ORIGINAL RESEARCH

Efficacy of phytoremediation technology in decontaminating the toxic effluent released during recovery of metals from polymetallic sea nodules

Huma Vaseem · T. K. Banerjee

Abstract Efficacy of phytoremediation using two macrophytes *Azolla pinnata* and *Lemna minor* in decontaminating the toxic effluent released during recovery of metals from polymetallic sea nodules was analysed by applying fish bioassay. The economically important fish, *L. rohita*, was exposed to both, the *Azolla*-phytoremediated effluent (APE) and *Lemna*-phytoremediated effluent (LPE) for assessment of metal bioaccumulation (Fe, Mn, Zn, Cu, Pb, Cr and Cd) and alterations in biochemical (proteins, lipids, glycogen, cholesterol, AST (aspartate amino transferase), ALT (alanine amino transferase) and ALP (alkaline phosphatase) composition of various tissues. Accumulation of metals (e.g. Mn, Zn, Cu and Fe) decreased in most of the tissues exposed to both the phytoremediated effluents perhaps due to decontamination of metals by the two macrophytes. The significantly recovered concentrations of different biomolecules included glycogen, lipids, cholesterol and proteins. The activities of three marker enzymes (AST, ALT and ALP) in phytoremediated effluent-exposed fish also decreased due to lowering of the toxicity of the decontaminated effluents achieved by phytoremediation. The improvement in different biomolecules and reduction in metal concentration in the fish tissues were better in APE exposed fish. However, their concentrations in both the phytoremediated effluent-exposed fish failed to reach the levels of control fish. This study points towards the efficacy of phytoremediation in detoxification of metal-contaminated effluents often released following industrial activities.

Keywords Phytoremediation · Polymetallic sea nodules · Metal bioaccumulation · Biomolecules

Introduction

Various domestic, industrial and mining toxic effluents are detoxified by several chemical, physical and mechanical processes for decontamination prior to their release into the aquatic ecosystem. Such types of detoxifying processes are not enough to contain pollution. Often due to cost factor there is a tendency to evade the mandatory treatment of the effluent for detoxification by the industrial houses. Hence biologists intervened and tried to evolve cheap and effective methods of phytoremediation for decontamination of the toxic effluents polluted with heavy metals (Mishra et al. 2008; Rai and Tripathi 2009; Vaseem and Banerjee 2012; Bharti and Banerjee 2012).

H. Vaseem · T. K. Banerjee (✉)
Department of Zoology, Banaras Hindu University (BHU), Varanasi 221005, India
e-mail: humavaseembhu@gmail.com

H. Vaseem
e-mail: beauty786waseem@gmail.com

Due to acute scarcity of certain economically useful metals like Cu, Cr, Co, Mn and Ni, etc., metallurgist at National Metallurgical Laboratory, Jamshedpur, India, are trying to develop indigenous technique to extract metals from polymetallic sea nodules which are abundantly found at sea bed. For the metal extraction purpose the nodules are processed through many metallurgical methods including reduction, ammonia leaching, solvent extraction, electro winning and smelting (Kumar et al. 1990; Jana et al. 1999; Agarwal and Goodrich 2008; Biswas et al. 2009). Such metallurgical processes also generate large amount of toxic effluent (PMN effluent). The toxicity of this waste water is due to contamination of toxic metals which cause extensive damage to the aquatic fauna (Vaseem and Banerjee 2013a, b). To lower the toxicity load of metals in this effluent, Vaseem and Banerjee (2012) successfully demonstrated decontamination of the effluent by phytoremediation technology.

There are numbers of reports related to application of phytoremediation in decontaminating the effluent released from various industries (Rai, 2008, 2010; Rai and Tripathi 2009). However, there are no bioassay data using fish to analyse the improvement in effluent quality following phytoremediation. Hence to bridge this gap the decontaminated effluents were subjected to fish bioassay analysis using a major carp (*Labeo rohita*) as bioindicator. Fish have widely been used as able bioindicator of variously contaminated waters hence selected (Gupta et al. 2009; Uysal et al. 2009; Maceda-Veiga et al. 2012). Six organs systems (e.g. muscles, liver, gills, kidney, brain and skin) of the fish exposed to the *Azolla*-phytoremediated effluent (APE) and *Lemna*-phytoremediated effluent (LPE) were examined for metal (Mn, Cu, Zn, Fe, Pb, Cr and Ni) bioaccumulation and alterations in biochemical (proteins, lipids, glycogen, DNA, RNA, cholesterol, AST (aspartate amino transferase), ALT (alanine amino transferase) and ALP (alkaline phosphatase) composition.

Materials and methods

Analysis of PMN effluent and its phytoremediation

Physicochemical parameters and concentrations of different metals (Fe, Mn, Zn, Cu, Pb, Cr and Cd) in PMN effluent were examined following the standard methods for evaluation of water and waste water (APHA-AWWA-WPCF, 1998) and atomic absorption spectrophotometer (AAS), respectively (Perkin-Elmer Model 2380, Inc., Norwalk, CT, USA). The details of the physicochemical characteristics of raw PMN effluent have been given by Vaseem and Banerjee (2012) and their finding shows that this effluent has been extremely toxic.

