

## Bioaccumulation of Metals in Tissues of *Solea Vulgaris* from the outer Coast and Ria de Vigo, NE Atlantic (Spain)

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**ABSTRACT:** Ría and coastal zone are, in particular, vulnerable to heavy metals pollution. Concentrations of 10 metals in liver, muscle and kidney of the sole (*Solea vulgaris*) from Ría of Vigo and its coastal zone (Spain) were measured from April 2006 to February 2007 and the relationships between fish size age and metal concentrations in the tissues was investigated. Concentrations of the heavy metals in examined fish ranged as follows: Cd (0.01- 0.7), Pb (0.1- 2.5), Hg (0.01- 0.7), Cu (3.3- 46.7), Zn (15- 274), As (3- 151), Se (0.9- 18.2), Mn (0.9- 9.76) µg/g dryweight. Kidney and liver showed the highest levels of Cd, Cu, Pb and Zn. The concentration of metals was significantly affected by the sampling site and fish tissues. Our results indicated that all heavy metals were found the highest in muscle tissue. A comparison of metals concentration in fishes from both sites showed higher bioaccumulation in the exemplars captured in Ría. This indicates that there is a trend of higher metal pollution level in the estuarine environment. Although, metals levels in the tissues in the area do not exceed contamination levels, measures are recommended for lowering heavy metals inputs into Ría of Vigo. Heavy metals in the edible parts of the investigated fish were in the permissible safety levels for human uses.

**Key words:** Assessment, Heavy metals, Fish, Vigo

### INTRODUCTION

Many marine areas in the world have been contaminated by heavy metals and the organisms living in contaminated waters have reflected the high metal concentrations (Bryan and Langston, 1992). These elements accumulated in fish organs may affect at last the quality and quantity of fishing and fish rearing. Although, fish muscle has been given more importance in investigation than other organs in the past (Förstner and Wittmann, 1983). Nowadays different tissues are commonly used as indicators of the degree of contamination of marine environment with the heavy metals (Phillips and Rainbow, 1993). Galicia, Spanish N-W, is a region with an important coastal fishing and shellfish production; it has a particular geography with a succession of estuarine bay systems called Rías which give adequate conditions for aquaculture. However the presence of the mussel-raft polygons can influence the

biogeochemical cycles of contaminants, including metals (Prego *et al.*, 2006). There is also a strong evidence that Ria of Vigo and other Galician Rias have undergone an increased contamination with trace metals, especially Cu, Pb and Zn in recent years and this may become a cause of concern if these elements are remobilized and enter the food chain. Flatfish species are among the most abundant and commercially important fishes for the coastal Vigo fisheries. They are particularly vulnerable to sediment contamination either through the direct contact with the substrate, benthic prey ingestion, or simply sediment particle ingestion (Moles *et al.*, 1994).

Since the metal pollution in aquatic ecosystems can be harmful to human health, it is necessary to understand and control the hazard levels of pollution in seafood. Therefore, present study has been undertaken to determine cadmium, mercury, copper,

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arsenic, zinc, lead, aluminium, argon, magnesium and manganese concentrations in the muscle, liver and kidney of fish and to investigate the relationship between the heavy metal load of fish and their age, size, and the seasonal variation. Also, to determine the differences between the concentrations of heavy metal accumulated by the sole in both studied sites.

## MATERIALS & METHODS

The sampling was performed in two sites of Ría de Vigo from April 2006 to February 2007 (Fig. 1). A number of 72 fishes were collected. The average length, weight and estimated age interval of fish samples are presented in Table 1. Before the analysis, a portion of muscle, the whole liver and kidney arches from each fish were removed. Samples of tissues were weighed, dried at 60°C in a Heraeus Hanau KB 500 oven for 48 h and preserved in desiccators until digestion. The digestion was performed according to ANTON PAAR standard procedures (Farkas *et al.*, 2000). The samples were subjected to a mixture of 65% HNO<sub>3</sub> and 30% H<sub>2</sub>O<sub>2</sub> under pressure in a microwave oven. Hg was analyzed by flameless atomic absorption spectrophotometry (Perkin- Elmer MAS-50A) after sulphuric acid digestion, as described by Joiris *et al.*, (1991). Zn concentrations were determined by atomic absorption spectrophotometry (SPECTRAA 250). Analysis for metals other Hg and Zn were performed using a Finnegan Element magnetic sector inductively coupled plasma mass spectrometer (ICP-MS) with a micro concentric nebulizer (MCN) and a standard

