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Metal concentrations in water, sediment, and fish from sewage-fed aquaculture ponds of Kolkata, India

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Abstract The concentrations of lead, cadmium, chromium, copper, and zinc were investigated in the sewage-fed pond water, sediment, and the various organs of *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Oreochromis mossambicus*, and *Cyprinus carpio* cultured in sewage-fed ponds, Kolkata, India. Among the metals, cadmium, lead, and zinc were detected in water and, except lead, were below the water quality guideline levels for the protection of freshwater aquatic life proposed by CEQG (Canadian Environmental Quality Guidelines) and AENV (Alberta Environment). Therefore, lead could pose danger to aquatic organisms. All the five metals were detected in the sediment and, except cadmium and lead, were below the sediment quality guideline levels for aquatic life proposed by EPA (Environmental Protection Agency). Therefore, these two metals could be toxic to aquatic organisms. Significant ($P > 0.05$) differences were observed among the five fish species for all these metals accumulation. Also, significant ($P > 0.05$) differences were noticed among these metals accumulation in fish organs. Cadmium showed the least bioaccumulation, while zinc showed the highest bioaccumulation in all the fish species. Though the metal concentration in the different fish tissues was variable, the highest concentration was found in kidney and the lowest in the muscle. Concentrations of these metals in the muscle tissue of all the fish species were well below the consumption safety tolerance in fish set by WHO/FAO, and thus, so far as these metals are concerned, these sewage-fed cultured fishes are safe and suitable for human consumption.

Keywords Sewage · Pond water · Sediment · Fish organs · Metals

Introduction

The use of excreta and wastewater in aquaculture is an age-old practice, particularly in Asia. Municipal waste like sewage water is disposed of through recycling in agricultural land by approved methods. Application of sewage to fish ponds is becoming a popular method for many cities because their disposal into fishponds is beneficial as they contain

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essential nutrients for fish. In India, there is over 130 sewage-fed aquaculture covering a total area of 12,000 ha (Strauss 1997). The Kolkata wetlands, which have been operational since about 1930, are the world's largest wastewater aquaculture, comprising an area of approximately 4,000 ha. In Kolkata, the wastewater aquaculture system receives 550,000 m^3 of untreated wastewater per day (Edwards and Pullin 1990). It is currently possible to achieve yields of approximately 5 t ha^{-1} of mainly two types of fish's, viz., Indian major carps and tilapia in ponds receiving wastewater. Fish produced in this system is sold at nearby markets in Kolkata, India and represents 16% of total fish sales in the municipality.

The sewage fed ponds, which are locally called "Bheries", are usually large and could be as big as 40 ha in size. These sewage-fed ponds are generally shallow, varying from 50 to 150 cm in depth. After pond preparation, the raw sewage drawn into the pond is conditioned for 20–30 days. Fish stocking is performed once the stabilization process is completed and water turns green. Most of the stocked fish are Indian major carps (70%) with other species, including common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), and tilapia. [Multiple stocking and multiple harvesting is the strategy adopted in these farms.] Each farm maintains its own nursing area, and normally healthy fish seeds are stocked into the pond continuously. While common carp and tilapia fry are produced on the farm, those of other species are obtained from local hatcheries. Fingerlings are generally more than 10–15 g in size when stocked. Fishes are grown mostly on the natural food produced in the pond, and sewage is regularly drawn in at a rate of 1–10% of the total volume of pond water throughout the culture period, using the color of the water as an indicator. In bigger ponds, continuous in- and outflow are maintained, allowing the same amounts of water to flow out of the pond. Aerators are used when oxygen depletion problems are noticed. Feeding of fish is generally not practiced, except when there is a heavy monsoon, resulting in shortfall of quality sewage (Nandeesha 2002).

Ponds managed for wastewater aquaculture are frequently subject to contamination with industrial pollutants (mainly tanneries, distilleries, paper, sugar, dye, and battery factories). The sewage effluents are also being mixed with heavy metals from tanneries, dye, battery, and chemical factories found at the eastern fringe of the city. Unfortunately, sewage effluent contains heavy metals as there is no running sewage treatment plant. Dilution and sedimentation of the solid particles reduce the content of heavy metals in fishpond water. However, they usually precipitate on the bottom sediment or are absorbed by aquatic plants including plankton, which are basically used by fish as feed sources. Fish analysis gives an indication of the available metals in the pond environment (mainly water and sediment), as well as the fish's own metal load, which is important when the fish is ingested as food. Due to worldwide awareness of metal enrichment in the aquatic organisms as a potential health risk, the present analysis investigates the levels of Pb, Cd, Cr, Cu, and Zn in muscle, gill, liver, intestine, and kidney of Indian major carps, tilapia, and common carp from the sewage-fed ponds of Kolkata. The reported data attempt to extend the available information on contamination levels in the investigated species and a means to assist the fish trade, as well as addressing health and safety issues.

Materials and methods

Study site

The present sewerage system of Kolkata consists of three main pumping stations and 14 intermediate smaller pumping stations. The system carried about 545 million liters per day (MLD) of domestic wastewaters, 227 MLD of industrial wastewater (mainly tanneries, distilleries, paper, and chemical factories), and storm water (during monsoons) and covers almost 90% of the city area. The terminal pumping stations discharge the wastewaters into the dry weather flow channel and storm water flow channel, which convey them further east through wetlands to the Kulti River. Some wastewaters from South Kolkata are carried to the Hooghly River through Tolly's Nullah (Drain). Those wastewaters are utilized for sewage fed fish farming on both sides of the channels and

Bhangare Khal (Canal) in the eastern fringe of the city. In the present investigation, samples were collected from five fishponds of sewage-fed ponds around the channels, East Kolkata (Fig. 1).

Collection of water samples

Water samples were collected just below the water surface at two different locations in each pond using acid-washed, distilled-water-rinsed polyethylene bottles. The two water samples were pooled together. The samples were collected from five ponds at quarterly interval over the 2-year period from January 1999 to December 2000. Samples were immediately filtered using 0.45-μm membrane filters. The filtered samples were used to analyze physico-chemical parameters (APHA 1992). For heavy metals analysis, 10 ml filtrate or dissolved fraction was acidified with 0.5 ml of concentrated HNO_3 and stored overnight at 4°C. The filtered materials were dried at 70°C and then digested with 5 ml concentrated HNO_3. Total concentrations of metals in water samples were obtained by adding the two fractions together (Smith et al. 1996).

