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Health Risks Associated with the Concentration of Heavy Metals in Sediment, Water, and Carp Reared in Treated Wastewater from a Slaughterhouse

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Abstract: The use of purified slaughterhouse wastewater in carp ponds, and the use of wastewater from the pond for the irrigation of agricultural fields, was the basis for the construction of an integrated system of agricultural production as a sustainable solution for the food and fish production industries. The negative side of such integrated production systems is the concern related to the safety of fish meat produced in such a system. The aim of this research was to determine the concentration of heavy metals and metalloids in the wastewater from the slaughterhouse, in the pond water and sediment, in the carp tissue and in the water leaving the pond, and to evaluate the effectiveness of the integrated system and the safety of the produced fish. Sampling was carried out in spring and autumn. The mean concentrations in all water samples (µg/L) were: As (12–125), Cd (0.12–4.2), Hg (1.14–14.21), Pb (<0.1–17.2), Cu (<0.1–44.6), Fe (17.02–425.2) and Zn (2.91–186.2), with the highest numbers in the wastewater, where it was above the prescribed limit values for the wastewater discharged from the slaughterhouses into natural recipients in both samplings. The efficiency of the wastewater treatment plant for heavy metals and metalloids was very high, in the range of 87% to 98%. The water from the pond corresponded to class 3 in terms of the concentration of heavy metals and metalloids both in spring and autumn, and can be used freely for breeding cyprinid fish species. The water from the irrigation canal corresponded to class 2/3 and can be used for irrigation. The mean concentrations of heavy metals and metalloids in the sediments (mg/kg) were: As (3.00–4.88), Cd (0.16–0.96), Hg (0.21–1.47), Pb (0.77–2.29), Cu (49.60–60.90), Fe (3.94–5.32) and Zn (92.8–115.20). The content of heavy metals in different organs of carp differed significantly depending on the season. The trend of heavy metal accumulation in common carp muscles in spring was: Zn > Fe > Cu > Pb > Hg > Cd > As, and in autumn: Zn > Fe > Cu > Pb > As > Cd > Hg. Metal concentrations in the examined fish samples were far below the WHO guidelines. It can be concluded that carp produced in a pond supplied with purified wastewater from the slaughterhouse industry, in terms of the concentration of residues of the tested heavy metals and metalloids, is safe for human consumption.

Keywords: integrated system; food safety; freshwater fish; sustainable aquaculture

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Citation: Pelić, M.; Mihaljev, Ž.; Živkov Baloš, M.; Popov, N.; Gavrilović, A.; Jug-Dujaković, J.; Ljubojević Pelić, D. Health Risks Associated with the Concentration of Heavy Metals in Sediment, Water, and Carp Reared in Treated Wastewater from a Slaughterhouse. *Water* **2024**, *16*, 94. https://doi.org/10.3390/w16010094

Academic Editor: Andrea G. Capodaglio

Received: 9 November 2023
Revised: 8 December 2023
Accepted: 19 December 2023
Published: 26 December 2023

1. Introduction

Fish production is characterized by specific water requirements, and water quality and availability are limiting factors in pond aquaculture [1]. The source of water for filling the pond must be free of harmful ingredients, and its quality, availability and quantity must be controlled. In many countries, the majority of domestic and industrial wastewater from urban, industrial and rural areas is still discharged into the environment without prior treatment [2–5], which represents a serious environmental problem. Treated wastewater is a sustainable water resource and has been used in aquaculture in many countries worldwide, including Israel [6,7], China [8], Vietnam [9] and India [10,11]. The negative side of such a concept is the concern regarding the safety of fish meat produced in such a system.

One of the concerns is the possible presence of heavy metals in fish and fish products, which threatens the reputation of fish as one of the leading foods when it comes to healthy nutrition. The anthropogenic influence on the occurrence of heavy metals in the environment is very large, and untreated wastewater that is discharged into waterways is their most significant source [12]. They can pose a significant risk to aquatic habitats and humans through the food chain [13]. It is known that heavy metals are hazardous pollutants of aquatic environments due to their bioaccumulative nature [14].

The examination of heavy metals in sediment, water and fish, and the different treatments in order to reduce their concentrations, has been of increasing interest to the scientific community in recent years [15,16]. This is mainly because heavy metals are nonbiodegradable, are toxic in relatively low concentrations and affect humans throughout the food chain [17]. There are only limited data on the bioaccumulation of heavy metals in fish farmed in treated wastewater, and the results obtained by several authors [7,18,19] suggest that farmed fish are safe in terms of the maximum permitted levels of heavy metals in fish intended for human consumption [20].

Heavy metals include those that are not needed by living organisms and have an exclusively toxic effect, namely: lead, mercury, cadmium and arsenic. Their presence in water above the prescribed concentrations directly affects plants, animals and people. There are also metals that are necessary for living organisms. This group includes zinc, iron, copper, molybdenum, manganese, cobalt and selenium [21], but when present in large concentrations can also have toxic effects. The adverse effects of heavy metals are usually cumulative and very rarely immediate. The danger of heavy metal poisoning through the consumption of aquatic organisms began to attract more attention after the case of industrial mercury pollution in Minimata Bay in Japan, and consequently the poisoning of people after the consumption of contaminated fish from the bay (Minimata syndrome) [22,23]. This event was followed by the poisoning of people with fish contaminated with cadmium (itai–itai disease) [24]. Arsenic is a metalloid that is part of the Earth's crust and water. It is found in nature in different forms. In fish, it is mainly found in an organic form [25], which is less toxic than the inorganic form. It is found in the human body in very low concentrations, and its physiological role is still not fully elucidated. Mutagenic and carcinogenic effects are attributed to it [26]. Cadmium is an element that rarely appears in nature, and it is characteristic that it accumulates during life. It is highly toxic and has negative effects on the reproductive, urinary and respiratory tract, as well as the locomotor system [27]. Mercury is a toxic element that is widely present in nature. The presence of mercury in fish represents a great potential danger for those who consume it. It exhibits toxic effects on the physiological and nervous system in humans. It is carcinogenic and mutagenic. Mercury is transferred to the fetus through the placenta [28]. Lead is a nonessential element and is ubiquitous in the environment. Iron is a very common element in nature, and zinc is a relatively widespread element in nature. Zinc is essential for the life of living organisms, but if it is introduced into the body in larger quantities than necessary, it becomes toxic. Copper is an element that is widespread in the environment.

Sediment is an important part of aquatic ecosystems and provides an abundance of nutrients and represents an area for spawning, but can also be a reservoir for pollutants, including heavy metals [29,30]. The content of heavy metals in fish tissues is observed from two aspects: ecologically and the safety of fish as food. When it comes to the hygiene–safety aspect, the content of heavy metals in meat is mainly considered, and in most countries, the content of heavy metals is legally defined [31]. Based on the content of heavy metals in the edible fish tissue, the correctness of the fish meat is evaluated; that is, the fulfilment of the prescribed conditions. However, no data are available on the concentration of heavy metals in the aquatic environment and fish produced in treated wastewater from slaughterhouses, although such systems represent sustainable solutions for the food industry, as well as fish farming.

The aim of this work was to investigate the occurrence and bioaccumulation of heavy metals (As, Cd, Hg, Pb, Fe, Cu and Zn) in the integrated system of the slaughterhouse and fish production based on samples collected in spring and autumn: in wastewater from the slaughterhouse before and after purification, in water from the prebasin, in water from the pond and water from the drainage channel (the channel after the pond melioration channel), in pond sediment, and finally in different tissues of carp (*Cyprinus carpio*) grown in this system. The implications of the accumulation of heavy metals on the safety of carp consumption were also assessed.

