



Biomonitoring toxic and essential element accumulation in trout in the As Pontes mine pit lake (NW Spain)

Marta López-Alonso^a, Marta Miranda^{b,*}, Inmaculada Orjales^b, Víctor Pereira^a, Belén Larrán^a, Ruth Rodríguez-Bermúdez^b, Cathaysa Veiras^a, Álvaro Robles^c, David Bravo^c, Ricardo Juncosa^d, Carlos Herrero-Latorre^e

^a Departamento de Patología Animal, Facultade de Veterinaria, Campus Terra, Universidade de Santiago de Compostela, 27002, Lugo, Spain

^b Departamento de Anatomía, Producción Animal e Ciencias Clínicas Veterinarias, Facultade de Veterinaria, Campus Terra, Universidade de Santiago de Compostela, 27002, Lugo, Spain

^c Profand Group, Avda. García Barbón, 62, 36201, Vigo, Spain

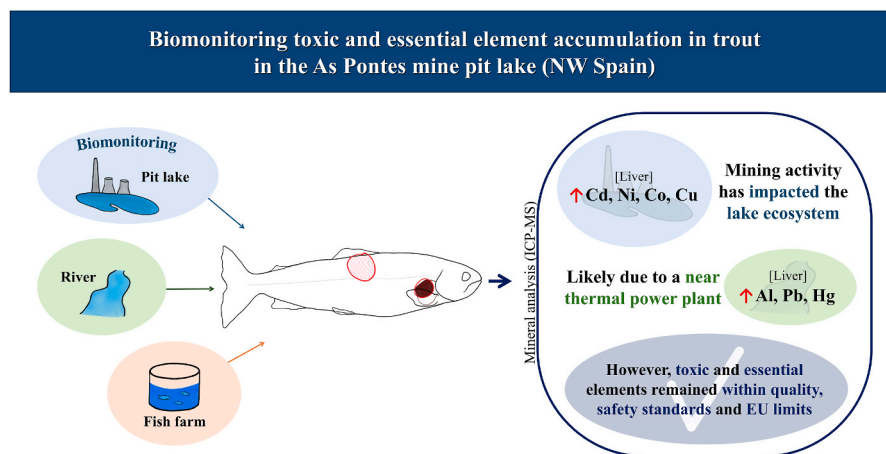
^d Civil Engineering School, University of Coruña, Campus de Elviña, La Coruña, 15071, Spain

^e Aquatic One Health Research Center (ARCUS), Departamento de Química Analítica, Nutrición e Bromatología, Facultade de Ciencias, Campus Terra, Universidade de Santiago de Compostela, 27002, Lugo, Spain

HIGHLIGHTS

- Mining activities have significantly impacted the aquatic ecosystem.
- Lake fish had higher liver levels of Cd, Ni, Co, and Cu, likely from mine run-off.
- However, metal levels found in water and fish meet legal safety standards.
- Biomonitoring of the pit lake must continue to assess the mining long-term impact.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Petra Petra Krystek

ABSTRACT

This study investigated the accumulation of toxic and essential elements in trout from a pit lake situated in the surroundings of the abandoned As Pontes de García Rodríguez lignite mine (NW Spain). The element concentrations were compared with those measured in fish from upstream of the River Eume and from a local fish farm. Liver and muscle samples from fish captured in the lake ($n = 16$), river ($n = 14$) and fish farm ($n = 10$) captured in March–April 2022 were acid digested and analyzed by ICP-MS. The mining activity in As Pontes was found to have significantly impacted the aquatic ecosystem. Concentrations of Cd, Ni, Co, and Cu in the livers of the lake

* Corresponding author.

E-mail address: marta.miranda@usc.es (M. Miranda).

<https://doi.org/10.1016/j.chemosphere.2025.144212>

Received 13 November 2024; Received in revised form 24 January 2025; Accepted 9 February 2025

Available online 18 February 2025

0045-6535/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

fish were significantly higher than in the livers of the other groups of fish, probably due to run-off from the spoil heap reaching the lake. Similarly, higher concentrations of Al, Pb, and Hg were observed in the river trout, associated with coal combustion at a near thermal power plant. Despite these variations, toxic and essential elements in both the pit lake and river remained low, within water quality standards, and do not pose an immediate threat to aquatic life. Concentrations of the metals in the muscle of wild fish, although higher than in farmed fish, were within EU limits. The study findings highlight the value of fish as indicators of environmental exposure and emphasize the ongoing need to monitor the pit lake to assess the long-term impacts of mining activities on aquatic health. Examination of liver tissue proved to be particularly useful in the assessments, providing a precise reflection of metal exposure and serving as a valuable means of evaluating environmental contamination and ecosystem health.

1. Introduction

Open-cast mining is a particularly controversial anthropological activity owing to the strong environmental impact (leading to soil contamination, loss of biodiversity and environmental degradation) and to the social and economic impacts on local communities, including conflicts with other types of land use and the loss of cultural heritage (Gillespie and Bennett, 2012; European Parliament et al., 2022). Once the mining activity ends, the pits are often transformed into artificial lakes. However, if the areas are not properly managed, the pit lake water can become contaminated with heavy metals and other toxic components. Consequently, biological activity in these environments may be strongly limited, with the subsequent risk of toxic effects in both wildlife and human local communities (Lund and Blanchette, 2023).

In order to minimise the impact of such projects, adequate environmental restoration plans must be established for areas affected by the mining, even before exploitation of the resources begins (Otcere et al., 2004; Young et al., 2022). One environmental remediation strategy used is the controlled formation of artificial lakes, which, if managed appropriately, can bring economic, aesthetic and welfare benefits to the population (Lund and Blanchette, 2023). The As Pontes pit lake, located in As Pontes de García Rodríguez (a town of approximately 10,000 inhabitants situated in the province of A Coruña, Galicia, NW Spain) was created as a result of the restoration and regeneration works of the open-cast mine that occupied the site, and from which lignite was extracted to supply the As Pontes thermal power station (1400 MW). The environmental restoration project was conducted by the owner of the power plant and the mine, the Empresa Nacional de Electricidad, S.A. (ENDESA) after closure of the site in 2008. The mine pit was filled by gravity with run-off water and water from the River Eume, and the work was completed in 2012. To guarantee the chemical quality of the water, several measures were taken, including sealing the outcropping materials and installing biological filters. The lake is the largest artificial lake in Spain, with a volume of 547 hm³, a maximum depth of 205 m and a perimeter of 17.8 km. The inner and outer mine spoil dumps were covered with topsoil, fertilised and planted with native vegetation (Aréchaga et al., 2011).

For these remediation projects to be environmentally sustainable, it is essential that the restored areas establish a natural ecosystem that tends towards biological balance (Miller et al., 2013; Young et al., 2022). According to data published by ENDESA, the process of colonisation of the lake by native flora and fauna has been developing naturally and there are currently three species of fish in the lake: trout, bream and stickleback (E.N.D.E.S.A., 2024). In these types of projects, conducting environmental monitoring studies is of utmost importance to determine any impact of the previous mining activity on the ecosystems. When any adverse effect is detected, remediation measures must be implemented. Assessment of the accumulation of toxic metals (such as mercury, cadmium, lead and arsenic, among others) within fish populations inhabiting these restored environments is particularly important. These elements have been shown to have detrimental effects on biological systems and may pose a public health risk (Balali-Mood et al., 2021; Di Bella et al., 2024; Sable et al., 2024). In addition, elevated

concentrations of essential elements such as iron, copper and zinc are frequently observed, fostering numerous organic-level interactions (Mazej et al., 2010). Consequently, fish serve as sentinel species for biomonitoring aquatic environments (Camizuli et al., 2014; Nava et al., 2023).

Studies conducted in recent years have shown that the As Pontes pit lake is in good ecological condition (López-Rodríguez et al., 2016) and that the physico-chemical quality of the water makes it suitable for the development of natural life and for carrying out leisure activities, including bathing. However, there are currently no data regarding the accumulation of toxic metals or essential elements in the fish that inhabit this restored environment. This information is essential for biomonitoring the project and also for guaranteeing the safety of activities as sport fishing and for the possible establishment of fish farms in the area. The primary objective of this study was to analyze the accumulation of toxic and essential elements in trout captured in the artificial lake, to contribute to the biomonitoring of the As Pontes pit lake. In addition, the element profiles were compared with those of trout collected from other sections of the River Eume and from a commercial fish farm in the same region, for comparative analysis.

2. Material and methods

2.1. Sample collection

Wild trout (*Salmo trutta*) were captured in the artificial pit lake at the As Pontes de García Rodríguez mine ($n = 16$) and upstream of the River Eume, at Muras ($n = 14$), in March–April 2022, as depicted in Fig. 1. Fishing in the lake is prohibited, and permission was therefore obtained from the environmental authorities of the Galician government (Consellería de Medio Ambiente, Territorio e Vivenda, Xunta de Galicia) to capture the fish. The capture procedure followed specialized fishing authorization protocols for various fish and invertebrate species in sport fishing. To mimic sport fishing conditions in the lake, fish were caught from the shore or using a “float tube” with light tackle or spinning with artificial lures. Water samples ($n = 3$) were also collected from the same locations. Upon capture, the weight and standard length of each trout were measured at the sampling sites with a digital balance (Smart Weigh GEM20) and measuring tape, respectively. The average size of individual fish captured in the lake (length 41 ± 10 cm; weight 837 ± 606 g) exceeded that of the fish captured in the river (length 27 ± 5 cm; weight 234 ± 101 g). The specimens were held under refrigerated conditions and transported immediately to the ImedA Research Group laboratory at the Facultade de Veterinaria, Campus Terra, Universidade de Santiago de Compostela, in Lugo. In the laboratory, all specimens underwent meticulous external and internal macroscopic examination for lesions; all animals were found to be healthy. Otolith analysis of the largest trout captured (length 60 cm, weight 2378 g) indicated that it was 5 years old. The fish were dissected to obtain samples of liver and muscle tissue (flesh) for analysis. Muscle tissue samples were extracted by making longitudinal incisions through the lateral line of the fish without skin and bones. Three subsamples of liver and muscle (1–5 g) from all sampled fish were stored in polyethylene vials at -20 °C until analysis.

Additionally, farmed rainbow trout (*Oncorhynchus mykiss*, $n = 10$) were obtained during the same period from a fish farm located in the River Rosende (Profand, Carballo, Fig. 1). Fish were sacrificed by administration of a lethal dose of anaesthetic. Similarly, the fish were measured (length 29 ± 7 cm) and weighed (weight 369 ± 198 g) and immediately transported to the laboratory under cold conditions, where they were processed using the same sample preparation protocol. The composition of the feed is described in Table S1. Water samples ($n = 3$) were also collected from the fish farm.