Sediment

An auger was used to collect sediment samples from the surface down to a depth of 10 to 20 cm at three different locations in each pond, and these three samples were pooled together. The sediment samples were collected from five fish culture ponds. All the sediment samples were sealed in polyethylene bags embedded in ice during transportation to the laboratory. They were then freeze-dried and passed through a 1-mm size sieve to separate the stones, leaves, and dead invertebrates. The sediments were then ground into powder of particle size less than 100 meshes (sieve size = 0.152 mm) using a mortar and a pestle. The sieved samples were used for physico-chemical parameters of sediment (particle size, pH, electrical conductivity, and calcium carbonate) that were determined according to Black (1965) and Jackson (1973). For heavy metal analysis, 0.1 g of sediment was digested using 5 ml of concentrated

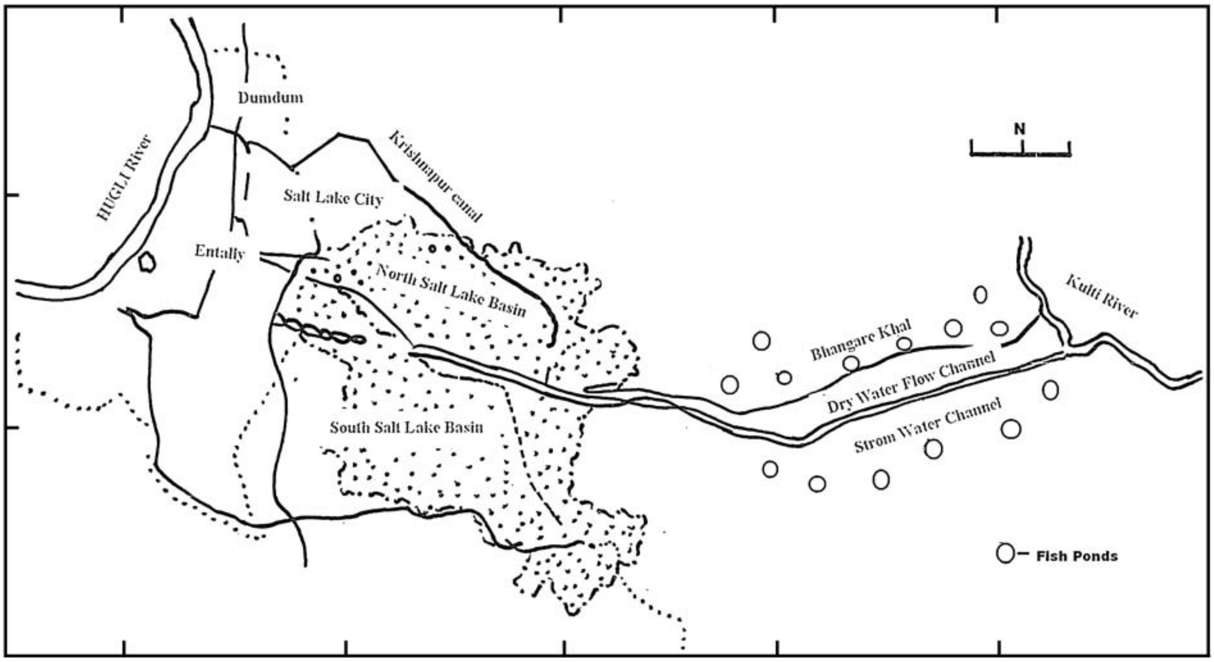

Fig. 1 Site map of Kolkata's waste recycling region and fish ponds

nitric acid at 135°C for 4 h. Hydrogen peroxide (2 ml; 30%) and concentrated perchloric acid were then added, and the temperature was maintained at 150°C until the liquor was clear and the particles turned white or gray (Zhou et al. 1998).

Fish

Five fresh fish of each species were collected from the sewage-fed pond near around the channels of Kolkata, India. The fish was collected from the same ponds where the water and sediment samples were also collected. The species include *Labeo rohita* (rohu), *Catla catla* (catla), *Cirrhinus mrigala* (mrigal), *Oreochromis mossambicus* (tilapia), and *C. carpio* (common carp). The mean weight of these fish species was 700 ± 50, 900 ± 100, 500 ± 50, 150 ± 25, and 1200 ± 150 g, respectively. The ages of the fishes were all around 1.0 year old. The fish samples were sealed in polythene bags and kept in the freeze at –20°C.

Rohu, catla, and mrigal were also collected from a freshwater polyculture pond to analyze metals contents as control (reference) data.

Preparation of tissues for heavy metal analyses

The fish samples were given about 2 h of defrosting before analysis. After the fish were rinsed with de-ionized water, each fish was dissected with the help of stainless steel implements on a clean petri dish to extract gill, muscle, liver, intestine, and kidney tissues. Polyethylene gloves were worn during dissection of fish tissues to reduce surface contamination of samples. After dissection, 2 g samples of each tissue was dried at 60°C overnight (12 h) to a constant weight. Dried tissues were ground, sieved, and transferred to a porcelain basin. One gram powdered sample was kept in 30 ml crucible and placed in the muffle furnace at 50°C, 150°C, and 450°C for 10 min, 1 h, and 8 h, respectively, by raising the temperature for complete ashing. Then they were transferred to 125-ml Erlenmeyer flask for digestion. Each tissue was digested separately with tri-acid mixture ($HNO_3/HClO_4/H_2SO_4 = 10:4:1$) at 5 ml per 0.5 g of sample and was placed on a hot plate at 100°C. Digestion was continued until the liquor was clear (AOAC 1990). All the digested liquors were filtered through Quantitative Whatman 541 filter paper and diluted to 25 ml in the volumetric flask with double-distilled water. They were stored in acid rinsed polyethylene bottles at 4°C prior to analysis.

Analyses of metals

The concentrations of lead, cadmium, chromium, copper, and zinc in the three matrices (water, sediment, and fish) were analyzed with an atomic absorption spectrophotometer (AAS, Perkin Elmer, Model 1025) by specific cathode lamp. The analyses were done in triplicate for each water, sediment, and fish sample. The wavelengths used for lead, cadmium, chromium, zinc, and copper measurement were 283.3, 228.8, 357.9, 213.9, and 324.7 nm, respectively. A standard reference material [Dogfish liver (DOLT-2), Canada] was also used to verify the accuracy of the metal determinations. The recovery rates for all heavy metals (Cu, Pb, Cd, Cr, and Zn) in the standard were within 100 ± 15% ($n = 3$). All these metals were measured in an air-acetylene flame.

