Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-023-28637-z

POST-COVID TRENDS AND NEW INSIGHTS FOR THE PHYSICAL-CHEMICAL-BIOLOGICAL TREATMENT OF WASTEWATERS AND SOILS

Common carp as an ecological indicator of environmental pollution in reservoirs of southern Spain: inferring the environmental risks of anthropogenic activities

Nestor Javier Mancera-Rodríguez[1,2] · Daniel Ruiz Galiano[3] · Antonio Jesús López-Montoya[2,4] · Eulogio J. Llorent-Martínez[5] · Lucía Molina-García[5] · Concepción Azorit[2,3]

Received: 16 January 2023 / Accepted: 2 July 2023

Abstract

Extraction and mineral processing, as well as the waste generated by old abandoned mining sites, are the main sources of contamination of water bodies and lands by potentially toxic elements (PTEs). The common carp (*Cyprinus carpio* Linnaeus 1758) has been reported to be a good ecological indicator of environmental pollution in water bodies. Hence, we evaluated the concentration of eleven PTEs (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in different tissues of common carp in two reservoirs of the province of Jaén, southern Spain: El Tranco de Beas (S1) and La Fernandina (S2). We also assessed the concentration of PTEs in water and sediment samples. We used inductively coupled plasma mass spectrometry for all the collected samples. We found high concentrations of As and Fe in water in the S2 reservoir, above the maximum limits allowed by the sanitary criteria in Spain; however, the analysis of sediments indicated low ecological risk in S1 and moderate ecological risk for As in S2. The concentration of PTEs in common carp was higher in the S2 reservoir, exceeding the permissible limits in the case of As, Cd, Pb, and Zn. As and Cd showed higher concentrations in the kidney; Cu, Fe, and Zn showed higher concentrations in the liver; and Pb and Mn presented higher concentrations in the gill and gill bone. There was a good correlation between the concentrations found in water/sediment samples and those in common carp, corroborating its usefulness as a good ecological indicator, allowing the detection of environmental pollution and inferring previous or current anthropogenic activities such as mining.

Keywords Monitoring pollution · Mining risk · Heavy metals · Potentially toxic elements (PTEs) · Common carp · ICP-MS

Introduction

Important and intensive mining activity was undertaken in the province of Jaén (Andalusia; southern Spain) until the end of the 1960s (Gutiérrez-Guzmán 2007), making the district of Linares-La Carolina, located in Jaén, the world's main producer of Pb during the first third of the twentieth century (Martínez et al. 2012). However, mines like the ones in the mentioned area, characterized by the presence of philonian deposits, essentially galena (PbS) with some silver content, were abandoned without any previous adaptation or subsequent remediation (Martínez et al. 2014, 2016; Mendoza et al. 2022a). This situation generated a potential risk of contamination of surface and groundwater by the leachate generated (Martínez et al. 2012, 2016).

The province of Jaén has 13 reservoirs, and it is of special interest to provide evidence on the state of concentration of potentially toxic elements (PTEs) in these water bodies in view of the activities or the use given to them. These reservoirs in most cases are a source of water for consumption, as well as being places for leisure activities, fishing, and tourism. Therefore, it is important to know the concentration of PTEs in water,

Highlights

- Eleven potentially toxic elements (PTEs) were analyzed in common carp
- Concentrations of most metals were higher in kidney, liver, and gill than in muscle
- High bioaccumulation of arsenic and cadmium was found in common carp kidney
- Common carp is a good ecological indicator of pollution in water reservoirs

Responsible Editor: Wei Liu

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sediments and in the species that inhabit these bodies of water. Different species of fish have been used as excellent indicators of contamination in water since they can bioaccumulate and biomagnify high concentrations of metals (Mancera-Rodríguez and Álvarez-León 2006). The levels of certain metals in fish tissues generally reflect those found in their biotic and abiotic environments (Wang and Rainbow 2008). The common carp *Cyprinus carpio*, an exotic invasive species native to Asia introduced to the Iberian Peninsula thousands of years ago, is present in almost all of these reservoirs in the province of Jaén (Fernández-Delgado 1990). Due to its typical bottom-feeding behavior, in which sediments are absorbed in the oral cavity and separated from food in the pharyngeal slits (Stergiou et al. 2014), common carp are of particular interest for monitoring contaminants. In this sense, the aims of this study are to evaluate the concentrations of eleven potentially toxic elements (PTEs) such as the following: arsenic (As), cadmium (Cd), cobalt (Co), chrome (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) in water, sediments, and different tissues of common carp (*C. carpio*). The studies were realized in two reservoirs with contrasting characteristics in the province of Jaén, Spain, and the levels of these chemicals were compared with similar environments and concentrations reported in other study areas for this species.

We used *C. carpio* as a possible bioindicator species of PTE concentration levels and evaluated the differences in concentration between different tissues in order to identify target tissues that can be used to identify the state of contamination of reservoirs and be included in the local management of reservoirs in the province of Jaén. We expected to find differences in the concentrations of PTEs between the two reservoirs. The La Fernandina reservoir, which was built on a former mining area (an old galena mine that was submerged) will present higher levels of PTEs concentration in water, sediments, and carp tissues, and the El Tranco de Beas reservoir located in in the central valley of the Sierras de Cazorla, in Segura and Las Villas Natural Park, will have lower PTE concentrations.

This research contributes to the knowledge of the levels of contamination in these two reservoirs, validating the use of *C. carpio* as a bioindicator species for potentially toxic elements and contamination in reservoirs of southern Spain and for inferring previous uses of the ecosystem, such as mining. We also inform whether the levels of contamination by PTEs reach the concentration limit values accepted for consumption in water, sediments, and fish, and finally we identify target tissues that can be used to identify the state of contamination. This is the first time that this type of study has been carried out in the reservoirs of this region. The information collected could be useful in taking measures to mitigate or remediate the potential level of contamination in the reservoirs of southern Spain or environments with similar conditions of intensive mining activity.

Methods

Study area

The El Tranco de Beas reservoir (S1) is located at the head of the Guadalquivir river basin, damming the waters of this river and those of its northern tributary, the Hornos river (38°10′17.7″N, 2°47′41.4″W). It has an average annual inflow of 177 hm^3, an area of 1500 ha and a storage capacity of 500 hm^3 (Fernández-García et al. 2008). The basin is located in limestone terrain. The reservoir is located in the Cazorla, Segura, and Las Villas Natural Park (Fig. 1), the second largest protected area in Europe. This reservoir is used for water supply, irrigation, electricity generation, fishing, bathing, and tourism. Several species were introduced: *Oncorhynchus mykiss* (Walbaum, 1792), *Salmo trutta* (Linnaeus, 1758), *C. carpio*, *Micropterus salmoides* (Lacépède 1802), and *Gambusia holbrooki* (Girard 1859). Also, autochthonous species are still detected such as *Luciobarbus sclateri* (Günther 1868), *Pseudochondrostoma willkommii* (Steindachner 1866), and *Cobitis paludica* (de Buen 1929).

La Fernandina reservoir (S2) is located in the Guarrizas river basin in the municipality of Vilches (38°10′52.7″N, 3°34′17.4″W), maximum depth 75.5 m, area 597.86 ha (Fig. 1). The basin of this reservoir has an average annual inflow of 98 hm^3 and a capacity of 245 hm^3. The basin is located in siliceous terrain. Under the waters of the reservoir lie the flooded galleries of the Palazuelos galena mine, already exploited even in pre-Roman times. Under the waters of the Fernandina lies the old Panzacola II reservoir, located over an old mine, the Tungsten Mine. This area is precisely the sampling point selected (see Fig. 1). The uses of the reservoir are the maintenance of ecological flow, irrigation, electricity generation, recreational activities, and drinking water supply to towns such as Linares and La Carolina. Introduced species: *Esox lucius* (Linnaeus 1758), *C. carpio*, *Alburnus alburnus* (Linnaeus 1758), *Lepomis gibbosus* (Linnaeus 1758), *Micropterus salmoides* and *Gambusia holbrooki*. Autochthonous species: *Luciobarbus sclateri*, *Cobitis paludica*, *Pseudochondrostoma willkommii*, *Iberocypris alburnoides* (Steindachner, 1866), and *Squalius pyrenaicus* (Günther, 1868) (Carrasco A., personal communication).