2. Materials and Methods

2.1. Experimental Facility Design

The facility was constructed in several stages. First, a system for the purification of slaughterhouse wastewater was built on the property of the meat industry in Pećinci (N 44.860783, E 19.957004). After that, a fish pond was built on the same property, which was supplied mainly with water from the wastewater treatment system from the slaughterhouse. The integrated production facility combines a slaughterhouse and fish production, i.e., in addition to the slaughterhouse, a system of carp production in earthen ponds is included. The used water from the slaughterhouse goes to the purifier, and then from the purifier outlet to the prepond. The efficiency of the purification was examined by the analysis of the water before and after the process of purification. The water from the purifier goes to the prepond, where the purified water is aerated, and then goes to the pond, where part of the nutrients from the purifier is used to feed the carp. The pond was aerated and the amount of dissolved oxygen in the water was measured daily. At the end of the process, the water from the pond goes into the melioration canal. The water is then used to irrigate the agricultural soil around the slaughterhouse. Fish samples were taken in April and October. Also, water samples and sediment samples were taken for analysis in both seasons. The detailed design, the scheme of the integrated system and the functioning of the system are described in the previous work of Pelić et al. [32].

2.2. Sampling

Water samples for testing heavy metals were taken in 500 mL plastic bottles. Sampling was carried out at five points: the water from the purifier, the water from the outflow of the purifier, the water from the prepond, the water from the pond and the water from the melioration channel. Sediment sampling was carried out according to the standard procedure, and was placed in a plastic container at a volume of 5 L. Fish were caught by pulling a net on the pond. The fish samples were placed in sterile plastic bags and delivered to the laboratory in the shortest possible time. The fish were sacrificed by a quick blow to the head. All samples were kept at refrigerator temperature during the transport to the laboratory. All specimens were dissected. Skinned dorsal muscles, skin, liver and kidneys were homogenized and prepared for analysis. Laboratory tests of water, sediment and fish were carried out in the laboratories of the Scientific Veterinary Institute "Novi Sad".

2.3. Sample Preparation

For the determination of toxic elements, the samples were prepared by the wet digestion method in the Ethos system, Microwave Labstation, Milestone (Milestone s.r.l., Sorisole, Italy).

2.3.1. Water Sample Preparation Procedure

A sample of 45 mL of water from the prepond, pond and amelioration canal, and 5 mL of wastewater was placed in the Teflon container for digestion. Reagents were then added to the vessel with the samples: 5 mL HNO_3 65% for the water from the prepond, pond and melioration canal, while 7 mL HNO_3 65% and 1 mL H_2O_2 30% were added for the wastewater. After the completion of the digestion process, the samples were cooled to room temperature, after which the solution was transferred to a suitable normal vessel and topped up to 50 mL (for the wastewater) or 100 mL (for the water from the prepond, pond and melioration canal). The microwave digestion program for the water from the prepond, pond and melioration canal lasted 10 min at a temperature of 160 °C with a power of up to 1000 W. The digestion program for wastewater took place in two steps: the first step lasted 10 min and the second step lasted 20 min, both at a temperature of 160 °C and a power of up to 1000 W.

2.3.2. Procedure for Preparing the Sediment and Fish Samples

A prehomogenized sample of 1 g was placed in Teflon cuvettes, after which 8 mL of diluted NHO_3 (Fisher Scientific, Loughborough, UK) and 2 mL of H_2O_2 (AppliChem GmbH, Darmstadt, Germany) were added. Digestion was carried out at a temperature of 180 °C for 30 min with a maximum power of 1000 W. After cooling to room temperature, the samples were diluted to a volume of 50 mL with deionized water (Smeg SpA., Guastalla, Italy).

2.4. Equipment and Analysis

The determination of the elements in the water, sediment and fish meat samples (As, Cd, Hg, Pb, Cu, Fe and Zn) was carried out by the inductively coupled plasma method with mass detection (ICP/MS) on an ICP/MS apparatus (inductively coupled (coupled) plasma with mass spectrometry), the Agilent 7700× (G3281A) series ICP-MS instrument (Agilent Technologies, Santa Clara, CA, USA). The measured values were then processed with the MassHunter Workstation G7200-90210 (Rev.A) software package. The detailed validation of the method, the quality control of the applied method and the instrumental analysis are described in the work of Novakov et al. [33].

2.5. Statistical Data Processing

The data were analyzed in the Excel (Microsoft Office 2013) software package with the Data Analysis add-on. Different statistical tests were used to analyze the results: one-factor ANOVA, the Tukey test and the *t*-test. Differences were considered significant at the level of $p < 0.05$.

3. Results

3.1. Concentrations of Heavy Metals in the Water Samples

The results of the analysis of the concentration of heavy metals and metalloids in the wastewater sampled before and after the purification treatment are shown in Tables 1 and 2.

Table 1. Heavy metals–water from the purifier.

Tested Parameter	Unit of Measure	Measured Spring Value	Measured Autumn Value	MDK-II [8]	MDK-III [9]	*p*-Value
As [1]	μg/L	112 ± 0.5	125 ± 0.2	10	50	<0.001
Cd [2]	μg/L	3.4 ± 0.2	4.2 ± 0.15	5	10	0.005
Hg [3]	μg/L	12.9 ± 0.1	14.2 ± 0.1	1	1	<0.001
Pb [4]	μg/L	16.3 ± 0.2	17.2 ± 0.1	50	100	<0.001
Cu [5]	μg/L	44.6 ± 0.4	41.6 ± 0.3	100	100	<0.001
Fe [6]	μg/L	420.6 ± 0.3	425.2 ± 0.2	300	1000	<0.001
Zn [7]	μg/L	183.4 ± 0.2	186.2 ± 0.2	200	1000	<0.001

Notes: [1] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc; MDK [8]—for the category II water; MDK [9]—for the category III water [34].

The order of elements in water from the purifier was the same in both season: Fe > Zn > As > Cu> Hg > Pb > Cd.

The sequence of elements in the water from the effluent was the same in both seasons: Fe > Zn > As > Cd > Hg > Pb and Cu, whereas in the prefishery pond, it was: Fe > As > Zn > Cu > Hg > Cd > Pb; it was also the same in both seasons.

Table 2. Heavy metals–water from the effluent from the purifier.

Tested Parameter	Unit of Measure	Measured Spring Value	Purifier Efficiency	Measured Autumn Value	Purifier Efficiency (%)	p-Value
As [1]	µg/L	12 ± 0.2	89	14 ± 0.2	88	<0.001
Cd [2]	µg/L	0.23 ± 0.04	93	0.24 ± 0.02	98	0.72
Hg [3]	µg/L	1.61 ± 0.03	87	1.69 ± 0.04	88	0.03
Pb [4]	µg/L	<0.1	100	<0.1	100	-
Cu [5]	µg/L	<0.1	100	<0.1	100	-
Fe [6]	µg/L	17.02 ± 0.16	95	18.3 ± 0.2	95	0.001
Zn [7]	µg/L	3.11 ± 0.11	98	3.42 ± 0.04	98	0.007

Notes: [1] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc.

In the water from the purifier, a statistically significantly higher concentration was found for all tested elements in the autumn sampling ($p < 0.05$), except for Cu, whose concentration was significantly higher in the spring sampling ($p < 0.05$) (Table 1). When it comes to the water from the purifier's outflow, that is, the water after purification, Pb and Cu were not detected either in the spring or in the autumn period. Concentrations of other elements were higher in the autumn, with statistically significant differences established for As, Hg, Fe and Zn ($p < 0.05$) (Table 2). The analysis of the results of the concentration of heavy metals in the water sampled before and after the purification treatment showed that the efficiency of the wastewater purification device within the slaughterhouse for As was 89% in spring and 88% in autumn; for Cd, 93% in spring and 98% in autumn; for Hg, 87% in spring and 88% in autumn; for Fe, 95% in spring and autumn; for Zn, 98% in both samplings (Table 2). The results of the analysis of the concentration of heavy metals in the purified wastewater sampled from the prepond and the pond within the integrated facility are presented in Tables 3 and 4.

Table 3. Heavy metals–water from the prepond.

