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Heavy Metal Content in Chilean Fish Related to Habitat Use, Tissue Type and River of Origin

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Abstract In this study, we analyze the concentration of ten metals in two freshwater fish—the benthic catfish *Trichomycterus areolatus* and the limnetic silverside *Basilichthys microlepidotus*—in order to detect possible accumulation differences related to fish habitat (benthic or pelagic), tissue type (gill, liver and muscle), and the river of origin (four different rivers) in central Chile. The MANOVA performed with all variables and metals, revealed independent effects of fish, tissue and river. In the case of the fish factor, Cu, Cr, Mo and Zn showed statistically higher concentrations in catfish compared with silverside for all tissues and in all rivers ($p<0.05$). In the case of the tissue factor, Al, Cr, Fe and Mn had statistically higher concentrations in liver and gills than in muscle ($p<0.05$). For the river effect, the analysis showed higher concentrations of Cr, Mn and Pb in the Cogoti river and the lower concentrations in the Recoleta river. These results suggest that not all metals have the same pattern of accumulation; however, some metals tend to accumulate more in readily catfish, probably due to their benthic habit, and in liver and gill tissue, probably as a result of accumulation from food sources and respiration.

Keywords Silverside (*Basilichthys microlepidotus*) · Catfish (*Trichomycterus areolatus*) · Fish organs · Heavy metals

The rapid development of human activities has resulted in increased pollution, which is a significant environmental hazard for invertebrates, fish and humans (Uluturhan and Kucukszgin 2007), especially heavy metals, which may have a natural or anthropogenic origin such as mining, wastewater, industrial discharges, agricultural runoff, air pollutants and deposition (Maceda-Veiga et al. 2012; Scanu et al. 2016). Most of these metals are essential for physiological functions (Taylor et al. 1985) and are considered as normal constituents of the fluvial or marine environment (Nieboer and Richardson 1980). However, when significant quantities of heavy metals are accumulated and biomagnified in aquatic food chains, the result could have lethal or sub-lethal effects on the local fish populations (Xu et al. 2004). Heavy metals can be assimilated by fish through different pathways: (i) from water passing through the gills, considered the most direct and important route (Evans 1987), (ii) from food, affecting the intestine and the metabolic organs (Hermenean et al. 2015), and finally (iii) from environmental contact with the skin (Amundsen et al. 1997). Further, studies have shown that gills are the main means of entry for dissolved substances from water, but the liver is most prone to accumulate heavy metals due to blood flow from gills (Clements and Rees 1997). Thus, different organs accumulate different amounts of metals; in this context gills and liver are proposed as indicators of pollution (Saltes and Bailey 1984).

As mentioned above, the metal concentration in freshwater systems depend on several factors, such as transport, velocity of the river (Wu et al. 2005) and human activities in the basin (Farag et al. 1998), furthermore, metal concentration

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may be heterogeneous in water and sediments of the same area (Copaja et al. 2016a). It is important to consider that only a small portion of free metal ions stays dissolved in water, whereas most are deposited in the sediment due to hydrolysis and adsorption (Horowitz 1991; Bradl 2004). Between 30% and 98% of the total metal load of a river can be sediment-associated (Gibbs 1973; Hua et al. 2016). This difference, observed in metal concentration between water and sediments and the presence of metals in the pore water (Tovar-Sanchez et al. 2006), could make a difference in the metal accumulation of species inhabiting the same area, but using different habitat niches. Overall, limnetic species are mainly exposed to the metals from water, while benthic species are exposed to metals present in the water column, sediment and pore water.

In Chile, high content of many metals has been reported among rivers (Schalscha and Ahumada 1998) and among sample sites within rivers (Vega-Retter et al. 2014). Considering this information, in the present study we analyzed the metal accumulation in three tissues of the silverside *Basilichthys microlepidotus* and the catfish *Trichomycterus areolatus*, both native and endemic fish species inhabiting rivers in Central Chile (Vila et al. 1999; Veliz et al. 2012). Initial analyses performed in *T. areolatus* reported differential metal accumulation in the tissue of the individuals located up and downstream of a reservoir dam (Copaja et al. 2016b). Interestingly, the silverside and the catfish present different ecological features, such as migration patterns (Quezada-Romegialli et al. 2010) and habitat use. The silverside is a limnetic species, thus it would be affected by the water quality and soluble metals in column water, while the catfish is a benthic species, probably more affected by direct contact with the sediment and suspended solids and metals from interstitial water. Furthermore, both species could be also affected by their food sources because they both feed on benthic invertebrate (Ruiz et al. 1993). Thus, we seek to detect a possible differential bioaccumulation related to the habitat use, tissues type and river of origin by quantifying heavy metals (Al, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Zn) in gills, liver and muscle tissues in the silverside *B. microlepidotus* and the catfish *T. areolatus* collected from four rivers in central Chile.