ICP-MS instrumentation and method validation

The concentrations of eleven PTEs: arsenic (As), cadmium (Cd), cobalt (Co), chrome (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) in water and sediments were measured using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7900ce, Germany) with low flow "micromist" concentric

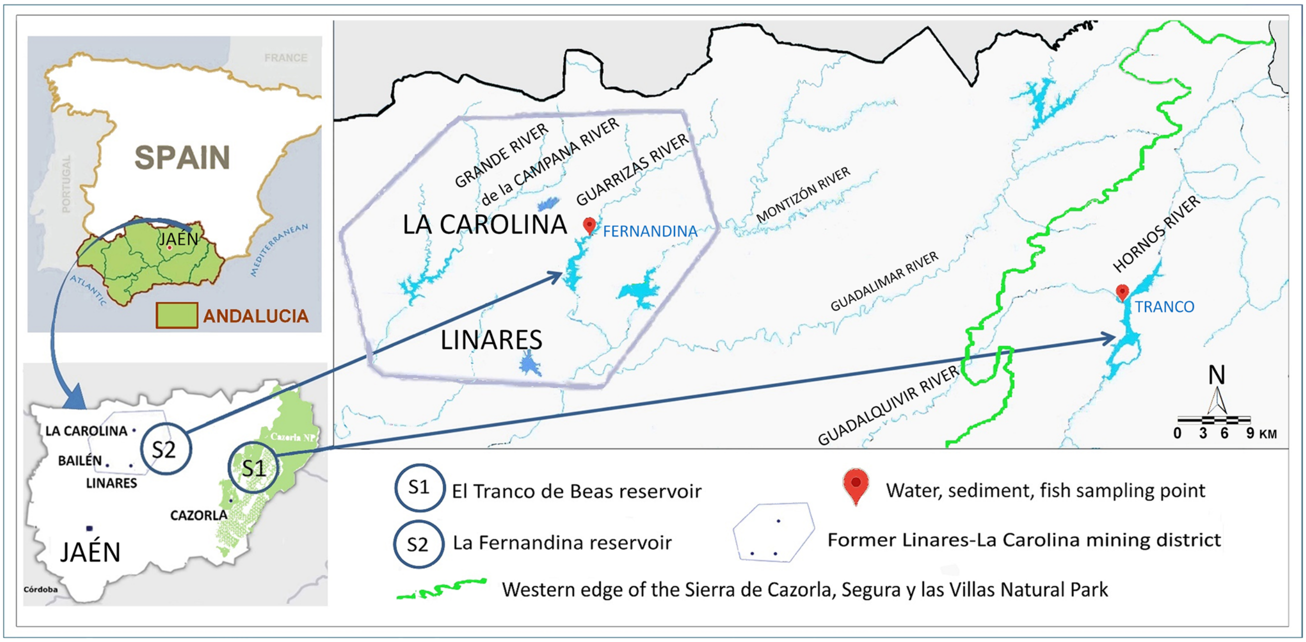

Fig. 1 Study area showing the El Tranco de Beas (S1) and the La Fernandina (S2) reservoirs from which samples were obtained. Linares-La Carolina mining district delimitation adapted from Azorit et al. (2012)

nebulizer (0.2 mL min^{-1}) and equipped with a double-pass spray chamber (Scott type) cooled using the Peltier system, with a temperature range of − 5 to 20 °C. ICP-MS. Operating conditions are presented in Supplementary Information SI 1. Samples were analyzed in collision mode with an He flow of 4.3 mL min^{-1}. Before starting the analytical session, a check of the state of the equipment was carried out (sensitivity, level of formation of oxides and doubly charged ions, and mass resolution) with a 10 ppb tuning solution containing 4 elements that cover the entire mass range (^{7}Li, ^{89} Y, ^{140}Ce, ^{205}Tl).

For quantification a calibration line was prepared in nitric at 0.5%, which covers the concentration range of the elements of interest, from a certified standard of known concentration. Analysis of blanks, patterns, samples, and controls in an automated way using the ISIS system in combination with the autosampler were carried out following a pre-established analysis sequence. Cleaning between samples with an appropriate solution was also performed. Finally, throughout the work session, in order to correct the drift or the instrument and the possible effects due to sample matrix, an online internal standard mixture was added containing 4 elements (^{45}Sc, ^{72}Ge, ^{103}Rh, and ^{193}Ir).

The detection limit (DL) and quantification limit (QL) were calculated as the concentration that provided 3 and 10 times the signal of the blank for each element. Method DLs (DLs in real samples, including simple treatment) are given in Supplementary Information SI 2.

Recovery experiments were performed to validate the analytical method. Samples of sediment and fish tissue were spiked with a multi-element standard of all the elements analyzed at different concentrations (similar to those found during the analysis of real samples). The spiked samples were kept overnight before performing the same sample treatment reported for fish/sediment samples the next morning. Blank samples and the same sediment and fish tissue samples were also analyzed during this study. The results are shown in Supplementary Information SI 3 and SI 4, where it can be observed that recovery yields were higher than 90% for most elements. In sediment samples Hg recovery was not satisfactory, so Hg was only analyzed in water and fish tissues.

Potentially toxic elements analysis in water and sediment samples

Two water samples and two bottom sediment samples were collected in the area with a high probability of containing mining waste (specifically at the height of the old Panzaciola dam) in the Fernandina reservoir and in an easily accessible point of the Tranco reservoir. The two water samples were collected in clean and dry polyethylene bottles of 40 mL at 0.5 m depth and 2 m distance from the shore in each reservoir in August 2017. The water samples were filtered through 45 µm membrane filters, acidified with 0.15 ml of HNO_3, and stored at 4 °C in 15 mL bottles.

Two bottom sediment samples were removed using an Ekman grab at 1 m depth and 2 m distance to shore in each reservoir, and collected in polyethylene bottles of 30 mL. Storage at 4°C. The sediment was dried at room temperature for 7 days. The sediment was sieved with a 63 µm Teflon sieve. About 0.4 g of sediment was weighed in duplicate from each sample and transferred to crucibles. Each sample of 0.4 g of sediment was taken and dried at 60 °C for 15 h for the determination of arsenic and mercury concentrations and at 105 °C for 24 h for the rest of the elements in a muffle furnace (Rosas 2005).

About 0.4 g of the lyophilized sediment was mixed with 5 mL of hydrochloric acid (HCl) and 15 mL 65% nitric acid (HNO_3) and heated at 80 °C for 3 h for As and Hg, and at 100 °C for 3 h for elements Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, and Zn. It was allowed to cool and diluted to 100 mL with Milli-Q water. It was centrifuged at 3000 rpm for 20 min and 10 mL of the supernatant liquid was taken and diluted to 100 mL prior to analysis, and 15 mL were used to determine the concentration of metals. For each element the concentration was calculated as the average of the two samples evaluated and expressed in mg L^{-1} for water samples, and in mg kg^{-1} for sediment samples. Finally, the concentration value determined in this study was compared for the metals for which maximum limits defined in the current regulations are available. In water the maximum limits were established by Royal Decree 140/2003, which establishes the sanitary criteria for the quality of water for human consumption in Spain (BOE 2003), and in sediments, the maximum limits were established by the regional government for trace elements in Andalusian soils for As and Pb (Junta de Andalucía 2015) (Table 1).