Tested Parameter	Unit of Measure	Measured Spring Value	Measured Autumn Value	MDK-II [8]	MDK-III [9]	p-Value
As [1]	µg/L	34 ± 0.2	36 ± 0.2	10	50	<0.001
Cd [2]	µg/L	0.13 ± 0.02	0.12 ± 0.01	-	-	0.16
Hg [3]	µg/L	1.14 ± 0.02	1.18 ± 0.06	-	-	0.34
Pb [4]	µg/L	<0.1	<0.1	-	-	-
Cu [5]	µg/L	9.12 ± 0.03	9.24 ± 0.02	-	500	0.004
Fe [6]	µg/L	103.2 ± 0.1	102.9 ± 0.8	500	1000	0.55
Zn [7]	µg/L	19.9 ± 0.2	20.25 ± 0.04	-	2000	0.04

Notes: [1.] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc; [8]—Limit values of the pollutants in the surface waters for the category II water; [9]—Limit values of the polluting substances in the surface waters for the category III water [35].

Table 4. Heavy metals–pond water.

Tested Parameter	Unit of Measure	Measured Spring Value	Measured Autumn Value	MDK-II [8]	MDK-III [9]	p-Value
As [1]	µg/L	24 ± 0.5	26 ± 0.2	10	50	0.003
Cd [2]	µg/L	0.69 ± 0.05	0.80 ± 0.02	-	-	0.02
Hg [3]	µg/L	1.59 ± 0.04	1.67 ± 0.02	-	-	0.036
Pb [4]	µg/L	2.11 ± 0.02	2.19 ± 0.01	-	-	0.008
Cu [5]	µg/L	<0.1	<0.1	-	500	-
Fe [6]	µg/L	19.22 ± 0.02	17.02 ± 0.03	500	1000	<0.001
Zn [7]	µg/L	2.91 ± 0.01	3.11 ± 0.01	-	2000	<0.001

Notes: [1.] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc; [8]—Limit values of the pollutants in the surface waters for the category II water; [9]—Limit values of the polluting substances in surface waters for the category III water [35].

In the spring, the water from the prepond had a higher concentration of Cd and Fe, but the differences were not statistically significant. The concentrations of As, Cu and Zn were statistically significantly higher in the autumn period in the water from the prepond ($p < 0.05$) (Table 3). Statistically significant higher concentrations of As, Cd, Hg, Pb and Zn were measured in the pond water in the autumn period ($p < 0.05$), while the concentration of Fe was significantly higher in the spring ($p < 0.05$). The concentration of Cu in the pond water was below the detection limit of the method (Table 4). The order of concentrations of the elements in the pond water was the same in spring and autumn: As > Fe > Zn > Pb > Hg > Cd > Cu. In addition to the results, the tables also show the reference values, i.e., MDK for heavy metals in the surface water for category II and III water according to the regulation on the limit values of pollutants in the surface and underground waters and sediment, and the deadlines for their achievement [35]. Table 5 shows the results of the analysis of the concentration of heavy metals in the water sampled from the irrigation canal. The order of elements was: Fe > As > Zn > Hg > Cd > Cu > Pb, which was the same in spring and in autumn.

Table 5. Heavy metals–irrigation canal.

Tested Parameter	Unit of Measure	Measured Spring Value	Measured Autumn Value	MDK [8]	*p*-Value
As [1]	µg/L	14 ± 0.2	16 ± 0.2	50	<0.001
Cd [2]	µg/L	0.52 ± 0.05	0.56 ± 0.01	10	0.57
Hg [3]	µg/L	0.94 ± 0.02	0.98 ± 0.04	1	0.14
Pb [4]	µg/L	<0.1	<0.1	100	-
Cu [5]	µg/L	0.4 ± 0.2	0.4 ± 0.1	100	0.45
Fe [6]	µg/L	131 ± 0.2	136 ± 0.5	-	<0.001
Zn [7]	µg/L	6.77 ± 0.02	6.88 ± 0.03	1000	0.006

Notes: [1.] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc [8] [36].

In the water from the irrigation canal, a statistically significantly higher concentration of As, Fe and Zn was measured in autumn ($p < 0.05$), as well as a higher concentration of Cd, Hg and Cu, but the differences were not statistically significant. The concentration of Pb in both samplings was below the detection limit (Table 5). In addition to these results, the limit values for the tested elements for water that can be used for irrigation, which are prescribed by the regulation on the permitted amounts of hazardous and harmful substances in soil and irrigation water [36], are also presented.

3.2. Concentrations of Heavy Metals in Sediment Samples

The results of the analyses of the heavy metal concentrations in the sediment samples taken in spring and autumn are shown in Tables 6 and 7.

Table 6. Heavy metals–sediment—spring.

Tested Parameter	Unit of Measure	Prepond	Pond 1	Pond 2	Irrigation Canal
As [1]	mg/kg	3.00 ± 0.05	4.71 ± 0.01	3.94 ± 0.05	4.88 ± 0.02
Cd [2]	mg/kg	0.16 ± 0.02	0.38 ± 0.02	0.96 ± 0.03	0.65 ± 0.01
Hg [3]	mg/kg	0.66 ± 0.02	0.54 ± 0.01	0.21 ± 0.06	1.47 ± 0.02
Pb [4]	mg/kg	1.50 ± 0.06	2.22 ± 0.04	1.67 ± 0.02	0.77 ± 0.02
Cu [5]	mg/kg	56.60 ± 0.06	55.10 ± 0.04	49.60 ± 0.35	60.40 ± 0.2
Fe [6]	mg/kg	5.23 ± 0.01	3.94 ± 0.03	4.86 ± 0.02	5.26 ± 0.04
Zn [7]	mg/kg	92.80 ± 0.05	113.0 ± 0.9	106.4 ± 0.3	114.5 ± 0.1

Notes: [1.] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc.

Table 7. Heavy metals–sediment—autumn.

Tested Parameter	Unit of Measure	Prepond	Pond 1	Pond 2	Irrigation Canal
As [1]	mg/kg	3.20 ± 0.05	4.86 ± 0.04	4.13 ± 0.02	5.21 ± 0.01
Cd [2]	mg/kg	0.26 ± 0.02	0.49 ± 0.08	0.99 ± 0.08	0.86 ± 0.04
Hg [3]	mg/kg	0.69 ± 0.03	0.62 ± 0.02	0.29 ± 0.03	1.49 ± 0.08
Pb [4]	mg/kg	1.60 ± 0.05	2.29 ± 0.03	1.69 ± 0.05	0.80 ± 0.02
Cu [5]	mg/kg	57.10 ± 0.05	55.80 ± 0.32	49.90 ± 0.1	60.90 ± 0.35
Fe [6]	mg/kg	5.32 ± 0.01	4.31 ± 0.01	4.95 ± 0.03	5.31 ± 0.02
Zn [7]	mg/kg	93.20 ± 0.05	114.0 ± 0.2	107.2 ± 0.2	115.2 ± 0.1

Notes: [1.] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc.

In the sediment samples from the prepond, the concentrations of all tested elements were higher in autumn. For As, Cd, Cu, Fe and Zn, the measured differences were statistically significant ($p < 0.05$), while for Hg and Pb, they were not (Table 8). In the sediment extracted from the pond, the determined concentration of all elements was higher in autumn, and the differences were significant for As, Hg and Fe ($p < 0.05$) (Table 8). In the sediment from the irrigation canal, element concentrations were higher in autumn, and the differences were significant for As, Cd and Zn ($p < 0.05$) (Table 8). A comparison of the average values of the content of heavy metals in the sediment sampled in the spring and in the autumn was also made, and the results and obtained *p*-values are shown in Table 9.

Table 8. Heavy metals–*p*-values for sediment—spring/autumn.

Tested Parameter	Unit of Measure	Prepond	Pond 1	Pond 2	Irrigation Canal
As [1]	mg/kg	0.008	0.003	0.004	<0.001
Cd [2]	mg/kg	0.004	0.071	0.329	<0.001
Hg [3]	mg/kg	0.223	0.003	0.009	0.716
Pb [4]	mg/kg	0.111	0.095	0.459	0.140
Cu [5]	mg/kg	<0.001	0.039	0.768	0.116
Fe [6]	mg/kg	<0.001	<0.001	0.010	0.064
Zn [7]	mg/kg	<0.001	0.133	0.028	0.002

Notes: [1.] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc.

