sustainability

MDPI

Article

Two Age Groups of Adult Pikeperch (*Sander lucioperca*) as Bioindicators of Aquatic Pollution

Dušan Nikolić [1,*], Vesna Poleksić [2], Aleksandra Tasić [3], Marija Smederevac-Lalić [1], Vesna Djikanović [4] and Božidar Rašković [2]

[1] Department of Biology and Inland Waters Protection, Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia; marijasmederevac@imsi.rs
[2] Institute of Animal Sciences, Faculty of Agriculture, University of Belgrade, Nemanjina 6, Zemun, 11080 Belgrade, Serbia; poleksic@agrif.bg.ac.rs (V.P.); raskovic@agrif.bg.ac.rs (B.R.)
[3] Institute of Veterinary Medicine of Serbia, Janisa Janulisa 14, 11000 Belgrade, Serbia; alekstasic79@gmail.com
[4] Department of Hydroecology and Water Protection, Institute for Biological Research "Siniša Stanković"—National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia; djiki@ibiss.bg.ac.rs
* Correspondence: dusan@imsi.rs; Tel.: +381-11-2078477

Abstract: Pikeperch of age classes 3+ and 4+ were collected from the Garaši reservoir (Serbia) to analyze their bioindicator potential and compare their possible differences. Concentrations of 26 elements were determined in gills, muscles, and liver by inductively-coupled plasma optical emission spectrometry (ICP-OES) and of 17 organochlorine pesticides (OCPs), and six polychlorinated biphenyls (PCBs) in muscle by gas chromatography with mass spectrometric detection (GC-MS). Histopathological changes in the liver and gills were analyzed as biomarkers of general fish health. Only the concentrations of Cd, Na, and P in the muscles differed significantly. The OCPs and PCBs concentrations were below the detection limits, so fish meat consumption does not pose a risk concerning these substances. Hg and Cd exceeded the maximum allowed concentrations in some 4+ individuals, probably due to biomagnification. Gills were the most affected by metal exposure in both age classes. Histopathological changes and indices were minor and did not differ significantly between age classes, suggesting that pollution did not affect the morphology and structure of gills and liver. There were no significant correlations between elemental accumulation and fish condition or between histopathological scores. Therefore, both age classes can be used as bioindicators of pollution.

Keywords: fish; age class; toxic elements; organochlorine pesticides; bioaccumulation; histological alterations

Citation: Nikolić, D.; Poleksić, V.; Tasić, A.; Smederevac-Lalić, M.; Djikanović, V.; Rašković, B. Two Age Groups of Adult Pikeperch (*Sander lucioperca*) as Bioindicators of Aquatic Pollution. *Sustainability* **2023**, *15*, 11321. https://doi.org/10.3390/su151411321

Academic Editor: Pablo Pita

Received: 12 June 2023
Revised: 8 July 2023
Accepted: 19 July 2023
Published: 20 July 2023

1. Introduction

One of the most attractive fish species, both in recreational and commercial inland fisheries in Europe, is the pikeperch (pikeperch, sander, zander, sudak), *Sander lucioperca* L. (Actinopterygii, Perciformes, Percidae). This pelagic species is found in freshwater and brackish water habitats. It inhabits rivers, lakes, reservoirs, and canals, as well as estuaries and coastal areas of the sea with lower salinity [1]. Pikeperch prefers hard substrates (gravel and sand) and plant roots for egg deposition [1]. As an ichthyophagous species native to Eastern Europe, it plays an important role in the regulation of phytophagous fish used for the improvement of water quality, especially in reservoirs and lakes [2]. Large predatory fish, such as pikeperch, are keystone species, and their status can show profound indirect effects on ecosystem functioning [3].

Despite pikeperch being one of the most valuable fish in freshwater fisheries, trends in European pikeperch population status vary, and stock assessments are scarce. Most stocks are targeted by recreational fisheries, and catch estimates are uncertain. Stock assessment methods are useful to evaluate the status of fish populations and determine

management measures that should be applied [4]. In addition to human pressure, the effects of climate change, environmental fluctuations, lack of reproduction habitats, lack of food availability for juveniles, especially during overwintering months, increasing predation pressure (cormorants), and pollution are the main threats for pikeperch populations [4].

Pikeperch has been an economically important species for commercial and recreational fishing in Serbia for decades. Official statistics show data on annual catches gathered since 1948 [5]. According to catch statistics, pikeperch accounts for about 8% of the total fish catch in Serbia. From 2006 to 2021, the total catch of pikeperch in open waters varies—137 in 2006, 258 in 2013, and 176 tons in 2021. Looking at the statistical data, there is generally a decreasing trend for this species, but officially reported catches may not reflect actual harvest trends. There has been a sharp decline in catches in the commercial fishery during the period cited [6]. Over the past decade, pikeperch catches in recreational fisheries have exceeded commercial fishing catches. It is an important food fish, and the entire harvest is used for human consumption. This is why sustainable fishing should be imposed as a priority.

As an important fish for human consumption, considerable efforts have been made to increase the stock in the ponds [7–9]. Even though pikeperch is considered an important species for intensive culture in Europe [9,10], there is no pikeperch production in Serbia, though sometimes it is stocked in common carp ponds to control pest fish [11].

As an apex predator in freshwater ecosystems, pollutants found in the water and biota are expected to accumulate in the various organs and tissues of this species [12,13]. Reservoirs and lakes are collectors of persistent pollutants. Accumulated elements can be resuspended from the sediment into the water column over time and transformed into bioavailable pollutants, such as methylmercury MeHg [14].

Toxic elements can affect the growth, physiological functions, and reproduction of aquatic animals. Certain elements (Fe, Cu, Zn, Cr) are essential but can be toxic in higher concentrations, while others (Pb, Hg, Cd, As) are not essential and are toxic even in trace amounts [15]. The level of organohalogen residues in the environment is changing slowly over time. Estimates of dietary intake show that the highest concentrations of most persistent organic pollutants (POPs) are ingested through food, especially organochlorine residues (OCPs) and polychlorinated biphenyls (PCBs), with fish being the most important source [16].

According to the literature [17], age classes 3+ and 4+ cover the expected maturation of both genders. The present study aims to determine the contaminant (both elements and organics) concentrations in the adult specimens of pikeperch, one of the most important fish species in recreational and commercial fisheries, as well as to compare the obtained results with histopathological (changes in the anatomy of the gills and liver) and organosomatic (condition index) biomarkers.

2. Material and Methods

In this study, we measured concentrations of 26 elements in different tissues (muscle, gills, and liver) and 17 organochlorine pesticides—OCPs and six polychlorinated biphenyls—PCBs in the muscles of age classes 3+ and 4+ pikeperch. The results obtained were compared with the condition of the fish, and it was estimated which age class is a suitable bioindicator of water pollution. Fish of age classes 3+ (3 years old) and 4+ (4 years old) were selected because fish of this age are most commonly available on the market for human consumption.

2.1. Sampling Location

The Garaši reservoir was selected for the assessment and sampling of this fish species. The reservoir was built in 1977 as a drinking water supply for the city of Aranđelovac (approx. 46,000 inhabitants) in central Serbia. According to the previously published results, the Garaši reservoir is constantly exposed to agriculture runoff and effluents from the wastewater treatment plant, which increases the nutrient load and concentrations of toxic elements [12,18].