Risk of PTE levels in sediments

In order to assess the ecological risk for PTE levels in sediments, the contamination factor (*Cf*) was determined; this factor is the ratio between the PTE content in sediments (Ci) and its background level (Cb) (Palacios-Torres et al. 2018). For PTEs, the reference value corresponds to the local background concentrations in the soils determined by Martínez et al. (2007) in the mining district of Linares (province of Jaén, Southern Spain). The values of the contamination factor (*Cf*) in the sediments were classified following the scale suggested by Hakanson (1980) and presented in Table 2.

The sediment concentration of seven environmentally-relevant elements (i.e., As, Cd, Cr, Ni, Pb, Cu, and Zn)

Table 1 Guideline values or Maximum limits established for water (mg L^{-1}), sediments (mg kg^{-1}), and tissues of fish (mg kg^{-1} fresh weight) or food products (mg kg^{-1}) in different regulations

PTE	Water		Sediments		Food products	
	Max.	Source	Max.	Source	Max.	Source
As	0.010	Boe 2003	36	Junta de Andalucía (2015)	0.200*	European Commission (2015)
Cd	0.005	Boe 2003			0.050	European Commission (2006)
Cu	0.002	Boe 2003				
Fe	0.200	Boe 2003				
Hg	0.001	Boe 2003			0.500	European Commission (2006)
Mn	0.050	Boe 2003				
Pb	0.010	Boe 2003	275	Junta de Andalucía (2015)	0.300	European Commission (2006)
Zn					50.000	(Vilizzi and Tarkan, 2016)

*For A: by the Regulation (EC) No. 2015/1006 is the maximum content of inorganic arsenic in food products (European Commission 2015)

Table 2 Significance criterion for Sediment quality guidelines (SQG) and corresponding threshold effect level (TEL) and probable effect level (PEL). Contamination factor (*Cf*), pollution load index (PLI), coefficient of the potential ecological hazard of each PTEs (Ei), and potential ecological risk index (RI)

TEL and PEL	*Cf*	PLI	Ei	RI	Color	Significance criterion
< TEL	< 1	< 1	< 30	< 50		Low ecological risk
	1 - 3		30 – 60.	50 - 100		Moderate ecological risk
TEL - PEL			60 – 120	100 - 200		Considerable ecological risk
	3 - 6		120 – 240	200 - 400		High ecological risk
> PEL	> 6	> 1	> 240	> 400		very high ecological risk

was compared to the threshold effect level (TEL), level below which adverse effects are not expected to occur; and the probable effect level (PEL), level above which adverse effects are expected to frequently occur; values for freshwater sediments defined by MacDonald et al. (2000). The pollution load index (PLI) was estimated according to the methodology described in Palacios-Torres et al. (2020). The sediments can be classified as unpolluted when PLI < 1, and polluted when PLI > 1 (Priju and Narayana 2006) (Table 2).

The potential ecological risk index (RI) (Hakanson 1980) was determined by the following equation:

$$RI = \sum Ei = \sum Ti * Cf = \sum Ti\frac{Ci}{Cb}$$

where E*i* is the coefficient of the potential ecological hazard of each PTE; T*i* is the toxic-response factor for each PTE; C*f* is the contamination factor; C_i is the measured concentration for PTE *i*; and C_b is the evaluation of reference value for PTE (Hakanson 1980; Jiao et al. 2015; Song et al. 2015). The values of the coefficient of the potential ecological hazard of each PTE (Ei) and the potential ecological RI in the sediments and the corresponding levels of ecological hazards were classified following the scale modified and suggested by Song et al. (2015) and Palacios-Torres et al. (2020) and presented in Table 2.

The toxic risk index (TRI) (Zhang et al. 2016) was determined by the following equation:

$$TRIi = \sqrt{\frac{(Ci/TELi)^2 + (Ci/PELi)^2}{2}}$$

The integrated toxic risks of metals in sediment are calculated using the following formula:

$$\sum_{i=1}^{n} TRIi$$

where *TRIi* represents the toxic risk index of a single PTE, C_i is the measured concentration for PTE*i*; in the sediment sample, *n* is the number of metals and TRI is the total TRI. TEL and PEL values for freshwater sediments defined by MacDonald et al. (2000) were used instead of TEL and PEL in this study. The following classification, modified from Kükrer (2018), was used for TRI: TRI ≤ 5 low toxic risk, 5 < TRI ≤ 10 moderate toxic risk, 10 < TRI ≤ 15 considerable toxic risk, 15 < TRI ≤ 20 high toxic risk, and TRI > 20 very high toxic risk.

PTE analysis in fish samples

Eighteen specimens from *C. carpio* were collected during the months of August to November 2017, eight specimens were collected in El Tranco de Beas reservoir and 10 in La Fernandina reservoir. These were transported to the laboratory of Zoology in the University of Jaén and the total length (TL) of each fish was measured to the nearest millimeter. In the El Tranco de Beas reservoir the total length of the *C. carpio* sampled ranged from 36.0 to 55.6 cm TL (44.3 ± 5.9), while the weight varied from 638.0 to 2666.1 g (1297.2 ± 634.3), and in the La Fernandina reservoir the total length of the *C. carpio* sampled ranged from 43.0 to 56.0 cm TL (49.9 ± 4.1), while the weight varied from 1176.4 to 2540.9 g (1868.8 ± 452.6).

A subsample of dorsal muscle, liver, kidney, heart, gill, and gill bone were removed from each fish using bistoury, and we stored the samples at − 4 °C. Then, 0.4 g of each tissue sample was taken in duplicate for each organ or tissue and digested with 7 mL of 65% HNO_3 at 90 °C overnight, cooled to room temperature, and then 3 mL of 33% H_2O_2 was added. Subsequently, the necessary digestion time was allowed for each sample. Once the samples had been properly digested, they were made up to a volume of 30 mL with Milli-Q water. Finally, 15 mL of this solution was taken and the concentrations of As, Cd, Co, Cr, Cu, Hg, Fe, Mn, Ni, Pb, and Zn in fish tissues were measured using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7900ce, Germany). For each element, the concentration was calculated as the average of the two samples evaluated, and expressed in mg kg^{-1}. The concentration value determined in tissues was compared for the maximum limits defined in (EC) Regulation No. 1881/2006 of the European Union (mg kg^{-1} fresh weight) for Pb, Cd, and Hg (European Commission 2006), for As with the maximum limit of inorganic arsenic in food products defined by Regulation (EC) No. 2015/1006 (European Commission 2015), and for Zn with the FAO/WHO permissible limits (Vilizzi and Tarkan 2016) (Table 1).

Differences in the mean concentration for the eleven PTEs studied (i.e., As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in eight specimens of *C. carpio* in El Tranco de Beas reservoir and 10 in La Fernandina reservoir were tested by Multivariate analysis of variance (Hotellings T2) and Games-Howell post-hoc multiple comparisons using R software version 4.1.3 (R Core Team 2022). The data for Hotelling's T2 comparisons have been linearized using natural logarithms, and we used the Doornik-Hansen multivariate normality test in our analysis.

Spearman's correlation method was used to reveal the correlations between PTE concentrations and the length (TL) and weights of specimens of *C. carpio* in both reservoirs using the R software packages. The level of statistical significance was determined at $p < 0.01$ and $p < 0.05$.

To establish significant differences in the concentrations of each PTE between tissues (muscle, liver, kidney, heart, gill, and gill bone tissue) in both reservoirs a Kruskall-Wallis non-parametric analysis of variance test and paired comparisons Dunn's test were used, since the data did not meet the assumptions of normality (Shapiro-Wilk normality test) and homoscedasticity (Levene's test). The significance level of 5% was used in all cases. We have plotted these Kruskal-Wallis post hoc comparison results together with the box-plot diagrams for each pollutant (box-plot with significant difference mark by letters) using R software version 4.1.3 (R Core Team 2022), for a better understanding of the comparisons between tissues.

