metals Concentration in Water Samples: The metals detected in the water samples of eight sites were Fe, Cu, Zn, Mn, Cd, and Ni. Test for Lead and Boron were negative. Fe, Zn and Mn were the most common metal pollutants detected in higher concentrations in the water bodies at the mining sites. However, the metal concentrations in the water bodies were within the permissible limits of Bureau of Indian Standards *(BIS)*.

b) Metal Concentration in the Body Tissues: Concentration of different metals and their distribution in the body tissues of *Puntius narayani* is indicated in Table 1. Different organs exhibited different patterns of metals accumulation in the freshwater fish *Puntius narayani*. Although the metals were in low concentration in the water bodies, the body tissues showed a higher concentration of these metals due to bioaccumulation. Of the body tissues, the gills showed a higher concentration (BAF = 301.6) of most of the metals as compared to liver and muscles. Gills were seen to accumulate higher concentration of Fe, Cu, Ni and Zn as compared to other body tissues. Liver showed a higher concentration of Mn and Cd. Muscle tissue was the least preferred tissue by most metals. However, muscle tissue was the second most preferred tissue by Cu (BAF= 745), Zn (BAF=63.2) and Ni (BAF=60).

2) Bioaccumulation Analysis

Heavy metals reaching the water bodies through the mining effluents were at levels below their toxic thresholds, whereas their concentrations in body tissues were higher. Due to

Table 1: Metal concentration in the water and body tissues of *Puntius narayani*

Metal →	*Metal concentration in mg/gm body weight of body tissue*					
Media/ ↓ Tissue	*Iron (Fe)*	*Copper (Cu)*	*Zinc (Zn)*	*Manganese (Mn)*	*Cadmium (Cd)*	*Nickel (Ni)*
Water (mg/Ltr)	0.126 ± 0.09	0.002± 0.002	0.028± 0.04	0.143± 0.11	0.001± 0.002	0.001± 0.001
Gill (mg/gm)	38.01±100.9	1.545± 2.09	23.34± 52.7	4.4± 7.8	0.004± 0.01	0.475± 0.9
Liver (mg/gm)	3.85± 9.3	0.69± 0.6	2.89± 2.1	9.93±17.8	0.058± 0.11	0.405± 0.74
Muscle (mg/gm)	0.014± 0.03	0.149± 0.16	1.77± 1.15	0.65± 0.57	0.006± 0.01	0.06± 0.1

their non-degradable nature, such sub-lethal concentrations may still pose a risk of damage through uptake and subsequent bioaccumulation in body tissues of organisms which cannot effectively metabolize and excrete the absorbed metals. Therefore, assessing bioaccumulation is essential. Bioaccumulation Factor (BAF) is expressed as the ratio of the contaminant in tissue to the concentration in the ambient environment at a steady-state, where the organism can take in the contaminant through ingestion with its food as well as through body surface. The present study showed bioaccumulation of most of the metals in the body tissue of the fish *Puntius narayani*.

a) Bioaccumulation of Different Metals: Similar pattern of metal preference by tissues was observed in all the eight sites. The study showed interesting findings of tissue preference of these metals. Cu, Zn and Fe showed a preference for accumulating many folds in the gills. Muscles showed a tendency to accumulate Cu. Liver showed affinity to concentrate Ni, Cu, and Zn. Tissue preference of different metals for bioaccumulation in is indicated in Table 2.

i)Bioaccumulation Factor of Fe: Higher concentration of Fe was observed in the gill tissues of *Puntius narayani*. The bioaccumulation factor was assessed for the concentration of Fe present in the water. The gills showed a manyfold increase in the concentration of Fe. Assessment of bioaccumulation factor (BAF) of Fe in fish tissues showed high BAF for gill tissue as compared to muscle and liver. Order of preference of bioaccumulation of Fe was Gill >Liver > Muscle.

ii) Bioaccumulation Factor of Cu: Cu was found to be having a preference of accumulating in the gills and muscles as compared to the liver. BAF of Cu was higher in all the three tissues. BAF for gill tissue was 775 as compared to 745 in muscles and 345 in the liver. Order of preference of bioaccumulation of Cu was Gill >” Muscle > Liver.

iii) Bioaccumulation Factor of Zn: Zn was found to be having a preference of accumulating