2.2. Fish Sampling

Pikeperch fishing in Serbia is regulated by a closed season (1 March–30 April) and a minimum landing size of 40 cm [19]. In recreational fishing, restrictions for daily bag limits are 3 specimens. Individuals of pikeperch (20 in total) were sampled in the summer of 2017 at Garaši reservoir (44.286876 N, 20.473896 E), using a set of standing gillnets (30 m × 2 m, 35–40 mm mesh size). The gillnets were left in the water overnight. Fish were sacrificed by a quick blow to the head. The age of each fish was determined by analyzing the scales between the dorsal fin and lateral line. Total body length (L, cm) and body weight (W, g) were measured, and the condition index or Fulton's condition factor (CF) of each fish was calculated [20]:

$$CF = WL^{-3} \times 100 \quad (1)$$

Samples were prepared and processed for elemental, OCPs, and histopathological analysis, according to Nikolić et al. [21].

2.3. Elemental Analysis

The concentrations of 26 elements: macroelements (Ca, K, Mg, P, S), essential (B, Co, Cu, Fe, Mo, Mn, Se, Si, Zn), and non-essential (Ag, Al, As, Ba, Cd, Cr, Hg, Li, Ni, Pb, Sr) microelements, were measured using ICP-OES (Spectro Genesis EOP II, Spectro Analytical Instruments DmbH, Germany) and expressed as μg g^{-1} dry weight (dw). The wavelength lines of the ICP-OES analysis are presented in the Supplementary Material. Three blank samples were run to determine the potential presence of the analyzed elements in the reagents used. The digested samples were diluted with distilled water to reach a volume of 25 mL. Bovine liver BCR-185R and lichen reference material IAEA-336 were used in the analysis to ensure that the detected concentrations were within 90–115% of the certified values for all elements analyzed.

The element concentrations in muscle tissue were compared with the maximum allowed concentrations (MACs) established in the national legislation of Serbia [22] and the European Union (EC) [23] to evaluate the health risks of pikeperch for human consumption. According to both legislations, the MAC for Cd is 0.05 μg g^{-1} ww, Hg is 0.50 μg g^{-1} ww, and Pb 0.3 μg g^{-1} ww. National legislation [22] also prescribes MACs for Cu, Zn, and As, which are 30.0, 100.0, and 2.0 μg g^{-1} ww, respectively.

The metal loading index (MPI) has been used to assess the total metal content in various tissues of pikeperch (Usero et al. 1997) [24]:

$$MPI = (C_1 \times C_2 \times \ldots \times C_n)^{1/n} \quad (2)$$

where C_n is the mean concentration of metal n in the analyzed tissue (μg g^{-1} wet weight).

2.4. OCPs and PCBs Analysis

Analysis of the presence of OCPs (aldrin, α-HCH, β-HCH, γ-HCH, δ-HCH, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, metoxychlor) in fish muscles was performed using gas chromatography with mass spectrometric detection (GC-MS) and an autosampler. In addition, the sum of six indicator PCB congeners (PCB 28, 52, 101, 138, 153, and 180) was determined simultaneously with the OCPs by GC/MS under the same preparation and determination conditions. Pesticide-grade acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ, USA) and used for sample extraction. Analytical standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). A mixture of OCP and PCB standards was prepared in acetonitrile at a concentration of 10 mg/L and stored in dark glass vials at −20 °C. Working solutions were prepared through appropriate dilution at concentrations of 0.01, 0.02, 0.03, 0.05, and 0.1 mg kg^{-1}. The internal standard triphenyl phosphate (ISTD) was used for calibration and quantification (Dr. Ehrenstorfer). Calibration curves were drawn using the ratio of the area of standard pesticides or PCBs to the

ISTD peak area (*y*-axis) relative to the concentration of the standard (*x*-axis) and fitted with an inverse-weighted (1/x) linear regression.

The system was a Clarus 680 gas chromatograph PerkinElmer with a Clarus SQ8T mass spectrometer. Chromatographic separation was performed using an Elite-CLPesticides capillary column (30 × 0.25 mm ID × 0.25 df, PerkinElmer part no. N9316662). The injection volume of the calibration standards and the tested samples was 2 µL. The system was operated with high-purity helium inert gas under a constant flow rate of 1 mL min^{-1}. The ion source temperature was 250 °C, and the inlet line temperature was 280 °C. The temperature of the injection port was isothermally set at 250 °C. The system was operated in electron impact mode at 70 eV. The oven temperature program was initially set at 80 °C for 2 min and increased to 150 °C at a rate of 25 °C min^{-1}, then to 200 °C at 3 °C min^{-1}, and finally to 280 °C at 9 °C min^{-1}, and held for 9 min. Turbo Mass v.6.1.0 software was used for data processing. Four ions of each analyte tested were scanned using the selected ion monitoring (SIM mode). Full scan mode was used continuously throughout the chromatographic run.

SANTE requirements were followed for method validation and quality assurance [25]. The latest version of the validation guidelines, SANTE 11312/2021, was used. A certified reference material blank sample of fatty fish (Fapas-Sand Hutton, York, UK) was used for blank matrices. The fatty fish sample was selected because it contains fat, i.e., a composition of fatty acids and proteins corresponding to the fish matrix. Blank matrices of oily fish were used to generate the matrix calibration curves to account for the matrix effect in the calibration, as well as in the quantification. The present modified QuEChERS method was validated for linearity, limit of detection (LOD), limit of quantification (LOQ), trueness, precision, and recovery on the spiked level 0.01 mg kg^{-1} and 0.02 mg kg^{-1}. For the recovery and precision experiment, we also used a 10 g blank sample of oily fish. A blank fish sample was added with a standard mixture of pesticides at 0.01 and 0.02 mg kg^{-1} (six replicates each). The performance parameters of the method are given in the Supplementary Material (Table S1).

All OCPs and PCBs concentrations are expressed as mg kg^{-1}. The concentrations of 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, heptachlor, and heptachlor epoxide in fish muscle were compared with the maximum allowed concentrations (MAC) in fish meat specified in the national legislation of Serbia [26]. The MAC for DDT and derivatives is 1.0 mg kg^{-1}, and heptachlor and heptachlor epoxide 0.1 mg kg^{-1}. The MAC for sum indicator PCB congeners (PCB 28, 52, 101, 138, 153, and 180) is 125 ng g^{-1}.

2.5. Histopathological (HP) Analysis of Gills and Liver

To assess the general health of the fish and the possible effects of toxic elements on the microscopic anatomy of the organs, we opted for a histopathological assessment of the gills and liver. This method is widely used in environmental toxicology and can detect the effects of contamination on various fish species [27–29]. For grading HP alterations in the gills and liver of all sampled individuals (20 in total), a semiquantitative scoring system published by Bernet et al. [30] was implemented. It is based on categorizing each lesion into one of four reaction patterns: circulatory, progressive, inflammatory, and regressive. Each HP alteration is assigned an importance factor (IF) ranging from 1 (minimal importance) to 3 (marked importance). A score value from 0 to 6 (0 = none, 2 = mild, 4 = moderate, and 6 = severe alteration) is given for the extent of a particulate lesion. IF and the score values were used to obtain values for various HP indices [30]:

(a) Reaction index of organ:

$$I_{\text{org rp}} = \sum_{\text{alt}} \left(a_{\text{org rp alt}} \times w_{\text{org pr alt}} \right) \quad (3)$$

(b) HP index of organ:

$$I_{\text{org}} = \sum_{\text{rp}} \sum_{\text{alt}} \left(a_{\text{org rp alt}} \times w_{\text{org pr alt}} \right) \quad (4)$$

(c) Total index for each individual fish:

$$I_T = \sum_{org} \sum_{rp} \sum_{alt} \left(a_{org\ rp\ alt} \times w_{org\ pr\ alt} \right) \quad (5)$$

where org stands for the organ (I_G-gills; I_L-liver), rp for the reaction pattern, alt for the alteration, *a* for a score value, and *w* for the importance factor.