Materials and Methods

Catfish and silverside were collected in four rivers from two basins during the winter of 2011. River names and sample sizes are shown in Table 1. Fish samples were obtained by using an electric fishing device and euthanized with tricaine methanosulfonate (MS222) at 250 mg L^{-1}. The gills, liver and a piece of dorsal muscle from each specimen were dried in an oven at 40°C to a constant mass in 10 mL beakers.

Dried tissues (0.5 g) were digested in a high-resolution microwave (Marsx press) (EPA method 3015) with 10 mL of 65% suprapur HNO_3 (Merck), the digestion conditions were: potency 1600 watts; (65%); time 15 min; temperature 200°C; duration 15 min; cooling 15 min. The digested samples were diluted ten times in 100 mL vials with deionized water Milli-Q (Millipore-simplicity) and stored in polyethylene plastic containers for analysis. Samples were analyzed using an atomic absorption spectrophotometer (AAS) (Shimadzu Spectrophotometer 6800, ASC-6100 auto sampler and graphite furnace gfa-ex7). Detection limits (mg L^{-1}) were: Al = 0.472; Cd = 0.016; Cr = 0.071; Cu = 0.0006; Fe = 0.509; Mn = 0.374; Mo = 0.0009; Ni = 0.056; Pb = 0.065; Zn = 0.076. Cu and Mo were analyzed using graphite furnace atomization and the other metals were analyzed using flame atomization. The reagents were of analytical grade. Glassware was soaked in 10% nitric acid and later rinsed with MilliQ grade water prior to use to avoid metal contamination. The quality of results was compared with reference material (Dolt-4: dogfish liver certified reference material for trace metals). Analytical results of the quality control samples indicated a satisfactory performance of heavy metal detection within the range of certified values (90%–110%) (Appendix Table 3).

Table 1 Sampling sites and sample size per site

River Basin	River	Coordinates	N° catfish	N° silverside
Limari	Recoleta	30°28′S; 71°06′W	5	8
	Cogoti	31°00′S; 71°05′W	6	8
	Paloma	30°44′S; 71°00′W	7	7
Choapa	Corrales	31°54′S; 70°54′W	7	6

To determine possible effects and interactions among the fish species, tissue and river (the independent variables) on the metal bioaccumulation (the dependent variables), a permutation MANOVA was performed using the vegan library implemented in R software (R Core Team 2017). Considering the significant level, permutation ANOVAs were performed for each metal independently. Finally, pairwise permutation analyses based on the difference of means were coded in R software to obtain the significance for pairs of comparisons when the factor contained more than two levels (e.g. tissue and river).

Results and Discussion

Mean and standard deviation per treatment are shown in Appendix Table 4. Cd and Ni were not detected or were below the detection limit, thus our results contain information on eight metals (Al, Cr, Cu, Fe, Mn, Mo, Pb, Zn). The permutation MANOVA showed statistical differences in the three main factors fish, river and tissue; the interaction of the factors showed no statistical significance (Table 2). In order to simplify the results and their discussion, we explain statistical significance by groups of metals demonstrating the same pattern of variation, namely: (i) fish effect (differences between fish species), (ii) river effect (with variation across rivers), (iii) tissue effect (differences among tissues).