When phytoremediated separately with two different macrophytes (*Azolla pinnata* and *Lemna minor*) for 7 days, the effluents were partially but significantly detoxified due to lowered concentration of different metal species (Vaseem and Banerjee 2012).

Experimental fish

Healthy specimens of the Indian major carp *L. rohita* were collected from the hatchery situated in Banaras Hindu University, Varanasi, India. The fish were acclimated to the laboratory conditions for 1 month in plastic tanks equipped with a continuous supply of well-aerated and dechlorinated water (room temperature: 24 ± 2 °C) and under natural photoperiod. During this period, fish were fed ad libitum with commercial fish pellets. The water was renewed after every 24 h with routine cleaning of the tanks. The examined water quality parameters were: dissolved oxygen (7.0–7.5 mg/l), pH (7.1–7.4), conductivity (125–130 μS/cm), alkalinity (35–43 mg/l as $CaCO_3$) and total hardness (39–50 mg/l as $CaCO_3$).

Experimental design

Acclimated fish were divided into four groups, each of 20 fish (weight of 28–30 g and length of 11–12 cm): one group exposed to 50 L of tap water; the second group to 50 L of the raw PMN effluent, third to 50 L of APE and the fourth to 50 L of LPE in a 80-L capacity plastic tubs for maximum period of 20 days (beyond which fish failed to survive) with regular renewal of water after every 5 days of interval (semi-static bioassay). Fish were regularly fed during experiment. Eight fish from each group were cold anesthetised and killed after 20 days of exposure. Three replicates of each experimental set were prepared. Entire brain, liver, kidney, gills,

small fragments of muscle and skin were dissected out and subjected for metal bioaccumulation (Mn, Cu, Zn, Fe, Pb, Cr and Ni) and biochemical (total proteins, total lipids, glycogen, cholesterol, AST, ALT and ALP) analyses.

Metal analyses

For analyses of metal accumulation six tissues (muscle, gills, liver, kidneys, brain and skin) of control, raw effluent-exposed and APE and LPE-exposed fish were collected in petri-dish and dried in an oven at 120 °C till there was no weight loss. Subsequently, the tissue samples were transferred to digestion flasks containing an acid mixture (nitric acid and perchloric acid (4:1 v/v). The digestion flasks were further heated on a hot plate at 120 °C till the tissues were dissolved. Double distilled water was added to the digestion samples to make their volume to 25 ml. Concentration of metals in the samples were analysed using atomic absorption spectro-photometer (AAS, Perkin Elmer Model 2380, Inc., Norwalk, CT, USA) (detection limits of AAS for metals in mg/kg have been given in Table 1). Concentration of Ni was found to be below the detectable level in all the tissues of APE and LPE exposed fish hence it was not shown in the Table 2.

Biochemical analysis

Total proteins Following Lowry's (Lowry et al. 1951) method total protein level (mg/g) was estimated using bovine serum albumin as standard.

Total lipids Following the method of Folch et al. (1957) total lipids from the tissue samples were extracted in chloroform methanol mixture (2:1)

Transaminase activities For measurement of the AST and ALT activities (μmole pyruvate formed/mg/h) method of Reitman and Franckel (1957) was used by using pyruvate as a standard.

Alkaline phosphatase activity The method of Bergmeyer (1956) was applied to determine the ALP activity (μmole PNP formed/mg/h) where sodium *p*-nitrophenylphosphate was used as substrate.

Cholesterol Zlatkis et al. (1953) method was used for estimation of cholesterol concentration (mg/g).

Glucogen Glycogen content (mg/g) was analysed following the method of Caroll et al. (1956) using anthrone reagent. Glucose was used as a standard.

Statistical analysis

For statistical analyses one-way analysis of variance (ANOVA) ($p < 0.05$) was performed followed by Duncan's multiple range test (DMRT). Values in the tables and figures are given in mean ± SD. In tables and figures alphabets denote the result of DMRT. Different alphabets show significant difference ($p < 0.05$) in the different values of control fish, raw effluent exposed fish, APE exposed fish and LPE exposed fish.

Results

Metal accumulation in the fish exposed to phytoremediated effluents

PMN effluent was found to be highly toxic due to its contaminated condition (BOD: 182 ± 3 mg/l, pH: 5.2, sodium: 130.26 ± 1.96 mg/l, potassium: 3.42 ± 0.07 mg/l, sulphate: 2300 ± 4.515 mg/l, carbonate: 296 ± 2.2 mg/l, Mn 4.9 mg/l, Cu 1.432 mg/l, Zn 0.816 mg/l, Fe 0.762 mg/l, Pb 0.655 mg/l, Cr 0.07 mg/l, Cd 0.018 mg/l) (Vaseem and Banerjee (2012). Following exposure of the fish to this toxic effluent the concentration of all the studied metals increased greatly in all the six tissues. Decontamination of effluent with both

Table 1 Detection limits for metals in tissues

Metals	Zn	Mn	Cu	Fe	Pb	Cr	Ni
Detection limit (mg/kg)	0.1	0.1	0.01	0.1	0.01	0.02	0.01

Table 2 Metal concentrations (mg/kg) in different tissues of the fish exposed to raw effluent, APE and LPE as well as control (wild) fish

