

Article

Pesticide and Toxic Metal Pollution in Waters, Fish and Wild Animals in Vojvodina, Serbia

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Abstract: High concentrations of pesticides and toxic metal pollutants in the environment, often present in surface waters in nature, can accumulate in wild animals and are a significant public health concern. Serbia is a moderately developed, south European country in transition, with a long tradition in agriculture, particularly in the northern part, Vojvodina. Our study aimed to assess the presence of pesticides and to measure concentrations of toxic metals in the district of Backi Petrovac, Vojvodina. Water samples were analyzed from canals, ditches, fish from the canals and wild animals caught in the Backi Petrovac district. We identified nine pesticides in water samples. Of all detected pesticides, one was a hormonal disruptor. There were no pesticides in animals, fish and organs in a concentration above the limit of detection (LOD) in observed periods. The concentrations of toxic metals detected in superficial water (Cd, Pb, Hg, As, Mn, Cu) were below the permitted limits, as well as the concentrations of Cd, Pb, Hg, As and Mn in the whole bodies of fish. Concentrations in kidneys and livers of wild animals (rabbit and roe deer) were below the permitted limits, with the exception of cadmium, whose concentrations in some animal samples were above the permitted limit. Based on the obtained results, we concluded that in the developed agricultural region unencumbered by heavy industry, pollution by pesticides and heavy metals can be controlled.

Keywords: pesticides; toxic metals; concentration in superficial water; whole bodies of fish; organs of wild animals

1. Introduction

Contemporary economies and agriculture generate large amounts of waste [1]. While in developed countries there are strict rules in waste management, in less developed countries this domain does not adequately follow the development. The amount of toxic waste that pollutes the soil, aquatic systems and endangers animals and plants is consistently rising [2].

The presence of pesticides and heavy metals in water is one of the primary environmental indicators, particularly in agricultural areas. High concentrations can severely affect fauna and are a significant public health concern [3,4]. Although pesticides are indisputably necessary to increase agricultural production, many of them are toxic to living organisms. Therefore, states limit by law the presence of pesticide residues in water, land

and living organisms [5]. Pesticide pollution is one of 28 agro-environmental indicators according to the European Commission [6]. Diverse living organisms, such as insects, fish, plankton, livestock and bacteria can be used as bioindicators for monitoring the health of the natural ecosystem.

Serbia is a middle developed south European country with a long tradition in agriculture, particularly in the northern part, Vojvodina, located in the fertile Pannonia basin, which until 1918 was part of the Austro-Hungarian Empire. It has developed agriculture. In the process of accession to the EU, Serbia has adjusted to EU laws and regulations in safe pesticide and waste management, with the determination of maximum residue levels (MRL) for pesticides and maximum permissible levels (MPL) for toxic metals [7]. However, the implementation of these laws in everyday practice is not consistent yet. Although pesticides, if not registered, cannot be bought in agricultural supply stores in Serbia, they can still be found on the black market. A special problem regarding the application of pesticides is frequency and amount of spraying, due to resistance to pesticides. Although in recent research farmers claimed they do not use more than the recommended concentrations, many of them thought the higher concentrations were more effective [8]. A special problem is the empty containers of pesticides. Although there are strict rules about management, the disposal in praxis has not yet been resolved, so the empty containers sometimes end up in watercourses. [8]. This can significantly contaminate the surface waters [3]. High numbers and concentrations of pesticides are predominantly detected in small- and medium-sized streams predominantly flowing through agricultural land [9].

The Serbian Environmental Protection Agency publishes annual reports on chemical pollution of surface and groundwater in Serbia. Pesticides have been detected every year, but only occasionally, with metolachlor, terbuthylazine and acetochlor detected among others [10]. Živančev [11] in four rivers in Serbia mainly detected atrazine, carbendazim, propazine and dimethoate, in low concentrations. Antić et al. [12] detected in the south-east part of the Danube carbendazim (269 ng/L), atrazine (188 ng/L), terbuthylazine (180 ng/L) and acetochlor (110 ng/L), while in the tributary Tisza river only terbuthylazine was present at high levels (130 ng/L). In other sampling campaigns, pesticides were present at low concentrations [12].