The permutation ANOVAs performed for the fish factor, independently for each metal, showed statistically higher concentrations of Cr, Cu, Mo and Zn in the catfish compared with the silverside ($p < 0.001$, Fig. 1). Other studies also found more metal concentration in benthic fish compared to pelagic fish; for example, Pb, Cd, Ni and Cr were found in high concentration in the benthic *Neogobius gorlap* compared to the pelagic *Rutilus rutilus caspicus* (Alipour et al. 2013), Cd and Cr were higher in *Triglia cuculus* (benthic) than in *Sardina pilchardus* (pelagic) (Canli and Atli 2003) and Cd, Cu, Zn, Pb and Fe were higher in *Mullus barbatus* (benthic) than in *Sparus aurata* (pelagic) (Kargin 1996), among others. This result suggests that sediment contributes to metal accumulation; a consistent finding with the idea that sediment is the most important reservoir of metals (Filgueiras et al. 2004). Previous studies have reported that metal concentrations in bottom sediments can be 1–3 orders of magnitude greater than in the overlying surface water (Bubb and Lester 1994; Copaja et al. 2016b). Our results are consistent with the findings of Copaja et al. (2016b), wherein catfish showed a higher metal concentration than silverside in the Rapel and Cachapoal rivers. This finding contributes to the hypothesis that the higher levels of bioaccumulation in catfish are due to its benthic habitat.

The permutation ANOVAs performed for the tissue factor, independently for each metal, showed statistically higher concentrations of Al, Cr, Fe and Mn in the liver and gills than in muscle in both fish species and in all rivers (Fig. 2). Other authors observed more metal in gills and liver compared to the muscle in the catfishes *Heteropneustes fossilis* (Bharti and Banerjee 2011), *Silurus glanis* (Squadrone et al. 2013), *Clarias gariepinus* (Tuncsoy et al. 2016) and the silversides *Atherina lagunae* (Ayed et al. 2013), *Odontesthes bonariensis* (Vazquez et al. 2015) and *B. microlepidotus* (Copaja et al. 2016b).

Considering the detoxifying function of the liver, this organ tends to accumulate metals which bind those elements

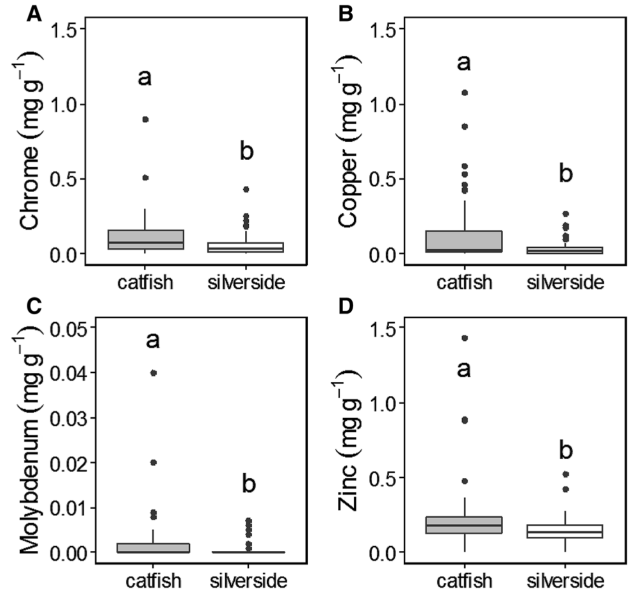

Fig. 1 Boxplot of the permutation ANOVA showing statistical differences in the fish factor. **a** Chrome, **b** Copper, **c** Molybdenum and **d** Zinc. Lettered bars denote statistical differences ($p < 0.05$)

to specific polypeptides called metallothionein (Hamilton and Mehrle 1986); for this reason, this organ has been identified as the best environmental indicator of water pollution and chronic exposure to heavy metals (Agah et al. 2009). Gills of fishes have several functions, such as exchange of gases, ion transport, excretion of ammonia and urea (Lawrence and Hemingway 2003), in direct interaction with water and pollutants. These functions, together with the high level of pollutants found in this organ, have led to the gills to often be considered an indicator of aquatic metal concentration, especially at the beginning of exposure (Dural et al. 2007). Authors relate the low concentration of metals in fish muscle to the affinity of the contractile proteins of muscle with calcium (Schiaffino and Reggiani 1996), an important characteristic of the general rule of organometallic chemistry (Palaniappan and Karthikeyan 2009).