2.6. Statistical Analysis

Normality and homoscedasticity of all data sets were tested with the Shapiro-Wilk and Levene tests, respectively. If the data sets did not show a normal distribution, comparisons of the element concentrations/HP scores were carried out using the Mann–Whitney *U* test. If the data sets met the assumptions of normality and homoscedasticity, significant differences between the groups were tested with the *t*-test. Similarly, significant differences in elemental concentrations for certain element between analyzed tissues was tested by the Kruskal–Wallis *H* test followed by the Mann–Whitney *U* test or one-way ANOVA followed by Tukey's HSD *post-hoc* test. Correlation tests between the condition of the fish and the element concentration, as well as between the HP scores for gills and liver, were examined using Spearman's rank correlation test. The significance level was set at 5%.

3. Results

3.1. Size and Condition of Analyzed Fish

The average body length and weight of the pikeperch individuals per age class, as well as Fulton′s condition factor, are given in Table 1. As expected and confirmed by statistical tests, 4+ individuals were longer and heavier than 3+ individuals.

Table 1. Number of individuals (n), total length (L), weight (W), and Fulton's condition factor (CF) of pikeperch individuals, as well as the metal pollution index (MPI), and element concentrations (μg g^{-1}dw) in muscle, gills, and liver tissue in two age classes of pikeperch individuals from Garaši reservoir. Values are presented as mean ± SD, while ND indicates values below the detection threshold.

		Age				
		3+	**BT**	**4+**	**BT**	**BG**
	n	8		12		
	L	37.4 ± 3.6		45.6 ± 3.5		
	W	422.6 ± 119.8		774.6 ± 157.7		
	CF	0.79 ± 0.03		0.82 ± 0.12		
	Tissue					
MPI	Muscle	3.38		2.15		
	Gills	6.29		7.60		
	Liver	5.60		4.08		
Ag	Muscle	0.01 ± 0.01		0.01 ± 0.01		
	Gills	0.04 †		0.01 †		
	Liver	0.01 ± 0.01		0.01 ± 0.01		
Al	Muscle	55.13 ± 60.40		32.09 ± 19.25		
	Gills	71.11 ± 88.28		228.71 ± 321.69		
	Liver	83.55 ± 82.00		44.23 ± 29.51		
As	Muscle	0.09 ± 0.11		0.21 ± 0.23		
	Gills	0.15 ± 0.15		0.16 ± 0.19		
	Liver	0.11 ± 0.16		0.16 ± 0.26		
B	Muscle	0.58 ± 0.60		0.28 ± 0.50 [b]		
	Gills	0.62 ± 0.27		1.91 ± 2.48 [a]	*	
	Liver	1.36 ± 1.17		0.99 ± 1.17 [a]		
Ba	Muscle	1.33 ± 1.11		1.56 ± 1.01		
	Gills	1.53 ± 1.30		1.18 ± 1.23		
	Liver	1.16 ± 1.08		1.25 ± 1.06		
Ca	Muscle	3503.81 ± 3448.33 [b]		1474.72 ± 958.03 [b]		
	Gills	23209.67 ± 5389.49 [a]	***	20661.45 ± 5731.74 [a]	***	
	Liver	599.71 ± 379.96 [c]		607.64 ± 442.34 [c]		

Table 1. *Cont.*

		Age				
		3+	**BT**	**4+**	**BT**	**BG**
Cd	Muscle	0.15 ± 0.02 $^{c, A}$		0.12 ± 0.02 $^{b, B}$		*
	Gills	0.27 ± 0.10 ^{a}	***	0.24 ± 0.07 ^{a}	**	
	Liver	0.18, 0.12 ‡,^{b}		0.28 ± 0.48 ^{a}		
Co	Muscle	0.05 ± 0.01 ^{a}		0.04 ± 0.02 ^{a}		
	Gills	0.04 ± 0.03 ^{a}	***	0.03 ± 0.02 ^{a}	***	
	Liver	ND ^{b}		0.004 ± 0.01 ^{b}		
Cr	Muscle	0.19 ± 0.08 ^{a}		0.22 ± 0.09 ^{a}		
	Gills	0.16, 0.06 ‡ ^{b}	***	0.04 ± 0.09 ^{b}	***	
	Liver	0.26 ± 0.08 ^{a}		0.23 ± 0.04 ^{a}		
Cu	Muscle	ND ^{b}		0.10 † ^{b}		
	Gills	ND ^{b}	*	ND ^{b}	*	
	Liver	7.46 ± 17.75 ^{a}		1.23 ± 2.00 ^{a}		
Fe	Muscle	55.16 ± 96.63 ^{c}		26.10 ± 20.01 ^{b}		
	Gills	153.20 ± 118.64 ^{b}	**	324.24 ± 291.66 ^{a}	***	
	Liver	401.10 ± 222.87 ^{a}		373.97 ± 354.04 ^{a}		
Hg	Muscle	1.49 ± 0.50 ^{a}		1.67 ± 1.02 ^{a}		
	Gills	0.43 ± 0.24 ^{b}	**	0.36 ± 0.31 ^{b}	**	
	Liver	0.90 ± 0.52 ^{b}		1.24 ± 1.00 ^{a}		
K	Muscle	9457.53 ± 688.14 ^{a}		8466.27 ± 1465.18 ^{a}		
	Gills	6075.07 ± 1215.45 ^{b}	***	6683.34 ± 571.74 ^{b}	***	
	Liver	6744.30 ± 750.41 ^{b}		6968.08 ± 815.62 ^{b}		
Li	Muscle	0.30 ± 0.32 ^{b}		0.41 ± 0.27 ^{b}		
	Gills	1.29 ± 0.77 ^{a}	*	2.07 ± 1.60 ^{a}	*	
	Liver	0.56 ± 0.40 ^{ab}		0.50 ± 0.97 ^{b}		
Mg	Muscle	1246.11 ± 183.56 ^{a}		1075.11 ± 224.32 ^{a}		
	Gills	1397.34 ± 379.14 ^{a}	**	1283.96 ± 451.70 ^{a}	***	
	Liver	659.34 ± 139.46 ^{b}		667.71 ± 131.36 ^{b}		
Mn	Muscle	0.15 ± 0.24 ^{c}		0.08 ± 0.15 ^{c}		
	Gills	16.29 ± 8.08 ^{a}	***	16.36 ± 7.63 ^{a}	***	
	Liver	4.46 ± 3.79 ^{b}		5.73 ± 2.88 ^{b}		
Mo	Muscle	0.09 ± 0.10 ^{b}		0.08 ± 0.07 ^{b}		
	Gills	0.13 ± 0.04 ^{b}	***	0.32 ± 0.65 ^{a}	***	
	Liver	0.48 ± 0.22 ^{a}		0.38 ± 0.16 ^{a}		
Na	Muscle	2065.22 ± 376.42 $^{b, A}$		1594.13 ± 269.53 $^{b, B}$		***
	Gills	3575.45 ± 744.28 ^{a}	***	3282.75 ± 181.35 ^{a}	***	
	Liver	3210.68 ± 391.50 ^{a}		3086.44 ±296.70 ^{a}		
Ni	Muscle	1.10 ± 0.19		1.12 ± 0.17		
	Gills	1.29 ± 0.38		1.16 ± 0.24		
	Liver	1.18 ± 0.28		1.21 ± 0.14		
P	Muscle	7698.28 ± 1892.31 $^{b, A}$		6121.35 ± 1423.87 $^{c, B}$		*
	Gills	28431.54 ± 10063.35 ^{a}	***	22684.94 ± 11497.63 ^{a}	***	
	Liver	8520.23 ± 2069.44 ^{b}		8854.67 ± 1619.43 ^{b}		
Pb	Muscle	0.10 ± 0.09		0.03 ± 0.06 ^{b}		
	Gills	0.45 ± 0.48		2.74 ± 4.58 ^{a}	***	
	Liver	0.09, 0.08 ‡		0.08 ± 0.08 ^{b}		
S	Muscle	9379.51 ±1935.98 ^{a}		8851.37 ± 2459.50 ^{a}		
	Gills	5052.70 ± 455.58 ^{b}	**	4935.37 ± 1925.17 ^{b}	***	
	Liver	6201.48 ± 1967.55 ^{b}		5418.45 ± 1593.82 ^{b}		
Se	Muscle	3.06 ± 1.87 ^{b}		2.65 ± 1.43 ^{b}		
	Gills	10.13 ± 4.21 ^{a}	**	7.54 ± 4.97 ^{a}	*	
	Liver	5.00 ± 1.75 ^{b}		3.38 ± 2.77 ^{b}		
Si	Muscle	67.47 ± 86.86		49.40 ± 30.82 ^{b}		
	Gills	125.52 ± 146.14		325.62 ± 412.45 ^{a}	*	
	Liver	64.71 ± 71.47		41.07 ± 33.61 ^{b}		
Sr	Muscle	ND ^{b}		ND ^{b}		
	Gills	42.13 ± 26.23 ^{a}	***	29.94 ± 27.22 ^{a}	***	
	Liver	ND ^{b}		ND ^{b}		
Zn	Muscle	17.62 ± 4.32 ^{b}		14.17 ± 2.32 ^{b}		
	Gills	64.26 ± 6.97 ^{a}	***	55.76 ± 10.68 ^{a}	***	
	Liver	59.44 ± 16.00 ^{a}		52.00 ± 12.44 ^{a}		