Statistical analysis

The Grand mean, standard deviation, and coefficient of variation were calculated following Alonge (1989). From the average values obtained for the metals in the pond water, bioaccumulation factors were calculated (Varshney 1991) as the ratio of concentration in fish organs (milligrams per kilogram) to concentration in water (milligrams per liter). Analysis of variance with the Duncan multiple range test was applied to find the significant differences among means of fish organs and fish species for various metals.

Results

Some physico-chemical parameters of these sewage-fed ponds water were measured during January 1999 to December 2000. The averages values of these parameters were as follows: pH (8.02 ± 0.23), total alkalinity (205 ± 25 mg l^{-1} as $CaCO_3$), total hardness (237 ± 23 mg l^{-1} as $CaCO_3$), electrical conductivity (0.86 ±

Table 1 The Grand range and Grand mean of trace metals status of sewage-fed fishpond water and sediment of Kolkata, W.B. during January 1999 to December 2000 ($N = 40$)

Water/sediment	Water (μg l^{-1})			Sediment (mg kg^{-1})				
Heavy metals	Pb	Cd	Zn	Pb	Cd	Cr	Zn	Cu
Grand range	10–90	10–120	10–70	9–140	5–23	32–192	33–273	10–49
Grand Mean	22	32	16	50.5	10.1	77.4	140.6	30.1

Cr and Cu were not detected in any water sample

0.17 dS m^{-1}), total ammonia (0.64 ± 0.16 mg l^{-1}), nitrate–nitrogen (0.55 ± 0.15 mg l^{-1}), nitrite–nitrogen (0.20 ± 0.05 mg l^{-1}), soluble orthophosphate (0.38 ± 0.17 mg l^{-1}), biological oxygen demand (13.2 ± 3.8 mg l^{-1}), chemical oxygen demand (63.6 ± 15.4 mg l^{-1}), dissolved organic matter (6.2 ± 2.8 mg l^{-1}), soluble sodium (97 ± 25 mg l^{-1}), and soluble potassium (13 ± 3 mg l^{-1}). During the same period, some physico-chemical characteristics of the sewage-fed ponds sediment were pH (7.32 ± 0.18), electrical conductivity (0.53 ± 0.07 dS m^{-1}), organic carbon (11.4 ± 0.26 g kg^{-1}), calcium carbonate (4.48 ± 1.12 g kg^{-1}), and texture (sandy loam).

The mean concentrations and the standard deviations of the metals in both the sediment and water are presented in Table 1. In the water, copper and chromium were not detected, while the concentration of cadmium was higher than that of lead and zinc. However, no significant differences were observed among the metals detected in the water. Among the metals detected in the sediment, zinc had the highest concentration, while cadmium had the lowest one.

Metal concentration (micrograms per gram dry weight) in different organs of Indian major carps, tilapia, and common carp are presented in Tables 4 and 5. Significant ($P > 0.05$) differences were observed among five fish species for different trace metals accumulation. In addition, significant ($P > 0.05$) differences were also noticed among different trace metals accumulation in muscle, gills, liver, intestine, and kidney of these fish species. Variation in levels of metals among the fish species are apparent, and this may be explained mainly in terms of the chemical forms of the elements and their concentrations in the local environment, microbiological activity, food habits of different fish species, and the age of the fish. Besides, differences in fish size are also responsible for the level of metals in fish body.

The bioaccumulation factors for the different metals in the fish species are given in Table 6. Since copper and chromium were not detected in water, bioaccumulation factors could not be calculated for these elements. For the five species analyzed, the order of bioaccumulation in all organs was zinc > lead > cadmium.

Metal concentrations in different organs of the Indian major carps, rohu, catla, and mrigal cultured in freshwater polyculture ponds are presented in Table 7. Lead, cadmium, and chromium were not detected in any organ of these fish.

Discussion

The sewage-fed pond water contained only detectable amounts of zinc, lead, and cadmium, with all the values lower than 120 μg l^{-1}. The surface water quality guideline levels were used to determine if the concentrations reported in the present study are toxic to freshwater aquatic life (CEQG 2002; AENV 1999). The comparison of Grand mean values of trace metals with CEQGs showed that Zn and Cd concentrations were below the maximum acceptable concentration (MAC)

Table 2 Comparisons of Grand Mean and maximum values of trace metals in water with MAC values

Metals	Grand Mean in water (μg/l)	Maximum values in water (μg/l)	CEQG guidelines, MAC values (μg/l)
Pb	22	90	7[a]
Cd	32	120	73[a]
Zn	16	70	30

[a] As per CEQG Guidelines, MAC values of Pb and Cd are hardness dependent: 7 μg/l at $CaCO_3 > 180$ mg/l for Pb; 73 μg/l at $CaCO_3$ 250 mg/l for Cd

(Table 2), and thus, toxic effects for these two metals would be rarely observed. The Grand mean value and also the maximum value of Pb showed high levels that exceed MAC, suggesting that adverse effects to aquatic organisms would frequently occur. In addition, the levels of the metals determined in water were much lower than those reported in the sediments.

The sewage-fed pond sediments contained detectable amounts of zinc, chromium, lead, copper, and cadmium. Zinc had the highest concentration, while cadmium was the least concentrated in the sediments. The sediment quality guideline (SQGs) levels were used to determine if the concentrations reported are toxic to aquatic life (EPA 1999; Table 3). The SQGs of threshold effects level (TEL) and probable effect level (PEL) were based on compilation of large toxicity databases for freshwater organisms. TELs represent chemical concentrations below which the probability of toxicity and other effects was minimal. While PELs represent mid-range above which adverse effects were more likely, although not always expected. TELs–PELs represent a possible-effects range, within which negative effects would occasionally occur. The comparison of Grand mean values of trace metals with SQGs showed that Cr, Zn, and Cu concentrations were below the TEL values, and thus, toxic effects for these three metals would be rarely observed. Both Grand mean value and the maximum value of Cd were above PELs, and thus, adverse effects were more likely to be expected. However, the maximum values of all these five metals were between TELs and PELs, and in this range, toxic effects would occasionally occur.