double-pass condensing spray chamber for sample introduction. Analytical quality control included the analysis of a 2% ultrapure nitric acid blank and one drinking water reference material, together with the procedural blank and a sample duplicate from the microwave digestion. The accuracy of the analytical procedure was checked by analyzing the standard reference materials DORM-2, DOLT-3 and TORT-2 in four replicates for each batch of 50 samples digested. Recovery rates ranged from 95% to 100% for all investigated elements.

Data analysis was performed using the statistical package SPSS v.16 for Windows. One-way analysis of variance (ANOVA) and Two-way ANOVA was used for checking differences between age, gender and sites, followed by a multicomparison Student Newman Kuels (SNK) test.

## RESULTS & DISCUSSION

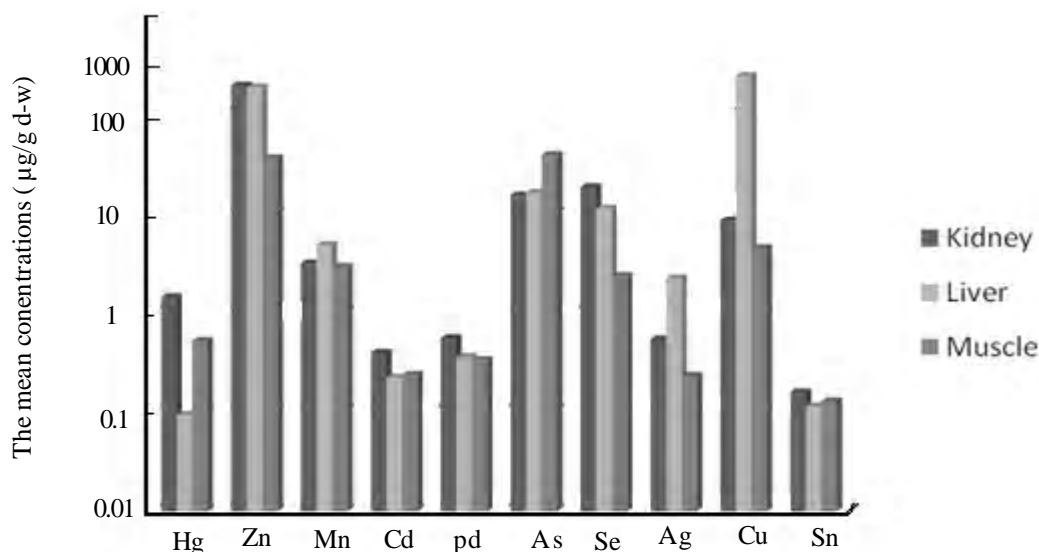
Muscle contained from 0.14 to 0.69 mg of Hg/Kg d.w., while values in the liver and kidney were in the range of 0.2- 0.77 and 0.015- 0.58 mg/Kg d.w. respectively (tab. 2). Hg content in muscle and liver of this species is higher than most values reported for the Mediterranean (Usero *et al.*, 2003). The EC Regulation (EC, 2006) has established a regulatory limit for muscle fish consumption. Sole has not exceeded this limit indicating a steady condition and good quality of the edible parts regarding this metal. Cd values were higher in liver than in the kidney and muscle (tab. 2, Fig. 2).



