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Hepatic metallothionein concentrations in the golden grey mullet (*Liza aurata*) - relationship with environmental metal concentrations in a metal-contaminated coastal system in Portugal

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Abstract

This field survey was designed to assess the environmental metal contamination status of Ria de Aveiro (Portugal). To achieve that goal, the concentrations of Cd, Hg, Cu and Zn in the sediments and water were assessed and *Liza aurata* hepatic metallothionein (MT) determined. The relationships between MT and environmental metal concentrations and hydrological factors were examined. Results revealed a wide distribution of metals both in water and sediments throughout the lagoon, mainly at Rio Novo do Principe (RIO) and Laranjo (LAR), at concentrations that may affect biota. MT concentrations were higher at the sites with high metal content (RIO and LAR). A significant positive correlation was found between MT and Cd in the sediments as well as with MT and Hg and Cu in the water. Moreover, a negative correlation between MT and salinity was found. Thus, the current data support MT use as a biomarker of metal exposure emphasizing the importance of hydrological parameters in its concentrations. Results suggest the continued monitoring of this lagoon system.

Keywords: *Liza aurata*; Metallothioneins; Cadmium; Copper; Zinc; Mercury; Aquatic pollution

33 1. Introduction

34 Metal contamination in aquatic systems is a critical environmental issue
35 due to metal uptake and toxicity in many aquatic organisms, as well as the
36 possibility of their trophic transfer along food chains, eventually reaching
37 humans. Most of these compounds have high affinity for particulate matter and
38 are readily adsorbed onto suspended particles eventually becoming part of
39 sediments where they will remain even after input cessation. Nevertheless,
40 metals may return to the water column or be transferred to biota through
41 physical, chemical and/or biological processes (Schneider and Davey, 1995;
42 Bervoets et al., 2005).

43 Normal fish cellular metabolism requires essential metals (such as Cu
44 and Zn) which may be taken up from water, food and/or sediment. However,
45 non-essential ones (such as Cd and Hg) may also be absorbed and
46 accumulated in tissues (Canli and Atli, 2003). Thus, environmental pollution
47 monitoring frequently includes assessment of metals in sediment, suspended
48 particulate matter or biota. However, the quantification of environmental or
49 tissue concentrations of a variety of metals does not take into account the
50 biological response or significance of the accumulated metals (Chan, 1995)
51 since metals can accumulate to high levels in organisms and yet be
52 metabolically unavailable, thus unable to induce toxicological effects (Vijver et
53 al., 2004; Banni et al., 2007). Metal exposure generally increases total tissue
54 concentrations of metals. However, the increase of essential metals in some
55 tissues may not be significant due to their regulated accumulation (Atli and
56 Canli, 2008).

Metallothioneins (MT) are low molecular weight cytosolic proteins rich in SH groups, known to be over-expressed in organisms from environments with high metal concentrations (Sarkar et al., 2006; Viarengo et al., 2007). In fish, the large amount of MT induction occurs in liver (Canli and Atli, 2003), an organ playing a key role in the storage and inactivation of metals. Considering that MT induction is one of the first detectable signs of the presence of metals (Filipovic and Raspor, 2003; Chesman et al., 2007; Van Campenhout et al., 2008) and the relationship found between metal concentrations in the environment and MT concentrations in fish tissues (Hylland et al., 1992), they have been used to characterize metal contamination in the environment (Chan, 1995; Linde et al., 2001).

Ria de Aveiro (Portugal), a coastal lagoon, provides an important habitat for a number of fish species, including the golden grey mullet (*Liza aurata*). The feeding habits of this species include benthic organisms and detritus, making it particularly adapted to accumulate sediment-associated contaminants and thus an important bio-indicator species. Previous studies concerning this species, in this complex aquatic system, revealed the existence of critical sites within Ria de Aveiro, capable of inducing oxidative and genetic damage (Oliveira et al., 2009a,b,c) as well as endocrine disruption (Oliveira et al., 2009d). High concentrations of metals have been detected in this aquatic system (Coelho et al., 2005; Monterroso et al., 2007) and MT inductions as well as inhibitions were observed in *L. aurata* gill, at some of those critical sites (Oliveira et al., 2009a). Thus, the hepatic MT assessment is highly relevant due to liver central role in metal storage and inactivation. In this perspective, this study assessed the relation between metal concentrations found in water and sediment

compartments of Ria de Aveiro and *L. aurata* hepatic MT. The influence of hydrological factors on MT concentrations was also studied.