$^{a–c}$ Values with different lowercase letters between tissues (BT) for certain elements are significantly different (*—$p < 0.05$, **—$p < 0.01$ and ***—$p < 0.001$, refers to overall values of Kruskal-Wallis H test or one-way ANOVA). $^{A, B}$ Values with different capital letters in the same row are significantly different between 3+ and 4+ groups (BG) (Mann-Whitney U test or t-test; *—$p < 0.05$, **—$p < 0.01$ and ***—$p < 0.001$). † Concentrations above the detection threshold only in one sample. ‡ Concentrations above the detection threshold only in two samples.

3.2. Element, OCPs, and PCBs Analyzes

The highest concentrations of K and S were detected in the muscles, Ca, Mn, P, Se, and Sr in the gills, and Cu and Mo in the liver in all study cases (Table 1). On the other hand, the lowest concentrations of MnNa, and Zn were observed in the muscles, Cr in the gills, and Ca and Co in the liver. According to the MPI, the gills were exposed to the highest pressure of metals in both age groups (Table 1). In both age classes, Al, Ba, and Ni in all tissues, as well as Hg in muscle and Sr in gills, greatly influenced heavy metal concentration (each of them with a concentration greater than 1.0 μg g^{-1} dw). The MPI values for gills and liver indicated that individuals in the 4+ age class were under higher pressure of metal pollution. We found no significant correlations between the elemental accumulation and fish condition.

The concentrations of Hg in muscle tissue of six individuals of age class 4+ and of Cd in two individuals of age class 4+ exceeded the MACs (concentrations given by both Serbia and the EU). Only 3, Cd (t = 2.296106; p = 0.033894), Na (t = 3.271997; p = 0.004235), and P (t = 2.129760; p = 0.047246) in muscle, out of 78 (26 elements × 3 tissues) possible differences in element accumulation between age class 3+ and 4+ pikeperch were found to be statistically significant (Table 1).

The optimization of data processing parameters is presented in the Supplementary Material. The LOQs of the analytes ranged from 0.003 to 0.007 mg kg^{-1}. The identical response of certain groups of polychlorinated derivatives of cyclohexane (lindane), polychlorinated cyclodiene (endosulfan), and polychlorinated biphenyls (DDT) confirms the narrow value of the angular coefficient from the calibration curves. The applicability of this modern modified approach to determine pesticides allowed a more accurate determination of the residues. The recovery was within the acceptable range of 70–120%. It can be concluded that the method used could effectively determine the desired analytes with satisfactory performance. When the real samples were analyzed, none had residues and PCBs above the levels of LOQ. None of the OCPs analyzed were detected in the pikeperch muscle.

3.3. Histopathological (HP) Analysis

In general, low histopathological scores for gills and liver were observed in both age classes (Table 2). The histopathological changes in gills and liver, as well as the histopathological indices, did not differ significantly between the age classes and are shown in Figure 1. In the gills, the only alteration with moderate/severe scores (mean alteration score >2) was edema of the primary epithelium. HP indices for the liver were higher than for the gills, indicating that the liver is a more sensitive organ to pollutants. This was also confirmed by higher scores for necrosis in the liver compared to the gills.

Table 2. HP scores presented as mean values ± SD, with importance factor (IF) for gills and liver alterations in two age classes of pikeperch individuals from the Garaši reservoir.

		Age Class	
Histopathological Alteration	**IF**	**3+**	**4+**
Gills			
Hyperaemia	1	2.0 ± 3.5	1.8 ± 1.8
Edema of primary epithelium	1	4.7 ± 1.2	3.0 ± 1.7
Edema of secondary epithelium	1	1.3 ± 2.3	1.0 ± 1.1
Hypertrophy of epithelial cells	1	2.0 ± 0.0	0.8 ± 1.0
Architectural and structural alterations	1	0.7 ± 1.2	0.2 ± 0.6
Presence of eosinophilic granular cell	1	0.7 ± 1.2	1.4 ± 1.9
Hyperplasia of epithelial cells	2	ND	0.4 ± 0.8
Leukocyte infiltration	2	0.7 ± 1.2	1.4 ± 1.9
Necrosis	3	ND	0.2 ± 0.6
I_{GP}		2.0 ± 0.0	1.6 ± 1.6
I_{GC}		6.6 ± 4.6	4.8 ± 2.9
I_{GR}		2.0 ± 2.0	1.8 ± 2.4
I_{GI}		2.0 ± 2.0	4.2 ± 4.8
I_{G}		12.7 ± 7.0	12.4 ± 8.0

Table 2. *Cont.*

Histopathological Alteration	IF	Age Class	
		3+	4+
Liver			
Sinusoidal congestion	1	4.0 ± 2.4	2.0 ± 2.3
Sinusoidal dilation	1	2.8 ± 3.0	1.0 ± 2.3
Fatty degeneration	1	2.3 ± 1.7	1.7 ± 2.4
Fibrosis of periportal and portal areas	2	0.5 ± 0.9	1.2 ± 1.6
Pyknosis of hepatocytes' nuclei	2	1.5 ± 1.8	1.3 ± 1.3
Vacuolation of hepatocytes	2	2.5 ± 2.6	2.2 ± 2.5
Stasis	2	3.5 ± 3.0	1.2 ± 2.3
Leukocyte infiltration	2	ND	1.0 ± 1.8
Necrosis	3	1.8 ± 2.0	0.7 ± 1.0
I_{LP}		1.0 ± 1.9	2.3 ± 3.2
I_{LC}		7.5 ± 5.3	3.2 ± 3.5
I_{LR}		18.3 ± 13.4	11.7 ± 7.1
I_{LI}		ND	2.0 ± 3.6
I_{L}		26.8 ± 16.5	19.2 ± 12.3
I_{T}		31.5 ± 16.2	29.5 ± 10.9

HP scores ranged from 0 (no alteration) to 6 (severe alteration) occurrence. Subscripted letters meaning: G—gills, L—liver, T—total, P—progressive, C—circulatory, R—regressive, I—inflammatory.

