

Bioaccumulation of Metals in the Edible Catfish *Heteropneustes fossilis* (Bloch) Exposed to Coal Mine Effluent Generated at Northern Coalfield Limited, Singrauli, India

Sandhya Bharti · Tarun Kumar Banerjee

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Abstract Metal accumulation in various tissues of *Heteropneustes fossilis* exposed to the effluent generated from an open cast coal mine was investigated. The contents of Fe, Mn, Cu, Zn, Ni, Pb, Cd and Cr in the effluent were above the permissible limits as suggested by the different pollution control agencies. Out of the eight metals investigated, accumulation (mg kg^{-1} dry weight of tissue) of Fe was maximum in every tissues followed by liver (265.88 ± 49.89) > kidney (153.0 ± 65.85) > gills (50.66 ± 23.923) > brain (49.303 ± 5.11) > air breathing organs (27.98 ± 10.93) > skin (19.56 ± 2.53) > muscles (8.74 ± 0.83). This was succeeded by Pb in brain (39.35 ± 5.79), Zn in kidneys (27.04 ± 2.31), Mn in the gills (20.69 ± 3.044), Cu (12.53 ± 1.01) > Cr (5.10 ± 2.87) in liver and Cd in kidneys (2.18 ± 0.084). Liver and ABOs showed significant uptake of all the metals. Except Cu and Cr, accumulation of most of the metals by kidneys and gills were also significant. Accumulation of Fe, Cd, Pb and Cr in most of the tissues of exposed fish were above the permissible limits indicating their potential hazardous impact on fish as well as on fish consumers. Even in the tissues of untreated fish the concentrations of Fe (12.26–428.47), Cd (0.2–1.22), Pb (0.02–9.42) and Cr (1.14–11.05) were above the permissible limits. This clearly demonstrates greater bioavailability of these metals in the area.

Keywords Bioaccumulation · Dudhichua open cast coal mine · Fish · Metals · Toxicity analyses

Effluent generated during excavation of coal is an important environmental pollutant having large number of metals as well as other toxic substances (explosives, washing reagents etc.), often used at the site of excavation. Discharge of coal mine effluent (CME) into the aquatic ecosystems threatens the aquatic flora and fauna of the area (Olsvik et al. 2000; Tiwary 2001). Some workers have investigated the contamination of aquatic ecosystem by metal mining effluents (Dube et al. 2005; Scmitt et al. 2007). However, the toxic impacts of CME on the fish are less investigated and our knowledge is mainly limited to the studies examining, bioaccumulation and toxicopathological impact of individual xenobiotics on different fishes (Kargin and Erdem 1991; Singh and Banerjee 2008). For the present study, *Heteropneustes fossilis* was chosen as the model organism because it is one of the most cherished fish next to murels in Asia. It is hardy fish and could survive in polluted aquatic ecosystem. Moreover, it is now declared as one the most threatened fish species in the world (Haniffa et al. 2008). The effluent was collected from Dudhichua, one of the main coal extraction sites under Northern Coal Field Limited (NCL), Singrauli (India). NCL is the fastest growing coal producers of India having 11 open cast coal mines located in District Sidhi, M.P. and partly in Sonebhadra District of U.P. (India) covering 2,202 km² area of Singrauli region and 80 km² area of Sonebhadra region (Bose and Leitman 1996). The waste effluents generated from these coal mines are generally discharged into the neighbouring Rihand and Son rivers causing extensive contamination. Therefore, in this paper efforts have been made to analyse the toxic impact of the

S. Bharti · T. K. Banerjee
Department of Zoology, Banaras Hindu University,
Varanasi 221005, India
e-mail: sandhyabharti54@gmail.com

T. K. Banerjee (✉)
Eco-Physiology Unit, Banaras Hindu University,
Varanasi 221 005, India
e-mail: tkbzool@yahoo.co.in

coal mine effluent in terms of bioaccumulation of different metals in certain vital organ systems (muscle, liver, kidneys, brain, gills, air breathing organs and skin) of *Heteropneustes fossilis* that might also help to establish the fish as an efficient biomonitor to detect CME contamination. Also, the purpose of this study was to examine the suitability of consumption of contaminated fish from human point of view.

