Analysis of metal concentration levels in water, sediment and fish tissues from Toledo municipal lake by applying SR-TXRF technique

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ABSTRACT

The main objective of this study was to evaluate the metal content in water and sediment from the Toledo municipal lake, as well as the concentration levels of heavy metals in muscle and liver of four fish species. A digestion procedure was performed in all fish samples. Metal analysis was performed by using the Synchrotron Radiation X-ray Fluorescence technique. The accuracy and validity of the measurements were determined by analysis of certified reference materials. The highest Cr, Cu and Se concentration levels above the maximum tolerance limit according to the Brazilian norms in fish tissue could be associated with the metal uptake and accumulation due to the direct contact with contaminated water and sediment.

Key words | fish tissue, metal concentration, sediment, SR-TXRF technique, water

NOMENCLATURE

BEL Brazilian Environmental Legislation for river water of class II (Brazil 2005)
BFL Brazilian Food Legislation (Brazil 1965)
C_{LP} element concentration in the liquid phase (mg L^{-1})
C_{SP} element concentration of the dry solid phase (mg kg^{-1})
C_Y yttrium concentration in the liquid phase (mg L^{-1})
I fluorescent intensity (c.p.s.)
K_z characteristic strongest K X-ray line
L_z characteristic strongest L X-ray line
M initial mass before acid digestion process (g)
S_K relative-to-yttrium fluorescent sensitivity for X-ray K series
S_L relative-to-yttrium fluorescent sensitivity for X-ray L series
V final volume after acid digestion process (L)
Z atomic number

INTRODUCTION

Rivers and lakes are being continually polluted by release of wastewaters containing inorganic and organic pollutants that are the worldwide concern. Many efforts have been directed toward making qualitative and quantitative decisions based on the monitoring water quality data and interpretation of results. Heavy metals are widely distributed in water, in mud at the bottom, in plants and in various living organisms. It is well-known that some heavy metals when in trace amounts are essential for life, but in excess they are toxic. Their distributions can also change among different environmental compartments, accumulating in advanced organisms through biomagnification effects in the food chain (Chi et al. 2007; Zhou et al. 2010). Sediment is an important environmental compartment in which heavy metal and organic pollutants accumulate, and under favourable conditions these pollutants can be released from it, causing detrimental effects to aquatic biota long after the initial input of pollution (Weng et al. 2008). Bioaccumulation and magnification are capable of leading to
toxic levels of heavy metals in fish, even when the exposure is low (Adeye et al. 1996). In fish, heavy metals may enter through the body surface, the gills or the digestive tract and their toxic effects may influence physiological functions, individual growth rates, reproduction and mortality (Amundensen et al. 1997; Birungi et al. 2007). Fish are notorious for their ability to concentrate heavy metals in their muscles that are the main source of dietary protein for human beings and thus heavy metals can ultimately jeopardise human health.

Due to the rapid dilution of wastewaters, monitoring of the pollutants has become a challenge and thus requires sensitive analytical techniques, intensive sampling programs and long collection time (Liscio et al. 2009). The analysis of heavy metals in solid samples is one of the most dynamically developing scientific areas, demanding the decomposition of samples such as soil and sediment by several conventional and microwave-assisted digestion methods. Nowadays, considerable development is observed in the sampling and analytical techniques, such as atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry (ICP-OES), ion beam analysis methods and X-ray fluorescence, for the non-destructive contamination analysis in a wide variety of applications (Alves et al. 2007; Sun et al. 2001; Braziewicz et al. 2002; Calza et al. 2004; Rizzutto et al. 2006; Khuder et al. 2007; Terra et al. 2008; Espinoza-Quinones et al. 2009a, b, 2010; Lattuada et al. 2009). Among these techniques, TXRF is mainly used for non-destructive surface-contamination analysis in the semiconductor industry and for chemical-trace analysis, as it offers limits of detection (LDs) in the fg region if synchrotron radiation (SR) is used (Meirer et al. 2010).

The present study deals with the identification and quantification of the following main elements (K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Rb, Sr, Zr, Ba, and Pb) that are found in three environmental compartments: water, sediment and fish tissues. It is difficult to determine whether bioaccumulation of heavy metals differs among fish species due to their differing feeding habits. An evaluation of the metal content in water and sediment from the Toledo municipal lake, as well as the concentration levels of heavy metals in muscle of four fish species (Piaractus mesopotamicus, Oreochromis niloticus, Cyprinus carpio, and Prochilodus scrofa), was performed by using the SR-TXRF technique.