Table 9. Comparison of average concentrations of heavy metals in sediment—spring and autumn.

Tested Parameter	Unit of Measure	Spring	Autumn	*p*-Value
As [1]	mg/kg	4.13 ± 0.86	4.35 ± 0.89	0.74
Cd [2]	mg/kg	0.54 ± 0.35	0.65 ± 0.34	0.66
Hg [3]	mg/kg	0.72 ± 0.53	0.77 ± 0.51	0.89
Pb [4]	mg/kg	1.54 ± 0.60	1.60 ± 0.61	0.90
Cu [5]	mg/kg	55.42 ± 4.48	55.92 ± 4.56	0.88
Fe [6]	mg/kg	4.82 ± 0.62	4.97 ± 0.47	0.71
Zn [7]	mg/kg	106.68 ± 9.90	107.40 ± 10.10	0.92

Notes: [1] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc; mean value ± standard deviation (n = 4); $p < 0.05$.

3.3. Concentrations of Heavy Metals in Different Fish Tissues

The results of the analysis of the heavy metals and metalloids in the different organs of carp (muscle, skin, gills, liver and kidney) that were sampled in spring and autumn are shown in Tables 10 and 11. Based on the results obtained, their comparative analysis was performed in relation to the season in which the sampling was carried out, as well as the analysis of their distribution in the different organs of carp.

Table 10. Heavy metals–common carp tissues—spring.

Tested Parameter	Unit of Measure	Muscle	Skin	Gils	Liver	Kidney
As [1]	mg/kg	0.003 ± 0.002 B	0.008 ± 0.007 B	0.004 ± 0.003 B	0.006 ± 0.003 B	0.016 ± 0.007 A
Cd [2]	mg/kg	< 0.001	< 0.001	< 0.001	0.018 ±0.009 A	0.037 ± 0.023 A
Hg [3]	mg/kg	0.013 ± 0.001 A	0.005 ± 0.001 A	0.004 ± 0.002 A	0.006 ± 0.003 A	0.016 ± 0.016 A
Pb [4]	mg/kg	0.036 ± 0.034 A	0.07 ± 0.06 A	0.078 ± 0.04 A	0.132 ± 0.11 A	0.114 ± 0.043 A
Cu [5]	mg/kg	0.148± 0.05 C	0.13 ±0.04 C	0.85 ± 0.33 B	2.11 ± 1.35 A	0.38 ± 0.28 C
Fe [6]	mg/kg	16.67± 6.74 C	17.24 ± 11.30 C	24.39 ± 7.58 BC	28.95 ± 3.92 B	71.29 ± 18.48 A
Zn [7]	mg/kg	21.86 ± 6.95 D	43.91 ± 7.58 C	90.5 ± 27.20 B	40.54 ± 23.55 CD	219.66 ± 75.93 A

Notes: [1] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc. Detection limit (mg/kg) of As, Cd, Hg и Pb = 0.001 mg/kg; Fe и Cu = 0.006 mg/kg; Zn = 0.007 mg/kg; the results are presented as the mean value± standard deviation (n = 7); A, B, C, D—values next to which there are different letters are statistically different at the level of significance, $p < 0.05$.

Table 11. Heavy metals–common carp tissues—autumn.

Tested Parameter	Unit of Measure	Muscle	Skin	Gils	Liver	Kidney
As [1]	mg/kg	0.020 ± 0.002 C	0.040 ± 0.009 B	0.009 ± 0.002 D	0.010 ± 0.003 D	0.590 ± 0.278 A
Cd [2]	mg/kg	<0.001	0.001 ± 0.001 C	0.001 D	0.040 ± 0.018 A	0.030 ± 0.004 B
Hg [3]	mg/kg	0.47 ± 0.09 A	0.047 ± 0.03 C	0.017 ± 0.006 D	0.090 ± 0.039 B	0.008 ± 0.005 E
Pb [4]	mg/kg	0.24 ± 0.10 B	0.32 ± 0.12 B	0.09 ± 0.04 C	0.823 ± 0.486 A	0.080 ± 0.031 C
Cu [5]	mg/kg	0.12 ± 0.02 C	0.010 ± 0.001 D	0.50 ± 0.33 B	3.92 ± 1.85 A	0.11 ± 0.02 C
Fe [6]	mg/kg	8.03 ± 1.15 D	30.94 ± 14.42 C	54.15 ± 7.55 B	36.00 ± 3.55 C	80.98 ± 13.82 A
Zn [7]	mg/kg	31.1 ± 8.01 C	58.14 ± 15.69 B	101.43 ± 12.52 A	20.220 ± 7.003 D	109.45 ± 29.13 A

Notes: [1] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc. Detection limit (mg/kg) of As, Cd, Hg и Pb = 0.001 mg/kg; Fe и Cu = 0.006 mg/kg; Zn = 0.007 mg/kg; the results are presented as the mean value± standard deviation (n = 7); A, B, C, D, E—values next to which there are different letters are statistically different at the level of significance, $p < 0.05$.

In spring, the average concentration of As in the organs of carp moved in the following decreasing order: kidney (0.016 mg/kg), skin (0.008 mg/kg), liver, gills and meat (0.003 mg/kg). In the spring, the concentration of Cd was the highest in the kidney (0.037 mg/kg), then in the liver (0.018 mg/kg), while it was the same in the meat, skin and gills, where it was lower than the detection limit of the applied method (<0.001 mg/kg). The average Hg concentrations had the following decreasing sequence: kidney (0.016 mg/kg), meat (0.014 mg/kg), liver (0.006 mg/kg), skin (0.005 mg/kg) and gills (0.004 mg/kg) (Table 10). The average Pb concentration decreased from the liver (0.132 mg/kg) through the kidneys (0.114 mg/kg), gills and skin, and was the lowest in the meat (0.036 mg/kg). In spring, the concentration of Cu was the highest in the liver (2.11 mg/kg), followed by the gills, kidney and meat, and was the lowest in the skin (0.13 mg/kg). The average Fe concentration decreased in the following sequence: kidney (71.29 mg/kg), liver (28.95 mg/kg), gills (24.39 mg/kg), skin and meat (16.67 mg/kg); for the Zn concentration: kidney (219.66 mg/kg), gills, skin, liver and meat (21.86 mg/kg) (Table 10).

In the spring, a statistically significant difference was found between the concentrations of As in the kidney compared to other organs ($p < 0.05$). The difference in the Cu concentration was statistically significant between the liver and gills and other organs ($p < 0.05$). The Cu concentrations in the meat and kidney compared to the skin were statistically significant ($p < 0.05$). A statistically significant difference was found in the concentration of Fe in the kidney compared to the other organs ($p < 0.05$), and in the liver compared to the concentration in the skin and meat ($p < 0.05$). Differences in the Zn concentration in the kidney compared to the other organs, the gills compared to the other organs, the skin compared to the meat, the gills and kidney and the liver compared to the kidney and gills were also statistically significant ($p < 0.05$). On the other hand, in the spring, it was determined that the differences in the concentration of As in the skin, liver, gills and meat were not statistically significant ($p > 0.05$), as well as the concentration of Cd in the kidney and liver, the differences in the concentrations of Hg and Pb in different organs, and also the Cu concentrations in the meat and kidney. There was no statistically significant difference between the amount of Fe in the liver and gills, as well as between the gills, skin and meat ($p > 0.05$). No statistically significant difference was found between the results of the Zn concentration in the skin and liver and in the liver and meat (Table 10).