Heavy metals are well-known environmental pollutants due to their toxicity, persistence in the environment, and bioaccumulative nature [13]. The heavy metals As, Cd, Pb and Hg are considered most toxic to humans, animals, fish and the environment. Excessive concentrations of heavy metals are detrimental. They destabilize ecosystems because of their bioaccumulation in organisms, and toxic effects on biota and cause death in most living beings [14]. Their natural sources include weathering of metal-bearing rocks and volcanic eruptions. The most common source of heavy metal pollution today is heavy industry. One of the most important anthropogenic sources, particularly in agricultural regions, includes agricultural activities—mainly fertilizing, mining and industrial activities. Phosphate fertilizers are produced from phosphate rock by acidulation. The final product contains all of the heavy metals present as constituents in the phosphate rock. Phosphate fertilizers are particularly rich in toxic heavy metals. Heavy metals are well-known environmental pollutants due to their toxicity, persistence in the environment, and bioaccumulative nature [13]. Based on comparison of actual concentrations of hazards substances, including the heavy metals, with average yearly concentrations as well as with maximal permitted concentrations in surface waters the Serbian Environmental Protection Agency classifies surface waters into five classes [10]. According to the yearly report, only arsenic, in two samples, detected in high concentrations in the northern part of Vojvodina, and iron, in different localities in Serbia, were classified in the fifth class.

In addition to everyday pollution with pesticides and heavy metals, in the past 20 years there have been incidents reported of poisonous waste accidentally spilling into natural watercourses, which in some cases had the scale of an ecological disaster [15]. The result was the mass death of fish, the destruction of animal and plant habitats, and the disturbance of the ecological balance.

The municipality of Backi Petrovac is a mostly agricultural area, with agricultural production in transition. While 20–30 years ago many farmers cultivated small amounts of land, up to 5–10 hectares, which involved a lot of manual work for controlling weeds, today the amount of land per farmer is increasing, and only mechanization and chemicals are used for weed control and for protection from pests. In the area, there are no industrial plants that would significantly pollute the environment. The closest upstream city is Vrbas, more than 30 km from the Backi Petrovac municipality, with mainly food producing industry.

Therefore in our research we wanted to examine the level and the threat of pollution of ecosystem-surface waters, fish and wild animals in this one developing agricultural environment, unburdened by nearby industry.

2. Materials and Methods

2.1. Place of Study

The study was performed in the Backi Petrovac district, a municipality with about 14,000 inhabitants, a traditionally agricultural region situated in the Vojvodina region, the most developed rural area in Serbia (Figure 1). Residents, primarily the Slovak minority, have traditionally engaged in agriculture since their movement within the former Austro-Hungarian Empire from the north part (today's Slovakia) to the south, Vojvodina (today's Serbia) about 250 years ago. In recent years, the number of small (up to 5 hectares) agricultural households has declined, and most smallholder farmers have around 25 hectares. The number of registered agrarian families in the district is 1313 [16].

