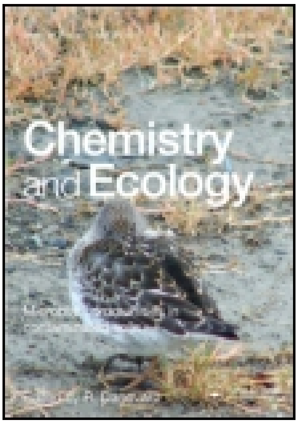

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Trace elements in fish and oysters from Sepetiba Bay (Rio de Janeiro – Brazil) determined by total reflection X-ray fluorescence using synchrotron radiation

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Trace elements were determined in fish and oysters from Sepetiba Bay, Brazil, by total reflection Xray fluorescence using synchrotron radiation (SRTXRF). Cr, Mn, Fe, Ni, Cu, Zn and Se were determined in fish muscles and organs and in oyster soft tissue. SRTXRF was shown to be a good tool for the analysis of trace elements from biological tissue samples. Overall, the levels of the analysed metals were higher in oysters than in the fish samples. Metals were not uniformly distributed throughout the body of the analysed fish. The detected concentrations of Cr, Zn and Se were very high in some samples, surpassing the maximum limits established by Brazilian legislation.

Keywords: trace elements; fish; oysters; heavy metals; contamination; Sepetiba Bay; TXRF; synchrotron radiation

1. Introduction

Industrial pollution and the discharge of potentially toxic trace metals in aquatic ecosystems cause serious environmental problems. Sepetiba Bay is an estuary in the state of Rio de Janeiro on the south-eastern coast of Brazil, where over recent years an increase in pollution levels has been reported [1–3], with metals being the main pollutants found in this ecosystem [2]. Aquatic animals in general gather contaminants from the environment, however, fish assimilate metals by ingestion of particulate material suspended in water, ingestion of food, ion-exchange of dissolved metals across lipophilic membranes, e.g. the gills, and adsorption on tissue and membrane surfaces [4]. Determination of the concentration of metals in different fish organs and oysters is important to identify areas with higher concentrations of these elements within the animals. Information about the levels of some toxic metals in fish and oysters also indicates the degree of pollution of the body of water in which these organisms live. Hence pollutants such as metals, are accumulated

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in marine organisms and sediments, and subsequently potentially transferred to humans via the food chain [5,6].

In this study, total reflection X-ray fluorescence using synchrotron radiation (SR-TXRF) was employed for the analysis of trace elements (Cr, Mn, Fe, Ni, Cu, Zn and Se) in muscles and different organs of carnivorous fish from the Sciaenidae family and in soft tissue of oysters from the *Crassostrea brasiliana* species captured in Sepetiba Bay, RJ, Brazil. These species are used as food by the local population.

Total reflection X-ray fluorescence (TXRF), a variant methodology from energy-dispersive X-ray spectrometry, has been used in various areas of science and technology [7]. It includes important characteristics such as: simultaneous detection of several elements, low detection limit, short measuring time and use of a small volume of sample. This method is based on the incidence of an X-ray beam at a small angle on the flat surface of a support or carrier on which the sample to be analysed is deposited. In this condition, the scattering effect is minimised and a better peak–background ratio is thus obtained, therefore reducing the detection limits. Multi-elemental analysis and low cost are two meaningful advantages of this method when compared with other methods such as atomic absorption spectrometry (AAS) and inductively coupled plasma techniques (ICP-AES and ICP-MS) [8]. Synchrotron radiation has been employed with great success in TXRF techniques for the analysis of small quantities of elements in biological applications [9,10]. SR shows peculiar characteristics in comparison with conventional sources of X-rays, being the ideal way to excite X-ray fluorescence. This radiation provides an exceptional glow, linear polarisation and natural collimation, and thus combines perfectly with TXRF [11].

2. Experimental procedures

2.1. *Sample and standard preparation*

Immediately after being captured from Sepetiba Bay, dozens of fish from the *Cynoscion acoupa* and *Micropogonis furnieri* species (Sciaenidae family) and oysters from the *Crassotrea brasiliana* species were taken to the Physical-Chemistry Control Laboratory at Fluminense Federal University for sample preparation. Samples of muscle, liver, intestines, gonads, kidneys and gills were removed from each fish, and samples of soft tissue were taken from each oyster.