2.2. Analytical procedures

Liver and muscle samples (1 g) were subjected to a microwave-assisted acid digestion protocol (ultraWAVE Milestone, Sorisole, Italy) at 240°C and 40–120 bar for 1 h prior to analysis. Briefly, 1 g of sample was mixed with 3 mL of nitric acid (69% TMA, Hiperpure, PanReac, Barcelona, Spain) and 1 mL of ultrapure water. The resulting digests were transferred to polypropylene tubes, and ultrapure water was added to a final volume of 50 mL. The water samples were filtered and acidified with nitric acid to a final concentration of 1% (v/v). The concentrations of fifteen toxic and essential elements were determined: Aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), sulphur (S), selenium (Se) and zinc (Zn). Analyses were carried out by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7900 \times ICP-MS system; Agilent Technologies, Tokyo, Japan). The sample introduction system consisted of an autosampler, a dual-step spray chamber with a Peltier system (Agilent Technologies, Tokyo, Japan), a concentric glass nebulizer (MicroMist, low flow, Glass Expansion, West Melbourne, Australia), and a quartz torch (Agilent Technologies, Tokyo, Japan). Elemental concentration quantification was performed using Agilent ICP-MS MassHunter 5.1 software (Version D.01.01, Agilent Technologies, Tokyo, Japan). Operational parameters were as follows: plasma flow at 15 L/min, nebulizer flow at 1.1 L/min, sample depth at 8, sample speed at 0.1 rpm, plasma RF power at 1550 W, and spray chamber temperature maintained at 2°C . Helium (He) (4 L/

min) or hydrogen (H_2) (4.2 L/min) gases were used to correct for interferences. In all cases the linear equation fits blanks, and the correlation coefficient was >0.999 . The samples were analyzed in triplicate.

Stringent quality control measures were implemented throughout the analysis. Thus, the limit of detection (LOD), the limit of quantification (LOQ) (calculated as three and ten times the standard deviation of the blanks, respectively), and analytical blanks and certified reference material (DORM-3 fish protein) were included. The results obtained are summarized in Table 1. The LODs of the different toxic and essential elements obtained by this analytical method were sufficient to allow their determination at the concentrations present in the samples. For those elements not certified by the certified reference material (Al, Co, Mo, and S), spiked samples were used at concentrations producing

Table 1

Analytical quality programme expressed as mean \pm standard deviation used in the determination of trace elements in this study.

Element	LOD ($\mu\text{g/L}$)	LOQ	DORM-3		
			Level measured (mg/kg)	Certified ^a level (mg/kg)	% of Recovery
Al	0.8	2.7	1.62 ± 0.23	–	–
As	0.3	1.0	6.47 ± 0.48	6.88 ± 0.30	94.0
Cd	0.2	0.7	0.286 ± 0.021	0.290 ± 0.020	98.6
Cr	0.3	1.0	1.54 ± 0.51	1.89 ± 0.17	81.5
Ni	0.2	0.7	1.47 ± 0.35	1.28 ± 0.24	114.8
Hg	0.1	0.3	0.358 ± 0.074	0.382 ± 0.060	93.7
Pb	0.1	0.3	0.347 ± 0.066	0.395 ± 0.050	87.8
Co	0.2	0.7	0.200 ± 0.014	–	–
Cu	3.3	11.0	15.0 ± 1.3	15.5 ± 0.63	96.8
Fe	8	26.7	325 ± 41	347 ± 20	93.7
Mn	1.1	3.7	3.06 ± 0.38	(4.6)	66.5
Mo	1.1	3.7	0.651 ± 0.014	–	–
S	1.2	4.0	11156 ± 2045	–	–
Se	0.9	3.0	3.81 ± 0.33	(3.3)	115.5
Zn	7	23.3	48.8 ± 1.8	51.3 ± 3.1	95.1

^a Values that are only informative are shown in parenthesis. LOD: limit of detection; LOQ: limit of quantification.

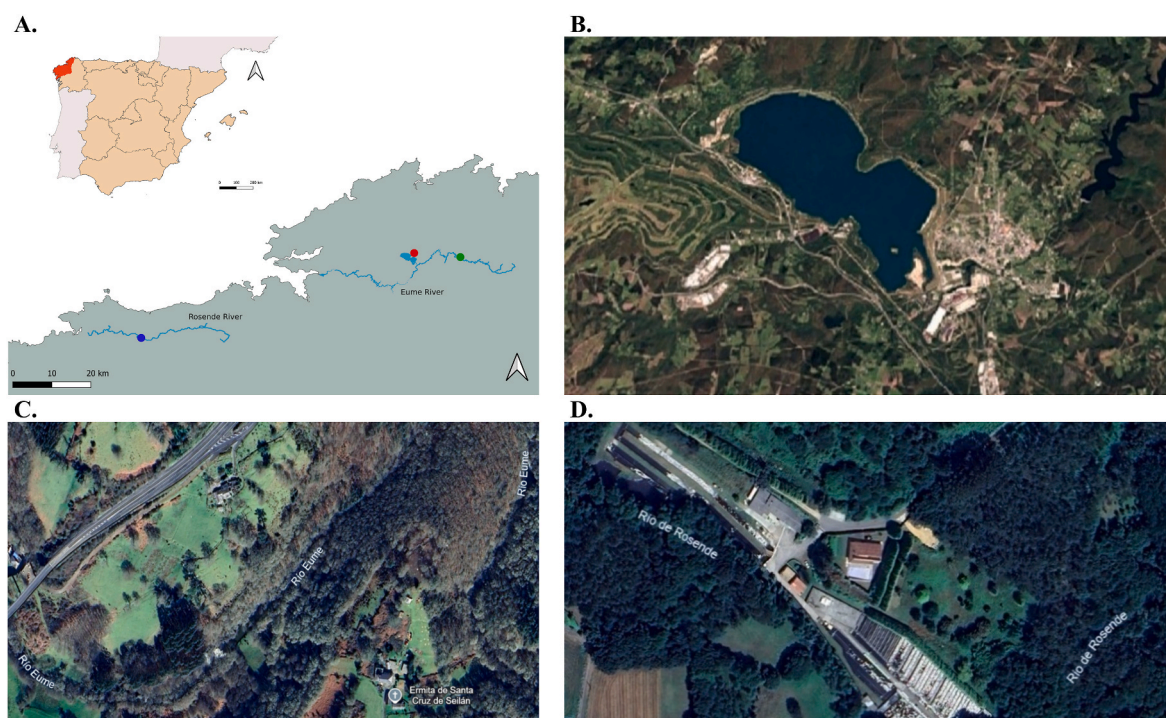


Fig. 1. (A) Location of study zones (B●), As Pontes lake (As Pontes de García Rodríguez) (C●), River Eume (Muras) and (D●) fish farm located in the River Rosende (Profand, Carballo).

absorbance values up to 2–10 times the usual concentrations in the tissues. The analytical recovery of these elements ranged between 92 and 106%. The results obtained for the reference material and the spiked samples indicated satisfactory levels of recovery, and therefore the accuracy of the determinations was acceptable.

2.3. Statistical and chemometric analyses

Statistical analysis was conducted to evaluate differences in metal concentrations between the three study groups (As Pontes lake, River Eume and fish farm). Analysis of variance (ANOVA) was used, with the

type of habitat (lake, river and fish farm) included as the main factor; fish length was also included as a covariate to account for potential size-related variations. A *post-hoc* Tukey test was used to identify significant differences between specific groups (a significance level of 0.05 was used in all cases).

Multivariate chemometric display techniques, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), were used to study the latent structure of the data set composed of the concentrations of fifteen elements in the forty trout samples ($X_{40 \times 15}$). PCA is a powerful statistical technique used for dimensionality reduction in datasets with many variables, as in the present case. The main goal of

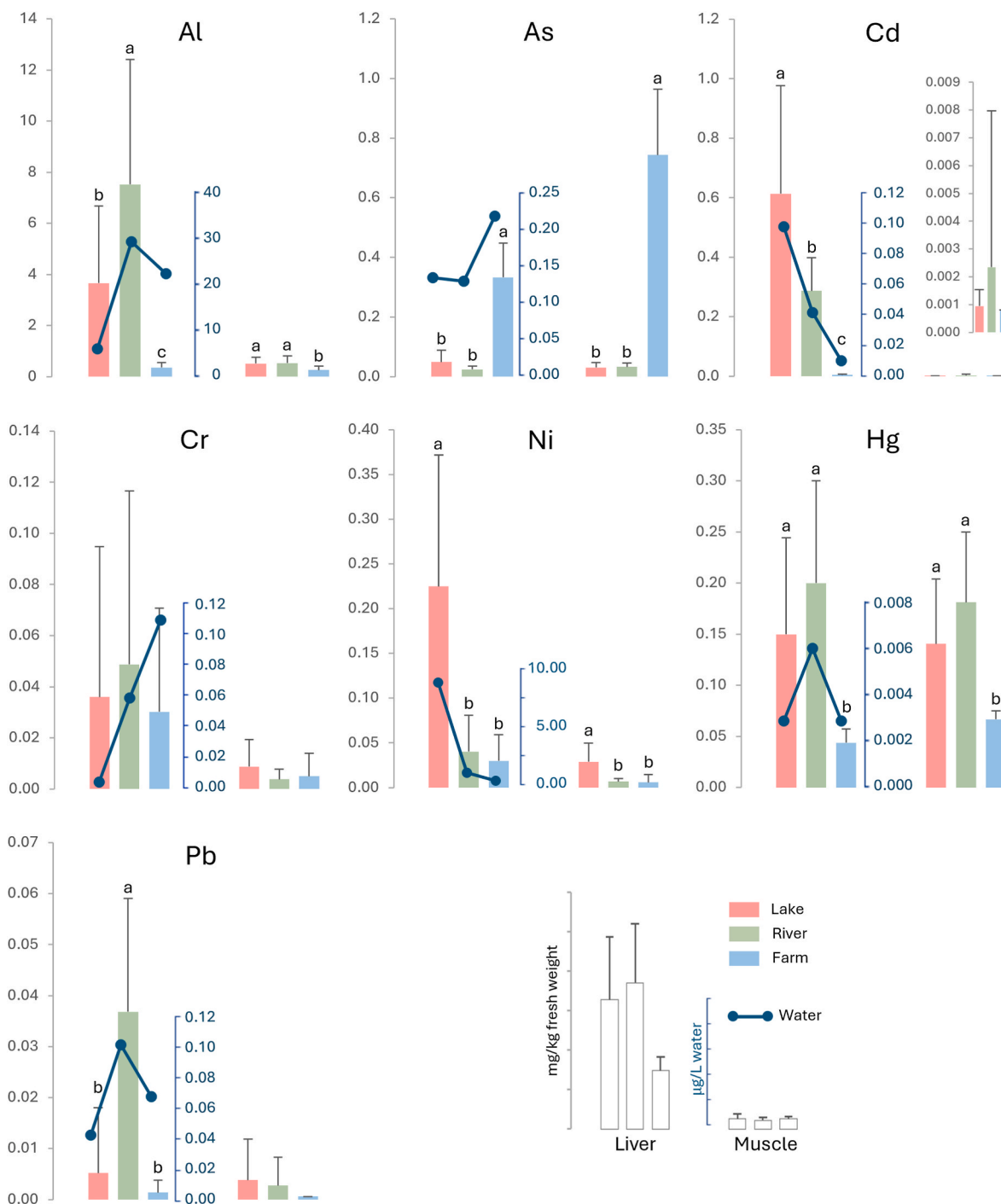


Fig. 2. Toxic element concentrations in liver and muscle of trout (expressed in mg/kg fresh weight) and water (µg/L) from the As Pontes lake, the River Eume and the fish farm. Different letters indicate statistically significant differences between groups.