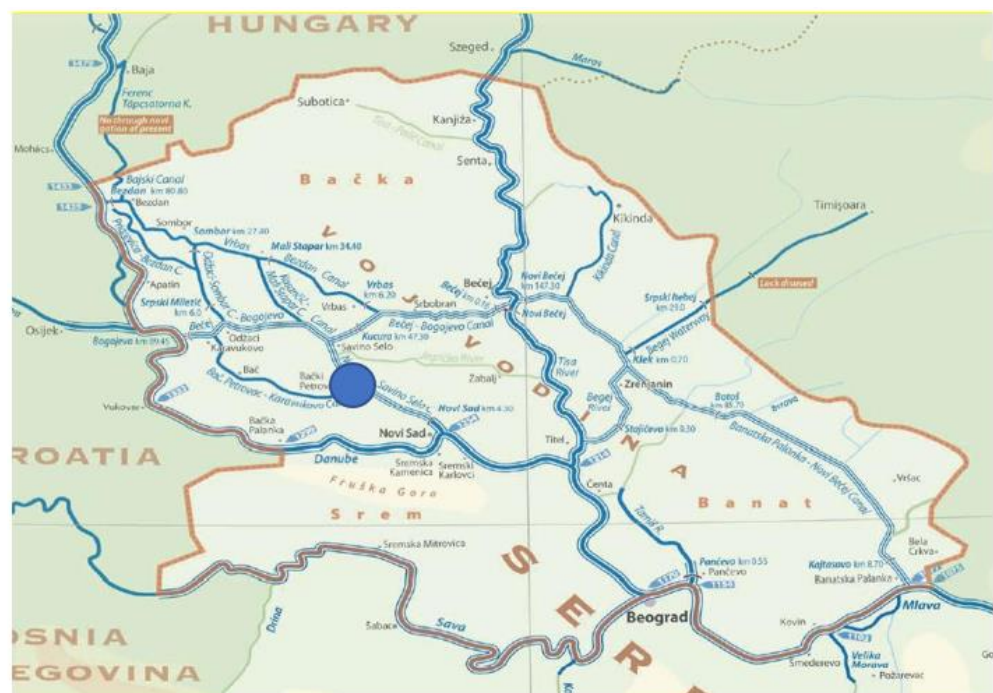


Figure 1. Vojvodina rivers and canals, and Backi Petrovac district (source: <https://www.superjoden.nl/vode-vojvodine-mapa.html> (accessed on 13 June 2021)).

2.2. Sampling

Water samples were collected in the period 2018–2020. The samples were collected in summer 2018, and in the spring of 2019 and 2020. Collection dates were chosen because pesticides were used for the most commonly cultivated crops at that time. Water samples were collected from 10 localities (Figure 2):

- The two canals passing through the region (from two points, 9 and 10 two empty in Figure 2). The Karavukovo Backi Petrovac (kanal KBP) is 52 km long, while the Mali Backi canal is 66 km long. The exact deepness of the canals is not known, unofficially

about 3 m. Around the canals there is high grass and particularly in summer, a lot of vegetables are grown in the canal (Figure 3). The most common agricultural species in the area are soybeans, corn, wheat, potatoes, paprika.

- From water ditches in the municipality, in different locations (seven points, 1, 2, 3, 5, 6, 7, 8 in Figure 2). The ditches in the field start from the canals and branch off the area. They are used mainly for drainage, only occasionally for irrigation. They are about 2 m wide and about 1–2 m deep. During the summer, some of them are water-free. At the time of sampling, water was in the ditches.
- From an artesian well (one point, 4), in the vicinity of the canal.

