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ORIGINAL PAPER

Metal bioaccumulation in fish *Labeo rohita* exposed to effluent generated during metals extraction from polymetallic sea nodules

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Abstract The aim of this study was to investigate the potential impact of effluent released during metals extraction from polymetallic sea nodules at the Pilot Plant at National Metallurgical Laboratory, India, by analysing different metal (Mn, Cu, Zn, Fe, Pb and Cr) concentrations in six tissues (skin, muscles, gills, liver, kidney and brain) of the fish *Labeo rohita*. Of the six analysed tissues of sea-nodule-effluent-exposed fish, liver accumulated highest concentration of most of the metals (2.91–287.36 mg kg^{-1}) while muscles (2.1–81.14 mg kg^{-1}) lowest. While the concentration of Fe was maximum (60.41–218.7 mg kg^{-1}) in all the tissue systems (except muscles and liver), Pb was minimum (0.8–2.91 mg kg^{-1}). Accumulation of most of the metals in all the tissues was above the safe limits as recommended by Food and Agricultural Organisation that indicate metal's potential hazardous impact on the fish. High bioaccumulation factors for these metals in the different tissues revealed that metals were extensively bio accumulated and bio concentrated. The time-dependent variation in metal pollution index illustrated increase in gross metal load ($p < 0.05$) in different tissue components with increase in exposure period. In conclusion, the sea nodule effluent was found to cause adverse health impact on the fish which in turn might also affect the human health when consumed.

Keywords Atomic absorption spectrophotometry · Heavy metal accumulation · Fish tissues · Health hazard

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Introduction

As the land-based resources of metals are depleting very fast, metallurgists have moved forward for their alternative sources of extraction. Ocean covering the 71 % of the earth's surface had always fascinated the scientists that made them to explore it, and metallurgists have found polymetallic sea nodules (PMN) as major source of a large number of important metals. PMNs are small, dark brown coloured balls, slightly flattened, measuring 5–10 cm in diameter. These nodules lay on the sea bed at 4,000–6,000 m deep. PMNs are made up of metal as well as nonmetal components. These nodules contain manganese, iron, aluminium, nickel, copper, cobalt and titanium as their major constituents along with some minor components such as sodium, magnesium, silicon, zinc, oxygen and hydrogen. Scientists at the National Metallurgical Laboratory, Council of Scientific and Industrial Research, Jamshedpur, India, have developed an indigenous technique to recover the metals from PMN. These nodules are passed through metallurgical processes including reduction, ammonia leaching, solvent extraction, electro winning and smelting (Jana et al. 1999; Kumar et al. 1990; Agarwal and Goodrich 2008; Biswas et al. 2009). During these processes, a large amount of highly contaminated effluent is generated which also retains substantial amount of the precious otherwise toxic metals. While analysing the physicochemical nature of the sea nodule effluent, Vaseem and Banerjee (2012) recently found that several toxic metals are present above their permissible limits in this effluent (EPA 2002). These highly toxic metals are the main cause of environmental pollution and are of concern due to their toxicity, persistence, bioaccumulation and biomagnifications into the food chain (Storelli 2008; Groth 2010; Mendil et al. 2010). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of the water-borne contaminants. Hence, fishes are often used as effective bioindicator for pollution monitoring (Olaifa et al. 2004; Clarkson 1998). Heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the aquatic organisms such as fish which make these toxicants deleterious to the aquatic environment and consequently to the humans who depend upon fish as a source of food. Hence, the accumulation of metals in the aquatic animals can be used as a reasonable measurement for public and animals health standard. The pattern of bioaccumulation of metals in different tissues or organ systems of animals varies according to their metabolic activities. The total metal load or gross metal accumulation in each tissue component or organ system has been illustrated by calculating metal pollution index (MPI). *L. rohita*, a major Indian carp, has been selected to know the detrimental effect of the sea nodule effluent on the aquatic organisms because of its regular use as important source of animal proteins.