The results of the analyses of the concentration of heavy metals and metalloids in the tissues of carp sampled in autumn showed that the concentration of As in the organs of carp decreased in the following sequence: kidney (0.59 mg/kg), skin, meat, liver and gills (0.009 mg/kg) (Table 11). In autumn, the concentration of Cd was the highest in the liver (0.04 mg/kg), then in the kidney (0.03 mg/kg), skin (0.0015 mg/kg) and gills (0.001 mg/kg). In the meat samples, it was below the detection limit of the test methods (0.001 mg/kg). The average Hg concentration decreased in the following sequence: meat (0.47 mg/kg), liver (0.09 mg/kg), skin (0.047 mg/kg), gills (0.017 mg/kg) and kidney (0.008 mg/kg). The average Pb concentration was in the following decreasing sequence: liver (0.82 mg/kg), skin (0.32 mg/kg), meat (0.24 mg/kg), gills (0.09 mg/kg) and kidney (0.08 mg/kg). The Cu concentration decreased in the following order: liver (3.92 mg/kg), gills (0.50 mg/kg), meat (0.115 mg/kg), kidney (0.11 mg/kg) and skin (0.01 mg/kg). The Fe level moved in the following descending sequence: kidney (80.98 mg/kg), gills (54.15 mg/kg), liver (36.001 mg/kg), skin (30.94 mg/kg) and meat (8.03 mg/kg). The concentration of Zn was highest in the kidney (109.45 mg/kg), then in the gills (101.43 mg/kg), skin (58.14 mg/kg) and meat (31.1 mg/kg), and the lowest was in the liver (20.22 mg/kg) (Table 11).

Analyzing the results of the heavy metal concentrations in the different organs of carp that were sampled in the fall, it was determined that the difference was statistically significant between the concentrations of As in the kidney compared to the other organs, in the skin compared to the other organs and in the meat compared to the other organs ($p < 0.05$). There were significant differences between the concentrations of Cd and Hg in the different organs. On the other hand, no statistically significant differences ($p > 0.05$) were found in the As concentration between the liver and gills, or between the Pb concentration in the skin and meat, as well as in the gills and kidney ($p > 0.05$). The difference in the Cu concentration in the meat and kidney was not statistically significant ($p > 0.05$), nor was the difference between the Fe concentration in the liver and skin. The difference in the Zn concentration was not statistically significant ($p > 0.05$) only between the kidney and gills (Table 11).

A comparison of the differences in the average values of the heavy metal concentrations between the seasons in which the sampling was performed (spring–autumn), and the obtained p-values for each organ, are shown in Table 12.

Table 12. Influence of the season on the concentration of heavy metals in the different organs of carp.

Tested Parameter	Unit of Measure	Muscle	Skin	Gils	Liver	Kidney
As [1]	mg/kg	$p < 0.0001$	$p < 0.0001$	$p = 0.001$	$p = 0.03$	$p = 0.0001$
Cd [2]	mg/kg	-	-	-	$p = 0.004$	$p = 0.28$ *
Hg [3]	mg/kg	$p = 0.0002$	$p = 0.04$	$p = 0.0002$	$p = 0.001$	$p = 0.10$ *
Pb [4]	mg/kg	$p = 0.0004$	$p = 0.0003$	$p = 0.05$ *	$p = 0.003$	$p = 0.10$ *
Cu [5]	mg/kg	$p = 0.26$ *	-	$p = 0.07$ *	$p = 0.06$ *	$p = 0.04$
Fe [6]	mg/kg	$p = 0.006$	$p = 0.07$ *	$p < 0.0001$	$p = 0.004$	$p = 0.29$ *
Zn [7]	mg/kg	$p = 0.04$	$p = 0.05$ *	$p = 0.35$ *	$p = 0.05$ *	$p = 0.004$

Notes: [1.] As—arsenic; [2] Cd—cadmium; [3] Hg—mercury; [4] Pb—lead; [5] Cu—copper; [6] Fe—iron; [7] Zn—zinc. $p < 0.05$; * $p > 0.05$—the difference is not statistically significant.

Based on the results shown in Table 12, the time of year in which the sampling was performed had a significant effect on the amount of As ($p < 0.05$) in all the examined organs of the carp. The concentration of Cd in the kidneys was not affected by the season, but the differences between the concentration of Cd in the liver were statistically significant in relation to the season ($p < 0.05$). Differences in the concentrations of Hg in the kidneys, Pb in the gills and kidneys, Cu in the meat, gills and liver, as well as Fe in the skin and kidneys and Zn in the skin, gills and liver were not statistically significant in relation to the season in which the sampling was performed (Table 12).

The order of the concentration of heavy metals in the different organs differed in spring and autumn, except in the gills, where it was the same in both seasons. In addition, the sequence of heavy metals was different in the different organs. In spring, the order of the elements in the meat was: Zn > Fe > Cu > Pb > Hg > As > Cd. The same sequence in the concentration of elements was in the skin and gills in the spring. In the liver and kidney, the order of the concentration of heavy metals and metalloids in spring was: Zn > Fe > Cu > Pb > Cd > Hg > As (Table 10). In autumn, the order of heavy metals in the meat was: Zn > Fe > Hg > Pb > Cu > As > Cd. The order of the concentration of elements in the skin in autumn was: Zn > Fe > Pb > Hg > As > Cu > Cd. In autumn, the liver contained the most Fe, followed by Zn, Cu, Pb, Hg and Cd, with the least being As. The sequence of elements in the kidney in autumn was: Zn > Fe > As > Cu > Pb > Cd > Hg (Table 11).

3.4. Assessment of the Exposure to Heavy Metals as a Consequence of Fish Consumption

The permissible weekly intake (PTWI) is an index that is used to calculate the concentration of heavy metals that a person can take into the body without having harmful consequences for his health. It is defined as the amount of a substance that can be taken into the body on a weekly basis during a person's life without the risk of negative effects on that person's health. Based on the data that the weekly consumption of freshwater fish per capita in Serbia is 29.4 g/week [37], and that carp is the most common freshwater fish, the weekly intake and the allowed weekly intake can be approximately calculated. Table 13 shows the PTWI values defined by the World Health Organization and the Food and Agriculture Organization of the United Nations (FAO/WHO) for certain metals for a person weighing 70 kg and the estimated weekly intake of the mentioned elements through the consumption of carp meat, which was analyzed in this paper.

Table 13. Permitted weekly intake and estimated average weekly intake of metals by consuming carp grown in a pond filled with purified wastewater from the slaughterhouse.

Parameter	PTWI	Weekly Intake for a 70 kg Person	Average Weekly Intake *
Cd [1]	5.75 µg/kg/weekly	0.403 mg/weekly	<0.001
Hg [2]	5 µg/kg/weekly	0.35 mg/weekly	0.02
Pb [3]	25 µg/kg/weekly	1.75 mg/weekly	0.01
Cu [4]	3.5 mg/kg/weekly	245 mg/weekly	0.03
Fe [5]	5.6 mg/kg/weekly	392 mg/weekly	0.57
Zn [6]	7 mg/kg/weekly	490 mg/weekly	1.20

Notes: [1] Cd—cadmium; [2] Hg—mercury; [3] Pb—lead; [4] Cu—copper; [5] Fe—iron; [6] Zn—zinc; mean value ± standard deviation (n = 4); $p < 0.05$. * The estimated weekly intake was calculated by taking into account the highest measured concentration of elements in the meat of carp grown in a pond filled with purified wastewater; the permitted weekly intake (PTWI) has been defined by the World Health Organization and the Food and Agriculture Organization of the United Nations (FAO/WHO) for individual metals for a person weighing 70 kg.

4. Discussion

4.1. Concentrations of Heavy Metals in the Water Samples

The obtained results for the concentrations of elements in the water were compared with the national water quality guidelines and literature values specified for ponds. By comparison, it can be determined that the concentration of As and Hg in the wastewater from the slaughterhouse before the purification was significantly above the MDK prescribed by the Regulation on Hazardous Substances in Water [34], which prescribes the concentration limit values of dangerous substances that may not be introduced into the waters directly or indirectly. It is very important to determine the quality and degree of pollution of wastewater and take appropriate measures accordingly. In the case of water coming out from slaughterhouses, it can be concluded that purification is a necessary measure before further use or discharge into waterways. The presented results of the analysis of the wastewater from the slaughterhouse obtained during our research are significantly lower compared to the results of the research by Dauda et al. [38], who analyzed the wastewater from a slaughterhouse in Nigeria. In the study by the aforementioned authors, the average concentration of Pb in the wastewater was 0.16 mg/L, which is about ten times higher than the result obtained in our study. The mean value for Zn in the water from a slaughterhouse in Nigeria was 79.5 mg/L, and for copper it was 1.61 mg/L, which is significantly higher than the results obtained in our research (Zn—184.8 μg/L, Cu—43.1 μg/L). Dankaka et al. [39] examined the wastewater from a slaughterhouse in Nigeria; during four weeks of testing, they obtained results according to which the concentration of Pb and Cu was below the detection limit, while the concentration of Fe was in the range of 0 to 0.64 mg/L. They determined that the mean value of the Fe concentration in the wastewater was 0.346 mg/L, which is lower compared to the results obtained in our study (420.6 μg/L in spring and 425.2 μg/L in autumn).