Materials and Methods

The effluent was collected in the month of July 2009 from Dudhichua open cast coal mine at NCL. Irrespective of sex, specimens of *Heteropneustes fossilis* (45 ± 5 g of body weight, 17 ± 5 cm in length) were collected from a single population from a local fish market, Chaukaghat, Varanasi, Uttar Pradesh. Fish were acclimated to the laboratory conditions for 1 month in tap water (having dissolved oxygen 5.5 mg L^{-1} , pH 7.2, water hardness 54 mg L^{-1} and water temperature $23 \pm 1^\circ\text{C}$) in large plastic aquaria. They were fed with minced goat liver. Water was renewed after every 24 h with routine cleaning of the aquaria. For the analyses of lethal toxicity, three groups of 15 fish were exposed separately to 25 Litre (L) of the coal mine effluent (having dissolved oxygen $5.56 \pm 1.54 \text{ mg L}^{-1}$, pH 6.2 ± 0.56 , water hardness $98.26 \pm 0.045 \text{ mg L}^{-1}$ and water temperature $22.5 \pm 1.05^\circ\text{C}$) for 26 days in the plastic aquaria (beyond which the fish died) without periodic renewal of the effluent. Parallel control groups of fish were retained in tap water. The temperature and pH of the coal mine effluent were measured at the collection site itself. Physicochemical analyses and metal concentrations of Fe, Mn, Ni, Zn, Cu, Pb, Cr and Cd in CME were studied following the standard methods for examination of water and waste water by atomic absorption spectrophotometer (AAS) (Perkin-Elmer Model 2380, Inc., Norwalk, CT, USA) outlined by American Water Public Health Association, American Water Works Association and Water Pollution Control Federation (APHA-AWWA-WPCF 1998) (Table 2). Different organ systems (muscle, gills, liver, kidneys, brain, skin and air breathing organs) of exposed as well as untreated (control) fish were subjected to metal analyses. One gram of each of the tissue samples was dried in petri dishes in an oven at 120°C till there was no 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. Using nitric acid the pH of the solution was maintained at 2.0. Amount of metals were analysed using AAS. The estimated detection limits of the metals in the effluent (mg L^{-1}) and tissue (mg kg^{-1} dry weight of tissue) are given in Table 1. The chemicals used for analysis were Merck analytical grade. Quality control measures were taken to assess contamination and reliability of the data. Blank and drift standards were run after five readings to calibrate the instrument. The heavy metal concentration in CME as well as fish samples were expressed as mean \pm SEM using excel 2007. Student *t* test was also performed to determine the level of significance of metal accumulation in exposed fish in comparison to untreated controls. Differences were regarded as significant at $p < 0.05$ and $p < 0.01$.

Results and Discussion

Metal concentration of Fe, Mn, Cu, Zn, Ni, Pb, Cr and Cd present in CME has been summarized in Table 2. Concentrations of all the eight elements in the CME were above the permissible limit recommended for any of industrial effluents (CPCB 1993; EPA 2002). Amongst these metals, the concentration of Fe was highest ($22.906 \pm 0.023 \text{ mg kg}^{-1}$ dry wt) while the concentration of Cd was lowest ($0.0598 \pm 0.008 \text{ mg kg}^{-1}$ dry wt) (Table 2). The absorption of above mentioned metals in the different tissue components of the exposed fish has been summarised in Table 3. Following 26 days of exposure the concentration of different metals increased in the various organ systems depending on their physiological role. Of the eight metals analysed in the different tissues, the concentration of Ni was below the detection level hence was not taken into further consideration. On the other hand the concentration of Fe was highest in every tissue investigated. The liver accumulated maximum concentration of Fe ($265.88 \pm 49.89 \text{ mg kg}^{-1}$ dry wt). The concentration of Fe in rest of the tissues followed the order: kidneys > gills > brain > air breathing organs > skin > muscle (Table 4). Following exposure although the concentration of Fe detected in muscle and skin were quite substantial,

Table 1 Detection limits for metals

| Metal | Fe | Mn | Zn | Cu | Pb | Cr | Cd | Ni |
|---|------|------|------|-------|------|-------|--------|-------|
| Detection limits in effluent (mg L^{-1}) | 0.08 | 0.06 | 0.05 | 0.001 | 0.01 | 0.002 | 0.0005 | 0.004 |
| Detection limits in tissues (mg kg^{-1}) | 0.1 | 0.1 | 0.1 | 0.01 | 0.01 | 0.02 | 0.05 | 0.01 |