The extraction of metals from PMN is an emerging technique that also needs sincere consideration to define the toxic impact of effluent released during PMN processing. The present study conducted from April 2011 in the laboratory at Banaras Hindu University is an effort in this direction to advise the metallurgist to take utmost care to reduce the toxic stress of this highly contaminated effluent before their disposal. The toxicity of the effluent was analysed by illustrating accumulation of the toxic metals, calculating bioaccumulation factor (BAF) and MPI of different organ systems (skin, muscles, gills, liver, kidney and brain) of the carp *L. rohita* exposed to sea nodule effluent for 20 days.

Materials and methods

Experimental design

Irrespective of sex, healthy specimens of the Indian major carp *L. rohita* were collected in the month of March 2011 from the hatchery situated at Banaras Hindu University, Varanasi, India. The fish were acclimated to the laboratory conditions for 1 month in plastic tanks containing well-aerated and dechlorinated water at 24 ± 2 °C under natural photoperiod. During this period, fish were fed ad libitum with commercial fish pellets (40 % of protein). The water quality parameters were kept nearly constant: dissolved oxygen (7.0–7.5 mg l^{-1}), pH (7.1–7.4), conductivity (125–130 µS cm^{-1}), alkalinity (35–43 mg l^{-1} as $CaCO_3$) and total hardness (39–50 mg l^{-1} as $CaCO_3$).

The effluent generated from the pilot plant at NML (CSIR), Jamshedpur, India, during recovery of metals from polymetallic sea nodule was brought to the laboratory at Banaras Hindu University, in large plastic containers having no contamination.

Batches of 20 fish (weight 28–30 g, length 11–12 cm) were exposed to the 50 l of the effluent (BOD: 182 ± 3 mg l^{-1}, pH: 5.2, Sodium: 130.26 ± 1.96 mg l^{-1}, Potassium: 3.42 ± 0.07 mg l^{-1}, Sulphate: 2,300 ± 4.515 mg l^{-1}, Carbonate: 296 ± 2.2 mg l^{-1}) (control fish retained in the laboratory water) for maximum period of 20 days (beyond which the fish died) with periodic renewal of the effluent. Eight fish each from experimental as well as control aquaria were cold anesthetized and killed after 10 and 20 days of exposure. Entire brain, liver, kidney, gills, small fragments of muscle and skin were dissected out for further processing. Three replicates of each experimental set were run simultaneously.

Heavy metal analysis

Six organ systems (muscle, gills, liver, kidneys, brain and skin) of exposed as well as untreated (control) fish were subjected to analyses of metal accumulation. One gram of each of the tissue samples was dried in petridishes in an oven at 120 °C till there was no further weight loss. Subsequently, they were put into digestion flasks containing a mixture of nitric acid and perchloric acid (4:1 v/v). The digestion flasks were further heated on a hot plate at 120 °C till the materials got dissolved. Later double distilled water was added to the digestion samples in volumetric flask to make the volume up to 25 ml. Amount of metals were analysed using atomic absorption spectrophotometer (AAS, Perkin Elmer). Detection limits of AAS for metals in mg kg^{-1} were 0.1 for Fe, 0.1 for Mn, 0.1 for Zn, 0.01 for Cu, 0.01 for Pb, 0.02 for Cr and 0.01 for Ni. The concentration of the metals in tissues has been given in mg kg^{-1} dry weight of tissue.

Bioaccumulation factor

The bioaccumulation factor (BAF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water (Authman and Abbas 2007). It was calculated using the following equation (Eq. 1):

$$\text{Bioaccumulation factor (BAF)} = \frac{\text{Pollutantconcentrationinfishorgan}}{\text{Pollutant concentration in water}} \quad (1)$$

Metal pollution index

Metal pollution index (MPI) was applied to compare the total metal accumulation level in various tissues of the fish. The MPI values were calculated using the equation proposed by Usero et al. (1997) (Eq. 2):

$$\mathrm{MPI} = (C_{f1} \times C_{f2} \times \ldots C_{fn})^{1/n} \quad (2)$$

where, C_{fn} is the concentration for the metal n in the sample.