FAO[m] (1983)	Mn (nl[n])	Cu (10)	Zn (50)	Fe (5.6)	Pb (nl)	Cr (1)
Skin						
Cont[o]	0.52 ± 0.22^a	7.53 ± 3.25^a	35.75 ± 0.77^a	21.73 ± 4.38^a	bdl	bdl
REE[p]	16.54 ± 0.27^b	37.99 ± 0.62^c	50.35 ± 0.82^c	135.00 ± 2.20^c	bdl	7.99 ± 0.13
APEE[q]	1.23 ± 0.62^a	9.19 ± 0.41^a	37.01 ± 1.85^a	45.51 ± 1.25^b	bdl	bdl
LPEE[r]	1.45 ± 0.81^a	13.14 ± 0.69^b	41.62 ± 1.24^b	44.74 ± 1.1^b	bdl	bdl
Muscles						
Cont	0.56 ± 0.01^a	1.8 ± 0.03^a	5.65 ± 0.09^a	38.45 ± 0.83^a	bdl	bdl
REE	10.35 ± 0.22^b	11.34 ± 0.51^c	81.14 ± 3.69^c	61.08 ± 2.78^c	2.1 ± 0.03	11.83 ± 0.25^a
APEE	0.77 ± 0.20^a	2.97 ± 1.30^{ab}	6.09 ± 0.56^a	47.29 ± 0.31^b	bdl	bdl
LPEE	0.82 ± 0.46^a	3.8 ± 1.07^b	20.12 ± 1.46^b	47.5 ± 0.59^b	bdl	0.64 ± 0.38^b
Gills						
Cont	0.61 ± 0.20^a	3.86 ± 0.17^a	5.69 ± 2.52^a	14.87 ± 2.79^a	bdl	bdl
REE	48.67 ± 0.79^b	60.55 ± 0.99^b	98.03 ± 1.60^c	129.36 ± 2.11^c	0.8 ± 0.02	12.70 ± 0.20^a
APEE	0.86 ± 0.08^a	4.58 ± 1.09^a	7.28 ± 0.69^b	22.66 ± 1.16^b	bdl	bdl
LPEE	0.92 ± 0.17^a	5.14 ± 1.25^a	17.36 ± 1.5^b	20.77 ± 1.02^b	bdl	1.54 ± 0.516^b
Liver						
Cont	0.33 ± 0.23^a	28.19 ± 1.06^a	38.79 ± 0.63^a	131.05 ± 5.96^a	bdl	bdl
REE	91.92 ± 4.18^b	287.36 ± 6.21^b	175.24 ± 3.79^b	264.16 ± 5.71^c	2.91 ± 0.15^a	52.77 ± 5.37^b
APEE	0.59 ± 0.17^a	28.9 ± 1.85^a	39.34 ± 0.99^a	143.82 ± 1.47^b	bdl	bdl
LPEE	0.45 ± 0.13^a	35.12 ± 1.69^a	54.66 ± 2.85^c	142.3 ± 1.15^b	0.01 ± 0.0^b	6.56 ± 1.16^a
Kidney						
Cont	2.28 ± 0.04^a	14.95 ± 0.76^a	22.69 ± 0.49^a	36.23 ± 0.59^a	bdl	bdl
REE	74.56 ± 1.22^b	44.36 ± 2.26^c	137.92 ± 7.03^c	254.93 ± 8.27^c	1.1 ± 0.02	40.32 ± 10.13^b
APEE	3.42 ± 1.26^a	15.91 ± 1.25^a	23.91 ± 1.61^a	36.43 ± 1.3^b	bdl	bdl
LPEE	2.30 ± 0.65^a	21.26 ± 1.76^b	34.29 ± 2.33^b	36.563 ± 1.98^b	bdl	1.83 ± 2.261^a
Brain						
Cont	0.92 ± 0.02^a	3.50 ± 0.16^a	4.38 ± 0.22^a	11.24 ± 1.89^a	bdl	bdl
REE	20.87 ± 0.45^b	19.33 ± 0.31^c	43.53 ± 0.71^c	71.66 ± 1.17^b	bdl	19.66 ± 0.42
APEE	0.85 ± 0.06^a	4.19 ± 0.42^{ab}	5.72 ± 1.24^a	13.10 ± 2.12^a	bdl	bdl
LPEE	0.87 ± 0.14^a	5.52 ± 1.64^b	13.97 ± 2.21^b	11.97 ± 1.38^a	bdl	Bdl

Values are given in mean $\pm$ SD

Different alphabets (a, b and c) show significant difference ($p < 0.05$) among different means

[m] FAO (1983): Food and Agricultural Organization

[n] No limit has been suggested by FAO (1983)

[o] Control wild fish

[p] Raw effluent-exposed fish

[q] *Azolla* phytoremediated effluent-exposed fish

[r] *Lemna* phytoremediated effluent-exposed fish

the macrophytes caused significant depletion in concentration of these metals. The concentration (mg/l) of metals in the *Azolla*-phytoremediated effluent was: Mn 0.19 ± 0.001, Cu 0.039 ± 0.000, Zn 0.019 ± 0.000, Fe 0.228 ± 0.08, Pb 0.026 ± 0.000, Cr 0.005 ± 0.000. Similarly *Lemna*-phytoremediated effluent had: Mn 0.301 ± 0.003, Cu 0.204 ± 0.001, Zn 0.306 ± 0.002, Fe 0.2 ± 0.001, Pb 0.1 ± 0.009 and Cr 0.026 ± 0.00. The percentage of decontamination of the metals reported after phytoremediation was in the order: 96 % of Mn, 97 % of Cu, 70 % of Fe, 96 % of Pb, 93 % of Cr and 78 % of Cd by *Azolla* and 94 % of Mn, 86 % of Cu, 62 % of Zn, 74 % of Fe, 84 % of Pb, 63 % of Cr and 78 % of Cd by *Lemna* phytoremediation (Vaseem and Banerjee 2012).