2. Material and Methods

2.1. Study Area

Ria de Aveiro is a lagoon permanently connected to the ocean (Figure 1), about 45 km long and 8.5 km wide. Sampling sites were selected on a geographic distribution basis taking into account the known and supposed sources of metal contamination as well as the selection of a (theoretically) unpolluted reference point. Sampling sites were: Torreira (TOR), an intermediate region of the longest channel (S. Jacinto-Ovar channel), far from the main polluting sources and thus assumed as reference site; Barra (BAR), the initial part of the Mira channel close to the lagoon entrance and subject to considerable naval traffic; Gafanha (GAF) situated in the vicinity of a deep-sea fishing port and dry-docks, also connected with the main channel coming from Aveiro city carrying domestic discharges; Rio Novo do Principe (RIO), located at the terminal area of the Vouga River, 6.5 km distant from a pulp/paper mill effluent outlet, that discharged to this water course during nearly five decades (until the year 2000); Laranjo (LAR), close to a chlor-alkali plant (6 km), an important source of metal contamination (mainly mercury); Vagos (VAG), located at the terminal part of the Ílhavo channel, receiving municipal and domestic effluents with high concentrations of polycyclic aromatic hydrocarbons (PAHs).

107

108 **2.2. Sampling:** *Liza aurata* was caught in October 2005, during low tide
109 using a traditional beach-seine net named “chinchá”. Juvenile specimens were
110 selected on the basis of their size, having an average length of 14.5 ± 2.5 cm and
111 weighing 21.6 ± 3.7 g. Immediately after catching, fish was sacrificed, liver
112 removed and frozen in liquid nitrogen. In the laboratory the samples were stored
113 at -80°C until further treatments.

114 At each sampling site hydrological parameters were assessed (Table I)
115 according APHA (1998) guidelines. Sediments were collected from the surface
116 (15 cm deep) with a grab and stored at -20°C until analysis. Sub-surface water
117 samples were collected by hand, using polyethylene gloves and glass bottles of
118 1 L.

119

120 **2.3. Metal determination in the water and sediments**

121 Total metal concentrations in the water samples were determined after
122 liquid-liquid extraction with ammonium pyrrolidine dithiocarbamate (APDC) and
123 methyl isobutyl ketone (MIBK) followed by graphite furnace atomic absorption
124 spectrophotometry (PerkinElmer). This procedure was adopted to pre-
125 concentrate the metals present in the water. Certified standard freshwater
126 reference material (SRM 1643d from NIST) was analyzed to check the accuracy
127 of metal determinations in the water samples.

128 Total metal concentrations in the sediments were determined in the < 63
129 μm fraction, to minimize the effect of grain size on metal distribution. Samples
130 were manually wet sieved using $63 \mu\text{m}$ nylon sieves and Milli-Q water. After
131 that, this sediment fraction was dried at 40°C . For analysis, approximately 2 g

132 of the sediment fraction were accurately weighed and placed into 50 ml
133 digestion vessels. After the addition of 5 ml 69% nitric acid (HNO_3), the samples
134 were digested for 2 hours at 80°C . The digestion mixture was cooled to room
135 temperature and 2 ml 30% hydrogen peroxide (H_2O_2) and 3 ml Milli-Q water
136 carefully added. The digestion vessels were heated for another hour. The
137 residue was allowed to cool and diluted with Milli-Q water to a final volume of 50
138 ml. Then samples were allowed to settle down for 24 hours and quantitatively
139 transferred to 50 ml vessels for analysis.

140 The concentrations of Cd, Cu, Hg and Zn were determined in the
141 supernatant using a Perkin-Elmer atomic absorption spectrophotometer (AAS)
142 model A Analyst 800. All metals except Cd and Hg were analyzed by flame
143 atomic absorption spectrophotometry. Cadmium was analysed by an
144 electrothermal atomic absorption spectrometry and mercury by cold vapour
145 atomic spectrophotometry with an automatic hydride generator system.