**Fig. 1.** Map showing the coastal area of Vigo and Ría de Vigo (Miño river estuary) with the selected sampling stations

**Table 1. Length, weight, age class and sex of the sampled fishes. I represents coastal area, II represents Ria de Vigo**

Season	Sampling sites	Length (mm; mean ± SD)	Weight (g; mean ± SD)	Age class (year)	sex	
					Male	female
Spring	I	28,55 ± 8,1	168 ± 64,85	2 - 7	2	2
	II	25,5 ± 7,17	155, 25 ± 75,35	0 - 3	2	4
Summer	I	28,55 ± 8,1	168 ± 64,85	1 - 8	5	5
	II	25,5 ± 7,17	155, 25 ± 75,35	0 - 6	4	6
Autumn	I	28,55 ± 8,1	168 ± 64,85	0 - 3	2	3
	II	25,5 ± 7,17	155, 25 ± 75,35	0 - 3	1	4
Winter	I	26,4 ± 7,22	187 ± 77,68	1 - 8	4	6
	II	22,85 ± 6,21	165 ± 64,17	0 - 5	5	5



**Fig. 2. Heavy metal concentrations in organs of sole collected from the coast and Ría de Vigo**

**Table 2. Mean and range concentrations of trace metals in sole (*S. vulgaris*) from the coastal waters and Ria de Vigo (Galicia). All values in mg/ Kg dry weight (d.w.)**

Metal	Kidney	Liver	Muscle
Hg	0.127 ± 0.06	0.45 ± 0.16	0.39 ± 0.12
Range	0.015 - 0.58	0.20 - 0.77	0.14 - 0.693
Zn	37.095 ± 36.8	192.08 ± 72.9	201.54 ± 106
Range	15.12 - 414.41	36.32 - 319.68	274.59 - 4.99
Mn	3.23 ± 2.06	2.95 ± 1.63	4.94 ± 3.61
Range	0.54 - 7.27	2.07 - 15.21	0.88 - 9.76
Cd	0.4 ± 0.29	0.22 ± 0.42	0.24 ± 0.12
Range	0.03 - 1.08	0.08 - 2.08	0.01 - 0.67
Pb	0.565 ± 0.8	0.36 ± 1.4	0.34 ± 0.39
Range	0.16 - 4.58	0.1 - 5.68	0.11 - 2.47
As	15.67 ± 9.21	16.65 ± 8.14	40 ± 27.29
Range	7 - 151.9	4.59 - 47.43	2.99 - 151.05
Se	18.915 ± 9.92	11.53 ± 8.09	2.395 ± 1.82
Range	2.01 - 43.41	2.46 - 34.02	0.89 - 18.18
Ag	0.55 ± 0.44	2.26 ± 1.47	0.23 ± 0.22
Range	0.08 - 1.66	0.11 - 5.81	0.05 - 1.65
Cu	8.71 ± 3.83	250.67 ± 151	4.58 ± 3.26
Range	18.7 - 0.42	31.03 - 739.08	3.26 - 46.71
Sn	0.16 ± 0.23	0.115 ± 0.17	0.13 ± 0.1
Range	0.03 - 0.83	0.03 - 0.95	ND - 0.36

**Table 3. Concentrations of trace metals (mg Kg<sup>-1</sup> dry wt.) in *S. vulgaris* from the coastal waters and Ria de Vigo (Galicia) sampled during July and February (2006/2007)**