Though copper and chromium were not detected in water, then the presence of copper and chromium in the fish could be due to the presence of these metals in the pond sediment by which the metal could diffuse into the overlying water depending on the sediment pH and redox potential. In the present study, the chromium content of the five fish species in descending order were *C. catla* > *L. rohita* > *C. carpio* > *O. mossambicus* > *C. mrigala*. The copper content followed the decreasing order as *C. carpio* > *O. mossambicus* > *C. catla* > *C. mrigala* > *L. rohita*. Copper is important for bone formation, maintenance of myelin within the nervous system, and synthesis of hemoglobin in animals. It is also a component of some important metalloenzymes. Copper also forms an important part of cytochrome oxidase and assorts other enzymes involved in redox reactions in cells (Sorensen 1991; Dallas and Day 1993). Copper reaches into aquatic systems through anthropogenic sources, such as industrial, mining, plating operations, use of copper salts to control aquatic vegetation, or influxes of copper-containing fertilizers (Nussey 1998).

Lead was detected in all the five fish species. Lead is toxic even in low concentrations and has no beneficial function in biochemical processes of fish (Adeyeye et al. 1996). Storage batteries, ammunition and type metal, cable sheaths, solder, pigments, and anti-knock compounds in petrol are the major sources of lead (Crosby 1977). The lead contamination in sewage-fed ponds of Kolkata could be mainly due to effluents of batteries and leaded gasoline. Lead could be responsible for inhibiting active transport mechanisms involving ATP, could depress the activity of the enzyme cholinesterase, could suppress cellular oxidation–reduction reactions, and could also inhibit protein synthesis (Waldron and Stofen 1974). The level of lead in fishes is an indication of the level of lead contamination in the water and sediment of the sewage-fed pond.

Table 3 Comparisons of Grand Mean of trace metals in sediments and TEL/PEL values

Metals	Grand mean values in sediments (μg/g dw)	Maximum values in sediments (μg/g dw)	EPA guidelines, TEL values (μg/g dw)	EPA guidelines, PEL values (μg/g dw)
Pb	50.5	140	46.7	218
Cd	10.1	23	1.2	9.6
Cr	77.4	192	81	370
Zn	140.6	273	150	410
Cu	30.1	49	34	270

The zinc content of various fish species in descending order are *C. catla* > *L. rohita* > *C. mrigala* > *O. mossambicus* > *C. carpio*. Zinc is present in all the fish species particularly in the muscles, which are useful to human being since this is the part of fish mostly consumed. Zinc is an essential element in trace amounts for animals. In mammals, it plays a vital role in the biosynthesis of nucleic acids, RNA polymerases, and DNA polymerases and, thus, is involved in the healing processes of tissues in the body. Other physiological processes, such as hormone metabolism, immune response, and stabilization of ribosome and membranes, also require zinc. Major sources of zinc in sewage in addition to galvanized materials and domestic products containing zinc are batteries, pigments, and paints.

Cadmium content of all the studied fish were more or less similar, and its concentration in the decreasing order were *O. mossambicus* > *C. catla* ≥ *C. mrigala* > *C. carpio* > *L. rohita*. Cadmium is thought to have no biological function (Thawley et al. 2004) and has toxic effects on proteins and ion channels (Kiss and Osipenko 1994). It has a strong affinity for sulphydryl-containing ligands, such as the amino acids cysteine and histidine (Vallee and Ulmer 1972). Cadmium produces toxic effects on the human kidney and causes Japanese Itai-itai disease (Satarug and Moore 2004). Cadmium enters into aquatic systems as effluents mainly through electroplating, pigments, plastic stabilizers, and batteries (Nriagu 1980).

There were significant differences among five fish species for different trace metals accumulation. However, significant differences ($P < 0.05$) were observed between *C. catla* and other four fish species for the chromium accumulation. For zinc accumulation, significant differences ($P < 0.05$) were observed between *C. catla*, *L. rohita*, and three other fish species. In the present study, *C. catla* showed higher tendency for the accumulation of metals than *L. rohita* and *C. mrigala*, while *L. rohita* had higher tendency than *C. mrigala* for the accumulation of metals, and similar trends of metal accumulation were also reported by Mahmood (2003) for these fish species collected from the stretch of river, Ravi, Pakistan. The difference in accumulation of metals in different fish species may be attributed to the feeding habit of the fish, physiological state of the tissues of the fish, and presence of ligands in the tissues having an affinity to metals and/or to the role of the tissue of the fish in the detoxification process (Table 4).

Maximum lead was found in fish kidney followed by intestine, liver, gill, and muscle (Table 5). Maximum cadmium was found in fish kidney followed by intestine, liver, gills, and muscle, and the same trend was found in fish for chromium accumulation. Maximum copper was observed in fish intestine followed by liver, kidney, gills, and muscle, while maximum zinc was detected in fish liver followed by intestine, kidney, gills, and muscle. Many workers (Robinson and Oldewage 1997; Mahmood 2003; Jayakumar and Paul 2006) reported about similar trends of metal accumulation in fish organs. In the present study, metal concentrations in the different tissues were variable, but it was clear that the highest concentrations were found in the kidney, liver, and gill tissues, followed by the muscle tissue. One of the main reasons attributed to the increased presence of these metals in these organs is their capacity to accumulate these metals brought by blood from other parts of the body and induce the production of the metal binding protein, metallothionein, which is believed to play a crucial role against the toxic effects of heavy metals by binding them (Bhattacharya et al. 1985). According to Klavercamp et al. (1984), the gill and the liver, along with kidney, are the main sites of metallothionein production and metal retention. This may be yet another main reason for the enhanced presence of metals in the gills, kidneys, and liver. Various reasons may be attributed to the lower rate of bioaccumulation of these metals in muscle. First, the muscle does not come into direct contact with the metal in the environment as it is totally covered externally by the skin, which in many ways helps the fish to ward off the penetration of the metal (Jayakumar and Paul 2006). Another probable reason may be the fact that even though muscle is the most valued edible tissue, it is not an active site for detoxification, and therefore, transport of metal from other tissues to muscle (as in the case of liver and kidney) does not arise.

Cadmium showed the least bioaccumulation followed by lead, while zinc showed the highest bioaccumulation in all the fish species (Table 6).