PCA is to transform the original set of correlated variables into a smaller set of uncorrelated variables called principal components. These components are linear combinations of the original variables and are ordered by the amount of variance they capture from the data, with the first principal component captures the most variance, the second principal component the second most variance, and so on. By focusing on the principal components capturing the most variance, PCA effectively reduces the complexity of the dataset while retaining most of its original information (Jolliffe, 2002). HCA is used to group a set of objects in such a way that objects in the same group (or cluster) are more similar to each

other than to those in other groups. It is commonly used in data analysis to identify patterns and structures within a data set (Kaufman and Rousseeuw, 2005). The primary objective is to segregate data into clusters that exhibit high intra-cluster similarity and low inter-cluster similarity, which helps to simplify the dataset and reveal any underlying patterns. In the case at hand, similarity between samples was determined on the basis of the squared Euclidean distance between each. The Ward method was used for establishing clusters (Everitt et al., 2011). Prior to the use of both PCA and HCA, the data were autoscaled to prevent the different size of variables affecting the outcome. Autoscaling

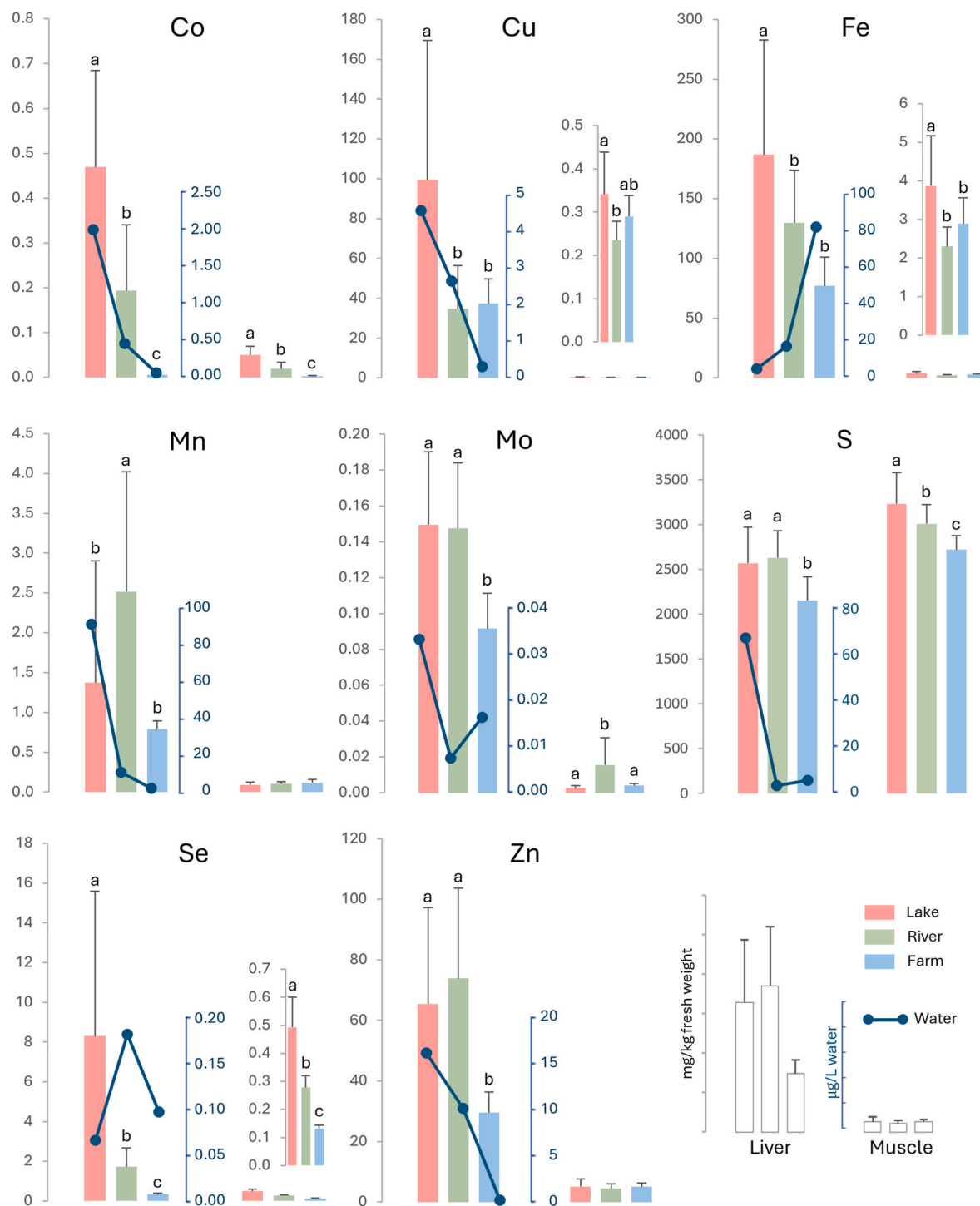


Fig. 3. Essential trace element concentrations in liver and muscle of trout (expressed in mg/kg fresh weight) and water (µg/L, except for S where it is expressed as sulphates in mg/L) from the As Pontes lake, the River Eume and the fish farm. Different letters indicate statistically significant differences between groups.

is a preprocessing technique used in chemometrics to standardize data by scaling each variable so that it has a mean of zero and a standard deviation of one. This procedure ensures that each variable contributes equally to the analysis. By transforming the data into a common scale, autoscaling improves the performance of multivariate statistical methods like PCA and HCA, leading to more accurate and interpretable results (Massart and Kaufman, 1983).

Statistical tests were performed with IBM SPSS for Windows ver. 29.0.1.0 (IBM, International Business Machines Corporation, Armonk, NY, USA). Chemometric techniques such as PCA and HCA were carried out using Statgraphics Centurion XVIII v18.1.12 software package (Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results

The concentrations of toxic and essential trace elements in the liver and muscle of trout (expressed in mg/kg fresh weight) and also in the water samples (in µg/L) are presented in Figs. 2 and 3, respectively. Location emerged as a significant factor for most elements (except Cr), especially in the liver, although fish length also had a significant influence on some elements (Table 2).

As anticipated, the concentrations of most toxic elements (except As and Hg) and essential elements (except S) were significantly higher in the liver than in the muscle. The most pronounced differences were between fish specimens collected from the lake and river relative to those from the fish farm. For most toxic elements (except Cr), there was a similar pattern of accumulation in the water and the residue levels in the liver. Cadmium and Ni concentrations were significantly higher in fish from the lake, while Al, Pb and Hg concentrations were higher in fish from the river. The As levels were much higher in the trout from the fish farm than in trout from the lake and river, although for the other

Table 2
Summary of the statistical model used to evaluate the influence of the origin (lake, river and fish farms) as a main factor and fish length as a covariate on toxic and essential trace element accumulation in the liver and muscle of trout. Statistically significant effects at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***), – not significant.

	Liver		Muscle	
	Origin	Fish length	Origin	Fish length
Al	F _{2,36} = 13.31 ***	F _{1,36} = 1.139 –	F _{2,36} = 4.535 *	F _{1,36} = 0.596 –
As	F _{2,36} = 81.54 ***	F _{1,36} = 0.901 –	F _{2,36} = 144.6 ***	F _{1,36} = 0.049 –
Cd	F _{2,36} = 10.79 ***	F _{1,36} = 5.418 *	F _{2,36} = 0.709 –	F _{1,36} = 0.001 –
Cr	F _{2,36} = 0.480 –	F _{1,36} = 0.374 –	F _{2,36} = 0.018 –	F _{1,36} = 3.451 –
Ni	F _{2,36} = 10.38 ***	F _{1,36} = 0.000 –	F _{2,36} = 13.00 ***	F _{1,36} = 3.636 –
Hg	F _{2,36} = 11.16 ***	F _{1,36} = 2.834 –	F _{2,36} = 13.07 ***	F _{1,36} = 3.086 –
Pb	F _{2,36} = 16.24 ***	F _{1,36} = 0.147 –	F _{2,36} = 1.315 –	F _{1,36} = 0.956 –
Co	F _{2,36} = 13.61 ***	F _{1,36} = 6.096 *	F _{2,36} = 22.09 ***	F _{1,36} = 0.107 –
Cu	F _{2,36} = 8.875 ***	F _{1,36} = 2.877 –	F _{2,36} = 8.992 ***	F _{1,36} = 2.467 –
Fe	F _{2,36} = 3.805 *	F _{1,36} = 4.351 *	F _{2,36} = 8.635 ***	F _{1,36} = 0.869 –
Mn	F _{2,36} = 5.488 **	F _{1,36} = 7.430 **	F _{2,36} = 1.654 –	F _{1,36} = 20.21 ***
Mo	F _{2,36} = 8.730 ***	F _{1,36} = 0.165 –	F _{2,36} = 5.365 **	F _{1,36} = 1.002 –
S	F _{2,36} = 8.166 ***	F _{1,36} = 3.139 –	F _{2,36} = 13.69 ***	F _{1,36} = 4.061 –
Se	F _{2,36} = 3.761 *	F _{1,36} = 3.647 –	F _{2,36} = 69.16 ***	F _{1,36} = 3.175 –
Zn	F _{2,36} = 8.398 ***	F _{1,36} = 0.213 –	F _{2,36} = 5.557 **	F _{1,36} = 15.82 ***

elements, the levels of residues were always lower (for Ni and Pb the differences were only numerical) than in the other two groups. By contrast, for essential elements there was no close association between the concentrations of elements in the water and the liver. Except for Fe and Se, essential element concentrations were higher in the lake water, although the specimens captured there only accumulated significantly higher levels of Co and Cu. Hepatic Mn levels were lower in lake trout than in river trout, but the Mo and S levels were very similar in both groups. As observed for the toxic elements, the concentrations of essential elements were lower in trout from the fish farm than in the other groups of trout, especially those from the lake (for Mn, the difference was only numerical).