Table 2 Results of the permutation MANOVA

Source of variation	Df	Pillai	Approx F	num df	den df	*p* value
Fish	1	0.244	5.275	8	131	<0.001*
River	3	0.452	2.945	24	399	<0.001*
Tissue	2	0.313	3.077	16	264	<0.001*
Fish × River	3	0.171	1.003	24	399	0.460
Fish × Tissue	2	0.175	1.580	16	264	0.074
River × Tissue	6	0.309	0.923	48	816	0.624
Fish × River × Tissue	6	0.351	1.058	48	816	0.371
Residual	138					

*Represent statistical significance ($p < 0.05$)

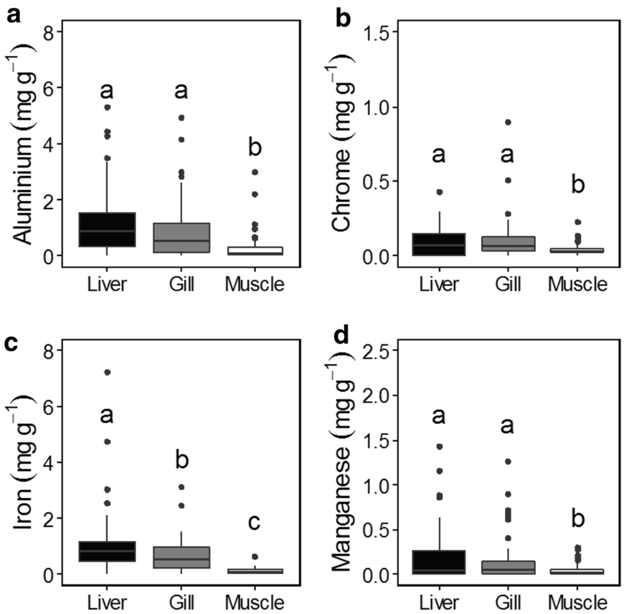

Fig. 2 Boxplot of the permutation ANOVA showing statistical differences in organ factor. **a** Al, **b** Cr, **c** Fe and **d** Mn in the tissues (gill, liver and muscle) analyzed for both fish species and all rivers. Lettered bars denote statistical differences ($p < 0.05$)

The permutation ANOVAs performed for the river factor, independently for each metal, showed statistical differences in Cr, Mn and Pb among rivers (Fig. 3). In this analysis, both fish present the highest concentrations in Cogotí and La Paloma rivers and the lowest values in Recoleta river. Similar variation among rivers was described for Cr in fish inhabiting rivers in Norway and Russia (Amundsen et al. 1997), Mn in fish species inhabiting Saudi Arabian drainages (Mahboob et al. 2014) and Pb concentration obtained from fishes collected in a Chinese River (Cheung et al. 2008). This evidence pointed out that the concentration of some metals in organ tissue is likely dependent on local availability.

Finally, the measured metals showed different concentrations in tissues, fish and rivers following some interesting patterns. From our data and the conclusions obtained from other studies, we observed three clear patterns related with metal accumulation: (i) the presence of higher concentration of metals in the catfish compared to the silverside suggest the habitat use as a determinant in metal uptake; (ii) the high concentration of metals in gill and liver suggests a predisposition of these organs with these elements, due to their direct interaction in the case of the gills and role the liver to bind pollutants to its metallothionein, compared to the low concentration of metals in muscle probably due to the high presence of Calcium that reduces the relationship of this organ with metals,, and (iii) the amount of metal fish accumulate could be greatly influenced by the local availability in the environment related to the dynamics of these systems.