Table 4 Species-wise distributions of trace metal concentrations (mean + SD, $N = 5$, micrograms per gram dry weight) in different organs

Fish/organ	Pb	Cd	Cr	Cu	Zn
Muscle					
L. rohita	0.35^{c} ± 0.08	0.07^{b} ± 0.05	0.44^{b} ± 0.05	0.25^{bc} ± 0.1	1.20^{a} ± 0.24
C. catla	0.48c ± 0.14	0.14^{c} ± 0.04	0.70^{a} ± 0.12	0.33^{b} ± 0.09	0.50^{b} ± 0.13
C. mrigala	0.09^{d} ± 0.03	0.30^{a} ± 0.14	0.08^{c} ± 0.04	0.51^{b} ± 0.18	0.50^{b} ± 0.09
O. mossambica	0.50^{b} ± 0.10	0.18^{a} ± 0.07	0.12^{c} ± 0.03	0.90^{a} ± 0.11	1.00^{a} ± 0.26
C. carpio	0.85^{a} ± 0.14	0.04^{b} ± 0.12	0.04^{c} ± 0.02	0.48^{b} ± 0.10	1.20^{a} ± 0.14
Gill					
L. rohita	2.28^{a} ± 0.16	0.44^{d} ± 0.13	1.21b ± 0.13	0.76^{c} ± 0.13	5.80^{a} ± 0.14
C. catla	1.60^{c} ± 0.11	0.40^{d} ± 0.12	1.60^{a} ± 0.10	0.85^{c} ± 0.12	5.80^{a} ± 0.46
C. mrigala	2.00^{b} ± 0.09	0.70^{c} ± 0.09	0.55^{c} ± 0.13	0.88^{c} ± 0.08	2.90^{b} ± 0.12
O. mossambica	1.20^{d} ± 0.12	1.60^{a} ± 0.10	0.25^{d} ± 0.08	1.90^{a} ± 0.09	1.76^{d} ± 0.12
C. carpio	2.50^{a} ± 0.15	1.00^{b} ± 0.09	0.50^{c} ± 0.12	1.35^{b} ± 0.09	2.30^{c} ± 0.17
Liver					
L. rohita	1.25^{c} ± 0.15	0.62^{c} ± 0.08	0.37^{d} ± 0.08	0.57^{b} ± 0.16	7.20^{a} ± 0.11
C. catla	0.80^{d} ± 0.09	1.00^{b} ± 0.09	4.00^{a} ± 0.10	3.00^{a} ± 0.13	7.30^{a} ± 0.16
C. mrigala	1.25^{c} ± 0.08	1.10^{b} ± 0.03	0.25^{d} ± 0.08	1.03^{b} ± 0.13	4.60^{b} ± 0.34
O. mossambica	7.00^{a} ± 0.13	1.40^{a} ± 0.09	0.70^{c} ± 0.10	0.43^{b} ± 0.08	3.54^{c} ± 0.14
C. carpio	1.70^{b} ± 0.10	0.63^{c} ± 0.08	1.00^{b} ± 0.15	3.30^{a} ± 0.17	5.00^{b} ± 0.10
Intestine					
L. rohita	2.04^{c} ± 0.12	0.69^{d} ± 0.09	2.80^{c} ± 0.23	1.23^{b} ± 0.14	4.60^{c} ± 0.12
C. catla	4.00^{a} ± 0.12	1.20^{b} ± 0.12	4.60^{a} ± 0.11	0.97^{a} ± 0.16	0.80^{a} ± 0.15
C. mrigala	1.40^{d} ± 0.11	0.93^{c} ± 0.12	1.40^{d} ± 0.08	0.85^{c} ± 0.12	2.00^{e} ± 0.24
O. mossambica	2.80^{b} ± 0.34	2.00^{a} ± 0.10	1.20^{d} ± 0.11	2.60^{a} ± 0.15	5.88^{b} ± 0.20
C. carpio	3.00^{b} ± 0.20	1.00^{b} ± 0.09	4.00^{b} ± 0.14	2.87^{a} ± 0.29	3.80^{d} ± 0.14
Kidney					
L. rohita	3.60^{d} ± 0.17	1.20^{c} ± 0.12	2.80^{b} ± 0.18	1.92^{b} ± 0.13	4.70^{c} ± 0.22
C. catla	6.10^{c} ± 0.21	2.00^{a} ± 0.16	13.0^{a} ± 0.12	0.45^{c} ± 0.11	6.20^{b} ± 0.12
C. mrigala	7.30^{b} ± 0.18	1.60^{b} ± 0.19	0.26^{d} ± 0.09	2.00^{b} ± 0.22	8.30^{a} ± 0.17
O. mossambica	ND	ND	ND	ND	ND
C. carpio	8.40^{a} ± 0.22	1.22^{c} ± 0.13	0.60^{c} ± 0.12	3.50^{a} ± 0.16	1.80^{d} ± 0.17

Means with similar letters in a column are statistically similar at $P < 0.05$

ND not detected

Table 5 Organ-wise distributions of trace metal concentrations (mean + SD, $N = 5$, micrograms per gram dry weight) in different fish species