Results and discussion

PTE concentrations in water samples

For the concentration of PTEs in water, Fe and As showed the highest values (0.310 mg L^{-1} and 0.022 mg L^{-1}, respectively) in the La Fernandina reservoir (Table 3), in potentially dangerous concentrations above the maximum limits allowed by Royal Decree 140/2003, which establishes the sanitary criteria for the quality of water for human consumption in Spain (BOE 2003). The other PTEs were found at low concentrations or below the detection limit in both reservoirs (Table 3).

These high concentrations of Fe in waters coincide with those found for nearby study areas in surface waters in the Linares-La Carolina district by Hidalgo et al. (2006) and Martínez et al. (2012) who found very high Fe concentrations, and Hidalgo et al. (2010) who reported Fe contents noticeably high in the water in the Grande River and La Campana River, with an average close to 3 g L^{-1}. According to Mendoza et al. (2020), the water in the Grande River carries high concentrations of PTEs (i.e., As, Cd, Pb, and Zn), especially in low water periods due to discharges from mine adits located in the headwater catchment area. In the same way, Martínez et al. (2016) found elevated contents of Fe (20 mg L^{-1}) and As (0.005–0.030 mg L^{-1}) in groundwater samples in the Linares-La Carolina district.

The lowest concentrations of Pb coincide with those reported by Hidalgo et al. (2010) in surface waters from the streams of the La Carolina district, despite the abundance of galena in the study area. In this regard, the authors suggest that this could occur due to absorption/precipitation processes that prevent lead from being released downstream (Frau et al. 2009).

PTE concentrations in sediment samples

The PTE concentrations (in mg kg^{-1}) found in bottom sediments in the El Tranco de Beas (S1) and La Fernandina (S2) reservoirs are shown in Table 3. For all PTEs the concentration in sediments showed higher values in the La Fernandina

Table 3 Average PTE concentrations in water (mg L^{-1}), sediments (mg kg^{-1}), and tissues of *Cyprinus carpio* (mg kg^{-1}) in the El Tranco de Beas (S1) and La Fernandina (S2) reservoirs, Jaén, Spain

PTE	Water[a]		Sediments[b]		*Cyprinus carpio*[c]	
	S1	S2	S1	S2	S1	S2
As	< 0.001	**0.022 ± 0.001**	1.515 ± 0.035	23.736 ± 0.028	**0.057 ± 0.005****	**0.249 ± 0.037****
Cd	bdl	bdl	0.047 ± 0.008	0.120 ± 0.036	**0.141 ± 0.024***	**0.717 ± 0.236***
Co	< 0.001	< 0.001	0.287 ± 0.001	1.626 ± 0.009	0.131 ± 0.043	0.218 ± 0.039
Cr	< 0.001	Bd	0.926 ± 0.044	2.768 ± 0.054	**0.303 ± 0.022***	**0.621 ± 0.129***
Cu	bdl	bdl	3.719 ± 2.344	9.024 ± 3.871	3.127 ± 0.462	3.184 ± 0.353
Fe	0.002	**0.310 ± 0.056**	841.176 ± 8.254	3410.181 ± 26.249	**117.179 ± 8.719***	**220.353 ± 32.256***
Hg	< 0.001	< 0.001	---	---	**0.014 ± 0.003***	**0.032 ± 0.006***
Mn	< 0.001	0.002	13.466 ± 0.035	44.390 ± 1.054	**1.987 ± 0.110****	**6.728 ± 0.420****
Ni	< 0.001	< 0.001	1.887 ± 0.600	5.362 ± 0.664	0.160 ± 0.015	0.211 ± 0.023
Pb	< 0.001	< 0.001	1.801± 0.165	12.774 ± 0.699	**0.088 ± 0.007****	**4.697 ± 1.197****
Zn	bdl	bdl	0.048 ± 0.047	15.820 ± 8.287	206.551 ± 40.220	144.034 ± 16.632

Underlined entries indicate values above the limit value. Results with statistical significance are shown in bold type

*Statistically significant effects between reservoirs ($p < 0.05$); **statistically significant effects between reservoirs ($p < 0.01$)

bdl below detection limit

[a]Maximum limits established by Royal Decree 140/2003, which establishes the sanitary criteria for the quality of water for human consumption in Spain: Mn: 0.050; Fe: 0.200; Cu: 0.002; As: 0.010; Cd: 0.005; Hg: 0.001; Pb: 0.010 (Boe 2003)

[b]Maximum limits established by the regional government for trace elements in Andalusian soils: Pb: 275; As: 36 (Junta de Andalucía 2015)

[c]Maximum limits allowed by Regulation (EC) No. 1881/2006 of the European Union (mg kg^{-1} fresh weight): Pb: 0.300, Cd: 0,050, Hg: 0.500 (European Commission 2006). For A: 0.200 by the Regulation (EC) No. 2015/1006 is the maximum content of inorganic arsenic in food products (European Commission 2015). For Zn, the maximum permissible limits defined by FAO/WHO: Zn: 50.000 mg kg^{-1} (Vilizzi and Tarkan 2016)

reservoir, with very high values of As (23.376 mg kg^{-1}), Fe (3410.181 mg kg^{-1}), Mn (44.390 mg kg^{-1}), Pb (12.774 mg kg^{-1}), and Zn (15.820 mg kg^{-1}). The concentrations of As and Pb in sediments did not exceed the maximum limits established by the regional government for trace elements in Andalusian soils (Table 3).

Considering that one of the main anthropogenic sources of As is the mining and metallurgy of minerals such as Cu, Pb and Ni ores, the high concentration values of As found in sediments in La Fernandina reservoir are associated with the fact that it is located on an old tungsten and galena mine, with a *Cf* value several times higher than the rest of the PTEs studied, indicating a high concentration in the sediments in relation to background level. Mendoza et al. (2020) found that practically all the samples collected in sediments in a nearby hydrographic basin in the old mining district of La Carolina (Jaén, Spain) presented As and Pb values above the limit set by the Andalusian regional standard, with the maximum values being found in the vicinity of the old abandoned mining operations.

The concentration in sediments in the El Tranco de Beas and La Fernandina reservoirs of Cd, Cu, Mn, Pb and Zn, contrast with those found in nearby study areas in the Linares-La Carolina district by Martínez et al. (2012), who found in sediments in abandoned mining dams high concentrations of these PTEs, which may flow into the streams that irrigate the area or into the aquifers of the sector. Even for Mn, Pb, and Zn, the values are several times higher than those found in the present study. The high values found by these authors are due to the fact that they sampled in mining dams where PTEs are discharged from mines. In addition, Mendoza et al. (2020) found high concentrations of As, Mn, Pb, and Zn in sediments of the live-bed and floodplain of the Grande and La Campana Rivers, as well as the Renegado River, and in particular, the As and Pb presented the highest contents with values much higher than those of the regional geochemical background, since they come from the ruins of old mining facilities dumpsites and fine tailing dams in the area. Mendoza et al. (2022b) found high concentrations of these same PTEs in the Federico mine (southeast of Spain), and emphasize that As and Zn are frequent elements in the study area because they are associated with mineralization.