Table 2 Concentration of metals in the Dudhichua mine effluent (mg L^{-1}) versus other effluents compared to the permissible level (mg L^{-1}) recommended for industrial effluent

| Heavy metals | Dudhichua, NCL present study | Bina, NCL Mishra et al. (2008) | Brunswick mines 100% Effluent Dube et al. (2005) | CPCB* 1993 | EPA [@] 2002 |
|--------------|------------------------------|--------------------------------|--|------------|-----------------------|
| Fe | 22.906 \pm 0.023 | 4.8 \pm 0.03 | 580.0 \pm 81.65 | 3.0 | 2.0 |
| Mn | 9.606 \pm 1.599 | – | 216.7 \pm 72.65 | 5.0 | 0.2 |
| Cu | 2.039 \pm 0.231 | 0.15 \pm 0.02 | 20 | 3.0 | 0.5 |
| Zn | 1.034 \pm 0.150 | 7.1 \pm 0.4 | 193.3 \pm 114.65 | 5.0 | 0.1 |
| Ni | 0.856 \pm 0.187 | 0.07 \pm 0.002 | – | 3.0 | 0.1 |
| Pb | 0.669 \pm 0.105 | – | 5 | 0.1 | 0.05 |
| Cr | 0.182 \pm 0.007 | 1.3 \pm 0.09 | – | 0.1 | 0.05 |
| Cd | 0.0598 \pm 0.008 | 0.09 \pm 0.004 | – | 2.0 | 0.01 |

Values are expressed as Mean \pm SEM, (n = 5)

* Central pollution control board 1993

[@] Environmental protection act 2002, General notice No. 44, February 2003

Table 3 The concentration of different metals in various tissue systems of *Heteropneustes fossilis*

| FAO [@] 1983 | Fe 5.6 | Mn ml [#] | Cu 10 | Zn 50 | Cd 0.5 | Pb 1.5 | Cr 1.0 |
|-----------------------|----------------------|--------------------|-------------------|--------------------|--------------------|--------------------|-------------------|
| Muscles | | | | | | | |
| Control | 12.26 \pm 0.32 | 0.48 \pm 0.06 | 1.56 \pm 0.19 | 3.42 \pm 0.57 | 0.26 \pm 0.01 | 0.26 \pm 0.08 | 1.14 \pm 0.05 |
| Exposed | 21.00 \pm 0.76** | 0.74 \pm 0.05 | 3.59 \pm 2.06** | 4.47 \pm 0.47 | 0.26 \pm 0.10 | 2.69 \pm 1.29** | 1.40 \pm 0.29 |
| Skin | | | | | | | |
| Control | 15.71 \pm 2.37 | 0.88 \pm 0.37 | 1.23 \pm 0.05 | 12.87 \pm 0.88 | 0.30 \pm 0.04 | 0.07 \pm 0.05 | 2.57 \pm 0.23 |
| Exposed | 35.27 \pm 2.00** | 1.23 \pm 0.34 | 2.94 \pm 0.58** | 30.82 \pm 0.48** | 0.44 \pm 0.03 | 3.47 \pm 0.66** | 2.65 \pm 0.34 |
| Gills | | | | | | | |
| Control | 133.05 \pm 25.03 | 17.32 \pm 3.23 | 7.51 \pm 1.2 | 29.92 \pm 4.35 | 1.22 \pm 0.24 | 0.02 \pm 0.01 | 5.00 \pm 0.85 |
| Exposed | 183.71 \pm 7.59** | 38.01 \pm 5.53** | 7.77 \pm 1.58 | 38.27 \pm 0.88** | 3.24 \pm 0.24** | 0.19 \pm 0.10* | 6.25 \pm 0.42 |
| ABOs | | | | | | | |
| Control | 70.60 \pm 10.93 | 2.37 \pm 0.48 | 3.79 \pm 0.24 | 12.39 \pm 1.78 | 0.23 \pm 0.02 | 3.96 \pm 0.96 | 3.56 \pm 0.55 |
| Exposed | 98.58 \pm 7.65** | 10.05 \pm 0.84** | 4.71 \pm 1.06* | 26.37 \pm 0.73** | 0.77 \pm 0.06** | 11.68 \pm 2.21** | 8.06 \pm 2.51** |
| Liver | | | | | | | |
| Control | 428.47 \pm 47.0 | 0.29 \pm 0.01 | 4.05 \pm 1.86 | 8.94 \pm 1.12 | 0.56 \pm 0.091 | 2.75 \pm 0.48 | 2.19 \pm 0.22 |
| Exposed | 694.35 \pm 20.11** | 1.53 \pm 0.03* | 16.58 \pm 0.54* | 23.39 \pm 0.80** | 1.00 \pm 0.101** | 6.38 \pm 1.24* | 7.29 \pm 2.87** |
| Kidney | | | | | | | |
| Control | 162.08 \pm 4.67 | 0.02 \pm 0.01 | 7.30 \pm 1.46 | 9.40 \pm 0.31 | 0.41 \pm 0.03 | 0.02 \pm 0.01 | 8.61 \pm 0.41 |
| Exposed | 315.07 \pm 45.19** | 4.15 \pm 1.13** | 7.38 \pm 0.14 | 36.43 \pm 2.01** | 2.58 \pm 0.09** | 26.81 \pm 4.48** | 8.84 \pm 0.61 |
| Brain | | | | | | | |
| Control | 84.42 \pm 5.82 | 0.02 \pm 0.00 | 4.81 \pm 0.41 | 4.29 \pm 0.10 | 1.2 \pm 0.04 | 9.42 \pm 1.45 | 11.05 \pm 0.20 |
| Exposed | 133.72 \pm 4.59** | 1.94 \pm 0.14* | 6.69 \pm 0.45** | 9.89 \pm 0.18** | 2.13 \pm 0.08 | 48.77 \pm 4.62** | 12.18 \pm 0.55 |