The samples were dried in a stove, ground and submitted to chemical digestion. Digestion was performed by incubating 100 mg of each sample in polyethylene tubes containing 2 mL of nitric acid (HNO_3-65%) (Merck®, Brazil) at 45 °C for 72 h. After dissolution, 500 μL of the samples were mixed with 50 μL of ICP standard gallium solution (Ga) (102.5 $\mu g \cdot mL^{-1}$), which was used as an internal standard. A small amount (5 μL) of the final solution was pipetted onto a clean Perpex™ sample support. Samples were dried in an infrared spot and processed in triplicate. Blank samples were made to identify any possible source of contamination. All reagents used were of analytical grade.

A calibration curve was made by using a multi-element standard solution containing the elements Al, K, Ca, Mn, Fe, Co, Ni, Cu, Sr, Mo for K-lines prepared at six different known concentrations (Table 1), with Ga addition as the internal standard.

2.2. *Instrumentation*

SR-TXRF measurements were performed at the Brazilian Synchrotron Light Laboratory in São Paulo, Brazil, using a polychromatic beam with a maximum energy of 20 keV for the excitation. A Si (Li) detector with a resolution of ∼140 eV at 5.9 keV was used. The reflector–detector distance

Table 1. Multi-elemental standard solution concentration for K-lines.

	Concentrations ($mg{\cdot}L^{-1}$)					
Elements	1K	2K	3K	4K	5K	6K
Al	50	40.9	36.36	31.82	27.27	22.73
K	100	81.82	72.73	63.64	54.54	45.45
Ca	10	8.2	7.27	6.36	5.45	4.5
Cr	50	40.9	36.36	31.82	27.27	22.73
Mn	10	8.2	7.27	6.36	5.45	4.5
Fe	10	8.2	7.27	6.36	5.45	4.5
Co	10	8.2	7.27	6.36	5.45	4.5
Ni	50	40.9	36.36	31.82	27.27	22.73
Cu	10	8.2	7.27	6.36	5.45	4.5
Zn	10	8.2	7.27	6.36	5.45	4.5
Sr	10	8.2	7.27	6.36	5.45	4.5
Mo	50	40.9	36.36	31.82	27.27	22.73

was 5 mm and the incidence angle (for Perspex™ sample carrier) 1.0 mrad. In order to obtain the X-ray intensities for each element, samples and standards were excited for 150 s and the obtained X-ray spectra were evaluated using the quantitative X-ray analysis system (QXAS) software [12] distributed by the International Atomic Energy Agency (IAEA).

2.3. *Quantitative analysis*

The correction of matrix effect is not necessary in the analysis by SR-TXRF for the type of samples analysed here, which is considered a thin film, since this effect does not happen.

The quantitative analysis was performed using Equation (1) because absorption and enhancement effects can be neglected since these samples are considered a thin film.

$$I_{\mathrm{i}} = s_{\mathrm{i}} \cdot C_{\mathrm{i}}, \tag{1}$$

where I_{i} represents the fluorescent intensity (cps) of the element i, C_{i} the concentration ($\mu g{\cdot}g^{-1}$), and s_{i} the sensitivity for this element ($cps{\cdot}\mu g{\cdot}mL^{-1}$).

The thin film formed on the Perspex™ support does not have a regular geometry and therefore the X-ray intensities depend on the thin film position. This geometric effect can be corrected by computing the relative intensity for each element in relation to an internal standard added in every sample and standard (Equation (2)).

$$I_{\mathrm{i}}/I_{\mathrm{Ga}} = s_{\mathrm{i}}/s_{\mathrm{Ga}} \cdot C_{\mathrm{i}}/C_{\mathrm{Ga}} \text{ or } I_{\mathrm{i}}/I_{\mathrm{Ga}} \cdot C_{\mathrm{Ga}} = s_{\mathrm{i}}/s_{\mathrm{Ga}} \cdot C_{\mathrm{i}}, \tag{2}$$

assuming $R_{\mathrm{i}} = I_{\mathrm{i}}/I_{\mathrm{Ga}} \cdot C_{\mathrm{Ga}}$ and $S_{\mathrm{i}} = s_{\mathrm{i}}/s_{\mathrm{Ga}}$, where I_{Ga} and C_{Ga} represent the fluorescent intensity (cps) and the concentration ($\mu g{\cdot}mL^{-1}$) of the internal standard in the sample, s_{Ga} the sensitivity for the internal standard ($cps{\cdot}\mu g{\cdot}mL^{-1}$), R_{i} the relative intensity of element i and S_{i} the relative sensitivity of element i.

By substituting the data from Equation (1) with those obtained from Equation (2) we obtain Equation (3), which was used in the quantitative analysis by TXRF.

$$R_{\mathrm{i}} = S_{\mathrm{i}} \cdot C_{\mathrm{i}}. \tag{3}$$

The relative sensitivity was determined by the measurements from the standards containing the elements Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn and Sr and Mo for the K-lines.