**MATERIAL AND METHODS**

**Sampling**

Sampling of water and sediments was performed in a plastic vessel as indicated in the American Public Health Association (APHA) Standard Methods (APHA 1998). All samples were collected at four collection sites from the Toledo municipal lake located at the Diva Paim Barths Ecologic Park, at the west region of the Brazilian Paraná state. Water sampling was performed by collecting 1 L of water in polyethylene vessels and preserving with 3.0 mL of HNO₃. Sediment samples were collected with an Eckman dredge and stored in plastic bags at −4°C. The physical–chemical analyses of aliquots of the representative samples included: pH, ammonium-nitrogen (NH₄⁺-N), nitrate-nitrogen (NO₃⁻-N), nitrite-nitrogen (NO₂⁻-N), dissolved oxygen (DO), and total ortho-phosphate (PO₄³⁻). Standard methods (APHA 1998) were adopted for all physical–chemical measurements.

In order to obtain a representative sample, at least three fishes of each fish species (O. niloticus, P. mesopotamicus, P. scrofa and C. carpio) from Toledo municipal lake were sampled for the analysis of heavy metals in fish tissues in August 2006. They are freshwater fish and are potential species for commercial fishing in Brazil, eating a vegetarian diet of water plants, but prefer to scavenge the bottom for insects, crustaceans (including zooplankton), and benthic worms. Fish species were labelled, stored on ice and on the same day transported to the laboratory for further treatment and analysis.

**Chemicals**

All the chemicals used in the study were of analytical-reagent grade. Milli-Q water was used throughout the sampling preparation. Standard stock solution (11.6 g L⁻¹) of yttrium was prepared by dissolving quantitatively a 50 g Merck package Y(NO₃)₃·6H₂O in ultra pure milli-Q water. The solution was stored in an acid-washed volumetric flask. Multi-element stock solutions have been also prepared from mono-element standard solutions (1.0 g L⁻¹ for AAS, Sigma-Aldrich) of some elements with low atomic number (K, Cr, Mn, Fe, Cu, Zn, Ga, and Br) and high atomic number (Sr, Mo, Ag, Cd, Sb, Cs, La, Sm, Dy and Pb), mixed in different concentrations, covering an energy wide region of K and L X-ray series, respectively, and obtaining sensitivity curves from the synchrotron radiation reflection X-ray fluorescence technique (SR-TXRF) (Yoneda & Horiuchi 1971; Aigner & Wobrauschek 1974).

**Sample digestion**

In lab, fishes were dissected using high quality stainless steel tools on a clean plastic working surface to separate some parts of their muscles and livers. Each dissected tissue
was weighed, then deep frozen and oven-dried by lyophilisation and weighed again to determine the moisture content. Collections of dried tissues for trace metal analysis were stored in clean, 8 mL PE bottles. Besides, the sediment samples were air-circulating oven-dried at 60°C for 48 h and finally pulverised before acid digestion. Both dried tissues and sediments were ground by manual grinding in an agate mortar with a pestle. The conventional hot plate digestion was performed for total decomposition of sediment samples (0.5 g dried sample) with a strong acid mixture (HF and HNO₃ in 2:1 (v/v) ratio) by using 100 mL Teflon beakers (Alves et al. 2001). On the other hand, the fish tissue digestion procedure was performed with 0.5 g of sample and 5 mL HNO₃ (65% nitric acid, Merck) and 0.5 mL H₂O₂ (30% hydrogen peroxide, Merck) and by heating on a hot plate (Pettersson & Olsson 1998). After cooling down, digestion vessels were vented and extractions were diluted to a final volume of 25 mL using milli-Q water.

**Metal determination by SR-TXRF**

For trace metals analysis by SR-TXRF technique, 10 µL of yttrium stock solution was added to each 10 mL sample (water, sediment or fish tissue). An aliquot of 5 µL was deposited on a pre-cleaned acrylic disk (Ø 30 mm, 3.0 mm thick). This reflector was left in a hood overnight to dry. Samples of multi-standard solutions were prepared using the same procedure in order to obtain the sensitivity curve of SR-TXRF for X-ray Kz and Lz lines. Procedural blanks (water and all reagents) were included.