The results of the quality of the water sampled after passing through the purifying system, from a few spots on the property of the meat industry, unequivocally show the efficiency of the purifiers and the integrated system. The analysis of the results obtained shows that the efficiency of the purifiers in terms of the content of heavy metals and metalloids is high, and ranges between 87 and 100%. As for the content of heavy metals and metalloids, the water from the prepool and pool in both the spring and autumn sampling corresponded to class 3, i.e., the tested parameters did not exceed the MDK for class 3 water according to the regulation on the limit values of pollutants in surface and underground waters and sediment, and the terms for their achievement [35]. Surface waters belonging to this class provide conditions for the life and breeding of cyprinids.

The comparison of the obtained values of the concentrations of heavy metals and metalloids in the water sampled from the melioration channel with MDK values prescribed for the tested elements for water that can be used for irrigation prescribed by the regulation on permitted amounts of hazardous and harmful substances in soil and irrigation water [36] showed that the water quality was class 2/3, and so could be used for irrigation. In this way, the pond affects the increase in the quality of the purified wastewater and the achievement of the limit concentrations set before entering the natural recipient.

In many countries, including Serbia, there is still pollution with untreated water, which is discharged into waterways and the environment. It should be borne in mind that, by applying proper procedures for wastewater treatment, 30 to 99% of contaminants can be removed [40].

4.2. Concentrations of Heavy Metals in the Sediment Samples

Most of the heavy metals in the aquatic environment are concentrated in the sediment, which was confirmed by our results, and due to the possibility of their remobilization, they represent a significant risk for the environment. The concentration of metals in the sediment depends on the pH value, the redox potential, the content of organic matter and the presence of elements in the water. Sediment is a matrix in which heavy metals are usually present, and their direct transfer from sediment to the aquatic environment and aquatic organisms is the main way of contaminating aquatic organisms [41]. In the aquatic environment, sediments have a very important role in the nutrient cycle, and it is known that sediments are responsible for the transport of nutrients as well as contaminants [42]. This is why the state of the ecosystem, i.e., water and sediment, directly affects the quality and safety of fish meat. So, by analyzing the sediments, we can obtain information about the contamination of an area at some point in time or in some historical period. The number of elements that could migrate from the sediment in the water is directly dependent on the pH, the oxidation-reduction potential of the environment, the ionic forces, etc. [43]. Microorganisms present in the sediment transform these heavy metals into biologically active or toxic organic or inorganic compounds that continue to circulate in biological cycles. According to the results obtained by Đinović et al. [44], the element content in the sediment directly influenced the concentration of these elements in the water, with the highest concentrations both in the sediment and in the water being measured for Fe and Mg. On the other hand, the results obtained in our research showed that the concentration of elements in the sediment did not directly affect the concentration of elements in the water.

The results of the analysis of the average concentrations of heavy metals in the sediment indicate that the concentration of the tested elements in our study was approximately the same in both samplings, with slightly higher values in the autumn period. By observing individual elements in individual sediment samples, differences were found that were statistically significant for individual elements, but by comparing the results between the average values of heavy metal concentrations in the sediment sampled in spring and autumn, it was determined that the difference was not statistically significant ($p > 0.05$) and that the season had no influence on the content of heavy metals in the sediment in the integrated carp production system. The measured concentrations did not exceed the concentrations prescribed for the sediment by the regulation on the limit values of polluting substances in surface and underground waters and sediment, and the deadlines for their achievement [35]. Based on the sediment evaluation criteria defined by the regulation, it can be concluded that the concentration of most of the investigated heavy metals in the sediment samples analyzed in our research is at the natural background level. The measured values of Cd in pond 2 (0.96 mg/kg in spring and 0.99 mg/kg in autumn) were higher than the target value (0.8 mg/kg). Hg concentrations in the sediment from the irrigation channel (1.47 mg/kg in spring and 4.49 mg/kg in autumn), prepond (0.66 mg/kg in spring and 0.62 mg/kg in autumn) and in the pond 1 (0.54 mg/kg in spring and 0.62 mg/kg in autumn) were higher compared to the target value of 0.3 mg/kg for Hg. The Cu concentration in the irrigation canal (60.4 mg/kg in the spring and 60.9 mg/kg in the autumn), the prepond (56.6 mg/kg in the spring and 57.1 mg/kg in the autumn) in the pond 1 (55.1 mg/kg in the spring and 55.8 mg/kg in autumn) and in pond 2 (49.6 mg/kg in spring and 49.9 mg/kg in autumn) were higher compared to the target value for copper, which is 36 mg/kg. The values of the pollutant concentrations that are higher than the target value and lower in relation to MDK (which was the case with all the tested elements in this research) show that the sediment was slightly polluted.

The mean concentrations of As, Cd, Hg and Pb in the sediment samples were below the proposed threshold effect concentrations (TEC), indicating that there were no adverse effects due to their presence.

4.3. Concentrations of Heavy Metals in the Different Fish Tissues

There is the possibility of the mobilization of pollutants from the ecosystem, especially toxic elements in fish tissues [45], which could reach the pond as a result of the pollution of the immediate environment. Existing pollutants from the environment can be bioaccumulated in fish meat and organs [46]. It is also important to note that carp is classified as a highly bioaccumulative fish species [47].

According to the regulations on the maximum concentrations of certain contaminants in food [48], the concentration of heavy metals detected in the muscle tissue of fish was well below the prescribed value. Furthermore, levels of heavy metals in common carp were below the Food and Agriculture Organization of the United Nations (FAO) and the European Union maximum permitted levels in fish intended for human consumption. Heavy metal levels in common carp in our study did not exceed the maximum permitted levels by the FAO/WHO Codex Alimentarius Commission [49]. The obtained results suggest that produced common carp comply with the standards of consumer safety, which is in line with previous results on the feasibility of using treated wastewater in fish production [50].

Previous findings have shown that the accumulation of heavy metals in fish tissues depends mostly on the concentration of heavy metals in water and the duration of exposure; however, some environmental factors, such as the salinity, pH, water hardness and temperature, also play a significant role in the accumulation of metals in fish tissues [51,52]. The bioaccumulation of metals from the surrounding environment occurs by the incorporation and retention in the fish bodies [53]. The fact that the content of heavy metals in fish meat does not have to be proportional to their content in water is also interesting. In the research conducted by Eneji et al. [54], the content of heavy metals in the meat of tilapia looked like this in a decreasing sequence: chromium > zinc > copper > iron > manganese > cadmium > lead, and in the water from which the fish was sampled, the sequence looked like this: iron > chromium > lead > manganese > zinc > copper > cadmium. This fact also indicates that the bioaccumulation of heavy metals is a complex and specific process that largely depends on the type of fish, and especially its place in the trophic system. This was confirmed by the observations of the relationship of the elements in the water and the fish in the research by Đinović et al. [44]. The mentioned authors stated that the concentration of elements in the water was not proportional to the content of the elements in the fish, which was also confirmed by our research, according to which the ratio of the elements in the water and fish also differed. According to our results, lead was not detected in the water from the pond, but it was detected in the meat of the carp, as well as in the liver, kidney, gills and skin. It can be concluded that this is a consequence of the bioaccumulation of lead from the sediment. The largest part of heavy metals enters the fish organism through the gills and skin (bioconcentration), but the share of heavy metals that reaches the fish organism through food (bottom fauna, zooplankton and phytoplankton) is not negligible either (biomagnification). It is known that carp feeds on bottom fauna and dives into the sediment when searching for food. The sediment samples in the study contained the most Zn, then Cu, Fe, As, Pb and Hg, and the least Cd, while the water contained the most Fe, then As, Zn, Cu, Hg and Cd, and the least Pb.