All values are expressed in mg kg^{-1} dry wt

Values are expressed in mean \pm sem, n = 15

[@] FAO Food and Agricultural Organization 1983

* Indicates a significant difference at $p < 0.05$, ** indicates a significant difference at $p < 0.01$. Significance was determined by Student's *t* test with reference to untreated (control) groups fish

they were significant at $p < 0.01$ (Table 3). The order of maximum concentration of other metal accumulation were $\text{Pb} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Cr} > \text{Cd}$. Highest concentrations of these metals were found in brain, kidneys, gills, liver,

liver and kidneys respectively. Significant accumulation of all the metals were detected in liver and ABOs. Except Cu and Cr, uptake of almost all the metals by kidneys and gills were also noteworthy. Although accumulation of Fe, Cu,

Table 4 Quantity of metals (total accumulated metals in exposed fish – metal already retained in unexposed fish) absorbed by the different tissues of *Heteropneustes fossilis* following exposure to the coal mine effluent

| Metals | Liver | Kidney | Gills | Brain | ABOs | Skin | Muscle | WHO [^] 1985 | FAO [@] 1983 |
|--------|-----------------------|----------------------|-----------------------|----------------------|----------------------|---------------------|--------------------|--------------------------|--------------------------|
| Fe | 265.88 ± 49.89 | 153.0 ± 65.85 | 50.66 ± 23.923 | 49.303 ± 5.11 | 27.98 ± 10.93 | 19.56 ± 2.53 | 8.74 ± 0.83 | – | 5.6 |
| Zn | 14.45 ± 1.11 | 27.04 ± 2.31 | 8.34 ± 3.51 | 5.61 ± 0.104 | 13.98 ± 1.12 | 17.95 ± 1.25 | 1.05 ± 0.89 | 10 – 75 | 50 |
| Mn | 1.24 ± 0.04 | 4.15 ± 1.13 | 20.69 ± 3.044 | 1.94 ± 0.135 | 7.68 ± 1.31 | 0.35 ± 0.07 | 0.26 ± 0.12 | 0.5 | nl [#] |
| Cu | 12.53 ± 1.01 | 0.08 ± 0.09 | 0.26 ± 0.069 | 1.88 ± 0.8 | 0.92 ± 0.09 | 1.70 ± 0.54 | 2.03 ± 1.71 | 3.0 | 10 |
| Pb | 3.63 ± 0.83 | 26.81 ± 4.99 | 0.183 ± 0.097 | 39.35 ± 5.79 | 7.72 ± 2.21 | 3.39 ± 0.62 | 2.43 ± 1.22 | 2.0 | 1.5 |
| Cr | 5.10 ± 2.87 | 0.23 ± 0.012 | 0.263 ± 0.444 | 1.08 ± 0.413 | 4.50 ± 2.092 | 0.08 ± 0.02 | 0.26 ± 0.24 | 0.15 | 1.0 |
| Cd | 0.44 ± 0.191 | 2.18 ± 0.084 | 2.021 ± 0.027 | 0.93 ± 0.057 | 0.54 ± 0.078 | 0.14 ± 0.07 | 0.00 ± 0.00 | 2.0 | 0.5 |
| Ni | BDL ⁺ | BDL | BDL | BDL | BDL | BDL | BDL | 0.5 | nl |