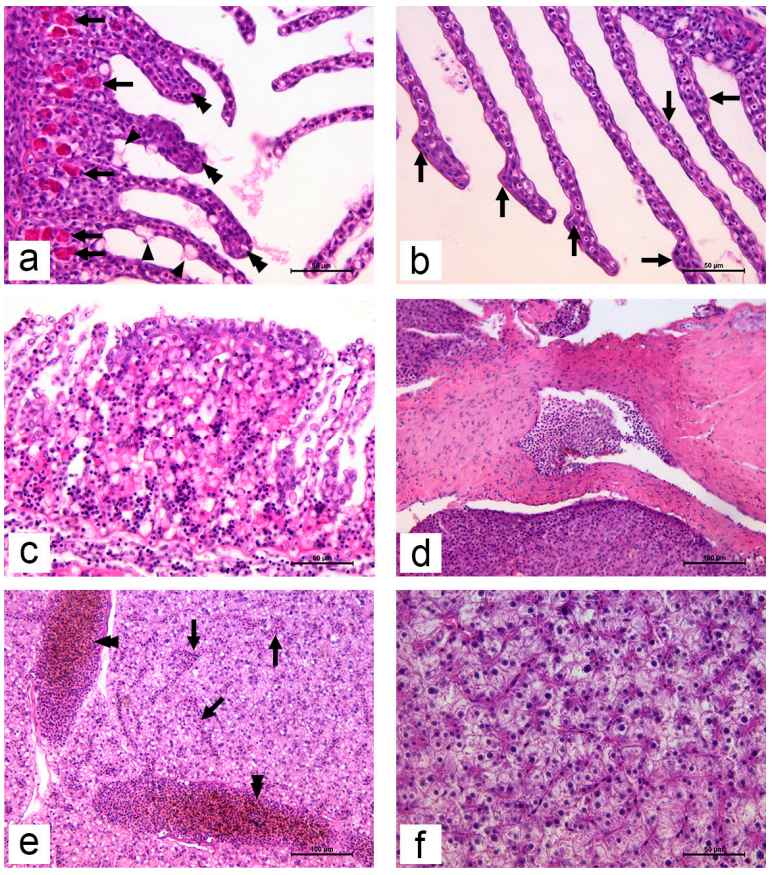

Figure 1. Some of the histopathological alterations found in gill (**a**–**c**) and liver (**d**–**f**) samples in the present study: (**a**) proliferation of the epithelium around one or two secondary lamellae (double arrowheads) and infiltration of eosinophilic granulocytes in the primary epithelium (arrows). Note mucous cells in primary and secondary lamellae emptying their contents in the environment (arrowheads) (HE × 400); (**b**) hypertrophy of respiratory epithelium (arrows), especially pronounced on the tips of secondary lamellae (HE × 400); (**c**) hyperplasia of the epithelium between the secondary lamellae and subsequent necrosis of the tissue with infiltration of leucocytes (HE × 400); (**d**) vascular fibrosis (HE × 200); (**e**) blood stasis in the vessels (double arrowheads) and congestion of sinusoid capillaries (arrows) (HE × 200); (**f**) vacuolization of hepatocytes in the liver parenchyma (HE × 400).

4. Discussion

4.1. Element, OCPs, and PCBs Analyzes

In both age classes, the gills were most affected by metal pollution, probably because this organ is in direct contact with the pollutants in the water [31]. The highest MPI values for gills were also recorded in pikeperch [12] as well as in other fish species, e.g., [21,28].

In contaminated ecosystems, toxic elements reach much higher levels than in fish sampled in less polluted environments [14]. The results of previous studies [14,32,33] state that the accumulation of toxic elements (Hg and Cd) can be two to four times higher in fish of higher trophic levels compared to omnivorous fish (i.e., common carp and mullet). This is especially true for Hg, as this element biomagnifies through the food chain [14]. The pikeperch is considered an apex predator in both lakes and rivers, with an estimated trophic position between 4 and 5 [34].

The concentrations of Hg in muscle tissue of six individuals of age class 4+ and of Cd in two individuals of age class 4+ exceeded the MACs (concentrations given by both Serbia and the EU). This could be due to the high accumulation of toxic elements in the sediments of the Garaši reservoir [35]. Cd has been classified as a category 1 carcinogen (IARC 1993) [36] for humans and can cause damage to the liver, lungs, and testes [37]. Although not directly mutagenic itself, it has been associated with lung, prostate, and kidney cancer [38]. Bioavailable contaminants, such as methylmercury MeHg, are responsible for stomatitis and kidney injuries in humans [39]. According to studies by Gochfeld [40] and Davidson et al. [41], chronic exposure to MeHg causes central nervous system damage, cerebral palsy and mental retardation, and blindness in infants born to mothers with high levels of Hg. Nabavi et al. [42] also reported that concentrations of Cd and Pb in pikeperch sampled from the Caspian Sea exceeded the MACs. Concentrations of toxic elements in pikeperch from the Danube River near Belgrade [43,44], the Gruža reservoir [45], and the Danube, Sava, and Tisa rivers [46] were below the proscribed MACs values. Noel et al. [47] found that muscle concentrations of As, Cd, Pb, and Hg in pikeperch from five French fishing areas were below MACs. Hg concentrations in this species collected from important fishing areas in the Czech Republic exceeded MAC value at only one (of 16) sampling sites [32].

In Tunisia, concentrations of Cd and Pb were higher in pikeperch muscle from a lake contaminated by industrialization and urbanization than in other locations [14]. Furthermore, concentrations of Hg and Cd in muscle were highest in pikeperch compared to common carp (*Cyprinus carpio*) and flathead grey mullet (*Mugil cephalus*) [14]. Higher Cd concentrations were found in pikeperch compared to common carp from Lake Beyşehir in Turkey [48], while Mazej et al. [49] found no difference between Cd concentrations in the muscles of fish species of different trophic levels, including common carp and pikeperch, from a Slovenian reservoir, contaminated with mine tailings. There were no differences between the Cd levels in the gills and liver of common carp and pikeperch, while differences in Cd concentrations in these organs were found in other fish species. In France, no differences in Cd concentrations in muscle were found between fish species at a few contaminated sites [14]. Concentrations of only three elements in muscle tissue were significantly different between 3+ and 4+ individuals. This could be due to the similar feeding and behavioral habits of the age classes studied. Similarly, significant differences between males and females of pikeperch were found for K, S, and Mg in muscles and Al, Ag, and Mn in the liver [12]. Milošković et al. [14] analyzed heavy metal concentrations in the muscle tissue of pikeperch, bream (*Abramis brama*), and catfish (*Silurus glanis*) from the Danube, Sava, and Tisa. They found that these rivers were slightly affected or even unaffected by direct pollution.

The absence of the OCPs and PCBs in the pikeperch muscle tissue of analyzed individuals is probably because these contaminants have not been used recently, as was the case for the European chub (*Squalius cephalus*) from the same reservoir [21]. At all study sites, concentrations of DDT and derivatives (ΣDDT < 0.3 mg kg^{-1}) in pikeperch muscle were

below MAC. HCB, α- HCH, β- HCH, and γ-HCH were low and generally below their limits of quantification, namely 0.00003, 0.00002, 0.00004, and 0.00003 mg kg^{-1}, respectively [32].