Statistical analysis

For statistical analyses, one-way analysis of variance (ANOVA) was performed to determine significance of differences ($p < 0.05$) between the pairs of means. Duncan's Multiple Range Test was also carried out to find out whether any mean is significantly different ($p < 0.05$) from others. The heavy metal concentrations in fish samples were expressed as mean ± SD. Regression analysis for MPI and exposure period were performed by using Microsoft Office Excel 2007. In case of Figs. (1, 2, 3, 4, 5, and 6) for MPI control values has been taken as 0 day.

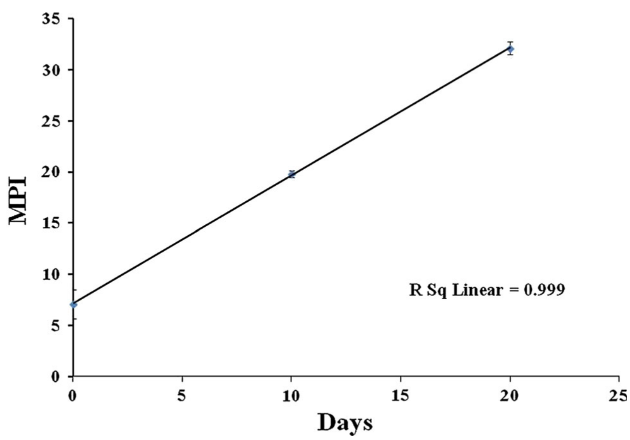

Fig. 1 Time-dependent variation in MPI of skin tissue

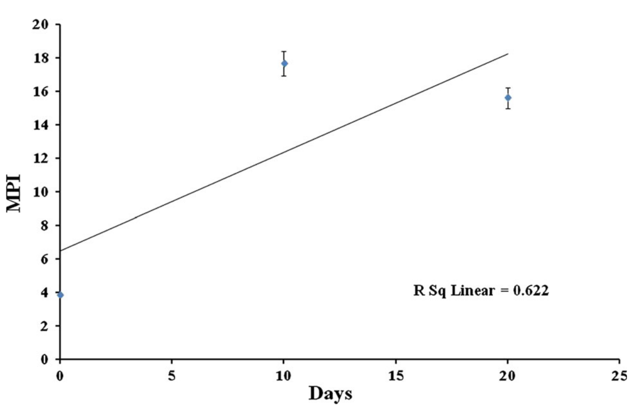

Fig. 2 Time-dependent variation in MPI of muscle tissue

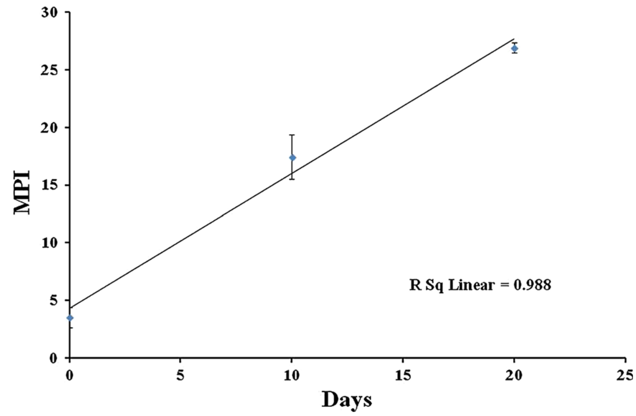

Fig. 3 Time-dependent variation in MPI of gill tissue

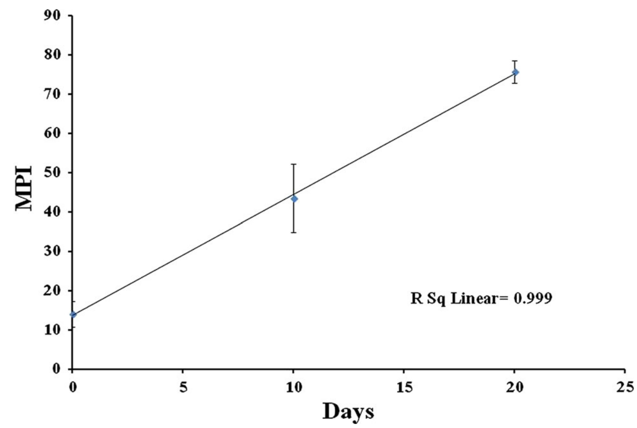

Fig. 4 Time-dependent variation in MPI of liver tissue

Results and discussion

The presence of a large number of toxic metals much above the safe limits in the sea nodule effluent made it highly toxic (Vaseem and Banerjee 2012). The same authors (Vaseem and Banerjee 2013) also observed marked deterioration in the biochemical constitution of six tissues of the fish exposed to PMN effluent.