Organ/fish	Pb	Cd	Cr	Cu	Zn
L. rohita					
Muscle	$0.35^{c} \pm 0.08$	$0.07^{c} \pm 0.05$	$0.44^{c} \pm 0.05$	$0.25^{d} \pm 0.10$	$1.20^{d} \pm 0.24$
Gill	$2.28^{b} \pm 0.16$	$0.44^{b} \pm 0.13$	$1.21^{b} \pm 0.13$	$0.76^{c} \pm 0.13$	$5.80^{b} \pm 0.14$
Liver	$1.25^{c} \pm 0.15$	$0.62^{b} \pm 0.08$	$0.37^{c} \pm 0.08$	$0.57^{c} \pm 0.16$	$7.20^{a} \pm 0.11$
Intestine	$2.04^{b} \pm 0.12$	$0.69^{b} \pm 0.09$	$2.80^{a} \pm 0.23$	$1.23^{b} \pm 0.14$	$4.60^{c} \pm 0.12$
Kidney	$3.60^{a} \pm 0.17$	$1.20^{a} \pm 0.12$	$2.80^{a} \pm 0.18$	$1.92^{a} \pm 0.13$	$4.70^{c} \pm 0.22$
C. catla					
Muscle	$0.48^{e} \pm 0.14$	$0.14^{d} \pm 0.04$	$0.70^{e} \pm 0.12$	$0.33^{c} \pm 0.09$	$0.50^{c} \pm 0.13$
Gill	$1.60^{c} \pm 0.11$	$0.40^{c} \pm 0.12$	$1.60^{d} \pm 0.10$	0.85b ± 0.12	$5.80^{c} \pm 0.46$
Liver	$0.80^{d} \pm 0.09$	$1.00^{b} \pm 0.09$	$4.00^{c} \pm 0.10$	$3.00^{a} \pm 0.13$	$7.30^{b} \pm 0.16$
Intestine	$4.00^{b} \pm 0.12$	$1.20^{b} \pm 0.12$	$4.60^{b} \pm 0.11$	$0.97^{b} \pm 0.16$	$9.80^{a} \pm 0.15$
Kidney	$6.10^{a} \pm 0.21$	$2.00^{a} \pm 0.16$	$13.0^{a} \pm 0.12$	$0.45^{c} \pm 0.11$	$6.20^{c} \pm 0.12$
C. mrigala					
Muscle	$0.09^{d} \pm 0.03$	$0.30^{c} \pm 0.14$	$0.08^{d} \pm 0.04$	$0.51^{d} \pm 0.18$	$0.50^{e} \pm 0.09$
Gill	$2.00^{b} \pm 0.09$	$0.70^{b} \pm 0.09$	$0.55^{b} \pm 0.13$	$0.88^{c} \pm 0.08$	$2.90^{c} \pm 0.12$
Liver	$1.25^{c} \pm 0.08$	$1.10^{b} \pm 0.03$	$0.25^{c} \pm 0.08$	$1.03^{b} \pm 0.13$	$4.60^{b} \pm 0.34$
Intestine	$1.40^{c} \pm 0.11$	$0.93^{b} \pm 0.12$	$1.40^{a} \pm 0.08$	$0.85^{c} \pm 0.12$	$2.00^{d} \pm 0.24$
Kidney	$7.30^{a} \pm 0.18$	$1.60^{a} \pm 0.19$	$0.26^{c} \pm 0.09$	$2.00^{a} \pm 0.22$	$8.30^{a} \pm 0.17$
O. mossambica					
Muscle	$0.50^{d} \pm 0.10$	$0.18^{d} \pm 0.07$	$0.12^{c} \pm 0.03$	$0.90^{c} \pm 0.11$	$1.00^{d} \pm 0.26$
Gill	$1.20^{c} \pm 0.12$	$1.60^{b} \pm 0.10$	$0.25^{c} \pm 0.08$	$1.90^{b} \pm 0.09$	$1.76^{c} \pm 0.12$
Liver	$7.00^{a} \pm 0.13$	$1.40^{c} \pm 0.09$	$0.70^{b} \pm 0.10$	$0.43^{d} \pm 0.08$	$3.54^{b} \pm 0.14$
Intestine	$2.80^{b} \pm 0.34$	$2.00^{c} \pm 0.10$	$1.20^{a} \pm 0.11$	$2.60^{a} \pm 0.15$	$5.88^{a} \pm 0.20$
Kidney	ND	ND	ND	ND	ND
C. carpio					
Muscle	$0.85^{e} \pm 0.14$	$0.04^{c} \pm 0.12$	$0.04^{d} \pm 0.02$	$0.48^{d} \pm 0.10$	$1.20^{e} \pm 0.14$
Gill	$2.50^{c} \pm 0.15$	$1.00^{a} \pm 0.09$	$0.50^{c} \pm 0.12$	$1.35^{c} \pm 0.09$	$2.30^{c} \pm 0.17$
Liver	$1.70^{d} \pm 0.10$	$0.63^{b} \pm 0.08$	$1.00^{b} \pm 0.15$	$3.30^{a} \pm 0.17$	$5.00^{a} \pm 0.10$
Intestine	$3.00^{b} \pm 0.20$	$1.00^{a} \pm 0.09$	$4.00^{a} \pm 0.14$	$2.87^{b} \pm 0.29$	$3.80^{b} \pm 0.14$
Kidney	$8.40^{a} \pm 0.22$	$1.22^{a} \pm 0.13$	$0.60^{c} \pm 0.12$	$3.50^{a} \pm 0.16$	$1.80^{d} \pm 0.17$

Means with similar letters in a column are statistically similar at $P < 0.05$

ND not detected

Table 6 Bioaccumulation factors of trace metals in the fish organs of the various fish species

Fish organs	*Labeo rohita*			*Catla catla*			*Cirrhinus mrigala*			*Oreochromis mossambicus*			*Cyprinus carpio*		
	Pb	Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn	Pb	Cd	Zn
Muscle	15.9	2.1	75.0	21.8	4.3	31.2	4.0	9.3	31.2	22.7	5.0	62.5	38.6	1.2	75.0
Gill	103.6	13.7	362.5	72.7	12.5	362.5	90.9	21.8	181.2	54.5	50.0	110.0	113.6	31.2	143.7
Liver	56.8	19.3	450.0	36.3	31.2	456.2	56.8	34.3	287.5	318.1	43.7	221.2	77.2	19.6	312.5
Intestine	92.7	21.5	287.5	181.8	37.5	612.5	63.6	29.0	125	127.2	62.5	367.5	136.3	31.2	237.5
Kidney	163.6	37.5	293.7	277.2	62.5	387.5	331.8	50.0	518.7	N.D.	N.D.	N.D.	381.8	38.1	112.5
Grand mean	86.5	18.8	293.7	117.9	29.6	369.9	109.4	28.8	228.7	130.6	40.3	190.3	149.5	24.2	176.2
SD	55.1	12.8	138.7	108.8	22.8	212.9	128.2	15.0	186.7	132.3	24.7	135.5	135.0	14.5	97.0
CV (%)	63.6	68.0	47.2	92.2	77.0	57.5	117.1	52.0	81.6	101.3	61.2	71.2	90.3	59.9	55.0

N.D. not determined

Table 7 Concentrations of various metals in organs of three different fish species from the freshwater polyculture fishponds