4.2. Histopathological (HP) Analysis

The histopathological changes in gills and liver, as well as the histopathological indices, did not differ significantly between the age classes and are shown in Figure 1. This is due to the similar behavior, diet, and physiology of the fish of both age classes analyzed in this study. In the gills, the only alteration with moderate/severe scores (mean alteration score >2) was edema of the primary epithelium. Lamellar edema results from ultrafiltration caused by increased arterial blood pressure in the gills [50]. It often occurs with metal exposure [51] and does not significantly limit the respiratory process. HP indices for the liver were higher than for the gills, indicating that the liver is a more sensitive organ to pollutants. This was also confirmed by higher scores for necrosis in the liver compared to the gills. The mechanism of metal toxicity for fish is well known. It is based on the ability to induce oxidative stress and produce an excess of reactive oxygen species (ROS) in the mitochondria [52,53]. Consequently, the presence of ROS in cells leads to an increase in the concentration of antioxidant enzymes and the occurrence of pyknosis of cell nuclei, apoptosis, and/or necrosis in hepatocytes [54,55]. Furthermore, the chronic presence of some metals (e.g., Cd) significantly alters liver physiology and induces fatty degeneration and vacuolization of hepatocytes in a dose-dependent manner [56]. A distinct pattern of circular alterations (congestion/dilatation of sinusoids and stasis in blood vessels) in fish liver has been reported in fish exposed to metals [57,58] and is usually more common in fish in summer [59], which may be due to higher metabolic activity and explain the higher scores of mentioned alterations.

5. Conclusions

Based on element and OCPs accumulation and histopathology of gills and liver, both 3+ and 4+ age classes of pikeperch could be used as bioindicators of water pollution. Only 3 (Cd, Na, and P in muscle) of 78 (26 elements × 3 tissues) possible differences in element accumulation between age classes were found to be significant. All OCPs and PCBs analyzed were below the detection limit; therefore, there is no threat from consuming fish meat regarding these pesticides. On the other hand, the concentrations of Hg and Cd exceeded the MACs in some individuals of age class 4+, probably due to biomagnification. Histopathological changes and histopathological indices were low and did not differ significantly between age classes, indicating that pollution did not affect the morphology and structure of the gills and liver. The alterations with the highest scores were edema of primary epithelium in the gills and sinusoidal congestion in the liver.

Environmental changes and increasing recreational fishing need to be monitored regularly using consistent and comparable methods, and the scientific community should continue to improve data sharing and collaboration in assessing population status. Fisheries monitoring and management appears to be one of the most important priorities for the sustainability of the pikeperch stock and the control of the health status of the pikeperch population.

Due to the low number of individuals involved in our research, the conclusions of this study should be viewed with caution. Further studies including a larger number of individuals, as well as an analysis of the influence of age, gender, and diet on the accumulation of elements in fish tissues, are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151411321/s1, Figure S1: Pikeperch individuals aged four (A) and three (B) years, sampled at Garaši reservoir; Table S1: Calibration curves of the matrix-matched standards (0.01–0.1 mg kg^{-1}), coefficients of determination (R^2), limits of detection (LOD), limits of quantification (LOQ), recovery and RSD for the spiked level 0.01 and 0.02 mg kg^{-1}.

Author Contributions: Conceptualization, D.N.; methodology, D.N., A.T. and B.R.; investigation, D.N.; formal analysis, D.N., V.P., A.T. and B.R.; resources, D.N. and B.R.; visualization, D.N. and

B.R.; writing—original draft preparation, D.N. and B.R.; writing—review and editing, V.P., A.T., M.S.-L. and V.D.; supervision, B.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (No. of contracts: 451-03-47/2023-01/200053, 451-03-47/2023-01/200116, 451-03-47/2023-01/200007, and 451-03-47/2023-01/200030).

Institutional Review Board Statement: There was no need for approval from an Ethical Committee for this study because this species is used in commercial fishing. Moreover, the authors had the approval of the Ministry of Environmental Protection and Environmental Protection Agency to conduct the study.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and its Supplementary Materials.

Acknowledgments: The authors wish to express their gratitude to Mirjana Mihailović for her contribution to the preparation of samples for elemental analysis, Ljiljana Kostić Kravljanac, who helped with the ICP-OES analysis, and Zorica Radović for preparing samples for histological analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kottelat, M.; Freyhof, J. *Handbook of European Freshwater Fishes*; Publications Kottelat: Berlin, Germany, 2007; pp. 534–535.
2. Bousseba, M.; Ferraj, L.; Ouahb, S.; Ouizgane, A.; El Moujtahid, A.; Droussi, M.; Hasnaoui, M. Food preferences of Pike Perch, *Sander lucioperca* (Linnaeus, 1758) in Morocco. *E3S Web Conf. EDP Sci.* **2020**, *150*, 02011. [CrossRef]
3. Mustamäki, N.; Bergström, U.; Ådjers, K.; Sevastik, A.; Mattila, J. Pikeperch (*Sander lucioperca* (L.)) in decline: High mortality of three populations in the northern Baltic Sea. *Ambio* **2014**, *43*, 325–336. [CrossRef] [PubMed]
4. Jakubavičiūtė, E.; Arula, T.; Dainys, J.; Tyrell Deweber, J.; Gorfine, H.; Härkönen, L.S.; Hyvärinen, P.; Hommik, K.; Kubecka, J.; Ložys, L.; et al. Status and future perspectives for pikeperch (*Sander lucioperca*) stocks in Europe. *bioRxiv* **2022**. bioRxiv:12.20.521162. [CrossRef]
5. Smederevac-Lalic, M. Socio-Ekonomske i Biološke Karakteristike Privrednog Ribolova na Dunavu [Doktorska Disertacija, Univerzitet u Beogradu, 2013]. NaRDuS—National Repository of Dissertations in Serbia. Available online: https://nardus.mpn.gov.rs/handle/123456789/2707 (accessed on 11 June 2023).
6. Statistical Office of the Republic of Serbia. Fish Catch in Rivers, Ponds and Channels. Fish Catch, 2000–2022. 2022. Available online: https://www.stat.gov.rs/en-US/oblasti/poljoprivreda-sumarstvo-i-ribarstvo/ribarstvo (accessed on 12 May 2023).
7. Frisk, M.; Skov, P.V.; Steffensen, J.F. Thermal optimum for pikeperch (*Sander lucioperca*) and the use of ventilation frequency as a predictor of metabolic rate. *Aquaculture* **2012**, *324*, 151–157. [CrossRef]
8. Steenfeldt, S.; Fontaine, P.; Overton, J.L.; Policar, T.; Toner, D.; Falahatkar, B.; Horváth, Á.; Khemis, I.B.; Hamza, N.; Mhetli, M. Current status of Eurasian percid fishes aquaculture. In *Biology and Culture of Percid Fishes*; Kestemont, P., Dabrowski, K., Summerfelt, R., Eds.; Springer: Dordrecht, The Netherlands, 2015; pp. 817–841. [CrossRef]
9. Stejskal, I.; Tran, H.Q.; Prokesová, M.; Zare, M.; Gebauer, T.; Policar, T.; Caimi, C.; Gai, F.; Gasco, L. Defatted black soldier fly (*Hermetia illucens*) in pikeperch (*Sander lucioperca*) diets: Effects on growth performance, nutrient digestibility, fillet quality, economic and environmental sustainability. *Anim. Nutr.* **2023**, *12*, 7–19. [CrossRef]
10. Policar, T.; Schaefer, F.J.; Panana, E.; Meyer, S.; Teerlinck, S.; Damien Toner, D.; Żarski, D. Recent progress in European percid fish culture production technology—Tackling bottlenecks. *Aquac. Int.* **2019**, *27*, 1151–1174. [CrossRef]
11. Marković, Z.; Stanković, M.; Dulić, Z.; Živić, I.; Rašković, B.; Spasić, M.; Poleksić, V. Aquaculture and fishery in Serbia-status and potentials. In Proceedings of the V International Conference "Aquaculture & Fishery" Faculty of Agriculture, Belgrade-Zemun, Serbia, 1–3 June 2011; pp. 36–40.
12. Nikolić, D.; Skorić, S.; Poleksić, V.; Rašković, B. Sex-specific elemental accumulation and histopathology of pikeperch (*Sander lucioperca*) from Garaši reservoir (Serbia) with human health risk assessment. *Environ. Sci. Pollut. Res.* **2021**, *28*, 53700–53711. [CrossRef]
13. Nikolić, D.; Skorić, S.; Mićković, B.; Nikčević, M.; Smederevac-Lalić, M.; Djikanović, V. Accumulation of 25 elements in gills, liver, gonads, and muscle of European chub (*Squalius cephalus*), Cactus roach (*Rutilus virgo*), and pikeperch (*Sander lucioperca*) from Zlatar reservoir (Serbia). *Environ. Sci. Pollut. Res.* **2022**, *29*, 50271–50280. [CrossRef]
14. Khemis, I.B.; Besbes Aridh, N.; Hamza, N.; M'Hetli, M.; Sadok, S. Heavy metals and minerals contents in pikeperch (*Sander lucioperca*), carp (*Cyprinus carpio*) and flathead grey mullet (*Mugil cephalus*) from Sidi Salem Reservoir (Tunisia): Health risk assessment related to fish consumption. *Environ. Sci. Pollut. Res.* **2017**, *24*, 19494–19507. [CrossRef]
15. Walker, C.H.; Sibly, R.M.; Hopkin, S.P.; Peakall, D.B. *Principles of Ecotoxicology*, 4th ed.; CRC Press: Boca Raton, FL, USA; Taylor & Francis Group: Abingdon, UK, 2012; pp. 1–352.

