Supplementary Materials

Seasonal differences in water pollution and liver histopathology of Iberian barbel (*Luciobarbus bocagei*) and Douro nase (*Pseudochondrostoma duriense*) in an agricultural watershed

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Table S1 – The analytical method, detection limit, and equipment used to determine the physicochemical parameters at the sampling points in the Vilariça River.

Parameter	Analytical method	Detection limit	Equipment
Total suspended solids (mg/L)	Gravimetry	-	
Nitrite (mg NO_2^-/L)	Spectrophotometry, sulfanilamide method Norma AFNOR NF T 90-013 (1975)	0.035	uni SPEC 2 Spectrophotometer UV-VIS
Nitrate (mg NO_3^-/L)	Spectrophotometry method, 2,6 - dimethylphenol (NP 4338-1)	1.25	uni SPEC 2 Spectrophotometer UV-VIS
Phosphate (mg P_2O_5/L)	Ascorbic acid method	0.23	uni SPEC 2 Spectrophotometer UV-VIS
Iron (μg Fe/L)	Atomic absorption spectrophotometry:electrochemistry	2.5	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Magnesium (mg Mg/L)	Atomic absorption spectrophotometry employing flame	0.50	Thermo Scientific ICE 3000 Series AA Spectrometer
Total aluminium (μg Al/L)	Atomic absorption spectrophotometry:electrochemistry	5	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Lead (μg Pb/L)	Atomic absorption spectrophotometry:electrochemistry	5	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Copper (μg Cu/L)	Atomic absorption spectrophotometry:electrochemistry	5	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Arsenic (μg As/L)	Atomic absorption spectrophotometry:electrochemistry	10	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Manganese (μg Mn/L)	Atomic absorption spectrophotometry:electrochemistry	2.5	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Zinc (μg Zn/L)	Atomic absorption spectrophotometry:electrochemistry	6	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Potassium (mg K/L)	Atomic emission spectrophotometry	1.0	Thermo Scientific ICE 3000 Series AA Spectrometer
Sodium (mg Na/L)	Atomic emission spectrophotometry	1.0	Thermo Scientific ICE 3000 Series AA Spectrometer
Calcium (mg Ca/L)	Atomic absorption spectrophotometry employing flame	0.50	Thermo Scientific ICE 3000 Series AA Spectrometer
Cadmium (μg Cd/L)	Atomic absorption spectrophotometry:electrochemistry	10	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Cobalt (μg Co/L)	Atomic absorption spectrophotometry:electrochemistry	5	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Nickel (μg Ni/L)	Atomic absorption spectrophotometry:electrochemistry	5	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Chromium (μg Cr/L)	Atomic absorption spectrophotometry:electrochemistry	2	UNICAM 939 AA Spectrometer Furmace UNICAM GF 90
Sulfates (mg SO_4^{2-}/L)	Turbidimetric method	5.0	uni SPEC 2 Spectrophotometer UV-VIS

Table S2 - The five classifications of surface water bodies for multiple uses according to Pereira [30], Rodrigues et al. [31], and SNIRH [32].

Parameter	A - no polluted	B - weakly polluted	C - polluted	D - very polluted	E - extremely polluted	Source
pH (Sorensen scale)	6.5–8.5	5.5–9	5–10	4.5–11	>11	SNIRH
Temperature (^{o}C)	≤ 20	25	28	30	> 30	Pereira
Conductivity (μS/cm,20°C)	≤ 750	1 000	1 500	3 000	>3 000	SNIRH
Total Suspended Solids (mg/L)	≤ 25	30	40	80	> 80	SNIRH
Dissolved oxigen (lab O_2 %)	≥ 90	70	50	30	< 30	SNIRH
Biochemical oxygen demand (mg O_2/L)	≤ 3	5	8	20	> 20	SNIRH
Chemical oxygen deficiency (mg O_2/L)	≤ 10	20	40	80	> 80	SNIRH
Oxidation (mg O_2/L)	≤ 3	5	10	25	> 25	SNIRH
Ammonium (mg NH_4^+/L)	≤ 0.5	1.5	2.5	4	> 4	SNIRH
Kjeldahl nitrogen (mg N/L)	≤ 0.5	1	2	3	> 3	SNIRH
Nitrate (mg NO_3^-/L)	≤ 5	25	50	80	> 80	SNIRH
Nitrite (mg NO_2^-/L)	≤ 0.01	0.02	0.15	0.3	> 0.3	Rodrigues
Orthophosphate (mg P_2O_5/L)	≤ 0.4	0.54	0.94	1	> 1	SNIRH
Total phosphorus (mg P/L)	≤ 0.2	0.25	0.4	0.5	> 0.5	SNIRH
Total coliforms (MPN/100ml)	≤ 50	5 000	50 000	$> 50 000$	-	SNIRH
Fecal coliforms (MPN/100ml)	≤ 20	2 000	20 000	$> 20 000$	-	SNIRH
Fecal Streptococci (MPN/100ml)	≤ 20	2 000	20 000	$> 20 000$	-	SNIRH
Iron (mg Fe/L)	≤ 0.5	1	1.5	2	> 2	SNIRH
Manganese (mg Mn/L)	≤ 0.1	0.25	0.5	1	> 1	SNIRH
Zinc (mg Zn/L)	≤ 0.3	1	3	5	> 5	SNIRH
Copper (mg Cu/L)	≤ 0.05	0.2	0.5	1	> 1	SNIRH
Chromium (mg Cr/L)	≤ 0.05	-	0.08	-	> 0.08	SNIRH
Cadmium (mg Cd/L)	≤ 0.001	-	0.005	-	> 0.005	SNIRH
Lead (mg Pb/L)	≤ 0.05	-	0.1	-	> 0.1	SNIRH
Mercury (mg Hg/L)	≤ 0.0005	-	0.001	-	> 0.001	SNIRH
Arsenic (mg As/L)	≤ 0.01	0.05	-	0.1	> 0.1	SNIRH
Cyanide (mg CN/L)	≤ 0.05	-	0.08	-	> 0.08	SNIRH
Phenol (mg C_6H_5OH/L)	≤ 0.001	0.005	0.01	0.1	> 0.1	SNIRH
Surfactants (LAS mg/L)	≤ 0.2	-	0.5	0.5	> 0.5	SNIRH

Table S3 – Liver histopathological changes observed in *L. bocagei* and *P. duriense* were grouped into five reaction patterns. An importance factor, ranging from 1 to 3, was attributed to each change.

Reaction pattern/Index	Histopathological alteration	Importance factor
Circulatory disturbances index	Congestion of blood vessels	1
Regressive changes index	Melanomacrophage centers	1
	Degenerative vacuolization	3
	Necrosis	3
	Endothelial rupture	3
	Periportal edema	2
	Necrosis of biliary epithelium	3
Progressive changes index	Vacuolization hepatocytes	1
	Fibrosis	3
	Hyperplasia of biliary epithelium	2
	Biliary duct epithelial detachment	2
Inflammation index	Exudate	1
	Lymphocyte infiltration	2

Table adapted by Bernet et al. [40]