In the fish exposed to both of these phytoremediated effluents, Mn concentration in all the tissues decreased significantly (Table 2). Similarly the concentration of Cu, Zn and Fe also decreased significantly in most of the tissues of exposed fish to both the phytoremediated effluents (Table 2). Cr was observed in all the raw effluent-exposed tissues and also in muscles, gills, liver and kidney of fish exposed to LPE (Table 2).

Biochemical investigation in the fish exposed to phytoremediated effluents

Following exposure to raw effluent the concentration of glycogen in the different tissues of fish decreased significantly (in muscle from 24 ± 1.09 to bdl, in liver from 142 ± 3.07 to 6 ± 0.13, in gills from 11.1 ± 0.18 to bdl, in kidney from 1 ± 0.01 to bdl) (Table 3). However, its concentration increased in the brain from 26.25 ± 1.30 to 46 ± 2.09. In the fish exposed to APE better recovery of glycogen was observed (muscles: 9.32 ± 0.58, liver: 24.69 ± 0.65 and gills: 4.98 ± 0.10) (Table 3). Similarly in LPE-exposed fish improvement in the concentration of glycogen was observed in these tissues (5.98 ± 0.37 in muscles, 15.01 ± 0.40 in liver, 1.62 ± 0.03 in gill (Table 3). In the kidney and skin the amount of the glycogen in the fish exposed to all the three types of effluents were below the detection limits. However, in the brain of both the phytoremediated effluent-exposed fish the elevated amount of glycogen continued (40.21 ± 2.23 in APE and 33.23 ± 1.85 in LPE (Table 3).

In comparison to wild control fish the loss of lipids (mg/g) following raw effluent exposure was highly significant in all the tissues (in muscles from 7.13 ± 0.32 to 0.2 ± 0.01, in liver from 8.99 ± 0.19 to

Table 3 Glycogen, lipid and cholesterol concentration (mg/g) in different tissues of the fish exposed to raw effluent, APE and LPE as well as control wild fish ($n = 3$)

	Control wild fish	REE fish[p]	APEE fish[q]	LPEE fish[r]
Glycogen				
Muscle	24 ± 1.09^a	Bdl	9.32 ± 0.58^b	5.98 ± 0.37^c
Liver	142 ± 3.07^a	6 ± 0.13^d	24.69 ± 0.65^b	15.01 ± 0.40^c
Gill	11.1 ± 0.18^a	Bdl	4.98 ± 0.10^b	1.62 ± 0.032^c
Brain	26.25 ± 1.30^d	46 ± 2.09^a	40.21 ± 2.24^b	33.23 ± 1.9^c
Kidney	1 ± 0.02	Bdl	Bdl	Bdl
Skin	Bdl	Bdl	Bdl	Bdl
Lipid				
Muscle	7.13 ± 0.32^a	0.2 ± 0.009^d	1.01 ± 0.05^b	0.60 ± 0.04^c
Liver	8.99 ± 0.19^a	1.42 ± 0.03^d	4.63 ± 0.10^b	1.23 ± 0.03^c
Gill	6.99 ± 0.11^a	Bdl	2.69 ± 0.04^b	2.38 ± 0.70^b
Brain	10 ± 0.45^a	2 ± 0.09^d	$4.86\ 0.22^b$	1.32 ± 0.07^c
Kidney	7.91 ± 0.13^a	0.98 ± 0.016^d	$5.11\ 0.08^b$	1.99 ± 0.040^a
Skin	0.32 ± 0.007	Bdl	Bdl	Bdl
Cholesterol				
Muscle	3.91 ± 0.18^a	1.71 ± 0.08^b	1.67 ± 0.03^b	1.79 ± 0.04^b
Liver	6.33 ± 0.14^a	2.4 ± 0.05^d	4.42 ± 0.12^b	3.16 ± 0.08^c
Gill	4.8 ± 0.08^a	1.23 ± 0.02^c	2.91 ± 0.06^b	2.62 ± 0.28^b
Brain	7.8 ± 0.35^a	Bdl	1.68 ± 0.09^b	Bdl
Kidney	4.1 ± 0.03^a	0.82 ± 0.01^d	2.02 ± 0.04^b	1.81 ± 0.04^c
Skin	Bdl	Bdl	Bdl	Bdl

Values are given in mean $\pm$ SD

Different alphabets (a, b, c and d) show significant difference ($p < 0.05$) among different means