Risk for PTE levels in sediments

Findings for the concentration factor suggest that the sediments can be categorized as low ecological risk for all PTEs studied in the El Tranco de Beas and La Fernandina reservoirs, except for As in the latter reservoir, PTE for which the sediments were categorized as a moderate ecological risk (Table 4). This is different from the findings in sediments in

Table 4 Threshold effect level (TEL), probable effect level (PEL), contamination factor (C*f*), pollution load index (PLI), potential ecological risk index (RI), and toxic risk index (TRI) values for the El Tranco de Beas (S1) and La Fernandina (S2) reservoirs, Jaén, Spain

					El Tranco de Beas (S1)				La Fernandina (S2)			
PTE	C_b	T*i*	TEL	PEL	Ci	C*f*	Ei	TRI	Ci	C*f*	Ei	TRI
As	10.00	10.00	9.79	33.00	1.515	0.152	1.515	0.114	23.736	2.374	23.736	1.788
Cd	0.20	30.00	0.99	4.98	0.047	0.235	7.050	0.034	0.120	0.600	18.000	0.087
Cr	50.00	2.00	43.40	111.00	0.926	0.019	0.037	0.016	2.768	0.055	0.111	0.048
Cu	37.00	5.00	31.60	149.00	3.719	0.101	0.503	0.085	9.024	0.244	1.220	0.206
Ni	20.00	5.00	22.70	48.60	1.887	0.094	0.472	0.065	5.362	0.268	1.341	0.184
Pb	116.00	5.00	35.80	128.00	1.801	0.016	0.078	0.037	12.774	0.110	0.551	0.262
Zn	50.00	1.00	121.00	459.00	0.048	0.001	0.001	0.001	15.820	0.316	0.316	0.096
				Pollution Load Index (PLI)		0.039				0.273	0,447	
				Potential Ecological Risk Index (RI)			9.655				45.274	
				Toxic Risk Index (TRI)				0.352				2.672

C_b, reference background level (mg kg^{-1}); T*i*, toxic-response factor for each PTE; TEL, threshold effect level; PEL, probable effect level; C_i, measured concentration for PTE; C*f*, contamination factor; E*i*, coefficient of the potential ecological hazard of each PTEs the mining district of La Carolina (Spain). Mendoza et al. (2020) found high values for Zn, As, Cd, and Pb, especially the latter, which is the main ore of the mining basin with a C*f* 30 times higher than those of the rest of the PTEs, and very high potential ecological risk (Ei) values for As, Cd, and Pb with respect to the rest of the PTEs studied (Mendoza et al. 2022b).

The results shown in Table 4 for levels for the seven PTEs evaluated (As, Cd, Cr, Cu, Ni, Pb, and Zn) in sediment samples in the El Tranco de Beas reservoir were below their respective TEL). For the La Fernandina reservoir the levels for all the PTEs evaluated except As were below their respective TEL. The level of As in this reservoir registered concentrations between TEL and PEL (Table 4).

According to the PLI based on the concentrations of As, Cd, Cr, Cu, Ni, Pb, and Zn, the sediment samples in both reservoirs were considered to be of low ecological risk. The incorporation of the Ei values produced RI scores that categorized the sediment samples in the El Tranco de Beas and the La Fernandina reservoirs as low ecological risk (Table 4), and the TRI scores categorized the sediment samples in the El Tranco de Beas reservoir (TRI: 0.352) and La Fernandina reservoir (TRI: 2.672) as low toxic risk (Table 4). Arsenic made the highest contribution to TRI in La Fernandina reservoir, followed by Pb, Cu, and Ni, respectively (Table 4). This low ecological risk and the value of TRI in the La Fernandina reservoir, which was built on a former mining area, contrasts with that reported by Mendoza et al. (2020) who found a high ecological risk in sediments in the mining district of La Carolina affected especially because the waste generated was accumulated without any preventive measures after abandonment (Mendoza et al. 2022b).

PTE concentrations in fish samples

Among the PTEs for which maximum allowable limits were available by European Union Regulation (EC) No. 1881/2006 (European Commission 2006) establishing maximum levels for certain contaminants in foodstuffs (i.e., Cd, Hg, Pb), only Hg was always below the threshold in the overall mean concentrations (i.e., averaged over muscle, liver, kidney, heart, gill, and gill bone tissue) (Table 3). For the eleven PTEs investigated, the values and the significant differences in the mean concentration for all *C. carpio* samples evaluated (post hoc Games-Howell test) are presented in Table 3. There were significant differences in the mean concentration of As ($p < 0.001$), Cd ($p < 0.046$), Cr ($p < 0.046$), Fe ($p < 0.015$), Hg ($p < 0.015$), Mn ($p < 0.001$), and Pb ($p = 0.005$) between the two reservoirs, with higher values of these PTEs for the La Fernandina reservoir. The Pb concentrations were above the threshold in the La Fernandina reservoir and Cd concentrations were above the threshold in both reservoirs. However, the concentration of Cd measured only in muscle was below the maximum permissible levels (Fig. 2). This coincides with the results of Vilizzi and Tarkan (2016), and Öztürk et al. (2009) who indicated that regarding the bioaccumulation of PTEs in *C. carpio* tissues for Turkey, the Cd, and Pb occurred at concentrations above maximum allowed limits, and pollution has reached levels hazardous to the health of humans. High levels of Pb that exceed those found in the present study have been reported for common carp in Turkey with concentrations between 7.04 and 14.23 mg kg^{-1} (Canli et al. 1998), and in Algeria by Derrag and Dali-Youcef (2014) with concentrations of between 6.33 to 10.81 mg kg^{-1}. However, the concentrations of Pb in carp tissues in the present study are among the highest reported in different waterbodies worldwide (Vilizzi and Tarkan 2016).

High levels of Cd that exceed those found in the present study have been reported for common carp in Algeria by Derrag and Dali-Youcef (2014) with concentrations of from 2.3 to 2.7 mg kg^{-1}, by Canli et al. (1998) and Özparlak et al. (2012), and in Turkey with concentrations of between 0.93–1.99 and 0.54 mg kg^{-1}, respectively. The present study showed high concentrations for the two reservoirs studied, exceeding the established limits. This is probably due to the bioaccumulation capacity of the common carp; its bottom-feeding habits make the carp available to access the cadmium stored in the sediments. In this regard, Kilgour (1991) found that bottom-feeding animals showed relatively high body concentrations of cadmium. Furthermore, the biomagnification factor may depend on feeding regimes, as the bioaccumulation calculated in experimental studies using lower feeding rates appears to be lower than those using higher feeding rates, as contemplated by Connolly et al. (2023). Thus, the fact that carp is also a fish with a high lipid content could play a role in the bioaccumulation of certain toxic compounds.

For As, the concentrations were above the threshold maximum content of inorganic arsenic in food products by Regulation (EC) No. 2015/1006 (European Commission 2015) in the La Fernandina reservoir (Table 3). However, it is emphasized that this maximum limit is only indicative since it corresponds to the maximum value of inorganic arsenic allowed in rice products, as there is no limit value established for fish. It is important to mention that in the current study we measured the concentration of total arsenic in tissues of *C. carpio*, but the arsenic in fish can mostly be found in the form of organic arsenic, which is less toxic (Fakhri et al. 2020). The elevated levels of As in the tissues of common carp in the La Fernandina reservoir are related to the high concentrations of this PTE in the reservoir water which exceeded the maximum limits established by Spanish legislation for water intended for human consumption and to the high As concentration values found in sediments. This can be associated with the