All values are expressed in mg kg⁻¹ dry wt

Values are expressed as mean ± sem, n = 15

[^] WHO – World Health Organisation 1985

⁺ BDL – below detection level

[#] nl – no limits was suggested by the concerned authority body

Values above the permissible limits are shown in bold letters

Pb were significant at $p < 0.01$ in muscles and skin, comparatively smaller concentration difference of remaining metals between exposed and untreated fish were found (Table 3). This is in agreement with findings of previous workers in other fishes (Gbem et al. 2001; Wagner and Boman 2003). In addition, it was found that accumulations of Fe and Cr in all tissues were above the permissible limit (FAO 1983) while the concentration of Pb was above the permissible limit (FAO 1983) in almost all the tissues (excepting gill). The concentration of Cd was above the permissible limit in most of the tissues except in skin and muscles. The concentrations of remaining metals (Zn and Ni) were below the permissible levels (FAO 1983). Our finding noticed that concentration of the metals in tissues were higher than the effluent perhaps due to biomagnification. While studying the trace metal concentration in the water, sediment and fish tissues from Lake Tanganyik, Chale (2002) also reported that concentrations of metals in the fish tissues were always higher than that of water. Although metals like Zn, Cu and Mn act as cofactor in several enzyme activities and that Fe being directly involve with haemoglobin formation in the blood tissues, excessive concentrations of these metals may also cause serious damage to normal metabolic activities of the fish (Zyadah and Abdel-Bakey 2000). A survey of Table 4 indicates that out of eight metals the concentration of Fe was found maximum in every tissue perhaps due to its contribution in composition of several tissue components. Metals like Fe and Mn play key role in uptake and accumulation in the tissues by competing with other toxic and/or bioinactive metal ions influencing their bioconcentration in fish organ systems (Ginneken et al. 1999). Differences in

metal accumulation in various fish organs have also been observed by Radhakrishnan (2010) (Table 5). The reason for increased Fe, Cu, Zn and Cr concentrations in liver might be because liver is the main site of synthesis of various proteins and other molecules which have high affinities for metals forming complexes (Fernandes et al. 2008). Being the main site for detoxification of various contaminants, metals are transported to the liver from different sites of absorption (Kargin and Erdem 1991). Out of the seven tissues, kidney showed the deposition of maximum numbers of toxic metals perhaps due to its excretory functions. On the other hand muscle was the site of least metal accumulation (Table 4). It is also reported that muscle is not an active tissue in accumulating metals (Wagner and Boman 2003; Radhakrishnan 2010) (Table 5). The other important organs showing active absorption of metals after liver and kidney are gills, ABOs and skin. Because of the proximity of gills to the external environment and the thinness of the barrier distance between the branchial blood and the aquatic environment, gills are the main site for the metals (like Cu, Zn and Cd) uptake from the water. The other reason for increased metal accumulation in gills is perhaps due to the increase in number of chloride cells which have the property of selective accumulation of metal ions. Heavy metal ions precipitate the mucous secretions of the gills. These precipitates occupy the intralamellar spaces and the movements of the gill filaments become arrested, surface area is reduced and respiration is prevented causing death of the fish (Roy 2010). The ABOs of *Heteropneustes fossilis* are modified gill structure. Blood is regularly transported to the ABOs for aerial oxygenation. In this process blood bound