Table 2: Tissue preference of metals for bioaccumulation

BAF of Metal	*Tissue of Puntius narayani*				
	Gill	>	Liver	>	Muscle
Iron	301.6		30.6		0.11
	Gill	>	Muscle	>	Liver
Copper	775		745		345
	Gill	>	Liver	>	Muscle
Zinc	833.6		103.2		63.2
	Liver	>	Gill	>	Muscle
Manganese	69.4		30.8		4.6
	Liver	>	Muscle	>	Gill
Cadmium	58		6		4
	Gill	>	Liver	>	Muscle
Nickel	475		405		60

in the gills as compared to liver and muscles. Zn bioaccumulation preference was found to be Gill > Liver > Muscle.

iv) Bioaccumulation Factor of Mn: The accumulation of Mn was higher in the liver and gill as compared to the muscle tissue of *Puntius narayani*. The BAF of Mn was 69.4 for liver tissue which was highest as compared to gill and muscle. Order of preference of Mn bioaccumulation in the tissues was Liver > Gill > Muscle.

v) Bioaccumulation Factor of Cd: Cd was found in very minute quantities in the water bodies of only two study sites. However, though the concentration in water was in minute quantity it showed a tendency to bio-accumulate in the liver (Fig. 1). Tissue preference of Cd was Liver > Muscle > Gill.

vi) Bioaccumulation Factor of Ni: Though Ni was found in body tissue from four sites, it was observed in the water sample of only one site. The BAF of Ni was higher in the Gills and liver. Tissue preference of Ni was Gill > liver >Muscle.

***b) Tissue-wise Distribution of Metals in Puntius narayani**:* When bioaccumulation of these metals in the different tissues was compared the present study showed metal specificity of deposition in gill, liver and muscle tissue. Bioaccumulation preference of different metals by Gill, Liver and Muscles of *Puntius narayani* is presented in Table 3.

Gills: Metal deposition in the gill tissue was higher as compared to the liver and muscles. Gill is preferred tissue for bioaccumulation of Zn, Cu, Ni, and Fe. The metal preference was Zn> Cu>Ni>Fe>Mn>Cd.

Liver: The present study showed that Ni has strong affinity to bioaccumulate in the liver, as compared to gill and muscle. The metal preference was Ni>Cu>Zn>Mn >Cd>Fe.

Muscles: Compared to the gill and the liver, the muscle tissue was the least preferred tissue

Table 3: Bioaccumulation preference of different metals by gill, liver and muscles of *Puntius narayani*

Gill	Zinc 833.6	>	Copper 775	>	Nickel 475	>	Iron 301.6	>	Manganese 30.8	>	Cadmium 4
Liver	Nickel 405	>	Copper 345	>	Zinc 103.2	>	Manganese 69.4	>	Cadmium 58	>	Iron 30.6
Muscle	Copper 745	>	Zinc 63.2	>	Nickel 60	>	Cadmium 6	>	Manganese 4.6	>	Iron 0.11

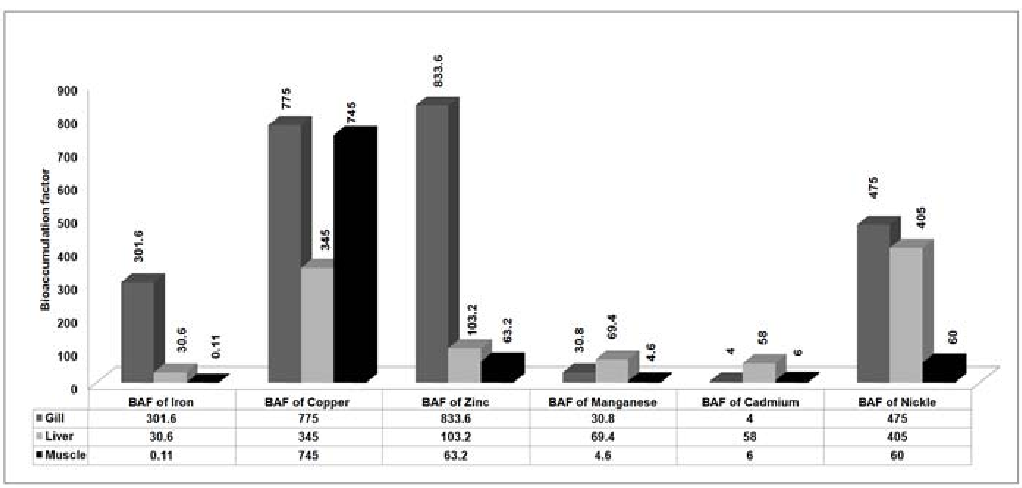

Fig. 1. Bioaccumulation factor of metals

for bioaccumulation of most of the metals. Cu however, showed a preference for muscle tissue for bioaccumulation. This is an important factor to be considered as muscle tissue of the fish is consumed by humans, which poses a risk of bioaccumulation of Cu in humans. The metal preference for muscle tissue was Cu>Zn> Ni>Cd>Mn>Fe.