146

147 **2.4. Metallothionein (MT) content**

148 After liver homogenization in 20 mM Tris buffer (pH 8.6) containing 150
149 mM of NaCl, the homogenates were centrifuged 45 minutes at 30000 g (4°C).
150 Supernatant aliquots were heat-treated at 80°C for 10 minutes and re-
151 centrifuged at 30000 g for 45 minutes (4°C). MT quantification was made by
152 differential pulse polarography (DPP) according to Bebianno and Langston
153 (1989) using a 646VA Processor autolab type II and an ECO Chemie IME663
154 mercury drop electrode. Comparisons of peak heights with those of standard
155 additions of purified rabbit MT enabled MT quantification. Accuracy of the DPP
156 analytical method was obtained by internal calibration using commercial rabbit

157 liver MT-I. Recovery studies of our cytosols spiked with the MT-I rabbit liver
158 standards revealed recovery rate from 97 to 103%. Results were expressed as
159 $\mu\text{g MT/g wet weight}$.

160

161 **2.5. Statistical analysis:** Results were expressed as means $\pm$ SE
162 (standard error) corresponding to experimental groups of six fish (n=6).
163 Statistical data analysis was done using Statistica software (StatSoft, Inc.,
164 Tulsa, OK, USA). Assumptions of normality and homogeneity of data were
165 verified. One way ANOVA was performed in order to assess significant effects,
166 followed by post-hoc Tukey test to signal significant differences between groups
167 (Zar, 1999). The significance of results was ascertained at $\alpha = 0.05$. Describing
168 factors were grouped using principal component analysis (PCA), reducing the
169 number of axis without losing relevant information. Principal components
170 (PCs) having eigenvalues of less than one were discarded, and varimax rotation
171 maximising the loading of a variable on one component was then applied on the
172 retained PCs. The resulting components were then used as independent factors
173 in multiple regression analyses taking MT as the dependent variable.

174 3. Results

175 3.1. Hydrological Parameters

176 The hydrological parameters, expressed in Table I, show differences in
177 terms of turbidity and salinity. Thus, waters were more turbid at LAR and VAG
178 (0.3 m) and less turbid at BAR (1.2 m). Salinity at RIO was 21 whereas the
179 other sites displayed values between 29 (LAR) and 34 (GAF and BAR).

180

181 3.2. Metals in Water and Sediment

182 The water analyses revealed that RIO displayed significantly higher
183 concentrations of Hg, Cu and Zn than TOR and the other studied sites (with the
184 exception of Zn concentrations which were not different from VAG) (Table I).
185 Concerning Cd, the highest concentrations in water were found at LAR
186 (significantly higher than in all other sites). BAR displayed lower concentrations
187 of Cd, Hg, Cu and Zn when compared to TOR (though not significantly in the
188 case of Cd). The metal concentrations in water at GAF were only significantly
189 different (higher) from TOR in the case of Hg. At LAR, the concentrations of all
190 the analysed metals were significantly higher than TOR whereas VAG displayed
191 lower Hg and Cu and higher Zn concentrations in water.

192 Concerning the concentrations of metals in the sediments (Table I), the
193 highest concentrations of Hg, Cu and Zn were found at LAR (significantly higher
194 than all other studied sites). Concerning Cd, the highest concentrations in the
195 sediments were found at RIO (significantly higher than all other studied sites)
196 and LAR (significantly higher than TOR, BAR, GAF and VAG). Hg
197 concentrations in sediments at BAR and RIO were also higher than in TOR,
198 GAF and VAG. The concentrations of Cu in sediments at RIO were also

199 significantly higher than TOR whereas Zn concentrations at BAR, GAF and
200 VAG were significantly lower than TOR.

201

202 **3.3. Metallothionein**

203 RIO displayed significantly higher MT concentration when compared to
204 all studied sites (Figure 2). Thus, compared to TOR, MT concentration was
205 256% higher at RIO. LAR also displayed significantly higher MT concentration
206 than TOR (107%), BAR, GAF, and VAG. At VAG, MT concentration was
207 significantly lower than TOR (74%).