Season	Tissues	Hg	Zn	Mn	Cd	Pb	As	Se	Ag	Cu	Sn
	Liver	0,416 ± 0,209	196,58 ± 82,24	8,105 ± 4,64	0,20 ± 0,09	0,645 ± 0,19	20,51 ± 11,25	11,49 ± 5,21	1,71 ± 1,14	253,53 ± 129,07	0,09 ± 0,08
Spring	Kidney	0,14 ± 0,18	157,34 ± 114,25	4,02 ± 1,79	0,42 ± 0,33	0,545 ± 0,34	27,78 ± 12,61	17,98 ± 9,21	0,48 ± 0,309	9,275 ± 2,19	0,44 ± 0,25
	Muscle	0,40 ± 0,12	56,32 ± 37,02	2,90 ± 1,19	0,27 ± 0,18	0,35 ± 0,19	48,75 ± 23,59	2,495 ± 1,19	0,215 ± 0,11	5,87 ± 1,406	0,13 ± 0,11
	Liver	0,339 ± 0,14	218,94 ± 67,31	4,785 ± 2,4	0,21 ± 0,1	0,23 ± 0,17	16,3 ± 5,41	12,33 ± 9,49	2,77 ± 1,43	196,18 ± 174,43	0,135 ± 0,08
Summer	Kidney	0,288 ± 0,18	150,63 ± 87,63	2,595 ± 2,00	0,145 ± 0,12	0,43 ± 0,23	13,13 ± 10,23	16,22 ± 7,83	0,55 ± 0,33	8,49 ± 04,48	0,08 ± 0,26
	Muscle	0,287 ± 0,13	37,445 ± 27,03	3,085 ± 1,82	0,215 ± 0,07	0,35 ± 0,51	25,11 ± 21,06	2,225 ± 1,08	0,245 ± 0,38	4,785 ± 3,64	0,08 ± 0,09
	Liver	0,462 ± 0,15	218,07 ± 66,57	4,805 ± 2,83	0,26 ± 0,12	0,28 ± 0,16	16,09 ± 6,84	12,27 ± 9,92	2,58 ± 1,43	231,97 ± 80,707	0,20 ± 0,19
Autumn	Kidney	0,157 ± 0,19	223,53 ± 91,89	2,98 ± 1,64	0,29 ± 0,18	0,525 ± 0,14	13,82 ± 7,23	19,59 ± 8,34	0,385 ± 0,23	7,085 ± 5,07	0,055 ± 0,24
	Muscle	0,311 ± 0,15	34,38 ± 18,31	3,165 ± 2,48	0,215 ± 0,09	0,33 ± 0,701	34,83 ± 13,55	2,29 ± 0,44	0,28 ± 0,04	4,335 ± 4,07	0,125 ± 0,09
	Liver	0,515 ± 0,15	115,165 ± 64,25	7,045 ± 4,14	0,265 ± 0,25	0,525 ± 0,48	17,715 ± 10,1	10,07 ± 5,53	1,48 ± 1,08	252,45 ± 128,94	0,11 ± 0,12
Winter	Kidney	0,069 ± 0,12	271,19 ± 101,35	4,42 ± 1,88	0,5 ± 0,25	0,89 ± 0,68	25,21 ± 13,48	23,01 ± 10,45	0,62 ± 0,51	8,83 ± 3,17	0,235 ± 0,19
	Muscle	0,418 ± 0,09	37,095 ± 23,31	2,795 ± 1,33	0,25 ± 0,14	0,34 ± 0,21	48,42 ± 27,11	3,075 ± 1,71	0,225 ± 0,11	4,445 ± 3,66	0,215 ± 0,09

Also, Cd values reported for this study are higher than those measured in fishes from the Mediterranean (Usero *et al.*, 2003) and lake Qarun, Egypt (Ali *et al.*, 2005). The regulatory limit for Cd in the fish muscle is 0.05 mg/Kg wet weight (EC, 2006), ~0.25 mg/Kg d.w. Although these mean values did not exceed the regulatory limit. Mean Cd in kidney and liver exceeded the limit this indicates a real potential of Cd in sole to become toxic for human consumption. The mean Pb concentrations were 0.36 in liver, 0.56 in kidney and 0.34 mg/kg d.w in muscle tissues. The statistical showed no significant difference among the studied organs. The muscle mean Pb in this study was higher than that observed by Usero *et al.*, (2003) in the Mediterranean, but consistent with the results reported by Ali *et al.*, (2005) in the lake Qarun, Egypt. The regulatory limit for Pb in muscle meat of fish is 0.3 mg/Kg wet weight, ~1.5 mg/Kg d.w. The mean Pb content in muscle and the other organs did not exceed this limit.