In the muscle tissue, the pattern of accumulation of toxic elements closely mirrored that observed in the liver, with a strong correlation between both tissues for most elements (e.g. Pearson correlation coefficient for the concentration of As in liver and in muscle was $r = 0.90$ and for Hg, it was $r = 0.92$) (see the corplot presented in Fig. S1 providing a visual exploration on correlation matrix). The only exceptions for the toxic elements were Cd, and Pb, where residues were below the detection limit in most samples (Cd only detected in 2 lake samples, Pb only in 3 river samples), with no significant differences between locations. Chromium concentrations in this tissue did not differ significantly in the three locations. Considering the essential elements, the concentrations of Co, Cu, Fe, Se and S were significantly higher in fish captured in the river, although a clear association with the concentrations in the liver was only observed for Co ($r = 0.87$) and Ni ($r = 0.75$). In the cases of Mn and Zn, there were no differences between groups, whereas Mo concentrations were only higher in the samples of fish from the river.

Fig. 4 illustrates the patterns of toxic and trace element accumulation in tissues relative to fish length for the elements for which this factor was significant. In the liver of fish captured from the lake and river, Cd, Co and Fe concentrations increased with fish length, while Mn concentrations decreased. However, these trends were not observed in trout from the fish farm. Both Mn and Zn concentrations in muscle tissue decreased with fish length across all groups.

A comprehensive chemometric analysis of the pattern of essential trace and toxic concentrations in fish by using PCA and HCA for the different tissues considered yielded interesting results. PCA was applied to the fifteen metals determined in the two tissues studied. The 3D-biplots of samples and variables for liver and muscle are shown in Fig. 5A and B respectively, in the space defined by the first three principal components (achieving for the 67.60% of total data variance in the first case of liver, and 56.76% in the case of muscle). The results were similar in both cases for both tissues, confirming the previous comment that location is a clear key for elemental profile. Samples of fish from fish farm, lake and river exhibited distinct patterns of metal accumulation. Fish farm samples constituted a homogeneous group in the 15-multidimensional space of the variables clearly separated from groups of lake and river samples. PCA revealed a strong association between As and the fish farm samples (see Fig. 5A and B); the association was evident in both tissues (although more pronounced in the liver than in the muscle) and can be attributed to the higher levels of As in the fish farm group, as previously noted. The fish from the lake and river were also different but not completely separate, with an evident overlap between them (more so in the muscle than in the liver). This result was corroborated by another chemometric display technique with different mathematical basis, i.e. cluster analysis. Application of HCA to the sets of 15 elements in both tissues yielded the dendrograms shown in Fig. 5C and D, for liver and muscle, respectively. As can be seen, the cluster profile obtained confirmed the previous result by PCA. Fish farm samples formed a clear separate and homogeneous cluster, distant from the samples in the other two groups. The homogeneity (high similarity between farm samples) can be explained by the fact that the trout in this group were all of the same age and they were also always fed under the same conditions. In other words, the factors length and origin were irrelevant in this group, while they were important in the fish from both the lake and the river.

Table 3

Toxic and essential trace elements concentrations in liver and muscle of trout (expressed in mg/kg fresh weight, unless indicated; dw = dry weight) from the literature.

Country	Main pollution source		Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	S	Se	Zn		
Liver																			
Spain	Unpolluted	dw	25		0.553			159	553									140	Legorburu et al. (1998)
	Organic pollution		nd		0.53			130	440									210	
	Agricultural area	dw			0.345			31.6						1.508					Linde et al. (1996)
	Urban area				2.01			26.0						0.635					
USA	Agricultural area	dw	4.39	0.4	3.88		0.67	194	445					nd		17.9		93.9	Heiny and Tate (1997)
Norway	Unpolluted					0.06												42.8	Brotheridge et al. (1998)
	Mining area					0.1												60	
Russia	Metallurgical and mining industry	dw	93	0.022	5.48	0.83		176		0.24	5.6		3.23	0.29				121	Moiseenko and Kudryavtseva (2001)
Czech Republic	Unpolluted				0.117		0.0515	55.8		0.145				0.21				30.9	Vítek et al. (2007)
	Distillery industry				0.213		0.0635	119		0.141				1.048				33.4	
Bosnia	Agricultural area			0.076	0.144		0.142	44.0		0.284				1.15				59.9	Has-Schön et al. (2008)
Argentina	Fish farms	dw								0.4						46.3			Arribére et al. (2008)
USA	Mining, industrial and urban area									0.027						3.05			Herrmann et al. (2016)
Croatia	Unpolluted		0.571	0.0204	0.112	0.0200		31.0	74.3		1.24	0.161				1.83	19.4	Dragun et al. (2018)	
	Urban area		0.429	0.0298	0.0125	0.0530		51.9	83.8		1.32	0.135				2.66	18.6		
Turkey	Farmed		2.37		0.006	0.1	6.09	77.04	120		1.02	0.7	3.33	0.06				9.62	Çilingir Yeltekin and Saglamer (2019)
	Unpolluted		2.66		0.02	0.22	9.33	55.6	161		1.53	1	4.66	0.04				14.4	
Country	Main pollution source		Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	S	Se	Zn		
Muscle																			
Spain	Unpolluted	dw			0.0636													21	Legorburu et al. (1998)
	Organic pollution		nd		0.05			nd	24					0.5				19	
Norway	Unpolluted					0.02		0.52										7.4	Brotheridge et al. (1998)
	Mining activity					0.04		11.5										20	
Russia	Metallurgical and mining industry	dw	12	nd	0.032	0.98		2.4		0.042	2.27		1.89	nd				34	Moiseenko and Kudryavtseva (2001)
Spain	Agricultural activity			0.0557	0.0014			0.446						0.0273				3.97	Bordajandi et al. (2003)
Czech Republic	Unpolluted				0.015		0.05	0.418		0.077				0.249				5.081	Vítek et al. (2007)
	Distillery industry				0.0145		0.0505	0.358		0.097				0.646				4.88	
Bosnia	Agricultural area			0.152	0.042		0.189	0.547		0.668				0.885				41.8	Has-Schön et al. (2008)
Argentina	Fish farm	dw								0.41							0.96		Arribére et al. (2008)
Turkey	Agricultural and industrial area	dw													4.7				Verep et al. (2012)
USA	Mining industrial and urban area									0.0368							1.04		Herrmann et al. (2016)
Turkey	Farmed		2.28		0.003	0.09	6.61	1.41	48.7		0.97	0.65	2.99	0.05				5.01	Çilingir Yeltekin and Saglamer (2019)
	Unpolluted		5.2		0.007	0.18	11.4	0.78	62.7		1.66	1.16	6.35	0.11				7.54	
Bhutan	Urban, industrial and agricultural area		0.557	0.023	0.005	0.03	0.241	0.335	56.5		0.088	0.013	0.119	0.046			nd	0.99	Tashi et al. (2022)

nd: not detected.

nd: not detected.

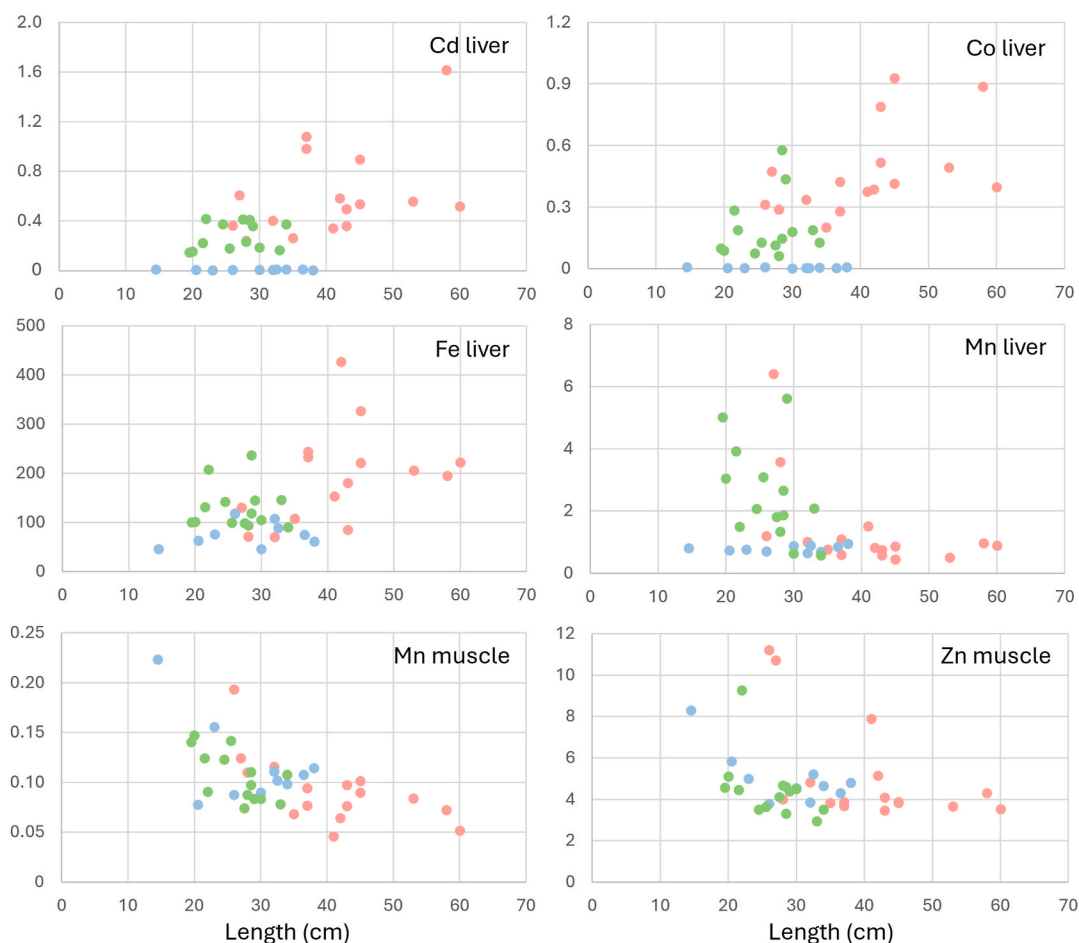


Fig. 4. Scatter plots showing the pattern of toxic and trace element accumulation related to length in trout captured in the As Pontes lake (●), the River Eume (●) and the fish farm (●).