Mkali et al. [55] investigated the heavy metal contamination of *Clarius garipenius*. They found Hg, Cd, Cr and Zn in the analyzed fish and water samples. The Pb, Mo and As content was below the limit of detection. They reported that the concentrations of the detected metals were higher in the fish than in the water, with the exception of Hg in two ponds, where it was above the levels permitted by the WHO.

Zaibel et al. [50] reported that the concentrations of heavy metals in guppy fish samples were lower than the limit of detection or the limit of quantification in a laboratory-scale experiment of fish-rearing in treated wastewater for four months. Feldlite et al. [7] also reported low concentrations of heavy metals in common carp (*Cyprinus carpio*)), tilapia hybrid (*Oreochromis niloticus* × *O. aureus*) and hybrid Chinese carp (a hybrid of silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*) reared in treated wastewater for five months and in secondary treated wastewater for two years. They detected Cd in the carp and tilapia liver and bone, and Pb in the bone of common carp reared in secondary treated wastewater.

Demirak et al. [56] noted that no correlation was found between the metal concentrations in the water and sediment or between the metal concentrations in the water and the muscle and gills of *L. cephalus*. Darko et al. [57] reported that the maximum concentrations of Pb, Cd and Hg in the water samples were 28.7, 18.2 and 150.0 μg/kg, respectively; the maximum concentrations of Pb, Cu and Hg in the sediment samples were 27.4, 323 and 150 μg/kg; the maximum concentrations of Pb, Cd and Hg in the fish samples were 48.6, 18.9 and 320 μg/kg. In the study conducted by Karadede and Ünlü [58], Cd, Co, Hg, Mo and Pb were not detected in the water, sediment and fish samples from the Atataurk Dam Lake, Turkey. Kuplulu et al. [59] reported that the metal concentrations decrease in the order As > Pb > Hg > Cd, and they found that order in all four of the seas of Turkey (the Black, Marmara, Aegean and Mediterranean Seas). According to our results, the metal concentrations in the muscle tissues were in the order Hg > Pb > As > Cd. Cd was reported as the lowest contaminant, which is in agreement with the study conducted by Kuplulu et al. [59].

There are conflicting opinions about the accumulation of heavy metals in certain organs. It is also assumed that certain organs have the ability to accumulate heavy metals and detoxify them. When it comes to Hg, it has been noticed that its concentration is higher in the meat of fish compared to the internal organs if the fish originates from flowing waters with a low content of this element. In contrast, if the Hg content in fish meat is greater than 1 mg/kg, it is observed that the content is higher in the internal organs compared to the meat [60]. According to the results of our research, the Hg content in autumn was higher in the meat compared to the organs, while in spring, the Hg content was slightly higher in the kidney compared to the meat, but it was higher in the meat compared to the other organs. According to the results of Đinović et al. [44], Fe (3.10–6.78 mg/kg) and Zn (3.10–6.78 mg/kg) were measured in the highest concentrations in fish fillets, and Fe and manganese were the most abundant in the water from Ečka pond. In our research, slightly higher values were found for the concentrations of Fe (8.03—16.67 mg/kg) and Zn (21.86—31.1 m/kg) in the muscles of carp compared to the results obtained by the mentioned authors for the same species.

The content of heavy metals in the different organs of carp in our research depended on the season. Higher average concentrations for most of the investigated heavy metals were measured in the samples taken in the autumn period compared to those taken in the spring, and could be connected with meteorological conditions, especially with a significantly higher amount of rainfall in April in comparison with October. These results are in agreement with previous findings [61,62]. The weight of the carp was higher in the autumn sampling, and there is probably a more intense accumulation of heavy metals. Positive correlations with age and body mass were previously reported for common carp [63]. It is known that numerous biotic and abiotic factors, both individually and jointly, affect the concentration of heavy metals in the different tissues of fish, water and sediment. The obtained results varied significantly under various ambient conditions and at different sampling times. In addition to the concentration and the form of metals in the water, their mutual synergistic and antagonistic action, the uptake of metals into the fish organism, is influenced by numerous physical and chemical parameters, among which are the water temperature, the concentration of soluble oxygen, the pH value and others, but also the physiological state of organisms, such as their age, size, sexual-cycle stage and others [62–64]. Therefore, in natural conditions, seasonal variations in the content of heavy metals in fish are most often encountered, which is the result of the combined action of all the mentioned factors.

The obtained results confirm the variations in the accumulation of heavy metals in the different tissues. According to the results of our research, most of the elements were accumulated either in the kidney (As, Cd, Hg, Fe and Zn) or in the liver (Cd, Pb and Cu). Karadede and Ünlü [58] reported that the highest concentrations of Cu, Fe, Mn and Zn were found in the liver, while the lowest concentrations were detected in the muscle tissue. In our study, the highest concentrations of Zn and Fe were observed in the kidney. Also, they reported that the highest level of Cu accumulation in common carp was found in the liver, which agrees with our results. Depending on the physiological role of each specific tissue, different metals have varied harmful effects in different fish tissues. The effects of heavy metals on different organ systems, such as the muscle tissue, liver, skin, gills, nervous system, skeleton and blood, are well described [63,65,66].

The maximum permitted level of Pb in the muscle meat of fish according to European and Serbian legislation [48,67] is 0.3 mg kg^{-1}. European legislation [67] set a maximum level of cadmium for the muscle meat of fish of 0.05 mg kg^{-1}, which is in accordance with the Serbian legislation [48].

According to previous investigations [44], the amount of harmful substances in the fish meat, water and sediment, which were collected from one of the biggest fish ponds in the Republic of Serbia, where production is taking place in a semi-intensive culture system, was within the prescribed range of the Serbian regulation and the European Union regulation of the maximum admissible concentrations of pollutants, which was not the case with fish from the wild [68,69]. Comparing the results obtained by examining the conventional pond and the results obtained in the present study, a lower content of pollutants in the samples from the integrated type of production has been noted. Although it is expected that the fish from aquaculture have a lower level of harmful substances than the fish from the wild, primarily due to the distance from facilities for growing the fish of the cities and big industrial polluters, it was not the case in all parts of the world according to the results of previous studies, which showed that the concentrations of some pollutants were higher in the fish from aquaculture in relation to wild fishes [70]. In the integrated system of fish production, there may also be an increased presence of various residues, which can enter the pond, and consequently the fish, either from the immediate environment or originating from the slaughterhouse and entering the pond with the water supplied to it. Certainly, in an integrated production system, it is necessary to evaluate the safety of the carp produced in this way for human consumption.

The degree of contamination of the fish meat can indirectly serve as a bioindicator of the degree of contamination of the ecosystem [62,71].

4.4. Assessment of the Exposure to Heavy Metals Because of Fish Consumption

The consumption of food containing large amounts of heavy metals can lead to poisoning. Intoxication can be acute or chronic. Those elements that tend to accumulate in certain organs over a long period of time lead to disease when their concentration reaches critical values in these tissues. Human exposure to heavy metals is most common through the consumption of contaminated food (about 90%) and is significantly more frequent than exposure through inhalation or through the skin [72]. When it comes to toxic elements that have an extremely harmful effect on the human body, even in very low concentrations, and show mutagenic, teratogenic and carcinogenic effects, the importance of the regular control of their concentration in food intended for human consumption must be emphasized.