Cu concentrations in liver were considerably higher than those in the muscle tissue and kidney (tab. 2 & 3). This is however not surprising as such values are common for most teleosts. Although, there is a tendency for kidney and liver to accumulate more Cu than in muscle. This indicates that sole may prove better edible qualities in terms of Cu accumulation. Sole shown Cu concentrations lower than the values reported for other areas such as the Seine estuary, France (Miramand *et al.*, 2001).

Zn concentrations were relatively high in comparison to other elements. Among organs, Zn concentrations in our study are significantly higher in liver and muscle tissues than in kidney (tab. 2 & 3). Zn values from this study were higher than the concentrations reported for fishes from Bay of Egypt and Gulf of Mexico (Tayel, 1995; Lewis *et al.*, 2002). This is an indication that Zn input into Ria de Vigo is not that severe. Highest Se concentrations are recorded in kidney and liver. There is no regulatory limit for the total Cu, Zn, Se in fishes and shellfishes as they are predominantly in a less toxic form. As levels were higher whereas for the other heavy metals. Significant differences ( $P < 0.05$ ) were recorded for all comparisons between the organs. However, in contrast to the other elements, Mn and As concentrations in common sole were higher in muscle tissue than in liver and kidney (tab. 2, 3 and fig. 2). This is consistent with the results reported by Usero *et al.*, (2003) in the Mediterranean. However, in the case of As, the results found in here were higher comparing with those in the Mediterranean. This is perhaps due to the highest anthropic activity and the presence of the mussel-raft polygons in the costal zones can influence the biogeochemical cycles of contaminants, including

metals (Prego *et al.*, 2006). As far as we were aware, there is no regulatory limit total As in fish products as this element is normally in organic form.

The dependence of heavy metals concentrations to fish body length relies mainly on the availability of such elements in the environment (Kljakovic *et al.*, 2002). The relationship between metals concentrations and fish age/length and gender was considered for sole on each season.

A significant ( $P < 0.01$ ) relationship of sole age was found for As in liver ( $r^2 = 0.337$ ), Cd ( $r^2 = 0.362$ ) and Mn ( $r^2 = 0.286$ ) in kidney and Cd ( $r^2 = 0.292$ ), As ( $r^2 = 0.409$ ) and Sn ( $P < 0.05$ ,  $r^2 = 0.235$ ) concentrations in muscle. It indicates that older sole fishes have the potential to accumulate more metals and this may become important for fish consumption (i.e. muscles) only if metal content in bigger fishes exceeds quality levels. Bioaccumulation differences in metal content between sole length groups were detected for Cu ( $F = 4.5$ ) in liver, Hg ( $F = 7.43$ ), Zn ( $F = 6.24$ ), Mn ( $F = 4.03$ ), Cd ( $F = 8.14$ ) and Se ( $F = 6.13$ ) in kidney and As ( $F = 7.79$ ), Cu ( $F = 4$ ) and Ag ( $F = 10.35$ ) in muscle. Furthermore, there was no significant correlation between concentrations of the remaining metals in the three organs and the length of studied fish ( $F = 5.45$ ). Similar dependencies between element enrichment and age or length of fish samples were described by Garcia-Montelongo *et al.* (1994) and Gibbs and Miskiewitz (1995). Significant seasonal variations in the heavy metal load of fish were detected for some elements analyzed. The variations in the heavy metal load of fish can be related only to the seasonal difference in their metabolic rate, which determines the physiological condition of fish (Farkas *et al.*, 2003).

## CONCLUSION

The results of heavy metal content in sole tissues revealed a differential bio-accumulation among organs. The metal content in fish liver was considerably higher than that in the muscle. Recognizing the inferential limitation of sample size, the presence of soles samples with metal concentrations exceeding quality criteria makes however potential toxic for human consumption, particularly for Cd and Pb. This specie is therefore suitable for use as bioaccumulation measure for indicating heavy metal pollution in the environment and the subsequent potential toxicity if consumed by humans. The results of this study indicate that the concentration of trace metals in fish vary significantly not only function of fish age and pollution load of the site, but is also influenced in a remarkable degree by the physiological condition of organisms, a fact that is essential to be considered further in comparative biomonitoring studies.