Table 5 Comparison of metal accumulation in fishes from different regions versus present study

| Medium | Tissue name | Fe | Zn | Mn | Cu | Pb | Cr | Cd | Ni | References |
|-----------------------|-------------|---------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------------------------|
| Brunswick mines | Liver | 980 ± 59.0 | 75 ± 14 | 6.1 ± 3.0 | 40 ± 24 | 0.73 ± 0.36 | 0.20 ± 0.18 | 0.24 ± 0.20 | 0.29 ± 0.04 | Wagner and Boman (2003) |
| | Muscles | 51 ± 5 | 29 ± 6 | 2.60 ± 1.0 | 1.80 ± 0.7 | <0.25 | 0.3 | 0.01 | 0.3 | |
| | Liver | | | | | 9 | 4 | 5 | 4.5 | |
| Synthesized medium | | | | | | | | | | Vinodhini and Narayanan (2008) |
| Chaliyar river Kerala | Kidney | | | | | 7 | 3 | 5 | 2 | Radhakrishnan (2010) |
| | Gills | | | | | 5 | 3.5 | 7 | 4 | |
| | Flesh | | | | | 2.5 | 2 | 1.5 | 1.5 | |
| | Liver | 325.6 ± 12.62 | 42.32 ± 2.18 | 21.5 ± 3.24 | 35.2 ± 1.72 | 0.2 ± 0.03 | 7.12 ± 1.3 | – | – | |
| | Gills | 110.25 ± 2.75 | 32.72 ± 3.5 | 26.0 ± 1.62 | 6.2 ± 0.3 | 0.18 ± 0.05 | 2.5 ± 0.1 | – | – | |
| | Muscles | 76.95 ± 2.1 | 17.42 ± 1.04 | 2.5 ± 0.22 | 0.62 ± 0.03 | 0.06 ± 0.01 | 0.12 ± 0.01 | – | – | |

contaminants continuously reach this tissue which lacks excretory property. Hence increase in concentrations of the various metals in this tissue is obvious. It is interesting to notice that even being boundary tissue, the accumulation of different metals in skin were not so high as observed in gills, ABOs, liver, kidney etc. perhaps due to continuous elaboration and sloughing of slime from the body surface (Singh and Banerjee 2008). Role of slime in eliminating metal ions is well documented. A difference in concentration of trace metals in various fish organs is indicated by the study of Vinodhini and Narayanan (2008). Further, Table 3 shows that accumulation of Pb was highest in the brain followed by kidneys > ABOs > liver > skin > muscle > gills. It may be due to the property of solubility and diffusibility of Pb in lipids present in large quantity in nerve fibers of the brain (Venugopal and Luckey 1977). It was noticed that even the fish directly brought from main local fish market, Chaukaghat, Varanasi contains toxic metals like Cr, Cd, Pb and Fe above the permissible limits in its various vital organ systems (World Health Organization 1985; FAO 1983) (Table 3). Thus, this finding is worrisome in view of metal potential danger to this threatened fish as well as to the fish consumers. Also, it alarms us towards their striving survivalship in present aquatic ecosystem hence needs immediate protective measures. Amongst the seven organ systems, gills accumulated maximum amount of Mn. Although no permissible limit was set by (FAO 1983) for Mn accumulation, Radhakrishnan (2010) has recently demonstrated the accumulation of significant amount of Mn in the gills of *Heteropneustes fossilis*. The cumulative toxic effects of various metals in different organ systems further aggravate the pathological effect of the effluent on fish. Cr, Cd and Pb are toxic elements that have no known biological role and exhibit their carcinogenic impact on aquatic biota and humans (Malik et al. 2010). Consumption of the aquatic food enriched with toxic metals may cause serious health hazards through food chain magnification. Finally, it is evident from the data that the coal mine waste water of Dudhichua, NCL, Singrauli, India contains higher concentrations of metals which also contaminate the neighbouring water bodies. Exposed *H. fossilis* shows significant accumulation of toxic metals (Cr, Cd, Pb and Fe) in their different organs. Also, concentration of these metals was above the permissible limit (FAO 1983). Lethal toxicity of the CME is confirmed by mortality of the fish (before 1 month, average after 26 days) following continuation of exposure. This might be due to excessive accumulation of toxic metals as demonstrated by our experiment. Extensive presence of different metals in various organ systems of untreated fish including their edible parts further makes them unsuitable for human consumption and also poses the danger of bioaccumulation of toxic metals at higher tropic levels.

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