Exposure to this effluent also caused bioaccumulation of metals in different organ systems of the fish. The data related to metal concentrations (Mn, Cu, Zn, Fe, Pb, Cr and Ni) in different tissue components of the control and

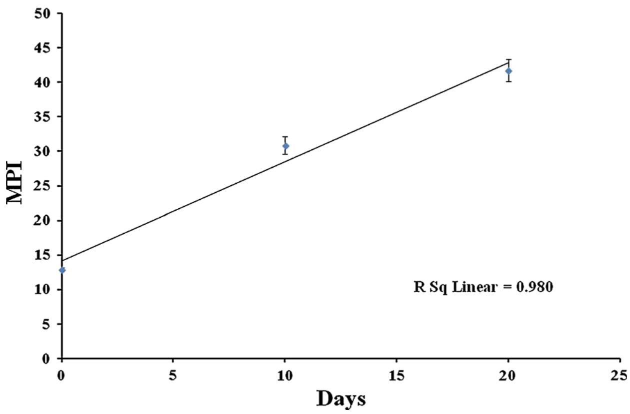

Fig. 5 Time-dependent variation in MPI of kidney tissue

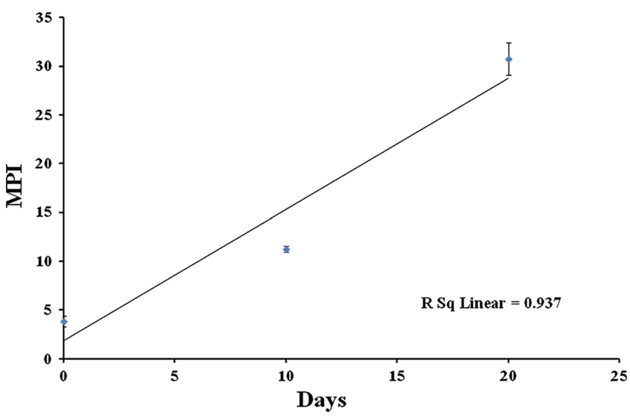

Fig. 6 Time-dependent variation in MPI of brain tissue

exposed fish have been summarised in Table 1. Of the different metals analysed, the concentration of Ni was below detectable levels in control as well as all the exposed tissues. Pb and Cr were also below the detectable level in all these tissues of the control as well as 10-day-exposed fish. In the 20-day-exposed fish, however, Pb was not detected in the skin and brain. Cr was present in all the 20-day-exposed tissues (Table 1). Following exposure, the liver accumulated highest amount of the metals, while muscles showed least deposition excepting Cr whose concentration was lowest in the skin. Trend of concentration of most of the metals present in tissues of exposed fish was liver > kidney > gills > brain > skin > muscles.