208

209 **3.4. Principal Component Analysis and Linear Multiple Regression**

210 Three principal components were defined as explaining the major
211 amount of total variance (89.97%) of the assessed parameters (except MT)
212 (Table II). Thus, the first component which includes salinity, temperature, Cd in
213 the sediments, Hg, Cu and Zn in the water explains 36.19% of total variance.
214 The second component including Hg, Zn and Cu in the sediments and Cd in the
215 water, explains 28.84%. The third components including turbidity, pH and
216 dissolved oxygen explain 24.93% of variance. According to the multiple
217 regression analyses the first two factors account for 98.2 % of MT variability and
218 the first factor alone is responsible for 77%. Cd in the sediments, Hg and Cu in
219 the water and salinity displayed the strongest (significant) correlations with MT
220 (Figure 3).

221

222

223

224 4. Discussion

225 Biological factors such as age, sex, reproductive status as well as
226 hormone levels may influence MT induction, increasing response variability
227 (Hamza-Chaffai et al., 1995). Moreover, it was demonstrated in *L. aurata* that
228 total length, body weight and age are biometric parameters correlated with MT
229 (Filipovic and Raspor, 2003). In this perspective, *L. aurata* specimens selected
230 for the current study were juvenile with the same age and reproductively
231 quiescent and the stress of capture and handling was reduced as much as
232 possible.

233

234 4.1. Metal concentrations

235 Metals mobility and reactivity in estuaries are highly influenced by the
236 organic matter content, salinity, redox conditions and pH (Sauve et al., 2000;
237 Liang and Wong, 2003). Sediment metal concentrations usually exceed those in
238 the water column. However, metals bound to the sediment may enrich
239 interstitial and overlying waters with metals (Bryan and Langston, 1992) upon
240 changes in the environment. In the current study, the concentrations of two of
241 the most dangerous metals to aquatic organisms, Cd and Hg, listed in the
242 priority substances of EU Directive 2008/105/EC, as well as two essential
243 metals, Cu and Zn, that in high concentrations may also induce toxic effects in
244 aquatic organisms (Oliveira et al., 2004) were quantified in the water and
245 sediments of Ria de Aveiro. The studied metals displayed a distribution
246 throughout the lagoon. Zn displayed the highest concentrations in sediments,
247 followed by Cu, Cd and Hg. The highest concentrations of metals in the
248 sediments were found in LAR (for Zn, Cu and Hg) and RIO (for Cd). At RIO,

LAR and BAR Hg was higher than the defined Water Framework Directive environmental quality standard for sediments ($0.36 \mu\text{g.g}^{-1}$). Most of the available studies on metals concentrations in Ria de Aveiro focused on Hg contamination in LAR, due to the emission history of a chlor-alkali plant (Ramalhosa et al., 2005). Thus, at LAR area, the concentration of Hg detected in the sediments reached values as high as $51.7 \mu\text{g.g}^{-1}$ (Coelho et al., 2005). In the current study, the highest concentration of Hg in the sediments was found in LAR ($2.33 \mu\text{g.g}^{-1}$) and was within the same order of magnitude of other studies (Pereira et al., 2006; Ramalhosa et al., 2006). The present data also revealed considerable Hg in the sediments of BAR and RIO (0.88 and $0.86 \mu\text{g.g}^{-1}$ respectively), probably due to tidal export from Laranjo area (Pereira et al., 1998) and/or from nonpoint sources. Despite most studies on metal concentrations at LAR focused on Hg, elevated concentrations of other metals were also found there by Monterroso et al. (2007). The concentrations of Cd, Cu and Zn found in the current study at LAR sediments were similar to those found by Monterroso et al. (2007).

The metal concentrations in water reported in the current study can only be compared with other studies in Ria de Aveiro in terms of Hg in LAR, being in the same order of magnitude of previously reported concentrations (Ramalhosa et al., 2006; Guilherme et al., 2008). Unlike sediments which displayed higher Hg, Cu and Zn concentrations at LAR, in water, RIO displayed the highest concentrations of Hg, Cu and Zn. Another clear difference to sediments was found in BAR that displayed very low Hg concentrations in water (below detection limit). Moreover, Cd concentrations at all studied sites were above the defined environmental quality standard for water of Water Framework Directive ($0.21 \mu\text{g.g}^{-1}$).