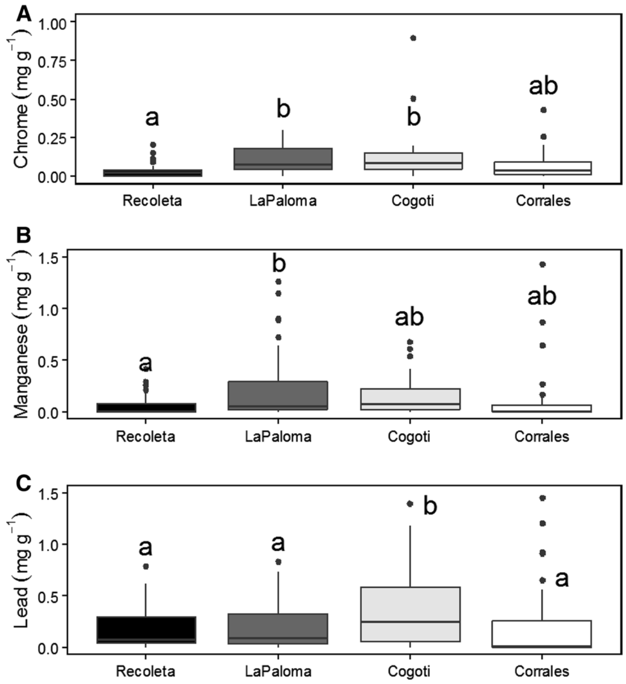

Fig. 3 Boxplot of the permutation ANOVAs showing statistical differences in the river factor. **a** Chrome, **b** Manganese, **c** Lead. Lettered bars denote statistical differences ($p < 0.05$)

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Compliance with Ethical Standards

Ethical Approval All procedures performed in this study were in accordance with the ethical standard of the Universidad de Chile.

Appendix

See Tables 3 and 4.

Table 3 Metal concentration (mg kg^{-1}) of the Dolt-4 reference material

Metal	Dolt-4 measured by the NRC (mg kg^{-1})	Dolt-4 measured in our ASS (mg kg^{-1})	Recovery (%)
Cd	24.3 (SD = 0.8)	27.3 (SD = 1.31)	112
Fe	1833 (SD = 75)	1657.8 (SD = 41.86)	90
Zn	116 (SD = 6.0)	119.1 (SD = 3.52)	103

This table contain the mean and standard deviation (SD) of Cd, Fe and Zn concentration measured by the National Research Council of Canada (NRC) and our measurement (three times) in an Atomic Absorption Spectrophotometer (AAS)

Table 4 Mean (mg g^{-1}) and standard deviation (SD) of each metal detected in the organs of the catfish and the silverside fish in the different rivers