Species	Organ	Metal concentrations (mean ± SD, $N = 5$, μg g^{-1} dry wt) in freshwater fishponds		Metal concentrations (mean ± SD, $N = 5$, μg g^{-1} dry wt) in sewage-fed fishponds	
		Cu	Zn	Cu	Zn
Labeo rohita	Muscle	0.30 ± 0.03	1.27 ± 0.04	0.25 ± 0.10	1.20 ± 0.24
	Gill	0.40 ± 0.12	4.62 ± 0.14	0.76 ± 0.13	5.80 ± 0.14
	Liver	0.44 ± 0.07	4.84 ± 0.11	0.57 ± 0.16	7.20 ± 0.11
	Intestine	1.22 ± 0.17	3.80 ± 0.13	1.23 ± 0.14	4.60 ± 0.12
	Kidney	0.80 ± 0.12	2.70 ± 0.18	1.92 ± 0.13	4.70 ± 0.22
Catla catla	Muscle	0.06 ± 0.01	0.41 ± 0.02	0.33 ± 0.09	0.50 ± 0.13
	Gill	0.46 ± 0.07	4.42 ± 0.21	0.85 ± 0.12	5.80 ± 0.46
	Liver	1.98 ± 0.11	5.62 ± 0.13	3.00 ± 0.13	7.30 ± 0.16
	Intestine	0.68 ± 0.21	6.71 ± 0.22	0.97 ± 0.16	9.80 ± 0.15
	Kidney	0.32 ± 0.12	4.10 ± 0.22	0.45 ± 0.11	6.20 ± 0.12
Cirrhinus mrigala	Muscle	0.16 ± 0.01	0.52 ± 0.04	0.51 ± 0.18	0.50 ± 0.09
	Gill	0.55 ± 0.11	1.70 ± 0.09	0.88 ± 0.08	2.90 ± 0.12
	Liver	0.91 ± 0.21	2.48 ± 0.12	1.03 ± 0.13	4.60 ± 0.34
	Intestine	0.43 ± 0.09	1.40 ± 0.16	0.85 ± 0.12	2.00 ± 0.24
	Kidney	1.40 ± 0.07	4.88 ± 0.21	2.00 ± 0.22	8.30 ± 0.17

Pb, Cd and Cr were not detected in any sample

Table 8 Daily dietary intake of trace metals by eating fish (mean values are in parenthesis)

Metals	Concentrations in the muscle of different fishes (μg g^{-1})	Daily intake (g/person)	Concentrations in daily intake (μg)	Acceptable daily intake limit (μg)	Remark
Pb	0.09–0.85 (0.45)	30.0	2.7–25.5 (13.5)	250	Normal for human consumption
Cd	0.04–0.30 (0.15)	30.0	1.2–9.0 (4.5)	57–72	Normal for human consumption
Cr	0.04–0.70 (0.28)	30.0	1.2–21.0 (8.4)	150	Normal for human consumption
Zn	0.50–1.20 (0.88)	30.0	15.0–36.0 (26.4)	19,000	Normal for human consumption
Cu	0.25–0.90 (0.49)	30.0	7.5–27.0 (14.7)	3,250–32,500	Normal for human consumption

Thus, these calculated values provide some indication of the bioavailability of metals to the fish from the water. The total concentration of the metal in the water does not play the major role in availability of that metal (Coetzee et al. 2002). This is also clear from the present study as higher bioaccumulation factors did not coincide with higher metal concentrations in the water. Metal species, as well as physico-chemical conditions of the waters, such as pH, alkalinity, hardness, humic acid, are very important as this determines the toxicity and speciation of the metal. It is also important to note that bioaccumulation factors would not give an accurate indication of the relative bioavailability of metals for uptake, if these metals were regulated in the fish, which might have been the case in the present study. In addition, the kinetics of uptake plays a crucial role. If a steady state is not reached, the bioaccumulation factor in itself is a function of time (Coetzee et al. 2002).

Copper and Zn were lower in all the fishes collected from the freshwater polyculture ponds than the corresponding values of the fish sampled in sewage fed ponds (Table 7). Lead, cadmium, and chromium were not detected in any organ of these fish collected from the polyculture ponds, while these trace metals were detected in the fishes of sewage-fed ponds.

Fish quality for human consumption

The lowest concentrations of the five metals studied were found in the muscle tissue of the five fish species. The metal concentrations in muscle are very important as this is the edible part of the fish. Zinc and copper plays an important role in the human metabolic activities (Chen and Chen 2001). The average daily fish consumption in India is 30 g/person. Daily trace metal intake through fish consumption was calculated based on the range and average metal content of these five fish species. The daily intakes of Pb, Cd, Cr, Cu, and Zn from fish were 13.5, 4.5, 8.4, 14.7, and 26.4 μg/person. Table 8 shows the provisional tolerable daily intakes of Pb, Cd, Cr, Cu, and Zn recommended by WHO/FAO and USA (CAC 1984; NRC 1989; Snyder et al. 1975; Chen and Chen 2001) for a reference weight of 70 kg. It is evident from the table that daily trace metal intake from these fishes did not exceed the provisional maximum daily intake recommended by WHO/FAO and USA. Therefore, the present study showed that consuming fish from the sewage-fed fishponds is not harmful to the human being as far as trace metals are concerned.

Conclusion

From the present investigation, it is evident that cadmium, chromium, copper, and zinc in sewage-fed pond water of Kolkata were detected and, except lead, were well below the maximum allowable levels for warm water fish culture. Concentrations of these metals in the muscle tissue of *L. rohita*, *C. catla*, *C. mrigala*, *O. mossambicus*, and *C. carpio* were far below than the consumption safety tolerance in fish set by WHO/FAO. Thus, from these metal points of view, all the five fish species cultured in Kolkata sewage-fed ponds are safe and suitable for human consumption.

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References

Adeyeye, E. I., Akinyugha, N. J., Fesobi, M. E., & Tenabe, V. O. (1996). Determination of some metals in Clarias gariepinus (Cuvier and Vallenciennes), Cyprinus carpio (L.) and Oreochromis niloticus (L.) fishes in a polyculture fresh water pond and their environments. *Aquaculture (Amsterdam, Netherlands), 147*, 205–214. doi:10.1016/S0044-8486(96)01376-2.

AENV (1999). *Surface water quality guidelines for use in Alberta* (p. 25). Edmonton, Alberta: Science and Standards Branch. Environmental Assurance Division. Alberta Environment.

Alonge, M. F. (1989). *Measurement and evaluation in education and psychology* (p. 152). Ado Ekiti: Adedayo Printing (Nig).