274 One of the outstanding difficulties encountered in environmental impact
275 assessments is to determine the biological significance of contaminants
276 (Langston et al., 2002). In this perspective numerical quality guidelines have
277 been developed, identifying concentrations of chemicals of potential concern
278 that may cause or contribute to adverse effects (MacDonald et al., 2000).
279 However, its use should be carefully interpreted since factors like organic
280 matter, grain size and percentage of clay may be confounding elements for
281 direct comparison. Nevertheless, the comparison of the present metal data with
282 some quality guidelines (Long et al., 1995; MacDonald et al., 1996) reveals that
283 the concentrations detected in the sediments mainly at LAR and RIO and BAR
284 may lead to frequent adverse effects in aquatic organisms. The concentrations
285 of metals in the water are highly dynamic, being influenced by abiotic factors
286 among which the water flow. Nevertheless, the water concentrations of Hg at
287 RIO as well as Cu at RIO, LAR and GAF, can lead to damage in aquatic
288 species considering they were higher than the US EPA (2006) estimated
289 highest concentration to which an aquatic community can be exposed
290 indefinitely without resulting in an unacceptable effect.

291 The bioavailability of a metal does not necessarily lead to harmful effects
292 in organisms since they evolved defence mechanisms which allow them to
293 decrease metal reactivity in cells. Among those defences, MT are considered as
294 the most suitable biomarker for Cd, Cu, Zn and Hg exposure (Hylland et al.,
295 1998), reflecting bioavailability and tissue pharmacokinetics of metal uptake
296 (Handy et al., 2003). In this perspective, the study of *L. aurata* liver MT
297 becomes highly relevant, taking into account that liver is the main metal
298 accumulating organ in this species (Usero et al., 2003). However, despite the

known presence of metals in Ria de Aveiro and the suitability of fish MT as biomarkers of exposure to metals, the studies focusing on environmental metal concentrations and fish MT concentrations are very scarce.

Measurable (constitutive) concentrations of MT are always expected even in uncontaminated sites, due to MT homeostatic role in transferring essential elements to and from metalloproteins. The observed MT concentrations ranged from 45.6 to 627.7 ($\mu\text{g}\cdot\text{g}^{-1}$ wet weight), being in the same order of magnitude of the MT concentrations found in other species. For example, Zorita et al. (2008) found MT concentrations in red mullets (*Mullus barbatus*) between 100 and 710 ($\mu\text{g}\cdot\text{g}^{-1}$ wet weight). However, the current hepatic MT concentrations were much lower than in *L. aurata* from Eastern Adriatic coastal zone (2320 $\mu\text{g}\cdot\text{g}^{-1}$ wet weight) (Filipovic and Raspor, 2003). Differences may be linked with different age, weight and size of the surveyed specimens. Supporting this hypothesis, a positive and statistically significant correlation between *L. aurata* total length and body weight with MT and metals in liver cytosol was found by the authors (Filipovic and Raspor, 2003).

In the current study, MT concentrations were higher at the sites displaying the highest metal concentrations, supporting its usefulness in environmental monitoring even in complex environments where interference of other xenobiotics can be found. MT use as biomarker of response to metals exposure is more sensitive to metals such Cd and Hg and to a much lower extent to essential metals (Olsvik et al., 2001; Banni et al., 2007; Atli and Canli, 2008;) which have their cytosolic concentrations closely regulated through homeostatic mechanisms often binding to structures other than MT (Laflamme et al., 2000; Langston et al., 2002; Linde et al., 2005). In this perspective, other

sulfhydryl group containing substances such as GSH may also play an important role in cellular protection against metal cytotoxicity (Viarengo and Nott, 1993; Oliveira et al., 2004). Nevertheless, a high positive correlation of MT and Cu cytosolic concentrations was found in liver of *L. aurata* (Filipovic and Raspor, 2003). Moreover, considering the complex environment that is an estuary, the combined effects of other xenobiotics should also be considered as demonstrated by Romeo et al. (1997) and van den Hurk et al. (2000). Despite the demonstration that simultaneous exposure to metals such as Cd and PAHs may yield higher MT concentrations than metal exposure alone, our study clearly shows MT induction in environments with high metal concentrations (RIO and LAR) and low concentrations in sites with low metal concentrations (TOR, BAR and VAG). The previous (and only available study) on MT concentrations of *L. aurata* caught at Ria de Aveiro (Oliveira et al., 2009a), showed higher gill MT concentrations at LAR and suggested that this site was the most contaminated with metals. Moreover, no significant differences to TOR were found in that study in MT concentrations from RIO. These results seem to confirm that the hepatic MT concentrations are more reliable to the environmental metal contamination assessment than gill MT.