Metal	Basin	Catfish				Silverside			
		N	Gill	Liver	Muscle	N	Gill	Liver	Muscle
Al	Recoleta	5	0.52 (0.50)	0.98 (0.56)	0.28 (0.34)	8	0.12 (0.09)	1.07 (1.98)	0.07 (0.04)
	Cogotí	6	1.16 (1.19)	1.23 (0.71)	0.20 (0.18)	8	0.92 (0.86)	1.53 (1.82)	0.40 (0.42)
	Corrales	7	2.26 (1.86)	0.94 (0.63)	0.10 (0.05)	6	0.63 (0.35)	1.40 (0.98)	0.91 (1.32)
	Paloma	7	1.02 (0.61)	1.68 (1.26)	0.27 (0.22)	7	0.44 (0.62)	0.77 (1.01)	0.18 (0.17)
Cu	Recoleta	5	0.05 (0.09)	0.11 (0.19)	0.01 (0.01)	8	0.02 (0.02)	0.04 (0.04)	0.04 (0.04)
	Cogotí	6	0.22 (0.24)	0.08 (0.05)	0.06 (0.07)	8	0.03 (0.02)	0.09 (0.08)	0.03 (0.02)
	Corrales	7	0.20 (0.19)	0.06 (0.05)	0.12 (0.20)	6	0.02 (0.01)	0.01 (0.01)	0.01 (0.02)
	Paloma	7	0.19 (0.30)	0.28 (0.39)	0.01 (0.01)	7	0.00 (0.01)	0.02 (0.01)	0.04 (0.09)
Cr	Recoleta	5	0.04 (0.05)	0.07 (0.09)	0.02 (0.01)	8	0.02 (0.02)	0.02 (0.02)	0.01 (0.01)
	Cogotí	6	0.28 (0.35)	0.11 (0.05)	0.07 (0.04)	8	0.04 (0.04)	0.11 (0.15)	0.03 (0.03)
	Corrales	7	0.11 (0.06)	0.07 (0.09)	0.02 (0.02)	6	0.08 (0.06)	0.09 (0.08)	0.06 (0.04)
	Paloma	7	0.17 (0.10)	0.19 (0.08)	0.07 (0.07)	7	0.08 (0.06)	0.08 (0.06)	0.04 (0.02)
Fe	Recoleta	5	1.07 (0.21)	1.30 (0.48)	0.15 (0.10)	8	0.27 (0.16)	1.75 (2.70)	0.09 (0.04)
	Cogotí	6	0.80 (0.50)	0.66 (0.25)	0.13 (0.05)	8	0.64 (0.35)	1.06 (1.19)	0.10 (0.06)
	Corrales	7	0.84 (1.09)	0.92 (0.47)	0.10 (0.08)	6	0.34 (0.28)	0.89 (0.51)	0.15 (0.26)
	Paloma	7	0.95 (0.46)	1.25 (0.55)	0.16 (0.11)	7	0.57 (0.85)	0.49 (0.30)	0.09 (0.07)
Mn	Recoleta	5	0.05 (0.13)	0.08 (0.18)	0.04 (0.09)	8	0.09 (0.09)	0.06 (0.07)	0.03 (0.03)
	Cogotí	6	0.23 (0.32)	0.25 (0.20)	0.03 (0.05)	8	0.04 (0.06)	0.12 (0.30)	0.02 (0.02)
	Corrales	7	0.11 (0.23)	0.25 (0.52)	0.03 (0.06)	6	0.12 (0.04)	0.22 (0.18)	0.04 (0.02)
	Paloma	7	0.36 (0.47)	0.41 (0.47)	0.07 (0.07)	7	0.19 (0.32)	0.10 (0.14)	0.12 (0.15)
Mo	Recoleta	5	0.00 (0.00)	0.01 (0.01)	0.01 (0.01)	8	0.00 (0.00)	0.01 (0.01)	0.00 (0.00)
	Cogotí	6	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	8	0.01 (0.01)	0.01 (0.01)	0.00 (0.00)
	Corrales	7	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	6	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Paloma	7	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	7	0.00 (0.00)	0.01 (0.01)	0.00 (0.00)
Pb	Recoleta	5	0.31 (0.13)	0.26 (0.21)	0.20 (0.23)	8	0.08 (0.09)	0.24 (0.30)	0.06 (0.06)
	Cogotí	6	0.91 (0.92)	0.17 (0.13)	0.14 (0.12)	8	0.09 (0.15)	0.23 (0.28)	0.17 (0.22)
	Corrales	7	0.64 (0.62)	0.12 (0.14)	0.04 (0.06)	6	0.28 (0.26)	0.63 (0.66)	0.64 (0.79)
	Paloma	7	0.25 (0.22)	0.31 (0.32)	0.13 (0.17)	7	0.15 (0.10)	0.19 (0.19)	0.03 (0.03)
Zn	Recoleta	5	0.18 (0.13)	0.32 (0.31)	0.12 (0.08)	8	0.13 (0.08)	0.12 (0.09)	0.09 (0.06)
	Cogotí	6	0.13 (0.13)	0.21 (0.16)	0.13 (0.03)	8	0.13 (0.05)	0.11 (0.08)	0.11 (0.03)
	Corrales	7	0.25 (0.28)	0.37 (0.55)	0.22 (0.11)	6	0.15 (0.14)	0.14 (0.07)	0.10 (0.07)
	Paloma	7	0.36 (0.47)	0.19 (0.04)	0.19 (0.04)	7	0.19 (0.16)	0.17 (0.08)	0.13 (0.06)

References

Agah H, Leermakers M, Elskens M, Fatemi SRM, Baeyens W (2009) Accumulation of trace metals in the muscle and liver tissue of five species from the Persian Gulf. Environ Monit Assess 157:499–514

Alipour H, Pourkhabbaz A, Hassanpour M (2013) Assessing of heavy metal concentrations in the tissues of *Rutilus rutilus caspicus* and *Neogobius gorlap* from Miankaleh international wetland. Bull Environ Contam Toxicol 91:517–521

Amundsen P, Staldvik FJ, Ludin AA, Kashulin NA, Popova OA (1997) Heavy metal contamination in freshwater fish from the border region between Norway and Russia. Sci Total Environ 201:204–224

Ayed N, Faure E, Quignard JP, Trabelsi M (2013) Determination of P, Ca, Zn, Cd and Pb concentrations in muscle, gills, liver gonads and skeleton of two natural populations of Atherina lagunae in north lake of Tunis (Tunisia). Bull Inst Natn Scien Tech Mer de Salammbô 40:141–149