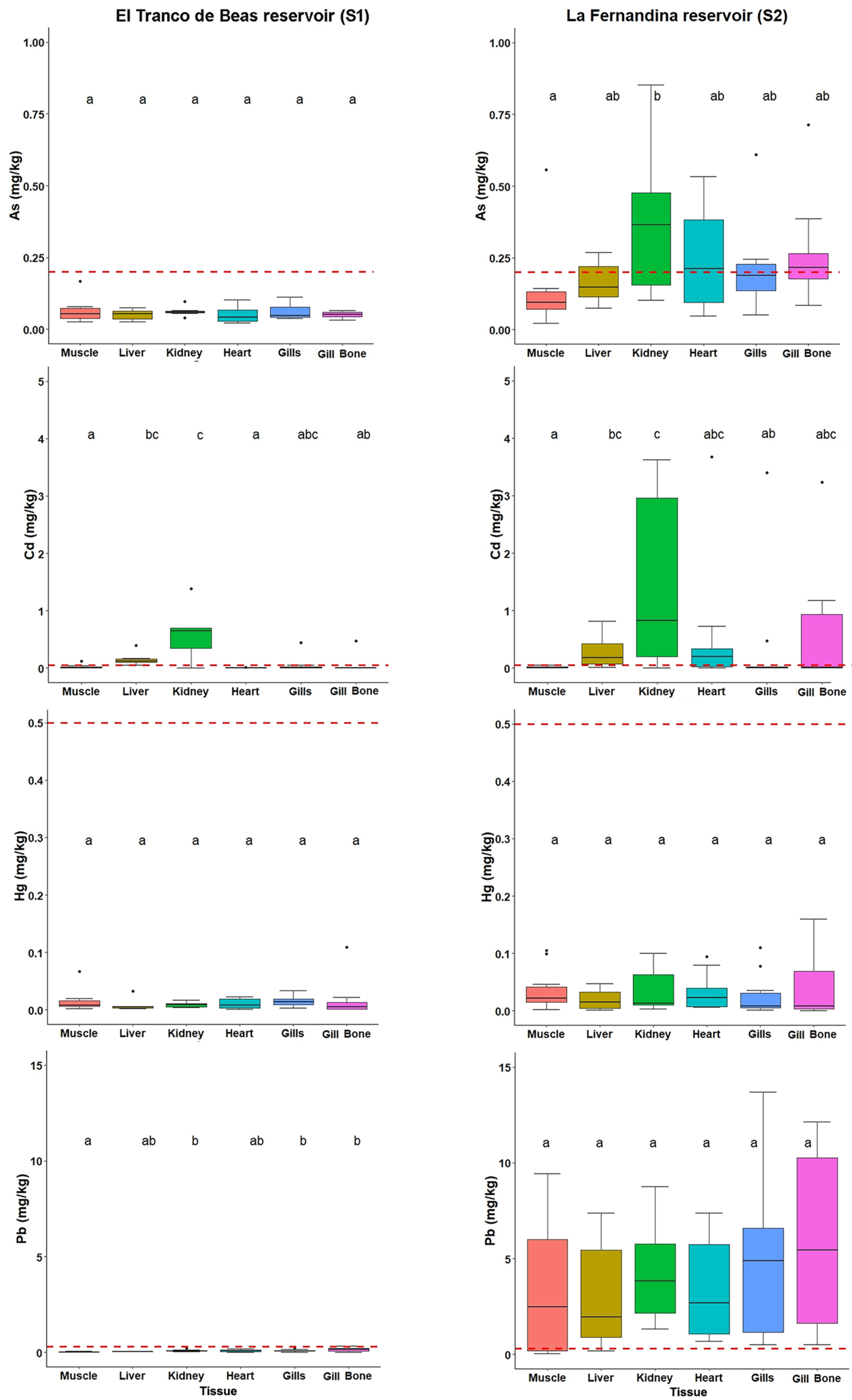

Fig. 2 Concentrations (mg kg^{-1}) of eight PTEs in the muscle, liver, kidney, heart, gill, and gill bone of *C. carpio* in the El Tranco de Beas (S1) and La Fernandina (S2) reservoirs, Jaén Spain. Red dash line indicates the maximum limits allowed by (EC) Regulation No. 1881/2006 of the European Union (mg kg^{-1} fresh weight): Pb: 0.300, Cd: 0.050, Hg: 0.500 (European Commission 2006). For As, the red dash line indicates the maximum content of inorganic arsenic in food products permitted by Regulation (EC) No. 2015/1006: As: 0.020 mg kg^{-1} (European Commission 2015). For Zn, the red dash line indicates the maximum permissible limits defined by FAO/WHO: Zn: 50.000 mg kg^{-1} (Vilizzi and Tarkan 2016). Pairwise statistically significant differences are indicated with different letters

reservoir being located over an old tungsten and galena mine. However, it is necessary to establish the different sources of arsenic contamination in the waters and sediments of the Fernandina Reservoir, because while this may be associated with the use of this PTE in former galena and silver mining it may also come from the recent use of chemicals in agricultural activities or industrial activities in the reservoir basin that may eventually

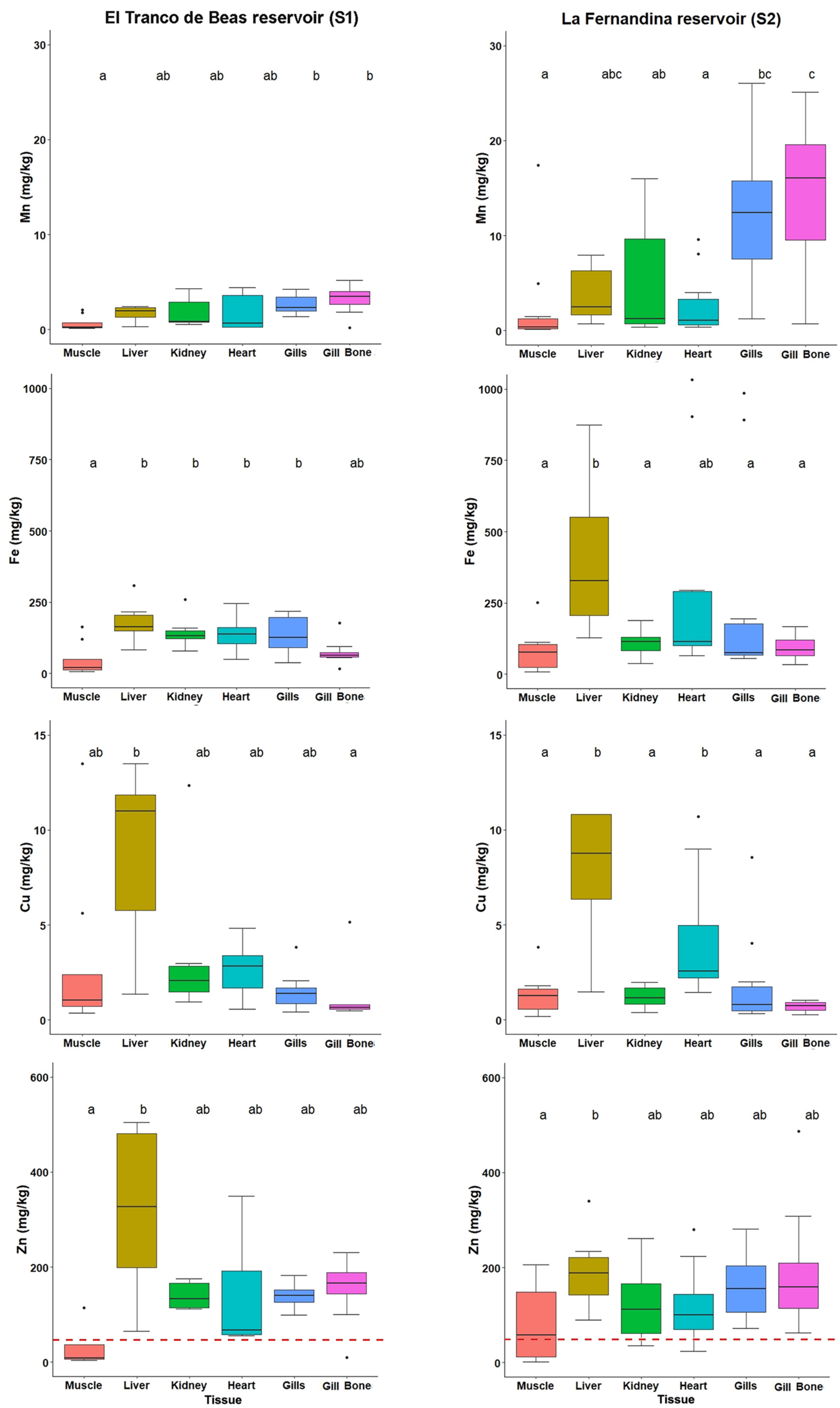

Fig. 2 (continued)

discharge effluents into the water. In this regard, Kandemir et al. (2010), Vilizzi and Tarkan (2016) and Köker (2022), who found arsenic levels at potentially dangerous concentrations in *C. carpio* in Turkish lakes, note that they are associated with chemical contamination from agricultural activities, and Tyokumbur and Okorie (2014) found that all mean concentration of As in the organs of common carp exceeded the World Health Organization guidelines limit in Nigeria due to discharges of preservatives used in industrial food and wood processing activities.