DISCUSSION

Mining effluents are the main source of metal contamination of the water bodies in the vicinity of mines. The metals detected in the water samples of the eight sites, evaluated in the present study were Fe, Cu, Zn, Mn, Cd, and Ni. The present study showed bioaccumulation of most of the metals in the body tissue of the fish *Puntius narayani*. Heavy metals released in the water bodies through the mining effluents maybe at levels below their toxic thresholds. Though the metals present in the water were in small quantities, the consequence of it on the aquatic organisms is of importance because of bioaccumulation. In the aquatic environment, these metals may exist as complex, diverse mixtures of soluble/insoluble forms. The respiratory and the integumentary systems become the main sites for entry of these metals from the water. Since these metals are non-degradable, even sub-lethal concentrations may pose a high risk of damage via uptake and subsequent bioaccumulation in the organisms. Earlier reports by Murugan et al. (2008) supports the findings of the present study, inferring that fishes exposed to high concentrations of trace metals in their habitat may take up substantial quantities of these metals. The bioaccumulation levels observed in any organism reflects the quantity of heavy metals ingested by them and also taken up through the body surface. It also depends on the manner in which the metal are distributed in the different tissues and the extent to which it gets deposited or retained in the body tissues of the organisms. Metal bioaccumulation and distribution in tissues of the fish is species specific. Biological factors such as age, size, feeding habits and reproductive cycle also influence metal uptake (Zhao et al. 2012). Environmental factors too, affect the uptake and accumulation of metals in aquatic organisms (Maurya et al. 2019). The altered physicochemical properties of water bodies also influence the rate of metal bioaccumulation in fish tissues(Fernandes 2015). The present study showed specificity of bioaccumulation of different metals in the gills, liver, and muscle tissue. Various metals may accumulate in fish body in different amounts, due to differential uptake, deposition and excretion rates (Tanee et al. 2013).

As compared to the liver and muscles, the gills are considered to be the main site of entry for the dissolved metals through surface exposure in an aquatic habitat. Thus, gills represent the target for the toxic action of most metals. Observations of the present study indicate that metals tend to not only get concentrated in the gill tissue, but also that gill is the preferred tissue for bioaccumulation of Zn, Cu, Ni and Fe. Findings of this study is in agreement with study of Shukla et al. (2007), which stated that gills are metabolically active sites, and therefore have the ability to accumulate higher concentration of specific metals as compared to the other body tissues. High concentration of metals in the gill tissue can also be because of excretion of mucous by this organ, which has affinity to be bound with metal ions. Surface of the gills serve as major pathways of metal ion exchange from water (Qadir and Malik 2011). Large surface area of the gills enables rapid metal uptake (Dhaneesh et al. 2012). From the present study it can be concluded that Zn, Cu, Ni and Fe have tendency to bioaccumulate in the gills, and that the entry of these metals may be mainly through the surface exposure.

The liver of the fish brings about not only storage of the metals, but also redistribution and subsequent detoxification of the metals. This study showed a higher rate of bioaccumulation of Ni, Cu, Zn and Mn in the liver tissue of *Puntius narayani*. Liver seems to be the preferred target organ for bioaccumulation of most heavy metals (Bawuro et al. 2018). Bioaccumulation of metals in the liver of the fish is of significance because it can bring about a broad range of physiological, biochemical, and behavioral dysfunctions through induction of oxidative stress in the fishes (Basha and Rani 2003). High Zn and Cu in the liver of fishes were recorded by Zhao et al. (2012) and M'Kandawire et al. (2017). However, the present study showed a higher rate of bioaccumulation of Ni in the liver besides Zn, Cu, and Mn. High Ni accumulation in the liver tissue of fishes was also reported in the studies of Ghosh et al. (2018). Accumulation in the liver tissue may be due to the transfer of these metals from phytoplanktons in the food chain and subsequent biomagnifications. Phytoplanktons readily incorporate metals in them and transfer it to the fishes through various trophic levels. Natural protein binding component, such as Metallothioneins are associated with increased levels of Zn, Cu and Ni in the liver (Gorur et al. 2012; Ghosh et al. 2018). *Puntius narayani* is a foraging omnivore feeding on worms, insects and other small invertebrates, as well as plant material, and organic detritus. In the present study, bioaccumulation of metals observed in these fishes may be attributed to: a) The cumulative effect of the amount of these metals ingested by the fishes through the food chain, b) The ability of the fishes to digest these metals or transport it to the liver for detoxification c) Adsorption through permeable membranes of the body.