Bdl below detectable level

[p] Raw effluent exposed

[q] *Azolla*-phytoremediated effluent exposed

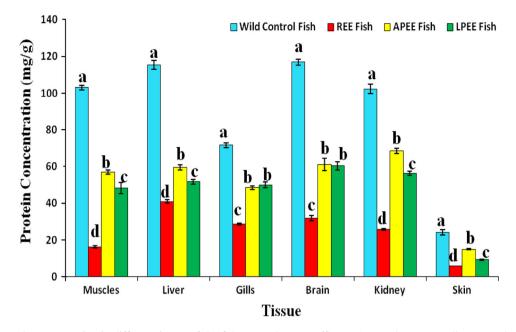[r] *Lemna*-phytoremediated effluent exposed

Fig. 1 Protein concentration in different tissues of the fish exposed to raw effluent, APE and LPE as well as control wild fish. *REE* raw effluent exposed, *APEE Azolla*-phytoremediated effluent exposed, *LPEE Lemna*-phytoremediated effluent exposed Different alphabets (*a*, *b* and *c*) show significant difference ($p < 0.05$) among different means

1.42 ± 0.03, in gills from 6.99 ± 0.11 to bdl, in brain from 10 ± 0.45 to 2 ± 0.09, in kidney from 7.91 ± 0.13 to 0.98 ± 0.02 and in skin from 0.32 ± 0.01 to bdl). There was marked improvement of this biomolecule in all tissues of the fish exposed to APE and the concentration of the lipids was 1.01 ± 0.05 in muscles, 4.63 ± 0.10 in liver, 2.69 ± 0.04 in gills, 4.86 ± 0.22 in brain, 5.11 ± 0.08 in kidney (Table 3). The recovery of lipids in the tissues of fish exposed to LPE was relatively less and their concentrations were 0.603 ± 0.04 in muscles, 1.23 ± 0.03 in liver, 2.38 ± 0.71 in gills, 1.32 ± 0.07 in brain, 1.99 ± 0.04 in kidney (Table 3). In the skin, however, there was complete loss of lipids in the fish exposed to all the three effluent types.

Following exposure to raw effluent the amount of cholesterol (mg/g) in different tissues of fish decreased substantially (from 3.91 ± 0.18 to 1.71 ± 0.08 in muscles, from 6.33 ± 0.14 to 2.4 ± 0.05 in liver, from 4.8 ± 0.08 to 1.23 ± 0.02 in gills, from 7.8 ± 0.35 to bdl in brain, from 4.1 ± 0.03 to 0.821 ± 0.01 in kidney) (Table 3). In the skin, however, the cholesterol level was beyond detectable limits in control as well as in all three effluent-exposed fish groups. The recovery of cholesterol in the muscular tissue in both the phytoremediated effluent-exposed fish was statistically insignificant in relation to raw effluent exposed fish. In liver, gills, brain and kidney significant recovery was noticed (4.42 ± 0.15 in liver, 2.91 ± 0.06 in gills, 1.68 ± 0.09 in brain, 2.02 ± 0.04 in kidney of APE exposed fish and 3.16 ± 0.08 in liver, 2.62 ± 0.278 in gills, bdl in brain, 1.81 ± 0.04 in kidney of LPE exposed fish (Table 3).

When exposed to raw effluent the amount of proteins present in the fish depleted extensively in all the tissues in comparison to the control ones (Fig. 1) (in muscles from 103.07 ± 1.19 to 16.28 ± 0.61, in liver from 115.35 ± 2.45 to 40.99 ± 0.89, in gills from 71.56 ± 1.14 to 28.61 ± 0.47, in brain from 116.97 ± 1.63 to 31.88 ± 1.45, in kidney from 102.36 ± 2.48 to 25.81 ± 0.42 and in skin from 24.13 ± 1.34 to 6 ± 0.00). Due to substantial decontamination of APE, its toxic activity also was lowered as manifested in the recovery of concentration of proteins in various tissues (in muscles 57.02 ± 1.14, in liver 59.48 ± 1.57, in gills 48.56 ± 0.97, in brain 61.23 ± 3.41, in kidney 68.49 ± 1.37, in skin $15 \pm .40$) from those of raw effluent-exposed fish (Fig. 1). Similarly concentration of proteins in the LPE-exposed fish also improved (48.34 ± 3.02 in muscles, 51.64 ± 1.37 in liver, 49.99 ± 1.71 in gills, and 60.6 ± 2.23 in brain, 56.42 ± 1.19 in kidney and 9.29 ± 0.25 in skin (Fig. 1).

PMN effluent exposure caused significant decrease in the AST activity (μmole pyruvate formed/mg/h) in all the tissues excepting skin (7.87 ± 0.13 to 1.02 ± 0.014 in muscle, 18.62 ± 0.95 to 3.43 ± 0.15 in liver, 5 ± 0.08 to 1.16 ± 0.01 in gills, 8.99 ± 0.07 to 2.08 ± 0.02 in brain, 15.91 ± 0.39 to 1.37 ± 0.02 in kidney

Table 4 AST, ALT and ALP activities in different tissues of the fish exposed to raw effluent, APE and LPE as well as control wild fish ($n = 3$)