To protect the health of consumers in most countries of the world, as well as in Serbia, the MDK of toxic metals in various foods are defined by law. According to the regulation of the Republic of Serbia, which is harmonized with EU regulations, MRLs in fish have been established for lead, cadmium and mercury [48]. In addition, there are also recommendations in the scientific literature for the permitted daily intake of heavy metals. It is recommended that the daily intake of cadmium be in the range of 10–60 µg. Excess cadmium can cause diarrhea, vomiting, growth retardation and deformities, kidney damage, anemia, hypertension, prostate and lung cancer, high body temperature, dizziness and headache [73]. Methylmercury is responsible for direct diffuse damage to the somatosensitive cortex of the brain, which is transferred to the fetus via the placenta [28]. The recommended daily intake of mercury for humans is up to 300 µg. After absorption, which is rapid, mercury is stored in the liver, kidneys, spleen and bones. An excess of mercury can lead to poisoning, which is manifested by diarrhea, vomiting, abdominal pain, dizziness, neurological disorders and others [74]. It is known that lead and its compounds are extremely toxic and that they easily enter the body through inhalation, ingestion and through the skin, so the daily intake should not exceed 100–429 µg. Excess lead leads to poisoning manifested by anemia, vomiting, inappetence, damage to the liver, brain and kidneys, headache and even coma. Chronic human exposure to lead leads to mental retardation, allergies, muscle weakness, kidney damage and damage to newborns [75]. The recommended intake of iron through food is from 50 to 400 µg per day [76]. Lack of iron in the diet leads to infections, anemia, pallor and anorexia. On the other hand, excess iron leads to damage to the liver, heart and pancreas, and causes hypoglycemia, vomiting, diarrhea, liver cancer and cyanosis [77]. The recommended daily intake of zinc is 100–200 µg. Lack of zinc leads to slower physical development, loss of the sense of touch and smell, loss of appetite and the reduced ability to heal wounds. Excess zinc leads to spasms, pancreatitis, dizziness, dermatitis and paralysis. The recommended daily intake of copper for humans is in the range of 150 to 600 μg. Lack of copper in the body leads to anemia, nephrosis and depigmentation, while excess copper leads to dizziness, diarrhea, hemolytic anemia, neurological and renal dysfunction, hypertension and dermatitis [76].

By comparing the results obtained in our study, it can be concluded that the concentrations of toxic metals were lower compared to the prescribed MDK. It can be concluded that the estimated weekly intake is far below the stated values for the recommended weekly intake. For the results to be more reliable, it is necessary to analyze a larger number of fish and to perform an analysis of both the meat and the various internal organs. Based on the results obtained in the research in this work, carp produced in a pond supplied with purified wastewater from the slaughterhouse industry is safe for human consumption from the point of view of the presence of residues of heavy metals and metalloids. Based on the presented results, it can be concluded that the concentration of heavy metals and metalloids in the wastewater from the slaughterhouse were above the prescribed limit values for wastewater from the slaughterhouses discharged into natural recipients in both the spring and autumn sampling. The purification of wastewater from the slaughterhouse is a necessary measure before the water is discharged into waterways. The efficiency of the wastewater treatment plant was very high, and for heavy metals and metalloids, it was in the range of 87% to 98%. The water from the pond corresponded to class 3 in terms of the concentration of heavy metals and metalloids both in spring and autumn. It can be used freely for breeding carp and other cyprinid fish species. The water from the irrigation canal corresponded to class 2/3 regarding the concentration of heavy metals and metalloids, and can be used for irrigation. No statistically significant differences were found for the tested heavy metals and metalloids in the sediment samples in relation to the season in which the sampling was performed. The measured concentrations of heavy metals in the sediment were generally close to or lower than the concentrations measured in the ponds or reservoirs where the carp were grown in Serbia and around the world. The measured concentrations corresponded to the pollutant concentrations in the sediment, either at the level of the natural background or at the level of the slightly polluted sediment. Carp produced in a pond supplied with purified wastewater from the slaughterhouse industry is safe for human consumption in terms of the concentration of residues of the tested heavy metals and metalloids. Additionally, the concentrations of detected metals in the muscle tissue were within the prescribed values for food samples. Continuous monitoring of the presence and concentration of heavy metals in integrated systems for fish production is very important, keeping in mind that fish is an important food source, but also an important indicator of environmental contamination. The presented results surely provide important data for the exposure assessment in the risk assessment of heavy metals for fish reared in purified wastewater. The content of heavy metals in the different organs of carp differed significantly depending on the season. The use of wastewater from slaughterhouses for fish production represents a completely new approach to finding solutions for the sustainability of the meat industry and the preservation of the environment. The application of this idea within the slaughterhouse is necessary from the aspect of solving environmental protection problems, bearing in mind the need to minimize environmental pollution as much as possible.

The permissible weekly intake (PTWI) is an index that is used to calculate the concentration of heavy metals that a person can take into the body without having harmful consequences for his health. It is defined as the amount of a substance that can be taken into the body on a weekly basis during a person's life without the risk of negative effects on that person's health. Based on the data that the weekly consumption of freshwater fish per capita in Serbia is 29.4 g/week [37], and that carp is the most common freshwater fish, the weekly intake and the allowed weekly intake can be approximately calculated. Table 13 shows the PTWI values defined by the World Health Organization and the Food and Agriculture Organization of the United Nations (FAO/WHO) [49] for certain metals for a person weighing 70 kg, and the estimated weekly intake of the mentioned elements through the consumption of carp meat, which was analyzed in this article. It can be concluded that the estimated weekly intake is far below the stated values for the recommended weekly intake.

In order for the results to be more reliable, it is necessary to analyze a larger number of fish and to perform an analysis of both the meat and the various internal organs. Based on the results obtained in the research in this article, carp produced in a pond supplied with purified wastewater from the slaughterhouse industry is safe for human consumption from the point of view of the presence of residues of heavy metals and metalloids.

5. Conclusions

Based on the presented results, it can be concluded that the concentration of heavy metals and metalloids in the wastewater from the slaughterhouse were above the prescribed limit values for the wastewater from slaughterhouses that is discharged into natural recipients in both the spring and autumn sampling. The efficiency of the wastewater treatment plant was very high, and for heavy metals and metalloids, it was in the range of 87% to 98%. The water from the pond corresponded to class 3 in terms of the concentration of heavy metals and metalloids both in spring and autumn, and can be used freely for breeding carp and other cyprinid fish species. The water from the irrigation canal corresponded to class 2/3 regarding the concentration of heavy metals and metalloids, and can be used for irrigation. No statistically significant differences were found for the tested heavy metals and metalloids in the sediment samples in relation to the season in which the sampling was performed. The measured concentrations of heavy metals in the sediment corresponded to the pollutant concentrations in the sediment either at the level of the natural background or at the level of the slightly polluted sediment. The content of heavy metals in the different organs of carp differed significantly depending on the season. Carp produced in a pond supplied with purified wastewater from the slaughterhouse industry is safe for human consumption in terms of the concentration of residues of the tested heavy metals and metalloids. The concentrations of detected metals in the muscle tissue are within the prescribed values for food samples. Continuous monitoring of the presence and concentration of heavy metals in integrated systems for fish production is very important, keeping in mind that fish is an important food source, but also an important indicator of environmental contamination. The use of wastewater from slaughterhouses for fish production represents a completely new approach to finding solutions for the sustainability of the meat industry and the preservation of the environment. The application of this idea within the slaughterhouse is necessary from the aspect of solving environmental protection problems, bearing in mind the need to minimize environmental pollution as much as possible. Future investigations should be directed towards further investigations into the long-term effects of using purified wastewater for rearing different fish species, exploring additional parameters for water quality assessment and also optimizing wastewater treatment processes.

Author Contributions: Conceptualization, D.L.P. and M.P.; methodology, Ž.M.; validation, Ž.M., N.P. and M.Ž.B.; formal analysis, N.P. and Ž.M.; investigation, M.P.; resources, M.Ž.B.; data curation, D.L.P.; writing—original draft preparation, D.L.P.; writing—review and editing, A.G. and J.J.-D.; supervision, D.L.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovation of Republic of Serbia by the contract of the implementation and funding of research work of NIV-NS in 2023, contract no: 451-03-47/2023-01/200031.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Jurica Jug-Dujaković worked at a few Croatian Universities, and he is an expert, still the guest lecturer in Croatia, and this manuscript has nothing to do with his work in the USA.

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