Table 2. Elemental concentration ($\mu g \cdot g^{-1}$) in a certified material – bovine liver, NIST1577b.

Elements	Certificate concentration	Measurement concentration
P	11000 ± 300	9130 ± 40
S	7850 ± 60	6235 ± 25
Cl	2780 ± 60	580 ± 10
K	9940 ± 20	8620 ± 20
Ca	116 ± 4	115 ± 6
Mn	10.5 ± 1.7	7.7 ± 0.2
Fe	184 ± 15	170 ± 1
Cu	160 ± 8	135 ± 0
Zn	127 ± 16	113 ± 0
Rb	13.7 ± 1.1	15 ± 1

2.4. *TXRF validation procedure*

The accuracy of the measurements was checked by determining the elemental concentrations in a standard solution prepared with bovine liver – NIST1577b, a certified material from the National Institute of Standards and Technology (NIST) (Table 2). The results obtained in the reference material for determining elements of higher atomic mass ($Z \geq 20$) showed a good recovery thus demonstrating the accuracy of the analytical methodology used in the study since the elements in the analysed samples are elements of high atomic number.

3. Results and discussion

SR-TXRF was shown to be a good method for the determination of trace elements in animal tissues.

The average values expressed in wet weight and the standard deviation of the concentrations of Cr, Mn, Fe, Ni, Cu, Zn and Se obtained from the fish and oyster samples are presented in Table 3. The results obtained per dry weight unit were converted into wet weight, by determining the percentage of water lost during the drying process. The concentrations of metals per wet weight unit were calculated to facilitate comparison with the maximum limits of metals allowed in foodstuffs for human consumption established by Brazilian legislation, which are expressed in fresh weight [13].

The results for the concentrations of metals in oyster and fish muscle were compared using analysis of variance (ANOVA). With the exception of Ni, the highest concentrations for all metals were detected in the oyster samples in comparison with the fish samples ($p < 0.01$). This result

Table 3. Mean concentration ($\mu g.g^{-1}$) in wet weight (± SD) of trace elements in muscle and organs of fish and oysters from Sepetiba Bay.

	Fish						Oysters
Elements	Muscle	Liver	Intestine	Gonads	Kidney	Gills	Soft tissue
Cr	0.50 (±0.13)	0.95 (±0.73)	0.48 (±0.18)	0.79 (±0.29)	0.86 (±0.52)	1.14 (±0.83)	4.5 (±4.09)
Mn	0.27 (±0.30)	0.85 (±0.59)	0.90 (±0.70)	0.52 (±0.27)	1.53 (±1.17)	2.82 (±2.45)	6.6 (±3.75)
Fe	6.12 (±1.54)	167.6 (±181.19)	21.7 (±9.65)	41.5 (±44.07)	91.8 (±52.51)	69.7 (±26.14)	219 (±228.61)
Ni	0.02 (±0.05)	0.09 (±0.15)	0.08 (±0.09)	0.22 (±0.33)	0.12 (±0.12)	0.32 (±0.39)	0.01 (±0.03)
Cu	0.18 (±0.15)	10.3 (±13.35)	0.95 (±0.81)	0.85 (±0.78)	10.4 (±11.08)	1.26 (±1.78)	17.5 (±8.89)
Zn	6.74 (±1.79)	38.5 (±25.32)	19.6 (±7.04)	110 (±122.91)	36.6 (±21.32)	28.0 (±5.43)	3 199 (±1 785.91)
Se	1.8 (±0.44)	11.9 (±8.94)	0.06 (±0.02)	0.88 (±0.44)	12.4 (±10.43)	7.0 (±4.54)	22.7 (±19.40)

was expected because oysters display filter-feeding habits by filtering particulate material in suspension, which is one of the compartments with the highest concentrations of metals in Sepetiba Bay. Furthermore, oysters, and other bivalve molluscs, possess detoxification mechanisms which involve the accumulation of high levels of heavy metals in metal–phosphate granules or granules linked to specific proteins [14].

In the fish samples, the concentration of the elements was not uniform with regard to the distribution of metals. Significant variations in the levels of Cr, Mn, Fe, Cu, Zn and Se were observed in the different fish organs when analysed using the Tukey's studentised range test ($p < 0.05$).