16. Darnerud, P.O.; Atuma, S.; Aune, M.; Bjerselius, R.; Glynn, A.; Petersson Grawé, K.; Becker, W. Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g., DDT) based on Swedish market basket data. *Food Chem. Toxicol.* **2006**, *44*, 1597–1606. [CrossRef] [PubMed]
17. Milardi, M.; Lappalainen, J.; Malinen, T.; Vinni, M.; Ruuhijärvi, J. Problems in managing a slow-growing pikeperch (*Sander lucioperca* (L.)) population in Southern Finland. *Knowl. Manag. Aquat. Ecosyst.* **2011**, *400*, 8. [CrossRef]
18. Karadžić, V.; Subakov-Simić, G.; Krizmanić, J.; Natić, D. Phytoplankton and eutrophication development in the water supply reservoirs Garaši and Bukulja (Serbia). *Desalination* **2010**, *255*, 91–96. [CrossRef]
19. Official Gazette of the Republic of Serbia No. 56/2015 & 94/2018 (2018a). Naredba o Merama za Očuvanje i Zaštitu Ribljeg Fonda. [Order on Measures for Conservation and Protection of Fish Stocks]. [In Serbian]. Available online: http://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/ministarstva/naredba/2015/56/1/reg (accessed on 11 June 2023).
20. Ricker, W.E. *Computation and Interpretation of Biological Statistics of Fish Populations*; Reprint; Department of the Environment, Fisheries and Marine Service, Bulletin Fisheries Research Board Canada, University of Michigan: Ann Arbor, MI, USA, 1975; Volume 191, pp. 1–382.
21. Nikolić, D.; Poleksić, V.; Skorić, S.; Tasić, A.; Stanojević, S.; Rašković, B. The European Chub (*Squalius cephalus*) as an Indicator of Reservoirs Pollution and Human Health Risk Assessment Associated with Its Consumption. *Environ. Pollut.* **2022**, *310*, 119871. [CrossRef] [PubMed]
22. Official Gazette of the Republic of Serbia No. 22/2018 & 90/2018 (2018b). Regulation on the Maximum Permitted Residue Levels of Pesticides in Food and Animal Feed and Feed and Animal Feed for Which Maximum Quantities of Residues of Pesticides Are Permitted. Annex 5—Regulation on Maximum Allowed Amounts of Certain Contaminants in Food and Feed for Animals of Plant and Animal Origin. (In Serbian). Available online: www.pravno-informacioni-sistem.rs/SlGlasnikPortal/prilozi/5.html&doctype=reg&abc=cba&eli=true&eliActId=427071&regactid=427071 (accessed on 11 June 2023).
23. European Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs (Text with EEA relevance). Official Journal of the European Union No. 1881/2006 364:5-24. Available online: https://eurlex.europa.eu/eli/reg/200-6/1881/oj (accessed on 23 January 2022).
24. Usero, J.; Gonzalez-Regalado, E.; Gracia, I. Trace metals in the bivalve molluscs *Ruditapes decussatus* and *Ruditapes philippinarum* from the Atlantic Coast of Southern Spain. *Environ. Int.* **1997**, *23*, 291–298. [CrossRef]
25. European Commission (EC) SANTE 11312/2021-Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. 2021. Available online: https://food.ec.europa.eu/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf (accessed on 11 June 2023).
26. Official Gazette of the Republic of Serbia Nos. 32/2002, 25/2010 & 28/2011 2011. Regulation on the Quantities of Pesticides, Metals and Metalloids and Other Toxic Substances, Chemotherapeutics, Anabolics and Other Substances That Can Be Found in Food. (In Serbian). Available online: https://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/slsrj/ministarstva/pravilnik/1992/5/1/reg (accessed on 11 June 2023).
27. Esmaeilbeigi, M.; Kalbassi, M.R.; Seyedi, J.; Tayemeh, M.B.; Moghaddam, J.A. Intra and extracellular effects of benzo [α] pyrene on liver, gill and blood of Caspian White fish (*Rutilus frissi kutum*): Cyto-genotoxicity and histopathology approach. *Mar. Pollut. Bull.* **2021**, *163*, 111942. [CrossRef] [PubMed]
28. Kostić-Vuković, J.; Kolarević, S.; Kračun-Kolarević, M.; Višnjić-Jeftić, Ž.; Rašković, B.; Poleksić, V.; Gačić, Z.; Lenhardt, M.; Vuković-Gačić, B. Temporal variation of biomarkers in common bream *Abramis brama* (L., 1758) exposed to untreated municipal wastewater in the Danube River in Belgrade, Serbia. *Environ. Monit. Assess.* **2021**, *193*, 465. [CrossRef] [PubMed]
29. Santos, R.M.B.; Monteiro, S.M.V.; Cortes, R.M.V.; Pacheco, F.A.L.; Fernandes, L.F.S. Seasonal differences in water pollution and liver histopathology of Iberian barbel (*Luciobarbus bocagei*) and Douro nase (*Pseudochondrostoma duriense*) in an agricultural watershed. *Water* **2022**, *14*, 444. [CrossRef]
30. Bernet, D.; Schmidt, H.; Meier, W.; Burkhardt-Holm, P.; Wahli, T. Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *J. Fish Dis.* **1999**, *22*, 25–34. [CrossRef]
31. Hamilton, P.B.; Rolshausen, G.; Uren Webster, T.M.; Tyler, C.R. Adaptive capabilities and fitness consequences associated with pollution exposure in fish. *Phil. Trans. R. Soc. B* **2017**, *372*, 20160042. [CrossRef]
32. Cerveny, D.; Zlabek, V.; Velisek, J.; Turek, J.; Grabic, R.; Grabicova, K.; Fedorova, G.; Rosmus, J.; Lepic, P.; Randak, T. Contamination of fish in important fishing grounds of the Czech Republic. *Ecotoxicol. Environ. Saf.* **2014**, *109*, 101–109. [CrossRef]
33. Ivanović, J.; Janjić, J.; Baltić, M.; Milanov, R.; Bošković, M.; Marković, R.V.; Glamočlija, N. Metal concentrations in water, sediment and three fish species from the Danube River, Serbia: A cause for environmental concern. *Environ. Sci. Pollut. Res.* **2016**, *23*, 17105–17112. [CrossRef]
34. Kopp, D.; Cucherousset, J.; Syväranta, J.; Martino, A.; Céréghino, R.; Santoul, F. Trophic ecology of the pikeperch (*Sander lucioperca*) in its introduced areas: A stable isotope approach in southwestern France. *Comptes Rendus Biol.* **2009**, *332*, 741–746. [CrossRef]
35. Nikolić, D.; Skorić, S.; Đikanović, V.; Mićković, B.; Hegediš, A.; Lenhardt, M.; Krpo-Ćetković, J. Toxic elements in water and sediment from six reservoirs in Serbia. *Water Res. Manag.* **2020**, *10*, 13–18.
36. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry*; IARC Scientific Publications WHO International Agency for Research on Cancer: Lyon, France, 1993; Volume 58, pp. 119–237.