On the other hand, the noted intersect between samples from lake and river was also observed in the distribution of the clusters (also with a greater overlap in muscle than in liver).

To further investigate the associations among variables based on location, HCA was applied to the set of variables considered across the three locations: fish farm, lake and river. From this point onwards, metal concentrations in the liver will be primarily discussed, as the liver was found to be a better bioindicator, providing clearer and more easily interpretable information than muscle tissue. The dendrograms obtained from the HCA of liver data are shown in Fig. 6A–C, and different association patterns according to the location can be seen. In the fish farm (Fig. 6A), the similarity between variables is greater than for the other groups as conditions in this environment are stabler than in the lake and river. The result reveals an association between essential elements that are supplemented in the diet (Mn, Cu and Zn), which are presented together in the cluster on the right side of the dendrogram. Lead appears associated with this cluster, which includes elements related to the diet and which may indicate a potential relationship of Pb with the mineral supplements supplied to the trout in the fish farm. In addition, elements with a pattern of renal elimination (S, Mo and Se) also appeared together in another single cluster. In relation to the toxic elements, Ni was very close to Cd, and Cr, while Hg and As were included in another group. In the lake samples (Fig. 6B), the distance between variables was higher than in the fish farm and river samples, with several groups of elements standing out. The first, in the cluster on the left, is composed of elements that are mostly present in high concentrations only in the fish from the lake, such as Co, Ni, Cd, Cu and Se. The very short distance between Cu and Se may indicate that Se exerts a

protective role. Molybdenum also appeared in this cluster, very close to Cd. Molybdenum is usually associated with kidney alterations, and Cd tends to accumulate with age in the kidney. The second cluster (in the middle of the dendrogram) includes high concentrations of elements, particularly in specimens from the river (Al, Hg and also Fe). Finally, the third cluster on the right, is formed by those elements with similar levels in specimens from the river and lake (S, Zn, As and Mn) in addition to Pb. In the dendrogram of samples from the river (Fig. 6C) the patterns are much less clear than in the previous two locations. The associations between Se and Hg (detoxification mechanism), S and Mo (renal elimination pattern) and Cr and Ni can be highlighted.

4. Discussion

Analysis of toxic and essential trace elements in the liver and muscle of trout captured from the As Pontes pit lake revealed concentrations consistent with previous findings in similar species from regions affected by anthropogenic activities such as mining, industrial processes and urban settlements (Table 3). However, comparison of our findings with these previous findings is challenging due to variations in detection limits, laboratory methodologies, sample types (wet or dry weight without specifying dry matter content) and differences in sizes of the specimens. Importantly, concentrations of toxic and essential trace elements in the lake water are clearly within the limits established in the Spanish environmental quality standards (As: 50, Cd: 0.2, Pb: 7.2, Hg: 0.07, Ni: 20, Cu: 22, Cr: 50, Se: 1, Zn: 200 $\mu\text{g/L}$; RD 817/2015, 2015) and well below those considered toxic to trout (for a detailed review, see Garai et al. (2021)). Additionally, concentrations of toxic elements in

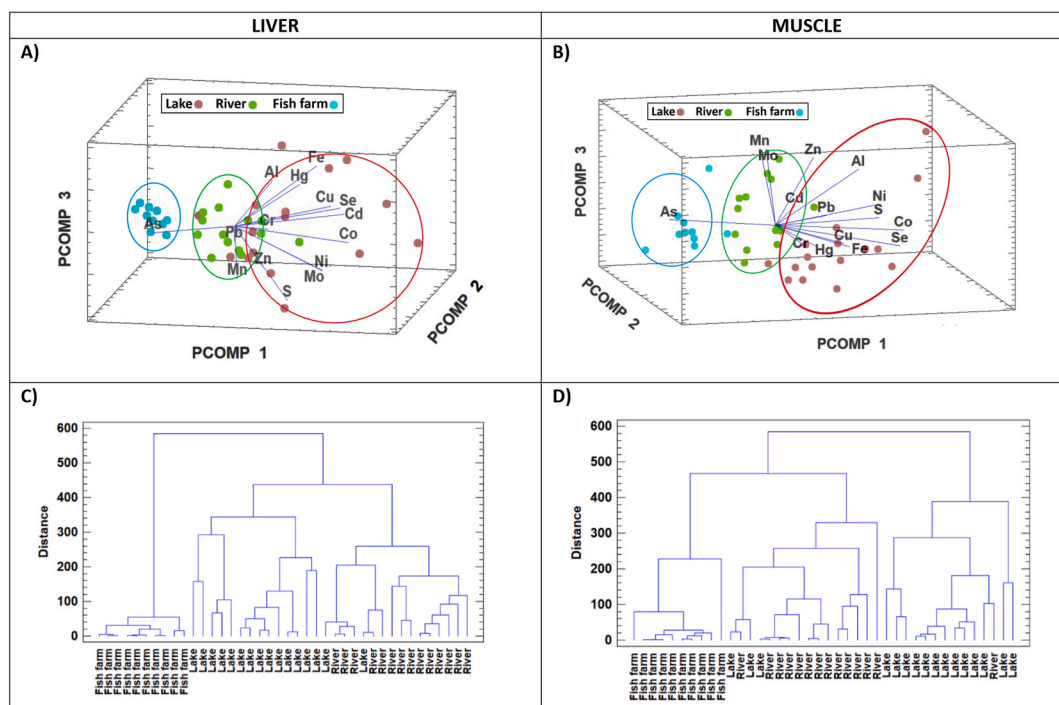


Fig. 5. A) Biplot of samples and variables in liver for the samples of the three locations (67.60% of total data variance). B) Biplot of samples and variables in muscle for the samples of the three locations (56.76% of total data variance). C) HCA dendrogram of the samples according to the metal content in liver. D) HCA dendrogram of the samples according to the metal content in muscle. Similarity between samples was measured on the basis of the squared Euclidean distance, and the Ward method was used to establish clusters.

trout muscle comply with European Union limits for fish intended for human consumption (Cd: 0.050, Hg: 0.5, Pb: 0.3 mg/kg fresh weight; [European Commission, 2023](#)), indicating that trout caught in the As Pontes pit lake would be safe for human consumption.

The study findings also indicate that the previous lignite mining activity appears to have an influence on the metal content in the water of the lake and, consequently, on the accumulation of toxic and essential elements in the fish living in the lake. Overall, although most toxic and essential trace elements were found at higher concentrations in the specimens captured in the lake, the mineral profile is similar to that found in trout captured in the upper reaches of the River Eume. These results are consistent with those of a previous study that examined the historical metal content in the sediments of the Ares estuary, into which the River Eume flows ([Álvarez-Vázquez et al., 2017](#)). This study showed a clear increase in the concentration of all elements, which were found in higher concentrations in the fish captured in the lake than in extracts from 1955, corresponding with the onset of the activity in the As Pontes open-cast mine ([Álvarez-Vázquez et al., 2017](#)). These results indicate that the mineral profile of the rocks underlying the basin, relatively rich in these minerals, strongly influences the toxic and trace element load found in the lake ecosystem and the basin environment, which is very different from that of the river where the fish farm included in our study is located. The geology of the area around the River Eume, near the lake and the area of Muras, mainly consists of black phyllites and schistose phyllites, which are metamorphic rocks. The most common minerals are phyllosilicates containing various metals. This area is within the “Ollo de Sapo formation”, one of the oldest geological formations on the peninsula. However, the fish farm is located in a geological environment of castoclastic granite, an igneous rock with a different composition and origin than metamorphic rocks. The passage of water over the mineralogical formations leads to the dissolution of the minerals and the particular composition of the water. Therefore, it is not surprising to find different cationic or anionic contents between the waters around the River Eume and the water in the River Rosende. It should be noted that

the water in both rivers complies with the limits prescribed in the current regulations on water quality ([RD 3/2023, 2023](#)), regulations transposed from the Water Framework Directive ([Directiva, 2000/60/CE](#)) and subsequent directives.

For more detailed investigation, it is important to recognize that the lake, of depth approximately 200 m, has established a thermocline at a depth of around 40 m ([Juncosa et al., 2018](#)), creating a distinct layer the warmer upper water and cooler lower water. The stable hypolimnion, impermeabilized with clay and in direct contact with the mine bed, does not significantly impact the habitat for trout. Conversely, the epilimnion, which is regularly renewed, plays a pivotal role in the ecosystem ([Juncosa et al., 2019](#)). This epilimnetic layer receives water from specific streams of the River Eume and run-off from the mine dump, alongside rainfall. Thus, differences in mineral concentrations between the lake water and the river water may be influenced by the metal profile (including concentration, bioavailability, and compound interactions) of run-off from the mine spoil heap. Studies conducted on the mine dumps during the restoration process indicate that the nature of the spoil heaps (predominantly carbonaceous clays and slates with varying sulfide content) and reclamation activities (such as topsoiling and fly ash amendments) led to higher levels of bioavailable metals compared to natural soils in the area yet remain below proposed toxicity limits for mine soils ([Monterroso et al., 1993, 1999](#)).

Among the elements found at higher levels in the lake water, two patterns of variation were observed in the trout tissues. On one hand, elements such as Co, Cu, Cd, and Ni accumulated significantly in the livers of fish captured in the lake compared to those from the river, with both being much higher than in fish from the fish farm. Conversely, elements like Mn, Mo, S, and Zn were present at similar or even lower levels in the livers of lake fish than in specimens caught in the river. Of the above-mentioned elements, a higher accumulation pattern was expected for the toxic elements, such as Cd and Ni, which tend to accumulate in organisms. However, for most essential elements, including Mn, Mo, S, and Zn, organisms exhibit homeostatic regulation, and when