	Control wild fish	REE fish[p]	APEE fish[q]	LPEE fish[r]
AST				
Muscle	7.87 ± 0.13[a]	1.02 ± 0.01[b]	4.56 ± 0.28[c]	4.22 ± 0.53[c]
Liver	18.62 ± 0.95[a]	3.43 ± 0.16[d]	10.24 ± 0.27[b]	7.16 ± 0.19[c]
Gill	5 ± 0.08[a]	1.37 ± 0.02[c]	2.1 ± 0.04[b]	2.03 ± 0.17[b]
Brain	8.99 ± 0.07[a]	2.08 ± 0.02[d]	6.18 ± 0.34[b]	2.22 ± 0.12[c]
Kidney	15.91 ± 0.34[a]	1.16 ± 0.02[d]	10.98 ± 0.22[b]	6.72 ± 0.13[c]
ALT				
Muscle	11.54 ± 0.17[a]	2.53 ± 0.04[b]	5.63 ± 0.35[c]	5.24 ± 0.23[c]
Liver	21.98 ± 0.47[a]	3.81 ± 0.08[d]	16.28 ± 0.43[b]	10.93 ± 0.29[c]
Gill	11.72 ± 0.20[a]	0.82 ± 0.01[d]	8.67 ± 0.17[b]	3.34 ± 0.07[c]
Brain	12.69 ± 0.11[a]	7.91 ± 0.06[c]	9.89 ± 0.55[b]	10.28 ± 0.57[b]
Kidney	4.81 ± 0.10[a]	0.13 ± 0.002[d]	1.68 ± 0.03[b]	0.61 ± 0.01[c]
ALP				
Muscle	0.53 ± 0[a]	0.20 ± 0.003[c]	0.39 ± 0.02[b]	0.36 ± 0.02[b]
Liver	1.29 ± 0.02[a]	0.56 ± 0.01[d]	0.96 ± 0.02[b]	0.73 ± 0.02[c]
Gill	0.71 ± 0.01[a]	0.26 ± 0.004[c]	0.53 ± 0.01[b]	0.53 ± 0.05[b]
Brain	0.97 ± 0.01[a]	0.24 ± 0.01[d]	0.76 ± 0.04[b]	0.54 ± 0.03[c]
Kidney	0.89 ± 0.01[a]	0.60 ± 0.009[c]	0.73 ± 0.01[b]	0.71 ± 0.01[b]

Values are given in mean ± SD

Different alphabets (a, b, c and d) show significant difference ($p < 0.05$) among different means

[p] Raw effluent exposed

[q] *Azolla*-phytoremediated effluent exposed

[r] *Lemna*-phytoremediated effluent exposed

(Table 4). Significant recovery in the enzyme activity in all the five tissues was noticed following exposure to both the decontaminated effluents. Following phytoremediation activity of the enzyme improved in liver (10.24 ± 0.27 in APE and 7.16 ± 0.19 in LPE), kidneys (10.98 ± 0.22 in APE and 6.72 ± 0.13 in LPE) and brain (6.18 ± 0.34 in APE and 2.22 ± 0.12 in LPE), in the muscles (4.56 ± 0.28 in APE and 4.22 ± 0.54 in LPE) and gills (2.1 ± 0.04 in APE and 2.03 ± 0.18 in LPE) (Table 4). In the skin, however, activity of this enzyme was neither demonstrated in the control nor in the phytoremediated effluent-exposed fish tissues. However, the activity of the enzyme in none of the tissues of the phytoremediated effluent-exposed fish reached the level of untreated control fish. The activity of the enzyme in the LPE showed similar pattern of fluctuation in different tissues even though its activity was comparatively less than APE exposed fish.

Like AST the activity (μmole pyruvate formed/mg/h) of the ALT also decreased in the raw effluent treated fish tissues (11.54 ± 0.173 to 2.53 ± 0.03 in muscles, 21.98 ± 0.47 to 3.81 ± 0.08 in liver, 11.72 ± 0.20 to 0.823 ± 0.01 in gills, 12.69 ± 0.11 to 7.91 ± 0.06 in brain and 4.81 ± 020 to 0.132 ± 0.002 in kidney) (Table 4). In both the phytoremediated exposed fish the enzyme activity recovered significantly in the muscle (5.63 ± 0.35 in APE and 5.23 ± 0.23 in LPE), liver (16.28 ± 0.43 in APE and 10.93 ± 0.29 in LPE), gills (8.67 ± 0.17 in APE and 3.338 ± 0.07 in LPE) and kidneys (1.68 ± 0.03 in APE and 0.612 ± 0.01 in LPE) (Table 4). Recovery in the ALT activity in the brain (9.89 ± 0.55 in APE and 10.28 ± 0.57 in LPE) of the phytoremediated effluent-exposed fish was not so remarkable. Excepting brain the efficacy of *Azolla* decontamination was better in rest of the four tissues as illustrated by improved recovery in this enzyme activity in these tissues.

The decrease in the ALP activity (μmole PNP formed/mg/h) in the different tissues was also extensive following exposure of the wild control fish to raw PMN effluent (0.53 ± 0.00 to 0.196 ± 0.003 in muscles, 1.294 ± 0.02 to 0.564 ± 0.01 in liver, 0.712 ± 0.01 to 0.263 ± 0.004 in gills, 0.970 ± 0.01 to 0.239 ± 0.01 in brain and 0.892 ± 0.01 to 0.599 ± 0.009 in kidney) (Table 4). In this case, also the recovery in the enzyme

activity was more following APE exposure (0.389 ± 0.02, 0.961 ± 0.02, 0.532 ± 0.01, 0.765 ± 0.04 and 0.728 ± 0.01 in muscles, liver, gills, brain and kidney, respectively) than LPE exposure (0.364 ± 0.02 in muscles, 0.729 ± 0.01 in liver, 0.53 ± 0.04 in gills, 0.539 ± 0.03 and 0.713 ± 0.01 in kidney) (Table 4). However, the difference was more pronounced in the liver and brain. In the skin the enzyme activity could not be identified in any of the control as well as experimental tissues.