Highest accumulation of metals in the liver tissue might be because it is the main site for synthesis of various proteins and other molecules which have high affinities for metals (Fernandes et al. 2008). Also liver being the main site of detoxification of various contaminants, different xenobiotics including metals are transported to this organ from different sites of absorption (Kargin and Erdem 1991). After liver, kidneys became the next important organ of bioaccumulation of metals from the effluent because of their excretory function.

Gills have large surface areas and remain always in direct contact with the aquatic environment; hence, chances of bioaccumulation of metals in the gills is also very high. Further, the barrier distance between the blood in the gills and ambient toxicants is very narrow causing easy movement of the metals into the gills. The other reason for increased metal accumulation in the gills is perhaps due to the increased density of chloride cells whose number increases in contaminated waters because of their role in picking up of anions including metals ions (Mazon et al. 1999, 2002; Costa and Fernandez 2002). The SH groups of glycoproteins of the slime of the fish gills are known to bind with metals causing increased metal concentration in the gills. Sloughing of the mucus from the gill's surface is not very extensive; hence, the concentration of metals in the gills continues to remain elevated. Further, the slime often remains trapped between neighbouring gill lamellae causing retention of the metals leading to their elevated concentration.

The concentration of different metal accumulation in the muscle was least perhaps due to its compact nature and structural configuration. The other reason might perhaps be due to less extensive blood circulation in the muscles than other vital tissues such as liver, kidneys and gills. Also muscle is a less metabolically active tissue (Adhikari et al. 2009; Wagner and Boman 2003; Radhakrishnan 2010). Even though the skin is a boundary tissue, the accumulation of metals is not so high as noticed in the gills, perhaps due to continuous elaboration and sloughing of slime from the body surface (Singh and Banerjee 2008) causing loss of metals bound with –SH groups of the slime. A difference in concentration of trace metals in various fish organs has also been indicated by the study of Vinodhini and Narayanan (2008).

In all the tissue systems, accumulation of Fe was maximum and Pb was minimum after 20 days (Table 1). Statistical analysis revealed that there were significant differences in metal concentration in 10- and 20-day-exposed fish (Table 1). However, concentration of Fe did not show significant alterations in the muscles of 10- and 20-day-exposed fish.

Survey of Table 2 revealed that concentration of Fe, Cr and Cu in all the tissues (except muscles) were above the safe limits (FAO 1983). However, Zn was above the permissible limits (FAO 1983) in most of the tissues excepting

Table 1 Metals concentration (mg kg^{-1}) in various tissues of control and sea-nodule-effluent-exposed fish