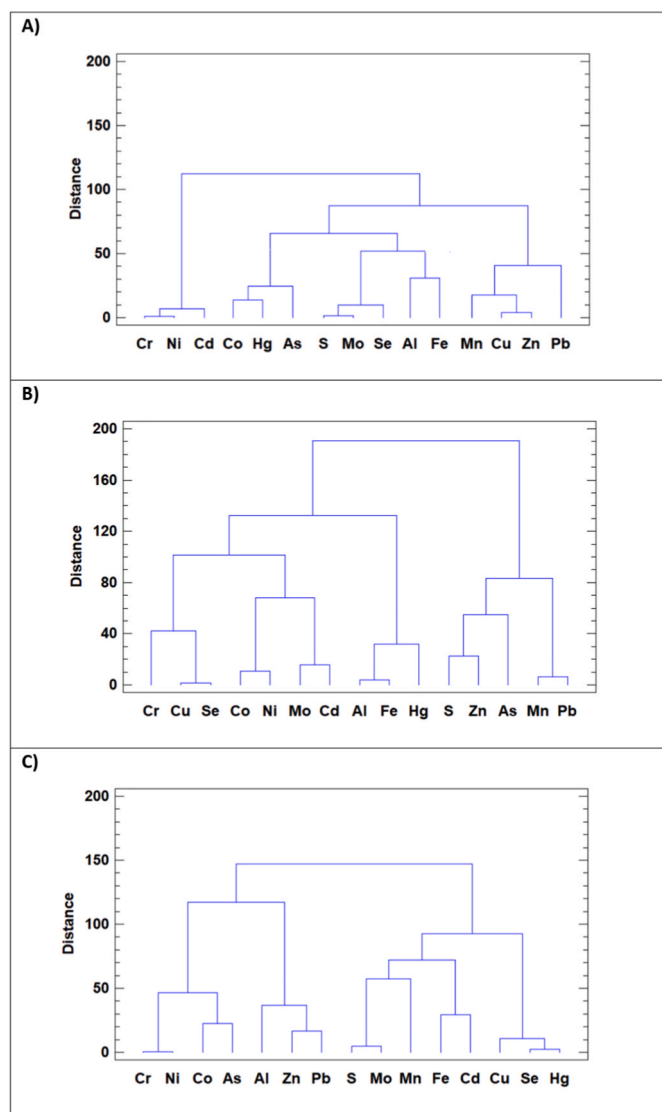


Fig. 6. Dendrogram from HCA of the 15 elements determined in liver of fish from the fish farm (A), As Pontes pit lake (B) and River Eume (C). In all cases the similarity between variables was measured on the basis of the squared Euclidean distance, and the Ward method was used to establish clusters.

exposed to concentrations above physiological requirements tissue concentrations remain within normal values; accumulation occurs only when the homeostatic regulation capacity is exceeded. In fact, our results are consistent with those of other studies that reported higher toxic residues but similar concentrations of essential trace elements in fish from areas affected by anthropogenic activity and control fish (Table 3). Among the essential trace elements, some exceptions to the general pattern of effective homeostatic regulation were observed. In the present study, accumulation of two elements, Cu and Co, was significantly higher in the livers of fish from the lake than in the livers of the other groups of fish. Unlike most terrestrial animals (excluding ruminants), fish have a limited ability to regulate Cu absorption when exposed to levels above their physiological requirements, leading to hepatic Cu storage (Garai et al., 2021). Similar rates of hepatic Cu accumulation in fish from polluted areas and in control groups are well documented (Table 3). Exposure to waterborne Cu induces oxidative stress responses in freshwater fish, leading to chronic toxicity that manifests as poor growth, reduced lifespan, decreased immune response and decreased fertility (Garai et al., 2021). Studies indicate that Cu concentrations in the range of 10–20 $\mu\text{g/L}$ can be toxic to river trout, affecting their

behaviour and physiology (Woody and O’Neal, 2012). Information on Co metabolism and accumulation in fish is more limited; however, it has been suggested that fish have a restricted capacity for homeostatic regulation of Co, which tends to accumulate with age (Mukherjee and Kaviraj, 2009). Our study findings corroborate this, with a clear trend for Co to accumulate with age in both lake and river fish, and larger specimens exhibiting higher Co concentrations in the liver.

Similarly to the lake, toxic elements found at higher concentrations in the waters of the River Eume—specifically Al, Hg, and Pb—accumulated at higher concentrations in the liver (and for Hg, also in the muscle) of trout caught in this area. The source of contamination is probably associated with the combustion of lignite coal in the As Pontes thermal power plant. The contamination of aquatic environments by toxic metals such as Hg, Pb and Al near coal-fired power plants is well documented (Singh et al., 2022; Vig et al., 2023), highlighting the need for stringent environmental monitoring and regulations to protect ecosystems and human health. Fish from the River Eume were collected 11 km northeast of the As Pontes thermal power plant, the area most affected by prevailing winds. A previous study carried out in the region had already shown the impact of the As Pontes chimney emissions in Hg exposure in cattle grazing (López Alonso et al., 2003) as far as 100 km downwind.

The mineral profile of fish from the fish farm was found to be very different from those of the fish from the lake and the River Eume, as demonstrated by PCA and HCA. These findings were partly anticipated as the river basin on which it sits (River Rosende) has a different geological origin, thereby conditioning a distinct profile of toxic and essential elements. Additionally, fish raised in fish farm systems are fed with unified standard diets, typically fortified with mineral supplements to meet nutritional requirements and often containing fishmeal as a source of protein, which may contain higher amounts of As than other raw materials (Dórea, 2006). Although the As content was higher in the fish farm (0.22 $\mu\text{g/L}$) than in the lake water and the River Eume (approximately 0.14 $\mu\text{g/L}$), it remained well below regulatory limits (EU, EPA, and WHO set a standard value of 10 $\mu\text{g/L}$ for As in drinking water; for a review see Frisbie and Mitchell, 2022). The significantly higher As residues in the liver and especially the muscle of fish from the fish farm can probably be explained by the As content in the feed. In this study, the As content in fish feed is very high (1.82 mg/kg of complete feed, Supplementary Table 1) due to the use of fishmeal as the primary source of protein. Elevated As levels in fishmeal-based feed (ranging from 1 to 6 mg/kg complete feed) have been reported, highlighting the potential for increased As accumulation in fish consuming these feeds compared to those fed with plant-based or other protein sources, typically containing As levels well below 0.5 mg/kg (Diljkan, 2023). Fortunately, although farmed fish may contain higher As residues than most foods, the As is usually organic and exhibits low toxicity to both humans and animals (Dórea, 2006; Jara and Winter 2014).

Finally, it is important to highlight that two essential elements, Fe and Se, were detected at higher concentrations in the liver and, to a lesser extent, in the muscle of fish from the lake, even though their overall concentrations in the lake water were remarkably low. This discrepancy may be attributed to interactions with other elements at the organic level or the increased bioavailability of these elements in the aquatic environment. In fish, the interactions between Se and toxic elements are well documented, with Se playing a protective role against these toxic elements (Hamilton, 2004; Talas et al., 2008; Kumar et al., 2020, 2024). One of the best-known interactions is between Se and Hg. Selenium plays a protective role by binding to Hg, thereby reducing the bioavailability and toxicity of this metal. Although this association occurs globally in all organisms, it is especially important in aquatic environments, mitigating the accumulation of Hg in fish and providing a crucial defence mechanism in aquatic ecosystems exposed to Hg pollution (Albuquerque et al., 2020, 2021). Although Hg exposure was low in the present case, an association between Se and Hg was observed in the liver of fish captured in the River Eume (see Fig. 6C). However, Se is

closely associated with Cu in fish from the lake, clearly demonstrating the protective power of Se against Cu accumulation, mitigating potential oxidative damage caused by Cu exposure (Fig. 6B). In the fish farm, where the level of exposure to these elements is low, Se is associated with elements such as Mo and S, which share a renal excretion pattern (Fig. 6A). The close association between Cd and Mo in the livers of the lake fish could also indicate some degree of compromised renal function in older fish. Cadmium is known to accumulate in the kidneys, causing damage, and Mo, which is excreted via the kidney, has been shown to be an early marker of renal function (Cedeño et al., 2020). In the case of Fe, the cluster analysis failed to identify any clear interactions with other elements that could explain why hepatic Fe concentrations are significantly higher in the fish from the lake than in the lake water, in which Fe levels are low. However, the robust correlation with Al (Fig. 6B), which is also scarce in the lake water, is evident, suggesting that both elements would occur in a more available form. Factors such as pH, the presence of other contaminants, and varying levels of organic matter can enhance the solubility and biological availability of Fe, thus facilitating its absorption by aquatic organisms (Stumm and Morgan, 1996; Cardwell et al., 2023). In the present study, hepatic Fe concentrations significantly increased with fish length, which may partly explain why the levels of Fe were significantly higher in liver of fish caught in the lake than in the other groups of fish.

Overall, our findings emphasize the importance of long-term tracking of pit lakes due to restored mine sites unique characteristics, while underscoring the value of fish biomonitoring to assess metal exposure and contamination in aquatic environments, consistent with several studies conducted in other aquatic habitats (Camizuli et al., 2014; Kalantzi et al., 2019; Varol and Sünbül, 2020; Varol et al., 2022; Nava et al., 2023, Table 3). Furthermore, the results also highlight the liver as a valuable bioindicator of metal exposure. Among the analyzed tissues, the liver proved to be the most informative, not only because it accumulated higher mineral levels than muscle, as previously reported in the literature (Kalantzi et al., 2019; Varol and Sünbül, 2020; Varol et al., 2022; Varol and Kaçar, 2023), but also because it provided clearer and more interpretable insights when using PCA and HCA techniques.

5. Conclusions

The study revealed that mining activities in As Pontes have strongly impacted the aquatic ecosystem. Significantly higher concentrations of Cd, Ni, Co, and Cu were found in the liver of fish from the lake, probably due to run-off from the mine spoil heap, while increased levels of Al, Pb, and Hg in trout from the river can be attributed to contamination to coal combustion from the thermal power plant. Nonetheless, the levels of toxic and essential elements in the water of the pit lake and river are low, adhering to legal quality standards and posing no threat to aquatic life. The muscle tissue content of toxic and essential metals in wild fish, although significantly higher than those of the farmed fish, was low and within EU limits. The findings highlight the value of fish as effective markers of environmental exposure and emphasize the value of liver tissue analysis for assessing metal exposure. Continuous monitoring of the pit lake is strongly recommended to evaluate to track the long-term impacts of the mining activity on the aquatic ecosystem. Future research should also consider broader geographic and temporal scales to understand cumulative and systemic environmental effects, ensuring the sustainability of these ecosystems.

CRedit authorship contribution statement

Marta López-Alonso: Writing – original draft, Supervision, Software, Funding acquisition, Data curation, Conceptualization. **Marta Miranda:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **Inmaculada Orjales:** Investigation. **Víctor Pereira:** Supervision, Funding acquisition. **Belén Larrán:** Investigation. **Ruth Rodríguez-Bermúdez:** Investigation. **Cathaysa**

Veiras: Investigation. **Álvaro Robles:** Conceptualization. **David Bravo:** Conceptualization. **Ricardo Juncosa:** Investigation. **Carlos Herrero-Latorre:** Writing – original draft, Software, Funding acquisition, Data curation.