The Zn concentrations in carp tissues exceeded by four times in the El Tranco de Beas Reservoir, and three times in the La Fernandina reservoir the permissible limit values of 50 mg kg^{-1} defined by FAO/WHO (Table 3, Fig. 2). This coincides with the high Zn contents reported by Kandemir et al. (2010) and Kaptan and Tekin-Özan (2014) in Turkey as well as Hettige et al. (2015) in Sri Lanka, who also found values higher than 150 mg kg^{-1} of Zn in this species. High levels of Zn have been associated with the fact that it is an essential element for many fish's physiological activities (Zhang et al. 2019) as a constituent of many enzymes and is responsible for certain biological functions. However, Calabrese et al. (1985) mention that high concentrations of Zn appear to have a protective effect against the toxicities of Cd and Pb, this being a possible explanation for the high value of Zinc in tissues in the present study, taking into account that the levels of Cd in the tissues of the common carp exceeded the maximum levels allowed in the two reservoirs and the level of Pb exceeded them in the La Fernandina reservoir. In this regard, Yilmaz et al. (2010) mentioned that Zn substantially reduces the toxic effect of Cd justified by the increase of metallothioneins induced by Zn, since Cd ions form complexes with metallothioneins which inhibits their toxic effect. Murphy et al. (1978) found the highest cadmium and zinc concentrations in fish from industrially contaminated lakes in the USA, with values of 13.60 and 820 mg kg^{-1}, respectively.

In general, none of the PTEs presented correlation with the length or weight of *C. carpio*. In the El Tranco de Beas reservoir the PTE concentrations in the tissues of *C carpio* showed a strong positive correlation between Ni with Cr ($p < 0.01$), a positive correlation between Pb with Fe ($p < 0.05$) and a negative correlation Pb with Hg ($p < 0.05$) (Fig. 3). In the La Fernandina reservoir the results indicated a strong positive correlation between Cu with Cr ($p < 0.01$), Pb with Cr ($p < 0.01$) and Pb with Cu ($p < 0.01$), and a weak positive correlation between Cd with Co ($p < 0.05$), As with Cu ($p < 0.05$) and As with Zn ($p < 0.05$), and a weak negative correlation between As with Co ($p < 0.05$) (Fig. 3). In this regard, Ariyaee et al. (2015) reported no significant correlation between length, weight, and heavy metal concentration in common carp fish tissues, but other studies on the muscle of common carp have reported negative correlations of total length and weight with As, Cd, Fe, and Mn (Tekin Özan and Aktan 2012) and positive correlations with total mercury concentration (Parang and Esmaeilbeigi 2022).

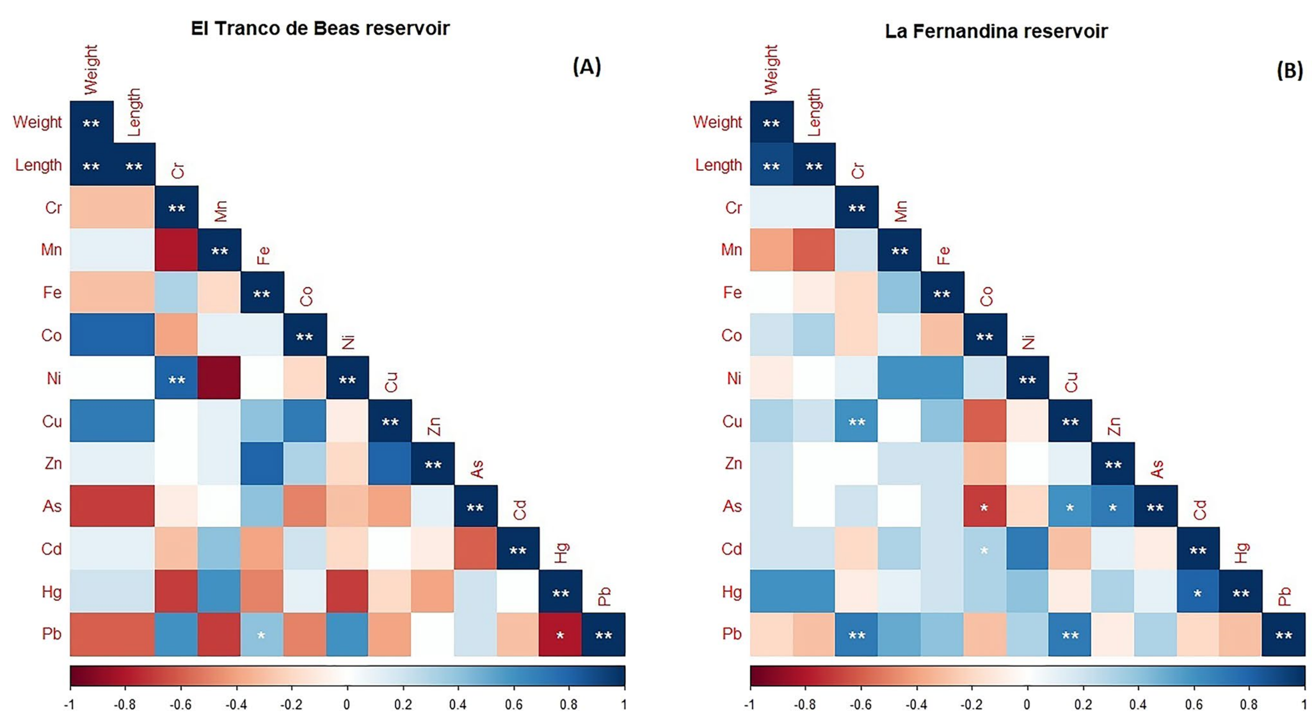

Fig. 3 The correlograms represent the correlations for all pairs of PTEs in *C. carpio* for **A** the El Tranco de Beas and **B** the La Fernandina reservoirs, Jaén Spain. Blue represents positive and red represents negative correlations. The color intensity is proportional to the coefficients (*$p < 0.05$, **$p < 0.01$). Arsenic (As), cadmium (Cd), cobalt (Co), chrome (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn)

Differences in PTE concentrations in tissues of the *C. carpio*

The values and the pairwise statistically significant differences in the mean concentration between each tissue of the *C. carpio* samples (muscle, liver, kidney, heart, gill, and gill bone tissue) are presented in Fig. 2.

As and Cd were mainly associated with the kidney and the concentration of most of the PTEs evaluated was lower in muscle than in other tissues. There were no significant differences in Hg concentration between tissues and the concentrations were always below the maximum limits established in the two reservoirs studied.

In the El Tranco de Beas reservoir, the Pb presented a higher concentration in gill and gill bone, although its values did not exceed the maximum limits established, and for the La Fernandina reservoir, although there were no significant differences, in all tissues the concentration exceeded the maximum limits established by European Union Regulation (EC) 1881/2006 in all tissues (European Commission, 2006) (Fig. 2). For both reservoirs, the gills exhibited a higher accumulation of Pb. This is in coincidence with the findings reported by Masoud et al. (2007), who found that gills showed a high accumulation of Pb due to the similarity of lead and calcium in their mobilization in the gills, and by Canli et al. (1998) who reported higher concentrations of Pb in gill and liver and the lowest concentration in muscle.

The concentration of As did not show significant differences between tissues in the El Tranco de Beas reservoir and its values were always below the limit of the maximum threshold content of inorganic arsenic defined in food products by Regulation No. 2015/1006 (European Commission 2015). In the La Fernandina reservoir, the mean concentration of As was significantly higher in kidney relative to other tissues, but liver, heart, gill, and gill bone also presented high values that exceeded the established limit value (Fig. 2). In the opposite way, Kandemir et al. (2010) found higher arsenic levels in muscle (2.462 mg kg^{-1}) than in the liver, and the arsenic levels could not be detected in the kidneys and gills, determining the source of As in *C. carpio* as a result of pesticides and herbicides present in agricultural effluents in the lakes of Turkey.