Tissue	Mn	Cu	Zn	Fe	Pb	Cr	Ni
Skin							
Cont	0.516 ± 0.223^a	7.51 ± 3.2^a	35.75 ± 0.77^a	21.73 ± 4.39^a	bdl	bdl	bdl
10D	4.57 ± 2.52^b	13.15 ± 0.21^b	39.57 ± 0.64^b	64.29 ± 1.04^b	bdl	bdl	bdl
20D	16.54 ± 0.27^c	37.99 ± 0.62^c	50.35 ± 0.82^c	134.98 ± 2.20^c	bdl	7.99 ± 0.130	bdl
Mus							
Cont	0.558 ± 0.01^a	1.8 ± 0.02^a	5.65 ± 0.09^a	38.45 ± 0.83^a	bdl	bdl	bdl
10D	8.30 ± 0.18^b	6.60 ± 0.142^b	29.51 ± 1.34^b	60.16 ± 2.73^b	bdl	bdl	bdl
20D	10.34 ± 0.22^c	11.34 ± 0.52^c	81.14 ± 3.68^c	61.08 ± 2.77^b	2.1 ± 0.034	11.83 ± 0.25	bdl
Gill							
Cont	0.608 ± 0.2^a	3.86 ± 0.17^a	5.69 ± 2.52^a	14.87 ± 2.79^a	bdl	bdl	bdl
10D	3.99 ± 1.41^b	11.79 ± 1.2^b	25.0 ± 1.76^b	82.27 ± 4.78^b	bdl	bdl	bdl
20D	48.67 ± 0.794^c	60.55 ± 0.98^c	98.03 ± 1.60^c	129 ± 2.11^c	0.8 ± 0.01	12.696 ± 0.20	bdl
Liv							
Cont	0.327 ± 0.22^a	28.19 ± 1.05^a	38.79 ± 0.63^a	131.05 ± 5.95^a	bdl	bdl	bdl
10D	8.88 ± 0.40^b	31.34 ± 1.42^b	102.6 ± 2.21^b	142.50 ± 3.07^b	bdl	bdl	bdl
20D	91.92 ± 4.17^c	287.36 ± 6.20^c	175.24 ± 3.78^c	264.16 ± 5.7^c	2.91 ± 0.148	52.74 ± 5.37	bdl
Kid							
Cont	2.28 ± 0.03^a	14.95 ± 0.76^a	22.69 ± 0.49^a	36.23 ± 0.59^a	bdl	bdl	bdl
10D	7.23 ± 0.11^b	36.37 ± 0.59^b	26.17 ± 1.33^b	131.72 ± 6.71^b	bdl	bdl	bdl
20D	74.57 ± 1.21^c	44.35 ± 2.26^c	137.92 ± 7.03^c	254.93 ± 8.27^c	1.1 ± 0.01	40.32 ± 1.83	bdl
Brain							
Cont	0.919 ± 0.01^a	3.50 ± 0.159^a	4.38 ± 0.22^a	11.25 ± 1.891^a	bdl	bdl	bdl
10D	10.49 ± 0.22^b	4.33 ± 0.196^a	7.7 ± 0.13^b	46.52 ± 0.76^b	bdl	bdl	bdl
20D	20.87 ± 0.45^c	19.33 ± 0.315^b	43.53 ± 0.71^c	71.66 ± 1.17^c	bdl	19.66 ± 0.424	bdl

Cont control, *10 D* 10-day-exposed fish, *20 D* 20-day-exposed fish, *Mus* muscles, *Liv* liver, *Kid* kidney, *bdl* below detectable level

In Table 1, alphabets denote the result of Duncan's multiple range tests. Different alphabets denote significant changes in the concentration of heavy metals in the fish with increasing exposure days ($p < 0.05$). Same alphabets denote nonsignificant changes

Table 2 Metals absorbed (mg kg^{-1}) in different tissues of fish exposed to sea nodule effluent after 20 days

Tissue	Mn	Cu	Zn	Fe	Pb	Cr
Skin	15.722	30.473	14.597	113.25	–#	7.992
Muscles	9.791	9.544	75.49	22.625	2.1	11.832
Gills	48.062	56.688	92.335	114.488	0.8	12.696
Liver	91.639	259.17	136.451	133.11	2.913	52.736
Kidney	72.286	29.409	115.229	218.696	1.1	40.32
Brain	19.951	15.833	39.148	60.41	–	19.66
FAO* (1983)	nl≠	10	50	5.6	1.5	1

*FAO (1983) Food and agricultural organisation, *nl*≠ no limit was suggested by the concerned authority body, # not accumulated

Table 3 Bioaccumulation factor in different organ systems of 20-day-exposed fish

Tissue	Mn	Cu	Zn	Fe	Pb	Cr
Skin	3.335	26.530	61.706	177.139	–	114.171
Muscles	2.088	7.922	99.436	80.157	3.206	169.028
Gills	9.818	42.284	120.134	169.763	1.221	181.371
Liver	18.544	200.670	214.759	346.667	4.447	753.371
Kidney	15.04	30.976	169.02	334.553	1.679	576
Brain	4.21	13.499	53.346	94.042	–	280.857