Declaration of competing interest

Although this study was supported by several grants, all authors declare that they have no conflicts of interest. This research was conducted as part of the Campus Terra collaborative projects, a partnership between the Universidade de Santiago de Compostela (USC) and the Culture Council of Xunta de Galicia, Spain. The authors Marta López-Alonso, Marta Miranda, Inmaculada Orjales, Víctor Pereira, Ruth Rodríguez, and Belén Larrán are affiliated with the “Grupo de Potential Crecimiento,” which is funded by GAIN (Axencia Galega de Innovación; grant number ED431B 2023/008). Additionally, Belén Larrán is the recipient of an FPU fellowship (Ref. FPU21/01742) from the Spanish Ministry of Education, Culture, and Sports.

Acknowledgements/Funding

This study was carried out within the Campus Terra collaborative projects, a collaboration agreement between Universidade de Santiago de Compostela (USC) and Culture counseling of Xunta de Galicia, Spain. Marta López-Alonso, Marta Miranda, Inmaculada Orjales, Víctor Pereira, Ruth Rodríguez and Belén Larrán belong to the “Grupo de Potential Crecimiento” funded by GAIN (Axencia Galega de Innovación; grant number ED431B 2023/008). Belén Larrán is in receipt of a FPU fellowship (Ref. FPU21/01742) from the Spanish Ministry of Education, Culture and Sports. The authors are grateful for the use of RIAIDT-USC analytical facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2025.144212>.

Data availability

Data will be made available on request.

References

- Albuquerque, F.E.A., Herrero-Latorre, C., Miranda, M., Barrêto Júnior, R.A., Oliveira, F.L.C., Sucupira, M.C.A., Ortolani, E.L., Minervino, A.H.H., López-Alonso, M., 2021. Fish tissues for biomonitoring toxic and essential trace elements in the Lower Amazon. *Environ. Pollut.* 283, 1–14. <https://doi.org/10.1016/j.envpol.2021.117024>.
- Albuquerque, F.E.A., Minervino, A.H.H., Miranda, M., Herrero-Latorre, C., Barrêto Júnior, R.A., Oliveira, F.L.C., Sucupira, M.C.A., Ortolani, E.L., López-Alonso, M., 2020. Toxic and essential trace element concentrations in fish species in the Lower Amazon, Brazil. *Sci. Total Environ.* 732, 1–12. <https://doi.org/10.1016/j.scitotenv.2020.138983>.
- Álvarez-Vázquez, M.Á., Caetano, M., Álvarez-Iglesias, P., Pedrosa-García, M. del C., Calvo, S., Uña-Álvarez, E., Quintana, B., Vale, C., Prego, R., 2017. Natural and Anthropocene fluxes of trace elements in estuarine sediments of Galician Rias. *Estuar. Coast Shelf Sci.* 198, 329–342. <https://doi.org/10.1016/j.ecss.2016.08.022>.
- Aréchaga, F., Ferreira, T., Gil, A., Menéndez, J.A., Valle, R., 2011. Riqueza restaurada: historia de la mina de As Pontes. Endesa Generación.
- Arribère, M.A., Ribeiro Guevara, S., Bubach, D.F., Arcagni, M., Vigliano, P.H., 2008. Selenium and mercury in native and introduced fish species of patagonian lakes, Argentina. *Biol. Trace Elem. Res.* 122, 42–63. <https://doi.org/10.1007/s12011-007-8059-6>.
- Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M.R., Sadeghi, M., 2021. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Front. Pharmacol.* 1–19. <https://doi.org/10.3389/fphar.2021.643972>.
- Bordajandi, L.R., Gómez, G., Fernández, M.A., Abad, E., Rivera, J., González, M.J., 2003. Study on PCBs, PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the River Turia (Spain). *Chemosphere* 53, 163–171. [https://doi.org/10.1016/S0045-6535\(03\)00417-X](https://doi.org/10.1016/S0045-6535(03)00417-X).
- Brotheridge, R.M., Newton, K.E., Evans, S.W., Taggart, M.A., McCormick, P.H., 1998. Nickel, cobalt, zinc and copper levels in brown trout (*Salmo trutta*) from the river Otra, southern Norway. *Analyst* 123, 69–72. <https://doi.org/10.1039/a704883a>.