The accuracy of the applied analytical method was checked with a multi-element standard reference material (Drinking Water Pollutants, Sigma-Aldrich) and a dogfish muscle tissue (DORM-2, National Research Council of Canada). For a standard preparation, 500 µL of Drinking Water Pollutants standard was mixed with 50 µL Y. About 0.5 g dried dogfish muscle tissue was weighed and mineralised according to the above described procedure for fish samples. Finally, the calculated data were compared with the certified values.

The SR-TXRF measurements were carried out by using a polychromatic X-ray beam, with beam energy from 2 up to 20 keV, from the D09-XRF beam-line, of the Brazilian Light Synchrotron Laboratory (Pérez et al. 1999). Each reflector disk containing the sample was positioned for the total reflection condition, and the acquisition time was set up at 100 s, except for multi-element standard reference materials that were set up at 500 s. For the detection, a Si(Li) detector was used with 160 eV FWHM at Mn-Kz line. The analyses of SR-TXRF spectra obtained from samples were made by using the AXIL software (Vekemans et al. 1994). Background-subtracted Kz (K, Cr, Mn, Fe, Cu, Zn, Ga, Br, Sr and Y) or Lz (Sr, Mo, Ag, Cd, Sb, Cs, Ba, Sm, Dy, and Pb) peak areas were used to determine the relative sensitivity yields of the STXRF spectrometer and to calculate the elementary concentrations of the samples.

**RESULTS AND DISCUSSION**

Based on the strong X-ray Kz and Lz peak intensities of light elements (K, Cr, Mn, Fe, Cu, Zn, Ga, and Br) and heavy metals (Sr, Mo, Ag, Cd, Sb, Cs, La, Sm, Dy and Pb), respectively, the relative sensitivity to yttrium values were determined for both X-ray K and L series of the SR-TXRF spectrometer. The exponential-type fits of sensitivity data for both X-ray K and L series are represented by the Equations (1) and (2). The fits have shown a good statistical quality.

\[
S_K(Z) = 0.0735\exp[-21.106 + 1.675 \times Z - 0.0274 \times Z^2]; \\
\begin{align*}
& r^2 = 0.9983 \\
& \chi^2 = 0.8245
\end{align*}
\]

\[
S_L(Z) = 0.0056\exp[-13.414 + 0.502 \times Z - 0.0034 \times Z^2]; \\
\begin{align*}
& r^2 = 0.9976 \\
& \chi^2 = 0.7150
\end{align*}
\]

The light elements, such as Si, Al, P, S and Cl among others, were identified in all SR-TXRF spectra, but their concentrations are not possible to determine because they are within a non-accessible region by the sensitivity curve of SR-TXRF spectrometer for X-ray K series. In addition, some elements were not possible to identify, such as P, S, Ag and Cd, because of their low concentrations in samples and the strong X-ray peak interference caused by the main elements in SR-TXRF spectra. Besides, the elements K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, and Ba were mainly identified in the compartments of water, sediment and fish tissue by their strong X-ray Kz and Lz lines. Apart from these elements, the arsenic X-ray spectral lines were identified in SR-TXRF spectra obtained from samples of water and muscle tissue, while the lead element was identified in sediment compartment only.
All relative-to-yttrium element concentrations in aqueous solutions, which were obtained from samples of water, digested sediment and fish tissues, were calculated by using Equation 3. However, the amount of element mass per dry sample (sediment, muscles and livers) was determined, taking into account the small dry digested sample and the final volume of the dilution. All element concentrations in dry fish tissue (muscle and liver) were converted to a wet weight by taking into account the samples’ moisture content (72–88% range).

\[ C_{LP}(z) = \frac{I_Z}{I_Y} \times \frac{C_Y}{S_z(z)} \]  

(3)

Table 1 summarises the measured and certified values for standard reference material drinking water pollutants and standard reference material (DORM-2). Measured values showed relative standard deviation lower than 10% for all elements in the analysed standards.