AOAC (Association of Official Analytical Chemists) (1990). *Official methods of analysis* (15th ed., p. 858). Washington, DC: Association of Official Analytical Chemists.

APHA (American Public Health Association. American Water Works Association and Water Pollution Control Federation) (1992). *Standard methods for the examination of water and wastewater* (18th ed.). Washington: American Public Health Association, APHA.

Bhattacharya, T., Ray, A. K., & Bhattarcharya, S. (1985). Response of Channa punctatus under short term and long term exposure to industrial pollutants. Induction of histopathology in the kidney. *Z.Mikrosk.Anat.Forsch (Leipz), 899*, 327–334.

Black, C. A. (1965). *Methods of soil analysis, Pt II*. Wisconsin: American Soc of Agron.

CEQG (Canadian Environmental Quality Guidelines) (2002). Canadian Council of Ministers of the Environment. Environment Canada. Hull, Quebec, 8 Chapters.

Chen, Y. C., & Chen, M. H. (2001). Heavy metal concentrations in nine species of fishes caught in coastal waters off Ann-Ping, S.W. Taiwan. *Journal of Food and Drug Analysis, 9*(2), 107–114.

Codex Alimentarius Commission (CAC) (1984). Contaminants: Joint FAO/WHO standards program (Vol. XVII, 1st ed., p1–33). Codex Alimentarius, Rome, Italy.

Coetzee, L., du Preez, H. H., & van Vuren, J. H. J. (2002). Metal concentrations in Clarias gariepinus and Labeo umbratus from the Olifants and Klein Olifants river, Mpumalanga, South Africa: Zinc, copper, manganese, lead, chromium, nickel, aluminium and iron. *Water S.A., 28*(4), 433–448.

Crosby, N. T. (1977). Determination of metals in foods—A review. *Analyst (London), 102*, 225–268. doi:10.1039/an9770200225.

Dallas, H. F., & Day, J. A. (1993). *The effect of water quality variables on riverine ecosystems: A review* (p. 240). Pretoria, South Africa: Water Research Commission Project No351. Water Research Commission.

Edwards, P., & Pullin, R. S. V. (1990). Wastewater-fed Aquaculture. Proceedings of the international seminar on wastewater recycling and rouse for aquaculture. Kolkata, 6–9 December.

Environmental Protection Agency (EPA) (1999). Sediment Quality Guidelines developed for the national status and trends program. Report number 6/12/99. http://www.epa.gov/waterscience/cs/pubs.htm (May 2004).

Jackson, M. L. (1973). *Soil chemical analysis*. New Delhi: Prentice Hall of India Pvt.

Jayakumar, P. V., & Paul, V. I. (2006). Patterns of cadmium accumulation in selected tissues of the catfish Clarias batrachus(Linn.) exposed to sub lethal concentration of cadmium chloride. *Vet Arhiv, 76*, 167–177.

Kiss, T., & Osipenko, O. N. (1994). Toxic effects of heavy metals on ionic channels. *Pharmacological Reviews, 46*, 245–267.

Klavercamp, J. E., McDonald, W. A., Duncan, D. A., & Wagenann (1984). Metallothionein and accumulation to heavy metals in fish, a review (pp. 99–113). In V. W. Cairns, P. V. Hodson, & J. O. Nriagu (Eds.), *Contaminant effects on fisheries*. New York: Wiley.

Mahmood, G. (2003). Lead and nickel concentrations in fish and water of river Ravi. *Pakistan Journal of Biological Sciences, 6*(12), 1027–1029.

Nandeesha, M. C. (2002). Sewage fed aquaculture systems of Kolkata: A century-old innovation of farmers. *Aquaculture Asia, 7*(2), 28–32.

National Research Council (NRC) (1989). *Recommended dietary allowances* (10th ed.). Washington, DC: National Academy.

Nriagu, J. O. (1980). *Production, uses, and properties of cadmium in the environment Part 1: Ecological cycling* (pp. 35–70). Chichester: Wiley.

Nussey, G. (1998). Metal ecotoxicology of the upper Olifants River at selected localities and the effect of copper and zinc on fish blood physiology. Ph.D.-thesis, Rand Afrikans University, South Africa.

Robinson, J., & Oldewage, A. A. (1997). Chromium, copper, iron and manganese bioaccumulation in some organs and tissues of Oreochromis mossambicus from the lower Olifants River, inside the Kruger National Park. *Water S.A., 23*, 387–403.

Satarug, S., & Moore, M. R. (2004). Adverse health effects of chronic exposure to low-lead cadmium in foodstuffs and cigarette smoke. *Environmental Health Perspectives, 112*, 1099–1103.

Smith, S., Chen, M. H., Bailey, R. G., & Williams, W. P. (1996). Concentration and distribution of copper and cadmium in water, sediments, detritus, plants and animals in a hardwater lowland river. *Hydrobiologia, 341*, 71–80. doi:10.1007/BF00012305.

Snyder, W. X., Cook, M. J., Nasset, E. S., Karhausen, L. R., Howels, G. P., & Tipton, I. H. (1975). *International* *commission on ideological protection*. Report on the task group on reference Man. New York: ICRP Publication 23.

Sorensen, E. M. B. (1991). *Metal poisoning in fish*. Boca Raton: CRC.

Strauss, M. (1997). Health (pathogen) considerations regarding the use of human waste in aquaculture. *Environmental Research Forum, 5/6*, 83–98.

Thawley, S., Morris, S., & Vosloo, A. (2004). Zinc and Cd accumulation in Potamonautes warreni from sites in the North-West Province of South Africa. *International Congress Series, 1275*, 180–188. doi:10.1016/j.ics.2004.09.036.

Vallee, B. L., & Ulmer, D. D. (1972). Biochemical effects of mercury, cadmium and lead. *Annual Review of Biochemistry, 41*, 91–129. doi:10.1146/annurev.bi.41.070172.000515.

Varshney, C. K. (1991). *Water pollution and managements* (pp. 88–89). New Delhi: Wiley Eastern.

Waldron, H. A., & Stofen (1974). *Sub-clinical lead poisoning* (pp. 1–224). New York: Academic.

Zhou, H. Y., Cheung, R. Y. H., Chan, K. M., & Wong, M. H. (1998). Metal concentrations in sediments and tilapia collected from inland waters of Hong Kong. *Water Research, 32*, 3331–3340. doi:10.1016/S0043-1354(98)00115-8.