Bharti S, Banerjee TK (2011) Bioaccumulation of metals in the edible catfish *Heteropneustes fossilis* (Bloch) exposed to coal mine effluent generated at northern coalfield limited, Singrauli, India. Bull Environ Contam Toxicol 87:393–398

Bradl HB (2004) Adsorption of heavy metals ions on soils and soils constitutuents. J Colloid Interface Sci 277:1–18

Bubb JM, Lester JN (1994) Anthropogenic heavy metal inputs to lowland river systems, a case study. The River Stour, UK. Water Air Soil Pollut 78:279

Canli M, Atli G (2003) The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ Pollut 121(1):129–136

Cheung KC, Leung HM, Wong MH (2008) Metal concentrations of common freshwater and marine fish from the Pearl River Delta, South China. Arch Environ Contam Toxicol 54:705–715

Clements WH, Rees DE (1997) Effects of heavy metals on prey abundance, feeding habits, and metal uptake of brown trout in the Arkansas River, Colorado. Trans Am Fish Soc 201:774–785

Copaja SV, Nuñez VR, Muñoz GS, Gonzalez GL, Vila I, Veliz D (2016a) Heavy metal concentration in water and sediments from affluents and effluents of mediterranean chilean reservoirs. J Chil Chem Soc 60:2797–2804

Copaja SV, Pérez CA, Nuñez VR, Muñoz GS, Vila I, Veliz D (2016b) Effect of Rapel reservoir in the distribution of heavy metals in two native fish. Bull. Environ Contam Toxicol 97:24–30

Dural M, Goksu MLZ, Ozak AA (2007) Investigation of heavy metal level in economically important fish species captured from the Tuzla Lagoon. Food Chem 1:415–421

European Community (2005) Commission regulation N° 78/2005 (pp: L16/43_L16/45). Official J European Union 2005 [20.1.2005]

Evans DH (1987) The fish gill: site of action and model for toxic effects of environmental pollutants. Environ Health Perspect 71:47–58

Farag AM, Woodward DF, Goldstein JN, Brumbaugh W, Meyer JS (1998) Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River Basin, Idaho. Arch Environ Contam Toxicol 34:119–127

Filgueiras AV, Lavilla I, Bendicho C (2004) Evaluation of distribution, mobility and binding behaviour of heavy metals in surficial sediments of Louro River (Galicia, Spain) using chemometric analysis: a case study. Sci Total Environ 330:115–129

Gibbs RJ (1973) Mechanisms of trace metal transport in rivers. Science 180:71–73

Hamilton SJ, Mehrle PM (1986) Metallothionein in fish: review of its importance in assessing stress from metal contaminants. Trans Am Fish Soc 11:596–609

Hermenean A, Damache G, Albu P, Ardelean A, Ardelean G, Ardelean DP, Horge M, Nagy T, Braun M, Zsuga M, Kéki S, Costache M, Dinischiotu A (2015) Histopatological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. Ecotoxicol Environ Saf 119:198–205

Horowitz AJ (1991) A primer on sediment-trace element chemistry. Lewis Publishers, Chelsea

Hua Z, Yinhui J, Tao Y, Mon W, Guangxun S, Mingjun D (2016) Heavy metal concentrations and risk assessment of sediments and Surface water of the Gan River, China. Pol J Environ Stud 25:1529–1540

Kargin F (1996) Seasonal changes in levels of heavy metals in tissues *of Mullus barbatus* and *Sparus aurata* collected from Iskenderun Gulf (Turkey). Water Air Soil Pollut 90:557–562

Lawrence AJ, Hemingway KL (2003) Effects of pollution on fish: molecular effects and population responses. Blackwell Science, Hoboken

Maceda-Veiga A, Monroy M, Sostoa A (2012) Metal bioacumulation in the Mediterranean barbel (*Barbus meridionalis*) in a Mediterranean River receiving effluents from urban industrial wastewater treatment plants. Ecotoxicol Environ Saf 76:93–101

Mahboob S, Alkahem HA, Al-Balawi F, Al-Misned F, Al-Quraishy S, Ahmad Z (2014) Tissue metal distribution and risk assessment for important fish species from Saudi Arabia. Bull Environ Contam Toxicol 92:61–66