342

343 **4.2. Relation between MTs and abiotic factors**

344 The multiple regression analyses showed that the assessed
345 metals and hydrological parameters grouped in components 1 and 2 could
346 explain 98.2 % of the variability of MTs. Thus, salinity, temperature, Cd in the
347 sediments Hg, Cu and Zn in water were responsible for 77% of MT variability.
348 Of these parameters, linear regression analyses signalled as more correlated
349 Cd in the sediments, Cu and Hg in water and salinity (inversely). It has been

generally accepted that the toxicity of certain metals to aquatic animals is influenced by ambient salinity. Thus, metals are usually more toxic at low salinities probably associated with increased uptake rates, as a result of an increase in free metal ion concentrations. In this perspective, a highly significant negative correlation between MT and salinity was also found in the Brazilian flounder (*Paralichthys orbignyanus*) collected from an estuary (Amado et al., 2006). Thus, a determinant role of salinity in the metal bioavailability can be on the basis of the negative correlation currently found between that abiotic parameter and hepatic MT. However, a definitive linkage between these variables is complicated by the occurrence of different patterns of contamination and salinity levels in the sites under comparison.

Thus, the usefulness of *L. aurata* hepatic MT as biomarkers of metal exposure was demonstrated in fish caught at Ria de Aveiro despite the known ability of other xenobiotics to interfere in MT concentrations and the known existence of different type of xenobiotics in Ria de Aveiro.

5. Conclusions

High concentrations of metals can be found in the sediments and water of Ria de Aveiro which may result in pernicious effects to aquatic organisms. The highest concentrations of metals in the sediments and water were found in LAR and RIO. Thus, in sediments, LAR for Zn, Cu and Hg and RIO for Cd displayed the highest concentrations. In water, RIO displayed the highest concentrations of Hg, Cu and Zn and LAR the highest concentrations of Cd.

374 *L. aurata* hepatic MT concentrations can be ranked as:
375 RIO>LAR>GAF>TOR>BAR>VAG. The highest hepatic MT concentrations
376 displayed at the most contaminated sites (RIO and LAR) reveal a strong *L.*
377 *aurata* defence capacity against metal toxicity.

378 The correlation between environmental concentrations of metals and *L.*
379 *aurata* hepatic MT, supports MT applicability as a biomarker of metal pollution in
380 contaminated aquatic systems, though the influence of abiotic factors namely
381 salinity should be considered.

382 The results of the current study suggest continued monitoring of Ria de
383 Aveiro.

384

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390

391

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554

555 **Figure 1** - Map of the sampling sites (■) in the Ria de Aveiro. The
556 respective coordinates (latitude, longitude) are: reference site – Torreira (TOR)
557 — 40°44'02 N, 008°41'44 W; Barra (BAR) — 40°37'42 N, 008°44'35 W;
558 Gafanha (GAF) — 40°38'38 N, 008°41'42 W; Rio Novo do Principe (RIO) —
559 40°41'08 N, 008°39'41 W; Laranjo (LAR) — 40°43'30 N, 008°37'43 W; Vagos
560 (VAG) — 40°33'59 N, 008°40'55 W.

561

562 **Table I** – Environmental characterization: hydrological parameters and
563 metals concentrations in water and sediments. Distinct letters indicate
564 significant differences between sites ($p < 0.05$).

565

566 **Figure 2** – Hepatic metallothionein (MT) of *L. aurata* collected at different
567 sites in Ria de Aveiro. Values represent mean $\pm$ standard error ($n=6$). Distinct
568 letters indicate significant differences between sites ($p < 0.05$).

569

570 **Table II** – Results of the Principal Component Analysis (PCA) on the
571 dataset: coefficients of the eigenvectors (loadings of the original variables) in
572 the linear combination of variates from which the PCs are computed (loadings
573 < 0.25 were omitted)

574

575 **Figure 3** - Correlation between *L. aurata* hepatic MT and A) Salinity; B)
576 Cu in water; C) Hg in water; D) Cd in the sediments. Statistical significance and
577 correlation coefficient are represented by p and r, respectively.