- Camizuli, E., Monna, F., Scheffler, R., Amiotte-Suchet, P., Losno, R., Beis, P., Bohard, B., Chateau, C., Alibert, P., 2014. Impact of trace metals from past mining on the aquatic ecosystem: a multi-proxy approach in the Morvan (France). *Environ. Res.* 134, 410–419. <https://doi.org/10.1016/j.envres.2014.07.008>.
- Cardwell, A.S., Rodriguez, P.H., Stubblefield, W.A., DeForest, D.K., Adams, W.J., 2023. Chronic toxicity of iron to aquatic organisms under variable pH, hardness, and dissolved organic carbon conditions. *ET&C* 42, 1371–1385. <https://doi.org/10.1002/etc.5627>.
- Cedeño, Y., Miranda, M., Orjales, I., Herrero-Latorre, C., Suárez, M., Luna, D., López-Alonso, M., 2020. Serum concentrations of essential trace and toxic elements in healthy and disease-affected dogs. *Animals* 10, 1–12. <https://doi.org/10.3390/ani10061052>.
- Çilingir Yeltekin, A., Saglamer, E., 2019. Toxic and trace element levels in salmo trutta macrostigma and oncorhynchus mykiss trout raised in different environments. *Pol. J. Environ. Stud.* 28, 1613–1621. <https://doi.org/10.15244/pjoes/90620>.
- Di Bella, G., El-Sorogy, A.S., Giacobbe, S., Nava, V., Al-Kahtany, K., Nour, H.E., 2024. Risk assessment of potentially toxic elements in intermittent rivers, “fiumara”, flowing in the Gulf of Milazzo (Sicily, Italy). *Environ. Earth Sci.* 83, 1–12. <https://doi.org/10.1007/s12665-024-11631-0>.
- Diljkan, M., 2023. Arsenic Speciation in Animal Feed and Feed Ingredients by ICP-MS and HPLC-ICP-MS Methods. The University of Bergen. Bergen (Norway). Available online: <https://hdl.handle.net/11250/3094688>.
- Directiva 2000/60/CE, 2000. Directiva 2000/60/CE del Parlamento Europeo y del Consejo, de 23 de octubre de 2000, por la que se establece un marco comunitario de actuación en el ámbito de la política de aguas. Diario Oficial de las Comunidades Europeas L 327, 1–73. Available online: <http://data.europa.eu/eli/dir/2000/60/oj>.
- Dórea, J.G., 2006. Fish meal in animal feed and human exposure to persistent bioaccumulative and toxic substances. *J Food Prot* 69, 2777–2785. <https://doi.org/10.4315/0362-028X-69.11.2777>.
- Dragun, Z., Filipović Marijić, V., Krasnići, N., Ivanković, D., Valić, D., Žunić, J., Kapetanović, D., Smrzlić, I.V., Redžović, Z., Grgić, I., Erk, M., 2018. Total and cytosolic concentrations of twenty metals/metalloids in the liver of brown trout *Salmo trutta* (Linnaeus, 1758) from the karstic Croatian river Krka. *Ecotoxicol. Environ. Saf.* 147, 537–549. <https://doi.org/10.1016/j.ecoenv.2017.09.005>.
- E.N.D.E.S.A., 2024. Presente, pasado y futuro de As Pontes. Available online: <https://www.endesa.com/es/la-cara-e/transicion-ecologica/presente-pasado-futuro-de-as-pontes>.
- European Commission, 2023. Commission Regulation (EU) 2023/915 of 25 April 2023 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006. DOCE: Diario Oficial de la Unión Europea. Available online: <http://data.europa.eu/eli/reg/2023/915/oj>.
- European Parliament, 2022. Directorate-general for internal policies of the union. In: Mononen, T., Kivinen, S., Kotilainen, J., Leino, J. (Eds.), *Social and Environmental Impacts of Mining Activities in the EU*. Publications Office of the European Union, pp. 1–83. <https://doi.org/10.2861/804163>.
- Everitt, B.S., Landau, S., Leese, M., Stahl, D., 2011. *Cluster Analysis*, fifth ed. Wiley, London.
- Frisbie, S.H., Mitchell, E.J., 2022. Arsenic in drinking water: an analysis of global drinking water regulations and recommendations for updates to protect public health. *PLoS One* 17, 1–42. <https://doi.org/10.1371/journal.pone.0263505>.
- Garai, P., Banerjee, P., Mondal, P., Saha, N.C., 2021. Effect of heavy metals on fishes: toxicity and bioaccumulation. *J. Clin. Toxicol.* 11, 1–10. <https://doi.org/10.35248/2161-0495.21.S18.001>.
- Gillespie, R., Bennett, J., 2012. Valuing the environmental, cultural and social impacts of open-cut coal mining in the Hunter Valley of New South Wales, Australia. *Journal of Environmental Economics and Policy* 1, 276–288. <https://doi.org/10.1080/21606544.2012.714970>.
- Hamilton, S.J., 2004. Review of selenium toxicity in the aquatic food chain. *Sci. Total Environ.* 326, 1–31. <https://doi.org/10.1016/j.scitotenv.2004.01.019>.
- Has-Schön, E., Bogut, I., Kralik, G., Bogut, S., Horvatić, J., Čačić, I., 2008. Heavy metal concentration in fish tissues inhabiting waters of “Buško Blato” reservoir (Bosnia and Herzegovina). *Environ. Monit. Assess.* 144, 15–22. <https://doi.org/10.1007/s10661-007-9627-0>.
- Heiny, J.S., Tate, C.M., 1997. Concentration, distribution, and comparison of selected trace elements in bed sediment and fish tissue in the south platte river basin, USA, 1992–1993. *Arch. Environ. Contam. Toxicol.* 32, 246–259. <https://doi.org/10.1007/s002449900182>.
- Herrmann, S.J., Nimmo, D.R., Carsella, J.S., Herrmann-Hoesing, L.M., Turner, J.A., Gregorich, J.M., Heuvel, B.D.V., Nehring, R.B., Foutz, H.P., 2016. Differential accumulation of mercury and selenium in Brown trout tissues of a high-gradient urbanized stream in Colorado, USA. *Arch. Environ. Contam. Toxicol.* 70, 204–218. <https://doi.org/10.1007/s00244-015-0241-9>.
- Jara, E.A., Winter, C.K., 2014. Dietary exposure to total and inorganic arsenic in the United States, 2006–2008. *Int J Food Contam* 1, 1–12. <https://doi.org/10.1186/s40550-014-0003-x>.
- Jolliffe, I.T., 2002. *Principal Component Analysis*, Second. Springer, New York.
- Juncosa, R., Delgado, J., Cereijo, J.L., García, D., Muñoz, A., 2018. Comparative hydrochemical analysis of the formation of the mining lakes of as Pontes and Meirama (Spain). *Environ. Monit. Assess.* 190, 1–13. <https://doi.org/10.1007/s10661-018-6880-3>.
- Juncosa, R., Delgado, J., Cereijo, J.L., Muñoz, A., 2019. Hydrochemical evolution of the filling of the mining lake of as Pontes (Spain). *Mine Water Environ.* 38, 556–565. <https://doi.org/10.1007/s10230-019-00612-6>.
- Kalantzi, I., Mylonka, K., Pergantis, S.A., Coli, A., Panopoulos, S., Tzapakis, M., 2019. Elemental distribution in the different tissues of brood stock from Greek hatcheries. *Aquac* 503, 175–185. <https://doi.org/10.1016/j.aquaculture.2019.01.004>.
- Kaufman, L., Rousseeuw, P.J., 2005. *Finding Groups in Data. An Introduction to Cluster Analysis*. Wiley. <https://doi.org/10.1002/9780470316801>.
- Kumar, N., Gupta, S.K., Chandan, N.K., Bhushan, S., Singh, D.K., Kumar, P., Kumar, P., Wakchaure, G.C., Singh, N.P., 2020. Mitigation potential of selenium nanoparticles and riboflavin against arsenic and elevated temperature stress in Pangasianodon hypophthalmus. *Sci. Rep.* 10, 1–17. <https://doi.org/10.1038/s41598-020-74911-2>.
- Kumar, N., Thorat, S.T., Patole, P.B., Gite, A., Reddy, K.S., 2024. Protective role of selenium and selenium-nanoparticles against multiple stresses in Pangasianodon hypophthalmus. *Fish Physiol. Biochem.* 50, 239–258. <https://doi.org/10.1007/s10695-023-01231-3>.
- Legorburu, I., Canton, L., Millan, E., Casado, A., 1998. Trace metal levels in fish from urala river (Spain) anguillidae, mugilidae and salmonidae. *Environ. Technol. Lett.* 9, 1373–1378. <https://doi.org/10.1080/0959338809384703>.
- Linde, A.R., Arribas, P., Sanchez-Galan, S., Garcia-Vazquez, E., 1996. Eel (*Anguilla anguilla*) and Brown trout (*Salmo trutta*) target species to assess the biological impact of trace metal pollution in freshwater ecosystems. *Arch. Environ. Contam. Toxicol.* 31, 1–6. <https://doi.org/10.1007/BF00212668>.
- López Alonso, M., Benedito, J.L., Miranda, M., Fernández, J.A., Castillo, C., Hernández, J., Shore, R.F., 2003. Large-scale spatial variation in mercury concentrations in cattle in NW Spain. *Environ. Pollut.* 125, 173–181. [https://doi.org/10.1016/S0269-7491\(03\)00073-3](https://doi.org/10.1016/S0269-7491(03)00073-3).
- López-Rodríguez, M.C., Leira, M., Valle, R., Moyá-Niell, G., 2016. El fitoplancton como indicador de calidad de masas de agua muy modificadas en la DMA. *El lago artificial de As Pontes (A Coruña. España). NACC: Nova Acta Cient. Compostelana* 23, 1–8.
- Lund, M.A., Blanchette, M.L., 2023. Closing pit lakes as aquatic ecosystems: risk, reality, and future uses. *Wiley Interdiscip Rev: Water* 10, 1–18. <https://doi.org/10.1002/wat2.1648>.
- Massart, D.L., Kaufman, L., 1983. *The Interpretation of Analytical Chemical Data by the Use of Cluster Analysis*, First. Wiley, NewYork.
- Mazej, Z., Al Sayegh-Petkovšek, S., Pokorny, B., 2010. Heavy metal concentrations in food chain of Lake Velenjsko jezero, Slovenia: an artificial lake from mining. *Arch. Environ. Contam. Toxicol.* 58, 998–1007. <https://doi.org/10.1007/s00244-009-9417-5>.
- Miller, L.L., Rasmussen, J.B., Palace, V.P., Sterling, G., Hontela, A., 2013. Selenium bioaccumulation in stocked fish as an indicator of fishery potential in pit lakes on reclaimed coal mines in Alberta, Canada. *Environ Manage* 52, 72–84. <https://doi.org/10.1007/s00267-013-0038-4>.
- Moiseenko, T.I., Kudryavtseva, L.P., 2001. Trace metal accumulation and fish pathologies in areas affected by mining and metallurgical enterprises in the Kola Region, Russia. *Environ. Pollut.* 114, 285–297. [https://doi.org/10.1016/S0269-7491\(00\)00197-4](https://doi.org/10.1016/S0269-7491(00)00197-4).
- Monterroso, C., Álvarez, E., Macías, F., 1993. Caracterización de los suelos de la escombrera de la mina As Pontes (La Coruña) en proceso de restauración ambiental. *Cuad. do Lab. Xeolóxico Laxe* 18, 307–316. Available online: <http://hdl.handle.net/2183/6148>.
- Monterroso, C., Macías, F., Alvarez, E., Fernández-Marcos, M.L., 1999. Geochemistry of aluminium and iron in mine soils from as Pontes, Galicia (NW Spain). *Water Air Soil Pollut.* 110, 81–102. <https://doi.org/10.1023/A:1005055704392>.
- Mukherjee, S., Kaviraj, A., 2009. Evaluation of growth and bioaccumulation of cobalt in different tissues of common carp, *Cyprinus carpio* (actinopterygii: cypriniformes: cyprinidae), fed cobalt-supplemented diets. *Acta Ichthyol Piscat* 39, 87–93. <https://doi.org/10.3750/AIP2009.39.2.02>.
- Nava, V., Di Bella, G., Fazio, F., Potorti, A.G., Lo Turco, V., Licata, P., 2023. Hg content in EU and non-EU processed meat and fish foods. *Appl. Sci.* 13, 1–11. <https://doi.org/10.3390/app13020793>.
- Otchere, F.A., Veiga, M.M., Hinton, J.J., Farias, R.A., Hamaguchi, R., 2004. Transforming open mining pits into fish farms: moving towards sustainability. *Nat. Resour. Forum* 28, 216–223. <https://doi.org/10.1111/j.1477-8947.2004.00091.x>.
- RD 3/2023, 2023. Real Decreto 3/2023, de 10 de enero, por el que se establecen los criterios técnico-sanitarios de la calidad del agua de consumo. su control y suministro. Available online: <https://www.boe.es/eli/es/rd/2023/01/10/3/con>.
- RD 817/2015, 2015. Real Decreto 817/2015, de 11 de septiembre, por el que se establecen los criterios de seguimiento y evaluación del estado de las aguas superficiales y las normas de calidad ambiental. Available online: <https://www.boe.es/eli/es/rd/2015/09/11/817>.
- Sable, H., Singh, V., Kumar, V., Roy, A., Pandit, S., Kaur, K., Rustagi, S., Malik, S., 2024. Toxicological and bioremediation profiling of nonessential heavy metals (mercury, chromium, cadmium, aluminium) and their impact on human health: a review. *Toxicologie Analytique et Clinique* 36, 205–234. <https://doi.org/10.1016/j.toxac.2024.03.096>.
- Singh, S., Dhyani, S., Pujari, P., 2022. Coal-fired thermal power plants and mercury risks: status and impacts to realize minamata convention promises. *Anthropocene Science* 1, 419–427. <https://doi.org/10.1007/s44177-023-00042-8>.
- Stumm, W., Morgan, J.J., 1996. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, Third. ed. Wiley, New York.
- Talas, Z.S., Orun, I., Ozdemir, I., Erdogan, K., Alkan, A., Yilmaz, I., 2008. Antioxidative role of selenium against the toxic effect of heavy metals (Cd+2, Cr+3) on liver of rainbow trout (*Oncorhynchus mykiss* Walbaum 1792). *Fish Physiol. Biochem.* 34, 217–222. <https://doi.org/10.1007/s10695-007-9179-9>.
- Tashi, S., Gurung, D.B., Dorji, U., Wangchuk, K., 2022. Trace elements in fish species: Schizothorax richardsonii, Salmo trutta and Neolissochilus hexagonolepis in relation to sizes and tissues of Punatsang Chhu. *Environmental Chemistry and Ecotoxicology* 4, 78–88. <https://doi.org/10.1016/j.ecence.2022.01.001>.
- Varol, M., Kaçar, E., Sünbül, M.R., Islam, A.R.M.T., 2022. Species, tissue and gender-related metal and element accumulation in fish species in a large reservoir (Turkey)

- and health risks and nutritional benefits for consumers. *Environ. Toxicol. Pharmacol.* 94, 1–8. <https://doi.org/10.1016/j.etap.2022.103929>.
- Varol, M., Kaçar, E., 2023. Bioaccumulation of metals in various tissues of fish species in relation to fish size and gender and health risk assessment. *Curr Pollution Rep* 9, 327–337. <https://doi.org/10.1007/s40726-023-00263-w>.
- Varol, M., Sünbül, M.R., 2020. Macroelements and toxic trace elements in muscle and liver of fish species from the largest three reservoirs in Turkey and human risk assessment based on the worst-case scenarios. *Environ. Res.* 184, 1–8. <https://doi.org/10.1016/j.envres.2020.109298>.
- Verep, B., Mutlu, C., Apaydin, G., Cevik, U., 2012. The trace element analysis in freshwater fish species, water and sediment in iyidere stream (rize-Turkey). *Pakistan J. Biol. Sci.* 15, 658–665. <https://doi.org/10.3923/pjbs.2012.658.665>.
- Vig, N., Ravindra, K., Mor, S., 2023. Heavy metal pollution assessment of groundwater and associated health risks around coal thermal power plant, Punjab, India. *Int. J. Environ. Sci. Technol.* 20, 6259–6274. <https://doi.org/10.1007/s13762-022-04284-8>.
- Vítek, T., Spurný, P., Mareš, J., Ziková, A., 2007. Heavy metal contamination of the loučka river water ecosystem. *Acta Vet.* 76, 149–154. <https://doi.org/10.2754/avb200776010149>.
- Woody, C.A., O'Neal, S.L., 2012. Effects of copper on fish and aquatic resources. Fisheries research and consulting. Available online: <https://www.conservationgateway.org/ConservationByGeography/NorthAmerica/UnitedStates/alaska/sw/cpa/Documents/W2013ECopperF062012.pdf>.
- Young, R.E., Gann, G.D., Walder, B., Liu, J., Cui, W., Newton, V., Nelson, C.R., Tashe, N., Jasper, D., Silveira, F.A., Carrick, P.J., 2022. International principles and standards for the ecological restoration and recovery of mine sites. *Restor. Ecol.* 30, 1–47. <https://doi.org/10.1111/rec.13771>.