Nieboer E, Richardson DHS (1980) The replacement of the nondescript term "heavy metals" by a biological and chemically significant classification of metal ions. Environ Pollut 1:3–26

Palaniappan PLRM, Karthikeyan S (2009) Bioaccumulation and depuration of chromium in the selected organs and whole pbody tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel. J Environ Sci 21:229–236

Quezada-Romegialli C, Fuentes M, Veliz D (2010) Comparative population genetics of *Basilichthys microlepidotus* (Atheriniformes: Atherinopsidae) and *Trichomycterus areolatus* (Siluriformes: Trichomycteridae) in north central Chile. Environ Biol Fish 89:173–186

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org

Ruiz VH, Lopez MT, Moyano HI, Marchant M (1993) Ictiología del alto Biobío: aspectos taxonómicos, alimentarios, reproductivos y ecológicos con una discusión sobre la hoya. Gayana 57:77–88

Saltes JG, Bailey GC (1984) Use of fish gill and liver tissue to monitor Zinc pollution. Bull Environ Contam Toxicol 32:233–237

Scanu S, Piazzolla D, Frattarelli F, Mancini E, Tiralongo F, Brundo MV, Tibullo D, Pecoraro R, Copat C, Ferrante M, Marcelli M (2016) Mercury enrichment in sediments of the coastal area of northern Latium, Italy. Bull Environ Contam Toxicol 97:593–600

Schalscha BE, Ahumada TI (1998) Heavy metals in rivers and soils of central Chile. Water Sci Technol 37:251–255

Schiaffino S, Reggiani C (1996) Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Phys Rev 76:371–423

Squadrone S, Prearo M, Brizio P, Gavinelli S, Pellegrino M, Scanzio T, Guarise S, Benedetto A, Abete MC (2013) Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. Chemosphere 90:358–365

Taylor D, Maddock B, Mance G (1985) The acute toxicity of nine "grey list" metals (arsenic, boron, chromium, copper, lead, nickel, tin, vanadium and zinc) to two marine fish species dab (*Limanda lianda*) and grey mullet (*Chelon labrosus*). Aquat Toxicol 7:135–144

Tovar-Sanchez A, Huerta-Diaz MA, Negro JJ, Bravo MA, Sanudo-Wilhelmy SA (2006) Metal contamination in interstitial waters of Donana Park. J Environ Manag 78:286–293

Tuncsoy M, Duran S, Ay O, Cicik B, Erdem C (2016) Accumulation of Copper in gill, liver, spleen, kidney and muscle tissues of *Clarias gariepinus* exposed to the metal singly and in mixture with chitosan. Bull Environ Contam Toxicol 97:486–489

Uluturhan E, Kucukszgin F (2007) Heavy metal contaminants in red Pandora (*Pagellus erythrinus*) tissues from the Eastern Aegen Sea, Turkey. Water Res 41:1185–1192

Vazquez FJ, Arellano FE, Fernandez-Cirelli A, Volpedo AV (2015) Monitoring of trace elements in silverside (*Odontesthes bonariensis*) from pampasic ponds, Argentina. Microchem J 120:1–5

Vega-Retter C, Muñoz-Rojas P, Vila I, Copaja S, Veliz D (2014) Living in a highly polluted environment: the case of the silverside *Basilichthys microlepidotus* (Teleostei: Atherinopsidae) in the Maipo River basin, central Chile. Popul Ecol 56:569–579

Veliz D, Catalan L, Pardo R, Acuña P, Diaz A, Poulin E, Vila I (2012) The genus *Basilichthys* (Teleostei: Atherinopsidae) revisited along its Chilean distribution range (21° to 40°S) using variation in morphology and mtDNA. Rev Chil Hist Nat 85:49–59

Vila I, Fuentes L, Contreras M (1999) Peces límnicos de Chile. Bol Mus Hist Nat 48:61–75

Wu Y, Falconer R, Lin B (2005) Modelling trace metal concentration distributions in estuarine waters. Estuar Coast Shelf Sci 64:699–709

Xu YJ, Liu XZ, Ma AJ (2004) Current research on toxicity effect and molecular mechanism of heavy metals on fish. J Mar Sci 28:67–70

Yousafzai AL, Chivers DP, Khan AR, Ahmad I, Siraj M (2010) Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem. Pak J Zool 42:537–544