As for the concentration of Ni, no statistical difference was observed, probably because the levels of Ni were very small and therefore not detected in many organs. Our results confirm differences in the accumulation of trace metals in different tissues which is in agreement with similar results reported in the literature [15,16]. The highest elemental concentrations were found in the liver, kidney and gills, and the lowest concentrations were detected in the muscles. Muscles are not active tissues in accumulating metals; however, the determination of trace metals in this tissue is important due to human consumption [17]. Many studies showed that heavy metals accumulate mainly in metabolic organs such as kidney and liver, which store metals in order to detoxificate by producing metallothioneins [18,19]. The concentration of metal in the gills could be due to complex formation between the elements and mucus normally present between the lamellae of this organ [19]. The concentrations of metals in the gills reflect the concentration in the water where the fish live, whereas the concentrations in the liver reflect exposure through diet. The results obtained in this study are comparable with results reported in the literature for studies worldwide. A study analysing the levels of trace metals (Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn) in two fish species (*Leuciscus cephalus* and *Lepomis gibbosus*) from Turkey, also showed higher accumulation of metals in liver and gills and lower accumulation in muscles [15]. Another study using 12 fish species from the Aegean and Mediterranean Seas reported that the metal levels (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) observed in muscles were significantly lower than in the liver [16].

Cr, Mn, Fe, Cu, Zn, Se and Ni are essential metals for human nutrition, however, when consumed in high levels can cause health problems [20].

The maximum permitted concentration (MPC) by the Brazilian legislation for chromium (Cr) in food ($0.1\,\mu g \cdot g^{-1}$) [13] was surpassed in all studied samples, including muscle samples. The mean chromium concentrations observed were 0.50 and $4.50\,\mu g \cdot g^{-1}$ for fish muscle and oyster soft tissues, respectively. Two decades ago, studies in Sepetiba Bay detected Cr values ($0.31\,\mu g \cdot g^{-1}$ in fish and $1.57\,\mu g \cdot g^{-1}$ in oysters) already above the MPC, yet lower than those detected in this study [21]. In the literature, Cr levels (wet weight) have been reported within the range 0.06–$0.84\,\mu g \cdot g^{-1}$ for fish muscles from the Black Sea coast [22]. This metal can be carcinogenic depending on its chemical form.

There is no reference to a MPC for manganese (Mn) in Brazilian legislation. The average Mn content observed in different fish tissues varied from 0.27 (muscle) to $2.82\,\mu g \cdot g^{-1}$ (gills). The average level of Mn observed in oysters was $6.6\,\mu g \cdot g^{-1}$. Manganese levels reported in the literature are within the range 2.76–$9.10\,\mu g \cdot g^{-1}$ (wet weight) for muscles of fish from the Black Sea [23] and 0.10–$0.99\,\mu g \cdot g^{-1}$ (wet weight) in seafood from the Aegean and Mediterranean Seas in Turkey [24]. The average values of Mn detected in this study are less than those observed in oysters from non-polluted areas of the Brazilian coast [25], which indicates that there are no sources of contamination by Mn in Sepetiba Bay.

High concentrations of iron (Fe) were detected in the fish liver samples ($167\,\mu g \cdot g^{-1}$). Fe can be accumulated in fish liver as porphyrin enzymes and the consumption of $200\,mg \cdot day^{-1}$ is considered toxic to humans [26]. The average iron content detected in the analysed fish muscle samples was $6.12\,\mu g \cdot g^{-1}$. Iron levels have been reported in the literature between 3.25 and $8.12\,\mu g \cdot g^{-1}$

(wet weight) for muscles of fish from a contaminated lake in Turkey [27]. The average iron content detected in the analysed oyster samples was 219 $\mu g{\cdot}g^{-1}$. Iron levels are reported between 44.21 and 170 $\mu g{\cdot}g^{-1}$ in oysters from Brazilian ecosystems without historical pollution [25]. Reported data for the mineral oyster composition indicate an iron concentration of 170 $\mu g{\cdot}g^{-1}$ for the oyster edible portion [28]. Because there is no established MPC for this metal for foodstuffs within Brazilian legislation it is difficult to interpret these results. According to the Joint FAO/WHO, the set limit for metal intake based on body weight by an average adult (60 kg body weight) should not exceed the provisional iron tolerable daily intake of 48 mg [29].

The average concentration of nickel (Ni) detected in any of the analysed samples did not surpass the MPC (5 $\mu g{\cdot}g^{-1}$) established by Brazilian legislation [13], hence the suggestion that Ni was the metal with the smallest discharge level in Sepetiba Bay [1]. The average Ni content observed in the analysed fish tissues ranged between 0.02 $\mu g{\cdot}g^{-1}$ in muscles and 0.32 $\mu g{\cdot}g^{-1}$ in gills. Ni levels have been reported within the range 0.01–0.05 $\mu g{\cdot}g^{-1}$ for muscles of fish from Sepetiba Bay in previous studies [1]. Another study reports nickel levels ranging from $<$0.01 to 2.04 $\mu g{\cdot}g^{-1}$ in muscles of fish from the Black Sea coast [22]. According to our results, Ni does not represent a source of pollutant contamination in Sepetiba Bay.