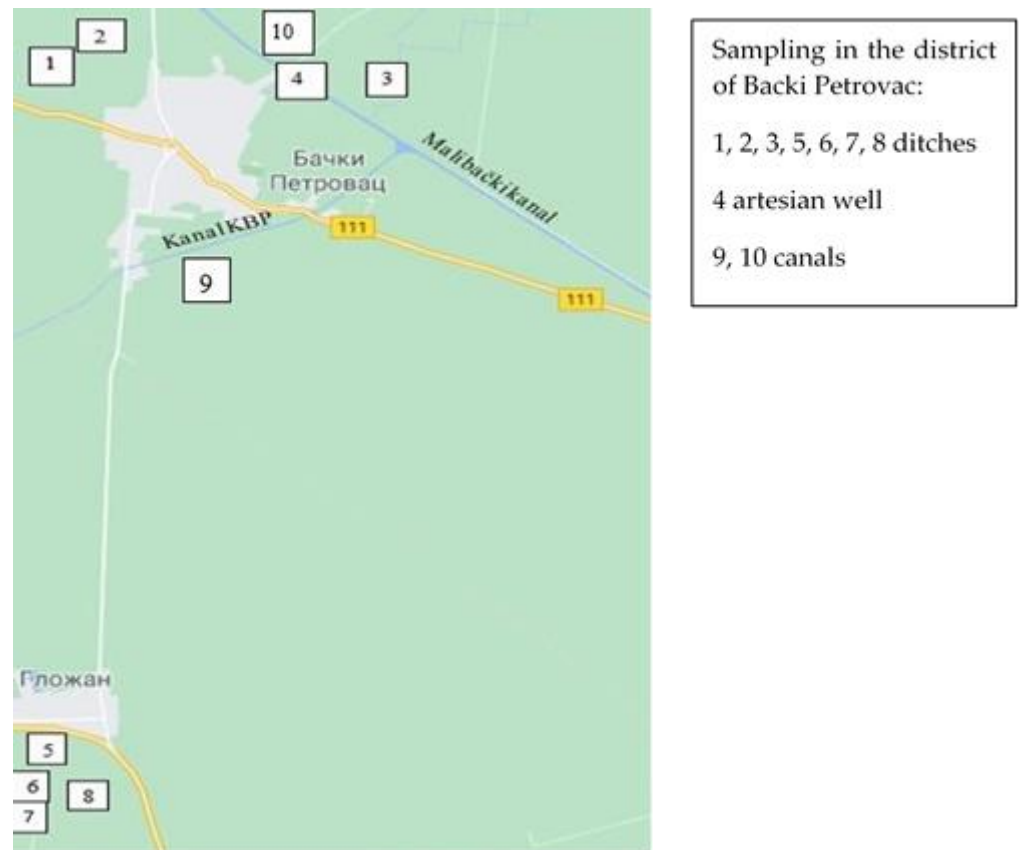


Figure 2. Sampling points in the district of Backi Petrovac.



Figure 3. Canal Karavukovo Backi Petrovac. (source https://commons.wikimedia.org/wiki/File:Kanal_Karavukovo-Ba%C4%8Dki_Petrovac_-_panoramio.jpg (accessed on 13 June 2021)).

A total of 30 samples were collected and analyzed.

Water sampling: All samples were taken from the water surface. All water samples for toxic metal analysis were sampled in accordance with SRPS EN ISO 5667-3: 2017; all samples were filtered using a membrane filter (nylon filter membranes, pore size 0.45 µm, diameter 47 mm) and had a turbidity of 1 NTU less than 1 NTU) measured turbidimetrically.

Fish samples were prepared from caught fish in both canals. Forty fish from the groups Cyprinidae (silver carp, *Carassius gibelio* and *Rutilus*, bream, *Abramis brama*, and redbfin, *Scardinius erythrophthalmus*) were analyzed. These species are herbivores. The number of the species was approximate. The homogenates of the whole bodies were analyzed.

Wild animal samples were mainly obtained from hunters during the 2019 and 2020 seasons (mostly rabbits, *Lepus europaeus*, and roe deer, *Capreolus capreolus*). Livers and kidneys were taken from the animals. Specimens from about 40 animals were analyzed (both kidneys and livers from 10 animals, and livers from 17 other animals, both rabbits and deer).

2.3. Qualitative Determination of Pesticide Residues

The presence of pesticide residues was determined by qualitative methods in the waters of the study area and in the organs of fish and animals killed or found in the study area.

The samples were prepared in the laboratory of the Department of Pharmacology and Toxicology of the Medical Faculty in Novi Sad.

For this type of search-qualitative analysis, the method EPA 525.2 (Method 525.2, Revision 2.0: Determination of Organic Compounds in Drinking Water by Liquid–Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry) was used. Concentration assessment was performed according to section 13.0 Method Performance, page 25 of the above standard method (EPA 525.2), the detection limit was 0.01 g/L.

2.4. Quantitative Determination of Toxic Metals

The presence of toxic metals (Cd, Pb, Hg, As, Mn and Cu in water, and Cd, Pb, Hg, As and Mn in fish and animals) was determined by quantitative methods.