Discussion

Exposure of fish to the raw PMN effluent caused accumulation of significant amount of different metals (Fe, Zn, Cu, Cr, Mn and Pb) in various tissues. Bioaccumulation of metals in the different tissues of the fish exposed to various waste water has also been reported by Vinodhini and Narayanan (2008), Dube et al. (2005) and Zyadah and Abdel-Bakey (2000). Recently, Vaseem and Banerjee (2012) successfully decontaminated this PMN effluent by phytoremediation technique. Decontamination of metals by phytoremediation is extensively been used (Rai 2008, 2010). To validate and quantify the degree of detoxification by phytoremediation, both the phytoremediated effluents (LPE and APE) were subjected to bioassay analyses using fish tissues as bioindicator.

Concentration of several metals in most of the tissues of both the phytoremediated effluent-exposed fish reached quite nearer to the levels of the control (wild) fish. Accumulation of Mn, Cu, Zn and Fe in all the tissues of both the phytoremediated effluent-exposed fish decreased significantly and their concentration reached nearer to the levels of the untreated control fish (Table 2). Significantly lowered levels of all these metals in both of the phytoremediated effluents exposed fish confirm the effectiveness of phytoremediation in decontamination of Mn, Fe, Cu and Zn by *Azolla pinnata* and *Lemna minor*. In APE-exposed fish accumulation of Cr was not detected in any tissues of the fish. It might be due to almost complete decontamination of this metal from the effluent by phytoremediation. But in case of LPE-exposed fish, significant accumulation of Cr was noticed in most of the tissues (Table 2). This indicates incomplete detoxification of Cr from the effluent by phytoremediation. Analysis of the Table 2 also indicates that the level of metal accumulation in the tissues of LPE exposed fish was higher than those of APE exposed ones. This also points towards the better efficacy of phytoremediation by *Azolla pinnata*. Accumulation of many metals in the tissues of phytoremediated effluent-exposed fish failed to reach the levels of untreated control (Table 2); however, the accumulation levels reached to the safe limits suggested by FAO.

Although the metal load decreased in the phytoremediated effluents, the toxic impact of the decontaminated effluents in the fish tissues continued. This was manifested by the decreased concentration of various biomolecules of the tissues in the fish exposed to the phytoremediated effluents and the reasons for continued toxicity of phytoremediated effluents might also be due to: (1) presence of the non-metallic toxicants and (2) bioconcentration of the metals by the fish tissues due to prolonged exposure even though under greatly lowered metallic stress. Hence fish raised in the phytoremediated effluent still may not be safe for consumption

Significant decrease in the concentration of different biomolecules was noticed in the raw PMN effluent-exposed fish. Exposure of fish to phytoremediated effluent caused significant improvement in the various biomolecules. Improvement in the concentration of protein, glycogen, lipids, DNA and RNA in the fish *Heteropneustes fossilis* exposed to phytoremediated coal mine effluent has also been reported by Bharti and Banerjee (2013). In the present study the concentration of the glycogen in the phytoremediated effluent-exposed fish tissues increased greatly from that of raw effluent exposed ones. However, the concentration of this carbohydrate moiety continued to be below the levels of untreated (wild) fish tissues. This might perhaps be due to continued utilisation of the glycogen molecules to meet additional energy requirement for combating the toxicity of the existing levels of the metals (Table 3) (Singh and Banerjee 2009 and Lin et al. 2011).

Due to decrease in severity of toxicity following phytoremediation by the macrophytes the total lipid (Table 3) and cholesterol concentrations (Table 3) increased significantly in comparison to the raw effluent exposed tissues. This might perhaps be due to lowered requirement of energy with consequent decreased utilisation of these biofuels. However, the lipid concentration did not reach the level of control ones. This might perhaps be due to incomplete detoxification of the effluent by both the macrophytes which caused continuation of additional requirement of energy of the fish even though in smaller amount.

The increase in protein level in several organ systems of both the phytoremediated effluent-exposed fish also suggests partial improvement in the water quality (Fig. 1) in which the fish were retained.

The activities of the three enzymes AST, ALT and ALP also decreased substantially in different tissues of the raw effluent exposed fish. In the phytoremediated effluent-exposed ones, the activities of these enzymes in many tissues increased (Table 4) substantially suggesting the improvement in the quality of the effluent due to bioremediation. However, due to incomplete detoxification by the macrophytes the activities of these enzymes continued to remain below the levels of untreated fish.

Conclusion

Due to significant depletion in metal concentration from the APE and LPE, the toxic stress of the effluent decreased causing significant reduction in metal accumulation and recovery of the various macromolecules in the different tissue systems of the fish. Recovery in amounts of different biomolecules and depletion in metal load were higher in APE exposed fish. However, due to incomplete phytoremediation of effluents, the metals continued to accumulate in the fish tissues. This was reflected in the quantitative alteration of various biomolecules and bioaccumulation of the toxic metals even in the tissues of APE and LPE-exposed fish.