No sample surpassed the MPC for copper (Cu) established for food consumption by Brazilian legislation, which is 30 $\mu g{\cdot}g^{-1}$ [13]. The average Cu content detected in the analysed fish tissues ranged between 0.18 $\mu g{\cdot}g^{-1}$ (muscles) and 10.4 $\mu g{\cdot}g^{-1}$ (kidneys). The average Cu content in the analysed oysters was 17.5 $\mu g{\cdot}g^{-1}$. These results are similar to those of other authors who did not find high concentrations of Cu in fish and oysters from Sepetiba Bay (0.54 $\mu g{\cdot}g^{-1}$ for fish muscle and 3.19 $\mu g{\cdot}g^{-1}$ for oyster tissues) [1,21]. Copper levels for fish muscles have been reported within the range 1.03–5.11 $\mu g{\cdot}g^{-1}$ (wet weight) for specimens from a historically environmentally contaminated lake in Turkey [27].

In the cae of zinc (Zn), the highest average concentrations were found in oysters (3199 $\mu g{\cdot}g^{-1}$) and in fish gonads, with levels in different tissues, specifically in fish, ranging from 6.74 $\mu g{\cdot}g^{-1}$ (muscle) to 110 $\mu g{\cdot}g^{-1}$ (gonads). The detected values in these samples were a lot higher than the MPC established by Brazilian legislation, which is 50 $\mu g{\cdot}g^{-1}$ for food consumption [13]. According to our results, of all the elements analysed, zinc presented the highest concentration, followed by iron, which is in agreement with results observed in other studies [5,30]. Samples of canned sardines and anchovies analysed in Turkey presented Zn levels ranging between 7.57 and 34.4 $\mu g{\cdot}g^{-1}$ (wet weight), which are higher than the amounts observed in fish muscles in our study [30]. Gonads are reported to be structures that can present high levels of Zn due to this element's participation in cell division and growth processes. Oysters also naturally accumulate Zn because the concentration of this metal is directly related to their reproduction and gonadal maturation. However, the levels of Zn detected in the oysters of Sepetiba Bay were extremely high, indicating environmental contamination. International legislations establish 1000 $\mu g{\cdot}g^{-1}$ of wet weight as the tolerable concentration limit of Zn in oysters [31]. The results obtained for the oysters from Sepetiba Bay in this study are among the highest described in the world [32] and are three times higher than the internationally established tolerable limit. Although Zn has a low degree of toxicity, cases of acute human intoxication due to the ingestion of oysters highly contaminated with Zn have been reported in literature [21]. Prolonged excessive dietary intake of zinc in humans can lead to deficiencies of iron and copper, nausea, vomiting, fever, headache, tiredness and abdominal pain [17].

The MPC for selenium (Se) in foodstuffs (0.30 $\mu g{\cdot}g^{-1}$) [13] was exceeded in the analysed oysters (22.7 $\mu g{\cdot}g^{-1}$), as well as in the majority of the analysed fish organs and tissues except in intestines (0.06 $\mu g{\cdot}g^{-1}$). The average Se content detected in fish muscles was 1.8 $\mu g{\cdot}g^{-1}$. Selenium contents have been reported in the literature within the range 0.73–2.34 $\mu g{\cdot}g^{-1}$ in fish and shellfish samples [33] and 0.16–0.85 $\mu g{\cdot}g^{-1}$ in fish from the Black Sea, Turkey [23]. Selenium is known as an essential element for most animals, however, it has a toxic effect if consumed in high levels. Considering the toxic aspect of this element when ingested in excess [34], the Brazilian legislation recommends the maximum consumption of 34 μg Se$\cdot$day^{-1} for adults.

4. Conclusions

The concentrations of most of the metals studied were higher in oysters than in fish organs and tissues. Among the fish samples, organs such as liver, kidneys and gills concentrated more metals than the muscles. The results demonstrated the accumulation of high concentrations of Cr and Se in fish muscles and Cr, Zn and Se in oysters from Sepetiba Bay. Oysters displayed particularly high levels of Zn. These results demonstrate the importance of monitoring the concentrations of metals in these species, which are consumed as food by the human population, thus representing risk of contamination.

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