Figure 1

Figure 2

Figure 3

Table I

			Sites					
			TOR	BAR	GAF	RIO	LAR	VAG
Water Physico-Chemical Parameters	Turbidity (m)		0.8	1.2	0.7	1	0.3	0.3
	Dissolved Oxygen (mg/L)		6.88	6.16	5.78	6.37	4.96	5.18
	Temperature (°C)		18.9	17.9	17.9	19	18.6	18.5
	pH		8.17	8.32	8.28	8.2	7.74	7.88
	Salinity		32.5	34	34	21	29	33
Metal Concentrations	Water ($\mu\text{g.L}^{-1}$)	Cd	$1.02 \pm 0.09^{\text{bc}}$	$0.39 \pm 0.12^{\text{c}}$	$1.52 \pm 0.10^{\text{b}}$	$0.92 \pm 0.13^{\text{bc}}$	$2.91 \pm 0.48^{\text{a}}$	$0.85 \pm 0.20^{\text{bc}}$
		Hg	$0.46 \pm 0.01^{\text{d}}$	BDL	$0.56 \pm 0.01^{\text{c}}$	$1.02 \pm 0.02^{\text{a}}$	$0.78 \pm 0.02^{\text{b}}$	$0.23 \pm 0.01^{\text{e}}$
		Cu	$2.80 \pm 0.36^{\text{c}}$	$1.25 \pm 0.11^{\text{d}}$	$3.82 \pm 0.24^{\text{bc}}$	$6.21 \pm 0.36^{\text{a}}$	$4.32 \pm 0.47^{\text{b}}$	$1.02 \pm 0.07^{\text{d}}$
		Zn	$12.05 \pm 0.33^{\text{c}}$	$4.07 \pm 0.47^{\text{d}}$	$10.10 \pm 0.12^{\text{c}}$	$18.72 \pm 0.42^{\text{a}}$	$14.52 \pm 0.39^{\text{b}}$	$18.05 \pm 0.71^{\text{a}}$
	Sediments ($\mu\text{g.g}^{-1}$ d.w.)	Cd	$0.16 \pm 0.01^{\text{c}}$	$0.14 \pm 0.02^{\text{c}}$	$0.15 \pm 0.02^{\text{c}}$	$0.91 \pm 0.03^{\text{a}}$	$0.31 \pm 0.00^{\text{b}}$	$0.12 \pm 0.03^{\text{c}}$
		Hg	$0.17 \pm 0.08^{\text{c}}$	$0.88 \pm 0.25^{\text{b}}$	$0.11 \pm 0.02^{\text{c}}$	$0.86 \pm 0.08^{\text{b}}$	$2.33 \pm 0.20^{\text{a}}$	$0.04 \pm 0.02^{\text{c}}$
		Cu	$14.28 \pm 0.34^{\text{c}}$	$14.96 \pm 0.62^{\text{c}}$	$15.15 \pm 0.36^{\text{c}}$	$23.48 \pm 0.53^{\text{b}}$	$30.11 \pm 0.37^{\text{a}}$	$13.71 \pm 0.34^{\text{c}}$
		Zn	$114.63 \pm 3.44^{\text{b}}$	$78.44 \pm 3.12^{\text{c}}$	$67.75 \pm 3.05^{\text{c}}$	$112.85 \pm 5.20^{\text{b}}$	$235.77 \pm 9.49^{\text{a}}$	$68.08 \pm 4.52^{\text{c}}$

Table II

	Component		
	1	2	3
Salinity	-0,932	-0,240	-0,118
Cd in Sediments	0,909	0,172	0,258
Hg in Water	0,850	0,399	-0,060
Temperature	0,827	-0,048	-0,243
Cu in Water	0,804	0,432	0,193
Zn in Water	0,770	-0,139	-0,614
Hg in Sediments	0,114	0,945	-0,110
Zn in Sediments	0,238	0,855	-0,318
Cu in Sediments	0,469	0,855	-0,191
Cd in Water	0,083	0,789	-0,491
Turbidity	0,032	-0,150	0,971
pH	-0,096	-0,372	0,897
Dissolved Oxygen	0,242	-0,397	0,739