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## REFERENCES

- Ali, M. H. H. and Fishar. M. R. (2005). Accumulation of trace metals in some benthic invertebrate and fish species relevant to their concentration in water and sediment of lake qarun, egypt. *Egypt journal of aqua research*, **31**, 289-301.
- Bryan, G. and, Langston, W. J. (1992). Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environ. Pollut.*, **76**, 89-131.
- Farkas, A., Salánki, J. and Varanka, I. (2000). Heavy metal concentrations in fish of Lake Balaton. *Lakes & Reservoirs; Res. and Manag.*, **5**, 271-279.
- Farkas, A., Salánki, J. and Specziár, A. (2003). Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. *Water Res.*, **37**, 959-964.
- Förstner, U. and, Wittmann, G. T. W. (1983). *Metal Pollution in Aquatic Environment*. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 486.
- Joiris, C. R., Holsbeek, L., Bouquegneau, J. M., & Bossicart, M. (1991). Mercury contamination of the harbour porpoise *Phocoena phocoena* and other cetaceans from the North Sea and the Kattegat. *Water Air Soil Pollu.*, **56**, 283-293.
- García-Montelongo F., Díaz, C., Galindo, L., Larrechi, M. S. and, Rius, X. (1994). Heavy metals in three fish species from the coastal waters of Santa Cruz de Tenerife (Canary Islands). *Sci. Mar.*, **58**, 179-83.
- Gibbs, P. J. and, Miskiewicz, A. G. (1995). Heavy metals in fish near a major primary treatment sewage plant outfall. *Baseline*, **30**, 667-73.
- Kljakovic Gaspic, Z. K., Zvonaric, T., Vrgoc, N., Odzak, N. and Baric, A. (2002). Cadmium and lead in selected tissues of two commercially important fish species from the Adriatic Sea. *Water Res.*, **36**, 5023-5028.
- Lewis, A., Scott, I., Bearden, W., Quarles, L., Moore, J., Strozier, D., Sivertsen, K. and, y Sanders, M. (2002). Fish tissue quality in near coastal areas of the Gulf of Mexico receiving point source discharges. *Sci. Total Environ.*, **284**, 249-261.
- Miramand, P., Guyot, T., Rybarczyk, H., Elkaim, B., Mouny, P., Dauvin, J. C. and, Bessineton, C. (2001). Contamination of the biological compartment in the Seine estuary by Cd, Cu, Pb and Zn. *Estuaries*, **24 (6B)**, 1056-1065.
- Mohamed Ali, H. H. and Fishar. M. R. A. (2005). Accumulation of trace metals in some benthic invertebrate and fish species relevant to their concentration in water and sediment of lake qarun, egypt. *Egypt journal of aqua research*, **31(1)**, 289-301.
- Moles, A., Rice, S. and, Norcross, B. L. (1994). Non-avoidance of hydrocarbon laden sediments by juvenile flatfishes. *Neth. J. Sea Res.*, **32 (3/4)**, 361-367.
- Phillips, D. J. H., and Rainbow, D. W. (1993). *Biomonitoring of Trace Aquatic Contaminants*. Environmental Managment Series. Alden Press, Oxford, 371.
- Prego, R., Otxotorena, U. and, Cobelo-Garcia, A. (2006). Presence of Cr, cu, Fe and Pb in sediments underlying mussel-culture rafts (Arosa and Vigo rias, NW Spain). Are they metal-contaminated areas?. *Ciencias Marinas*, **32, (2B)**, 339-349.
- Tayel, F. T. (1995). Trace metals concentrations in the muscle tissue of ten fish species from Abu-Qir Bay, Egypt. *Int. J. Environ. Health Res.*, **5**, 321- 8.
- Usero, J., Izquierdo, C., Morillo, M. and Gracia, I. (2003). Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern Atlantic coast of Spain. *Environ. Int.*, **29**, 949- 956.