37. Bertin, G.; Averbeck, D. Cadmium: Cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). *Biochimie* **2006**, *88*, 1549–1559. [CrossRef]
38. Waalkes, M.P. Cadmium carcinogenesis. *Mutat. Res.* **2003**, *533*, 107–120. [CrossRef] [PubMed]
39. Tchounwou, P.B.; Ayensu, W.K.; Ninashvili, N.; Sutton, D. Environmental exposure to mercury and its toxicopathologic implications for public health. *Environ. Toxicol.* **2003**, *18*, 149–175. [CrossRef]
40. Gochfeld, M. Cases of mercury exposure, bioavailability, and absorption. *Ecotoxicol. Environ. Saf.* **2003**, *56*, 174–179. [CrossRef] [PubMed]
41. Davidson, P.W.; Myers, G.J.; Weiss, B. Mercury exposure and child development outcomes. *Pediatrics* **2004**, *113*, 1023–1029. [CrossRef] [PubMed]
42. Nabavi, S.F.; Nabavi, S.M.; Latifi, A.M.; Eslami, S.; Ebrahimzadeh, M.A. Determination of trace elements level of pikeperch collected from the Caspian Sea. *Bull. Environ. Contam. Toxicol.* **2012**, *88*, 401–405. [CrossRef] [PubMed]
43. Subotić, S.; Spasić, S.; Višnjić-Jeftić, Ž.; Hegediš, A.; Krpo-Ćetković, J.; Mićković, B.; Skorić, S.; Lenhardt, M. Heavy metal and trace element bioaccumulation in target tissues of four edible fish species from the Danube River (Serbia). *Ecotoxicol. Environ. Saf.* **2013**, *98*, 196–202. [CrossRef] [PubMed]
44. Jovanović, D.A.; Marković, R.V.; Teodorović, V.B.; Šefer, D.S.; Krstić, M.P.; Radulović, S.B.; Ivanović Ćirić, J.S.; Janjić, J.M.; Baltić, M.Ž. Determination of heavy metals in muscle tissue of six fish species with different feeding habits from the Danube River, Belgrade—Public health and environmental risk assessment. *Environ. Sci. Pollut. Res.* **2017**, *24*, 11383–11391. [CrossRef] [PubMed]
45. Milošković, A.; Branković, S.; Simić, V.; Kovačević, S.; Ćirković, M.; Manojlović, D. The accumulation and distribution of metals in water, sediment, aquatic macrophytes and fishes of the Gruža Reservoir, Serbia. *Bull. Environ. Contam. Toxicol.* **2013**, *90*, 563–569. [CrossRef] [PubMed]
46. Milošković, A.; Dojčinović, B.; Kovačević, S.; Radojković, N.; Radenković, M.; Milošević, D.; Simić, V. Spatial monitoring of heavy metals in the inland waters of Serbia: A multispecies approach based on commercial fish. *Environ. Sci. Pollut. Res.* **2016**, *23*, 9918–9933. [CrossRef]
47. Noël, L.; Chekri, R.; Millour, S.; Merlo, M.; Leblanc, J.C.; Guérin, T. Distribution and relationships of As, Cd, Pb and Hg in freshwater fish from five French fishing areas. *Chemosphere* **2013**, *90*, 1900–1910. [CrossRef] [PubMed]
48. Altındağ, A.; Yiğit, S. Assessment of heavy metal concentrations in the food web of lake Beyşehir, Turkey. *Chemosphere* **2005**, *60*, 552–556. [CrossRef]
49. Mazej, Z.; Sayegh-Petkovšek, A.; Pokorny, B. Heavy metal concentrations in food chain of Lake Velenjsko jezero, Slovenia: An artificial lake from mining. *Arch. Environ.* **2010**, *58*, 998–1007. [CrossRef]
50. Perry, S.F.; Davie, P.S.; Daxboeck, C.; Ellis, A.G.; Smith, D.G. 10 Perfusion methods for the study of gill physiology. In *Fish Physiology*; Hoar, W.S., Randall, D.J., Eds.; Academic Press: Cambridge, MA, USA, 1984; Volume 10, pp. 325–388. [CrossRef]
51. Delahaut, V.; Rašković, B.; Salvado, M.S.; Bervoets, L.; Blust, R.; De Boeck, G. Toxicity and bioaccumulation of Cadmium, Copper and Zinc in a direct comparison at equitoxic concentrations in common carp (*Cyprinus carpio*) juveniles. *PLoS ONE* **2020**, *15*, e0220485. [CrossRef]
52. Olsson, P.E.; Kling, P.; Hogstrand, C. Mechanisms of heavy metal accumulation and toxicity in fish. In *Metal Metabolism in Aquatic Environments*; Langston, W.J., Bebianno, M.J., Eds.; Springer: Boston, MA, USA, 1998; pp. 321–350. [CrossRef]
53. Sun, Q.; Li, Y.; Shi, L.; Hussain, R.; Mehmood, K.; Tang, Z.; Zhang, H. Heavy metals induced mitochondrial dysfunction in animals: Molecular mechanism of toxicity. *Toxicology* **2022**, *469*, 153136. [CrossRef]
54. Velma, V.; Tchounwou, P.B. Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, *Carassius auratus*. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2010**, *698*, 43–51. [CrossRef]
55. Javed, M.; Ahmad, I.; Usmani, N.; Ahmad, M. Studies on biomarkers of oxidative stress and associated genotoxicity and histopathology in *Channa punctatus* from heavy metal polluted canal. *Chemosphere* **2016**, *151*, 210–219. [CrossRef]
56. Zhu, Q.L.; Li, W.Y.; Zheng, J.L. Life-cycle exposure to cadmium induced compensatory responses towards oxidative stress in the liver of female zebrafish. *Chemosphere* **2018**, *210*, 949–957. [CrossRef]
57. Atamanalp, M.; Sisman, T.; Geyikoglu, F.; Topal, A. The histopathological effects of copper sulphate on rainbow trout liver (*Oncorhynchus mykiss*). *J. Fish. Aquat. Sci.* **2008**, *3*, 291–297. [CrossRef]
58. Younis, E.M.; Abdel-Warith, A.A.; Al-Asgah, N.A.; Ebaid, H.; Mubarak, M. Histological changes in the liver and intestine of Nile tilapia, *Oreochromis niloticus*, exposed to sublethal concentrations of cadmium. *Pak. J. Zool.* **2013**, *45*, 833–841.
59. Kostić, J.; Kolarević, S.; Kračun-Kolarević, M.; Aborgiba, M.; Gačić, Z.; Paunović, M.; Višnjić-Jeftić, Ž.; Rašković, B.; Poleksić, V.; Lenhardt, M.; et al. The impact of multiple stressors on the biomarkers response in gills and liver of freshwater breams during different seasons. *Sci. Total Environ.* **2017**, *601*, 1670–1681. [CrossRef] [PubMed]