The values of the mean element concentrations of the samples from lake waters are summarised in Table 2. Besides, the results of the mean element concentrations in sediments and wet weights of muscles and livers are summarised in Table 3. In comparison with the Brazilian environmental norms for river water quality (Brazil 2005), all detected metals in superficial water are below the maximum tolerance limits for them. Lead was not detected in superficial water due to its low content, but sediment lead was observed (Table 3). In benthic environment, small organisms such as worms, crustaceans and insect larvae can be affected and die by sediments contaminated with heavy metals, reducing the food available to larger animals such fish (EPA 2005). High concentrations were found for sediment Cr and Pb (19–52 and 16–26 mg kg\(^{-1}\) dry weight, respectively), which are above the maximum recommended limits for contaminated sediment in water (EPA 2005), being a possible source of accumulation in the aquatic fauna.

The muscle analysis of O. niloticus, P. mesopotamicus, P. scrofa and C. carpio was carried out to evaluate the possible accumulation of metals and their possible transfer to humans through fish consumption. The Brazilian legislation (Brazil 1965) that establishes the maximum tolerance limits for inorganic contaminants in food considers the elements As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sb, Se, Sn and Zn as toxic elements for humans. The Cr concentrations found in fish muscle samples of O. niloticus, P. mesopotamicus, P. scrofa, and C. carpio were 1.1, 0.14, 0.7 and 0.2 mg kg\(^{-1}\), respectively, which are above the 0.1 mg kg\(^{-1}\) maximum tolerance limit for Cr (see Table 3). In addition, the muscles of O. niloticus have shown the highest Cu (54 mg kg\(^{-1}\)) and Se (1.7 mg kg\(^{-1}\)) concentrations above the 30 and

\begin{table}[h]
\centering
\footnotesize
\begin{tabular}{|l|l|l|l|l|}
\hline
| Reference material | Element | BEL (μg L\(^{-1}\)) | Site 1 | Site 2 | Site 3 | Site 4 |
\hline
| DWP | Cr | 9.09 ± 0.45 | 9.2 ± 0.7 | & & & |
| DWP | As | 9.09 ± 0.45 | 8.9 ± 0.6 | & & & |
| DWP | Se | 4.54 ± 0.23 | 4.2 ± 0.4 | & & & |
| DORM-2 | Cr | 34.7 ± 5.5 | 32.7 ± 8.3 | & & & |
| DORM-2 | Mn | 3.66 ± 0.34 | 3.91 ± 0.63 | & & & |
| DORM-2 | Fe | 142 ± 10 | 152 ± 15 | & & & |
| DORM-2 | Ni | 19.4 ± 3.1 | 18.3 ± 4.0 | & & & |
| DORM-2 | Cu | 2.34 ± 0.16 | 2.4 ± 1.6 | & & & |
| DORM-2 | Zn | 25.6 ± 3.2 | 27.1 ± 3.6 | & & & |
| DORM-2 | As | 18.0 ± 1.1 | 17.0 ± 1.5 | & & & |
| DORM-2 | Se | 1.40 ± 0.09 | 1.50 ± 0.15 | & & & |
| DORM-2 | Pb | 0.065 ± 0.007 | 0.060 ± 0.013 | & & & |
\hline
\end{tabular}
\caption{Mean value of element concentrations (in μg L\(^{-1}\)) in Toledo municipal lake water samples collected from four collection sites, including their maximum tolerance limits recommended by the Brazilian environmental legislation (BEL) for river water of class II (Brazil 2005).}
\end{table}
The metal uptake by fish can be considered as a direct consequence of urban and industrial residues release. The results of physical–chemical parameter values are summarised in Table 4. A strong positive and significant Spearman’s correlation coefficient (Hamilton et al. 1977) was found between most physical–chemical parameters values at collection sites in Toledo Lake, while no statistically significant correlation coefficient was found between physical–chemical and element concentration values. The fact has shown that the main source of pollution in Toledo municipal lake could be attributed to industrial residues that were released in the past.

### CONCLUSION

The results of this study supply valuable information on the metal contents in fish from Toledo Lake. The analytical procedure of SR-TXRF technique used in this study has
allowed obtaining heavy metal values with good precision and accuracy in water, sediment and fish tissues from Toledo municipal lake. The highest Cr, Cu and Se concentration levels above the maximum tolerance limit according the Brazilian norms in fish muscles could be associated with the metal uptake and accumulation due to the direct contact with water and sediment.

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REFERENCES