The concentration of Cd was significantly higher in kidneys relative to other tissues and also presented high values that exceeded the limit established by European Union Regulation (EC) No. 1881/2006 (European Commission 2006) in liver in both reservoirs, and in the heart and gill bone in the La Fernandina reservoir (Fig. 2). However, the muscle was the only tissue with Cd concentrations below the maximum permissible levels. Similar results have been found in different fish species showing that muscle is not an active tissue in accumulating PTEs (Karadede and Ünlü, 2000; Uysal et al. 2008; Ardakani and Jafari 2014). Canli et al. (1998) in Turkey reported higher concentrations of Cd in gill and liver and the lowest concentration in muscle. Our results are similar to those found by authors such as Moiseenko and Gashkina (2020) who find that the Cd concentrations in the kidneys of all the fish species analyzed are practically always more than double that in the liver. Cd concentrations in the muscles are two to three orders of magnitude lower than in the kidneys. The kidneys are organs in which exchange processes are active and Cd in particular circulates to the kidneys and is accumulated in the renal tissue, causing nephrotoxicity (Moiseenko and Gashkina 2020). The kidney is a target organ in heavy metal toxicity for its capability to reabsorb and concentrate divalent ions and metals, and Cd is among the most common metals implicated in kidney toxicity, depending on the nature, the dose, and the time of exposure (Lentini et al. 2017).

We found no significant differences in Cr and Ni concentrations between tissues in either of the two reservoirs. This is similar to the results reported by Vilizzi and Tarkan (2016) for *C. carpio* in water bodies of Anatolia, Turkey.

In the present study Cu, Fe, and Zn were mainly associated with the liver and Mn with the gill and gill bone, and always significantly higher relative to muscle tissue (Fig. 2), which is similar to the results reported by Tekin-Özan and Kir (2008) and Vilizzi and Tarkan (2016) for *C. carpio* in water bodies of Turkey, and by Ardakani and Jafari (2014) who found higher levels of Cu, Fe and Zn content in liver tissue. In this regard, Zhang et al. (2019) have highlighted that the higher concentrations of PTEs in the liver could be due to *C. carpio* obtaining its food from the bottom, as benthic feeding fish may have higher PTE concentrations (Yi et al. 2011).

The content of Cu in the liver was high in both reservoirs, being almost 3 to 4-fold higher than the level in other tissues (Fig. 2), which agrees with the findings of Zhang et al. (2019) who found a similar pattern in common carp in the upper Mekong River in China. The highest concentration of Cu in the liver is consistent with that reported in *C. carpio* in lakes of Turkey by Yaramaz (1986), who found the highest concentration in liver, by Öztürk et al. (2009) who found the highest concentration in heart followed by liver, and by Papagiannis et al. (2004) in the lakes of Greece who reported the highest level of Cu in the liver and the lowest levels in the muscle.

All fish tissues had significantly high Zn levels and they exceeded by three or four times the values reported as toxic, above the limit of 50 mg kg^{-1} body weight, according to the FAO/WHO (Vilizzi and Tarkan 2016). A similar result was reported by Zhang et al. (2019) who found higher levels of zinc in gills with respect to liver and muscle. Furthermore, the levels of Mn were significantly higher in gill and gill bone in both reservoirs in contrast to the other organs, this due to the fact that the gills might be a route of ingestion. In this sense, Tekin-Özan and Kir (2008) also found the highest Mn concentrations in gills followed by liver and muscle.

Conclusions

As and Fe showed the highest values in water in the S2 reservoir, above the maximum limits allowed by the sanitary criteria for the quality of water for human consumption in Spain. Similarly, the concentration of all PTEs in sediments was also higher in S2 than in S1, observing very high values of As, Fe, Mn, Pb, and Zn. The environmental indices calculated suggest a low ecological risk from PTEs in the basin sediments in S1 and moderate ecological risk for As in S2. After a preliminary study of PTEs in water and sediment samples, we analyzed the potential of using common carp as an ecological indicator of pollution. High concentrations of As, Cd, Pb, and Zn were found in tissues of common carp in S2, as well as high concentrations of Cd and Zn in S1, exceeding the maximum limits established for human consumption. We found significantly high bioaccumulation levels of As and Cd in kidney, Fe, Cu, and Zn in liver, and Pb and Mn in gill tissues, and these are suggested as target tissues for assessing this PTE accumulation. The results support the idea that the common carp is a good ecological indicator of environmental pollution in the reservoirs of southern Spain. Even though water or sediment analyses may not reflect high levels of contamination, the bioaccumulation of PTEs in carp species can become a reliable indicator of the presence of PTEs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-023-28637-z.

Acknowledgements The authors are grateful for the support of the Universidad de Jaén (Programa Operativo FEDER Andalucía 2014-2020, Plan Andaluz de Investigación, Desarrollo e Innovación PAIDI2020), Baltasar Deutor from the Centro de Instrumentación Cientifico-Técnica (CICT) and Research Groups RNM175. Néstor Javier Mancera-Rodríguez is grateful for the scholarships awarded by the Asociación Universitaria Iberoamericana de Postgrado – AUIP and Consejería de Economía, Conocimiento, Empresas, Junta de Andalucía, for a postdoctoral research position in 2022. Special thanks to Antonio Carrasco who provided updated information on the location and presence of fish species in each reservoir.

Author contribution Nestor Javier Mancera-Rodríguez: conceptualization, data interpretation, validation, writing—original draft preparation, writing—reviewing and editing. Daniel Ruiz Galiano: sampling design, data acquisition, investigation. Antonio Jesús López-Montoya: data curation, formal analysis, methodology. Eulogio J. Llorent-Martínez: investigation, methodology, resources. Lucía Molina García: investigation, methodology. Concepción Azorit: funding acquisition, conceptualization, validation, writing—reviewing and editing, and corresponding author. All authors agreed to the submitted manuscript with all its contents.

Funding This study has been developed with the support of the Research Group "*Biodiversity and sustainable development RNM175–PAIDI–*", University of Jaén, Spain and the scholarship awarded by the Asociación Universitaria Iberoamericana de Postgrado – AUIP and Consejería de Economía, Conocimiento, Empresas, Junta de Andalucía, Spain.

Data availability The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The present study has been carried out conforming to the legal requirements of the Spanish Government. The authors confirm that this manuscript is their original work. In this manuscript, all data collected during the study are provided, with no data published separately from the study.

Consent to participate The authors declare their consent to participate in this article.

Consent for publication The authors declare their consent to publish this article.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Nestor Javier Mancera-Rodríguez[1,2] · Daniel Ruiz Galiano[3] · Antonio Jesús López-Montoya[2,4] · Eulogio J. Llorent-Martínez[5] · Lucía Molina-García[5] · Concepción Azorit[2,3]

✉ Concepción Azorit
cazorit@ujaen.es

Nestor Javier Mancera-Rodríguez
njmancer@unal.edu.co

Daniel Ruiz Galiano
daniruizga@hotmail.com

Antonio Jesús López-Montoya
amontoya@ujaen.es

Eulogio J. Llorent-Martínez
ellorent@ujaen.es

Lucía Molina-García
Lucymolgar@gmail.com

1 Departamento de Ciencias Forestales, Grupo de Investigación Ecología y Conservación de Fauna Silvestre, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Sede Medellín, Bogotá, Colombia

2 PAIDI Research Group RNM175, Junta de Andalucía, Sevilla, Spain

3 Departamento de Biología Animal, Vegetal y Ecología, Área de Zoología, Facultad de Ciencias Experimentales, Universidad de Jaén, Jaén, Spain

4 Departamento de Estadística e Investigación Operativa, Facultad de Ciencias Experimentales, Universidad de Jaén, Jaén, Spain

5 Departamento de Química Física y Analítica, Área de Química Analítica, Universidad de Jaén, Jaén, Spain