Bioaccumulation in fish muscle is a matter of grave concern, as it is consumed as food by humans. These heavy metals which reach the humans through the food chain can have serious consequences of renal failure, liver damage, cardiovascular diseases, and in severe cases even death in humans (Busaidi et al. 2011; Rahman et al. 2012). Metals induce oxidative stress in the humans by initiating series of physiological changes, biochemical alterations, and behavioural dysfunctions. This oxidative stress developed in response to the heavy metals needs to be viewed seriously as it leads to damage to biomolecules and disrupts the signalling pathways. As a result, it leads to the pathogenesis of multiple human diseases (Arif et al. 2015). The muscle tissue of fishes is reported to accumulate significantly fewer metals as compared to other tissues such as gills and liver (Yilmaz et al. 2010). The present study also shows a similar effect. However, an interesting observation of the present study is the preference for Cu accumulation by muscle tissue of *Puntius narayani*. BAF of muscles for Cu was 745 folds higher. However, there was no significant accumulation of Fe, Mn and Cd in the muscle tissue. Accumulation of specific metals in the muscle of fishes may be a major health concern for humans in general. The high affinity of Cu(I) and Cu(II) for protein sites having cysteine, methionine, and histidine side chains act as potential ligands is the cause of misfolding of proteins due to displacement of essential metal ions from their active sites. Hence, uptake, distribution and utilization of Cu and finally its excretion from the body needs to be tightly regulated (Rae et al. 1999; Halloran and Culotta 2000). Bioaccumulation of Cu facilitates the generation of highly reactive oxidative species (such as hydroxyl radicals), which can cause DNA damage and oxidation of proteins and lipids (Halliwell 1990). Ni induced DNA damage in gill cells and erythrocytes of *Prochilodus lineatus* was indicated in the studies of Palermo et al. (2015). Study by Fernandes (2018) showed 11.1 percent DNA damage in the fishes which was attributed to the synergistic effect of multiple heavy metals, and alteration of water quality due to mining effluents. This warrants source control and treatment measures of effluents released by mines to prevent and reduce the discharge of metals and other pollutants into the water bodies. If this is regulated, it will help prevent heavy metal risks to human health. We need proper implementation of laws and change in attitude to safeguard the purity and quality of our water bodies from being polluted by mining effluents. Besides, assessing water quality, DNA damage studies and bioaccumulation studies should be used as an index to estimate the level of aquatic pollution.

CONCLUSION

Constant exposure of the fishes to the metals on account of the continuous leaching of the metals from mining effluents leads to significantly high levels of these metals which can have a chronic effect on fishes. Since fish is one of the major components of the human diet, bioaccumulation of metals observed in the fish tissue is a matter of grave concern. In humans, the presence of even trace heavy metals in diets can cause serious health problems if ingested over a long period of time. The present study infers that bioaccumulation studies in aquatic organisms should be mandatory besides physicochemical evaluation of water in determining water safety and preventing health hazards in humans.

RECOMMENDATIONS

For evaluation of water quality, assessment of physicochemical parameters should be supplemented by bioaccumulation studies in aquatic organisms, preferably fish. This is because, even if water samples are detected with heavy metals within permissible limits in the aquatic environment, they have a high risk of getting accumulated in body tissues of fishes due to bioaccumulation. Therefore, to declare a water body as safe assessing BAF is essential.We recommend that mining effluents should be treated specially to remove metals even if they are in traces and bioremediation need to be adopted before releasing it into the water bodies as it may lead to health hazards on account of bioaccumulation and biomagnifications. The environmental impact assessment should include bioaccumulation studies and DNA damage studies for renewal of mining licences.

ACKNOWLEDGEMENTS

The author wishes to express her deep appreciation and gratitude to the fund provided by the Department of Science and Technology (DST- SERB). The author also acknowledges the help rendered by Shreya, Vaibhavi, Gautami, Meika and Siddhavi.

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Paper received for publication in September, 2019
Paper accepted for publication in November, 2019