skin and brain. Leaving apart the gills and kidney, Pb was above its permissible limits (FAO 1983) in all the tissues. No permissible limit has been set by FAO (1983) for Mn accumulation. Hence, from Table 2, it is clear that most of the metal accumulations in the different tissue systems of the exposed fish were above their permissible limits (FAO 1983). Even though metals like Zn, Cu and Mn act as cofactor in several enzyme activities and that Fe is directly involved with haemoglobin formation in the blood tissues, their presence in excessive concentration might be toxic to the fish (Zyadah and Abdel-Bakey 2000; Ikem et al. 2003). Pb is also a toxic element and has no known biological function and shows its negative health effect on aquatic biota and humans (Shahtaheri et al. 2007; Gulser and Erdogan 2008). Chromium is considered as essential trace elements. As it is involved in insulin function and lipid metabolism, it is of great importance, but its higher concentration may cause deleterious effect on the health. Even though our knowledge on the toxic impact of different metals in the human subjects is vast, very little information is available on the deleterious effects of PMN effluent on mammals including human beings. The cumulative and synergistic toxic effects of various metals in different organ systems further aggravate the pathological effect of the effluent on the fish. Most of the metals were found to be above their permissible limits in muscles (edible part) of the exposed fish; hence, these fishes are not suitable for human consumption because consumption of the aquatic food enriched with toxic metals may cause serious health hazard.

To understand the status of bioaccumulation of metals by different organ systems following prolonged exposure of 20 days, the BAF values for these metals in the examined tissue were calculated (Table 3). The highest value of BAF was noticed in the liver for every metal type. Muscles and skin had least BAF values. Amongst the metals studied, Fe showed highest BAF value and Pb lowest. The present finding noticed that concentrations of most of the metals in tissues were higher than those of the effluent (Table 3) perhaps due to their bioconcentration. It is already reported that aquatic organisms accumulate metals to concentrations many times higher than present in the water (Olaifa et al. 2004). While studying the trace metal concentration in the water, sediment and fish tissues from the lake Tanganyik, Chale (2002) also noticed that concentration of metals in the fish tissues were always higher than that of water. The bioaccumulation factors (BAF) of all the metals in different organs in the present study were too high in relation to their highest value of 1.00 which is the nontoxic limit for a metal. The higher BAF values observed for all the metals were due to their bioaccumulation and bioconcentration in the fish tissues. Iron showed the maximum value of BAF amongst all the analysed tissues except muscle due to their maximum bioaccumulation amongst all the metals. This might perhaps be due to its contribution in composition of a large number of tissue components. Amongst the various tissue components examined, highest value of BAF for liver indicated its role of concentrating metals which is several times higher than their concentration in water. It is perhaps due to trapping of these metals in the metallothionein proteins which were also highest in the liver (Al-Yousuf et al. 2000; Ikem et al. 2003; Kent 1998).

The grossly altered concentrations of the various metals in different tissues in relation to increased period of exposure have been properly illustrated in the Figs. 1, 2, 3, 4, 5, and 6 as regression analysis. Figures 1, 2, 3, 4, 5, and 6 showed the time-dependent variation in metal pollution index for skin, muscle, gills, liver, kidney and brain having R^2 0.999, 0.622, 0.988, 0.999, 0.980, and 0.937, respectively, indicating the fact that with increased exposure period, concentration of metals increased in all the tissues examined. Due to meagre deposition of metals, the linear relationship in muscle was not properly depicted.

Conclusion

Fish exposed to sea nodule effluent showed significant bioaccumulation of different metals, most of which were above their permissible limits. MPI values for different organ systems showed linear dependency with the exposure period excepting muscles. Finally, it was found that the sea nodule effluent is very toxic and has negative health impact on the fish. Discharge of this toxic effluent in neighbouring water bodies should be restricted; otherwise, accumulation of different metals in the fish may make them unsuitable for human consumption posing the danger of bioconcentration of the metals at higher tropic levels.

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