References

Agarwal HP, Goodrich JD (2008) Extraction of Copper, Nickel, and Cobalt from Indian ocean polymetallic nodules. Can J Chem Eng 81:303–306

APHA/AWWA/WPCF. Standard methods for the examination of water and wastewaters (1998) Twentieth ed. American Public Health Association, American Water Work Association and Water Pollution Control Federation, Washington (DC), New York

Bharti S, Banerjee TK (2012) Phytoremediation of the coalmine effluent. Ecotoxicol Environ Saf 81:36–42

Bharti S, Banerjee TK (2013) Analysis of fish tissues under stress of coal mine effluent. Department of Zoology, Banaras Hindu University, Thesis, pp 53–73

Biswas A, Chakraborti N, Sen PK (2009) Multiobjective optimization of manganese recovery from sea nodules using genetic algorithms. Mater Manuf Process 24:22–30

Caroll WV, Longly RW, Roe JH (1956) The determination of glycogen in the liver and muscle by the use of anthrone reagent. J Biol Che 220:583–593

Dube MG, MacLatchyb DL, Kiefferb JD, Glozierc NE, Culpd JM, Casha KJ (2005) Effects of metal mining effluent on Atlantic salmon (*Salmo salar*) and slimy sculpin (*Cottus cognatus*): using artificial streams to assess existing effects and predict future consequences. Sci Total Environ 343:135–154

FAO (1983) Compilation of legal limits for hazardous substances in fish and fishery products. Food Agric Organ Fish Circ No 464

Folch JL, Sloane M, Stanley GH (1957) A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–5075

Gupta A, Rai D, Pandey R, Sharma B (2009) Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. Environ Monit Assess 157:449–458

Jana RK, Pandey BD, Premchand (1999) Ammoniacal leaching of roast reduced deep-sea Manganese nodules. Hydrometallurgy 53:45–56

Kumar V, Pandey BD, Akerkar DD (1990) Electrowinning of nickel in the processing of polymetallic sea nodules. Hydrometallurgy 4:189–201

Lin YS, Tsai SC, Lin HC, Hsiao CD, Wu SM (2011) Changes of glycogen metabolism in the gills and hepatic tissue of Tilapia (*Oreochromis mossambicus*) during short-term cd exposure. Comp Physiol Biochem 48:517–527

Lowry OH, Rosenbrough NG, Farr AL, Randall RG (1951) Protein measurements with folin phenol reagent. J Biol Chem 193:265–275

Maceda-Veiga A, Monroy M, De Sostoa A (2012) Metal bioaccumulation in the Mediterranean barbell (*Barbus meridionalis*) in a Mediterranean river receiving effluents from urban and industrial wastewater treatment plants. Ecotoxicol Environ Saf 76:93–101

Mishra VK, Upadhyaya AR, Pandey SK, Tripathi BD (2008) Heavy metal induced due to coal mining effluent on surrounding aquatic system and its management through naturally occurring aquatic macrophytes. Bioresour Technol 99:930–936

Rai PK (2008) Phytoremediation of Hg and Cd from industrial effluents using an aquatic free floating macrophyte *Azolla pinnata*. Int J Phytorem 10:430–439

Rai PK (2010) Phytoremediation of heavy metals in a tropical impoundment of industrial region. Environ Monit Assess 165:529–537

Rai PK, Tripathi BD (2009) Comparative assessment of *Azolla pinnata* and *Vallisneria spiralis* in Hg removal from G.B. Pant Sagar of Singrauli industrial region. India Environ Monit Assess 148:75–84

Singh AK, Banerjee TK (2009) A study on carbohydrate moieties of gills and air-breathing organs of the walking catfish *Clarias batrachus* (Linn.) following exposure to arsenic. Toxicol Environ Chem 91:43–52

Uysal K, Kose E, Bulbul M, Donmez M, Koyun M, Erdogan Y, Omeroglu A, Ozmal F (2009) The comparison of heavy metal accumulation ratios of some fish species in Enne Dame Lake (Kutahya/Turkey). Environ Monit Assess 157:355–362

Vaseem H, Banerjee TK (2012) Phytoremediation of the toxic effluent generated during recovery of precious metals from polymetallic sea nodules. Int J Phytorem 14:457–466

Vaseem H, Banerjee TK (2013a) Effect of effluent released during recovery of precious metals from polymetallic sea nodules on biochemical changes in freshwater fish *Labeo rohita*. Clean Soil Air Water 41:1–6

Vaseem H, Banerjee TK (2013b) Metal accumulation in fish *Labeo rohita* exposed to effluent generated during recovery of precious metals from polymetallic sea nodules. Int J Environ Sci Technol. doi:10.1007/s13762-013-0381-2

Vinodhini R, Narayanan M (2008) Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). Int J Environ Sci Tech 5:179–182

Zlatkis A, Zak B, Boyle AJ (1953) A new method for direct determination of serum cholesterol. J Lab Clin Med 41:481–492

Zyadah MA, Abdel-Bakey TC (2000) Toxicity and bioaccumulation of copper, zinc and cadmium in some aquatic organism. Bull Environ Contam Toxicol 64:740–747