The samples were prepared at the Institute of Public Health of Vojvodina, Department of Hygiene.

The samples were mineralized by wet acid digestion according to the procedure of the Start D Microwave Digestion System (manufactured by Milestone) for quartz inserts (cuvettes) (QS-50 quartz inserts for trace and ultra trace analysis). An inner solution of 5 mL of 65% ultra-pure HNO₃ was used and a mixture (outer solution) of 5 mL of deionized water (ASTM 2 purity) and 1 mL of H₂O₂ (for metal trace analysis) (shown in Figure 3), as the outer solution had no physical contact with the sample contained in the quartz cuvette. The laboratory uses certified reference materials in the quality control system. Quality assurance/quality control was externally evaluated.

Mineralization was performed according to SRPS EN ISO 15587-2: 2009 and technical instructions of the manufacturer (Milestone; Digestion Application Note DG-CL-03) with the following parameters: temperature 180 °C, applied power max. 1000 W and time 25 min.

A Start D Microwave Digestion System (manufactured by Milestone) with an HPR 1000/10 rotor (for high pressure) with Teflon cuvettes and 30 mL QS-50 quartz inserts was used.

ICP-MS method was used to determine the level of toxic metals (surface water SRPS EN ISO 17294-2:2017; SRPS EN ISO 15587-2:2009). The values of limit of quantitation (LOQ) for toxic metals are presented in Table 1a.

The values of limit of quantitation (LOQ) for pesticides are presented in Table 1b.

All data were coded and analyzed using SPSS version 20 (IBM SPSS, 2020) and Microsoft Office Excel 2010.

Table 1. (a) Limit of quantitation (LOQ), $\mu\text{g/L}$, in surface water, in samples of whole fish and in livers and kidneys of wild animals. (b) Limit of quantitation (LOQ), ng/g sample in surface water, in fish and in wild animal organs (livers and kidneys).

| (a) | |
|---------------------------------------|-----------------------------|
| Toxic Metal | LOQ ($\mu\text{g/L}$) |
| Cd | 0.04 |
| Pb | 1.0 |
| Cu (depends on hardness of the water) | 0.1 |
| As | 0.1 |
| Mn | 0.2 |
| Hg | 0.08 |
| (b) | |
| Pesticide Group | LOQ (ng/g Sample) |
| organophosphorus pesticides | 1 |
| organochlorine pesticides | 0.5 |
| carbamates | 3 |
| triazines | 5 |
| sulfonilurea | 3.16 |
| metolachlor | 2 |

Maximal permitted concentrations (MPC) in surface water: Cd 0.45–1.5 ($\mu\text{g/L}$), depending on hardness of water; Pb 14 ($\mu\text{g/L}$) Hg 0.07 ($\mu\text{g/L}$); Cu 5–112 * ($\mu\text{g/L}$) depending on hardness of water. LOQ * (Limit of quantitation) and limit values in drinking water [14] for the analyzed metals in ng/mL were as follows: Cd < 0.04; Pb < 1.0; Hg < 0.16; As < 0.1; Mn < 0.2; Cu < 0.1.

The results are shown as frequency and percentage, analyzed at level of $\alpha \leq 0.05$ to determine statistical significance. The chi-square test (χ^2) was used to measure the possible correlation between nominal variables, with Yates' correction.

A one-factor ANOVA test determined the statistical significance of the difference between the liver and kidney concentrations.

3. Results

The detected pesticide residues are shown in Table 2.

The most frequently found pesticide in the canals was dichlorobenzene (five samples in 2018), followed by one sample of nicotine in 2018 and one sample of metolachlor in 2019 and phenylphenol in 2020. In ditches, nicotine (metabolite of nicotinoids, three samples in 2018) dichlorobenzene, metolachlor, bornanon and 2,3 dipyridyl were detected. In 2019 terbutylazine, metolachlor, bromoanalin and bromophenylisocyanate were found while in 2020 one sample each of phosphonic and benzoic acid was detected. In the artesian well, there were no pesticides detected.

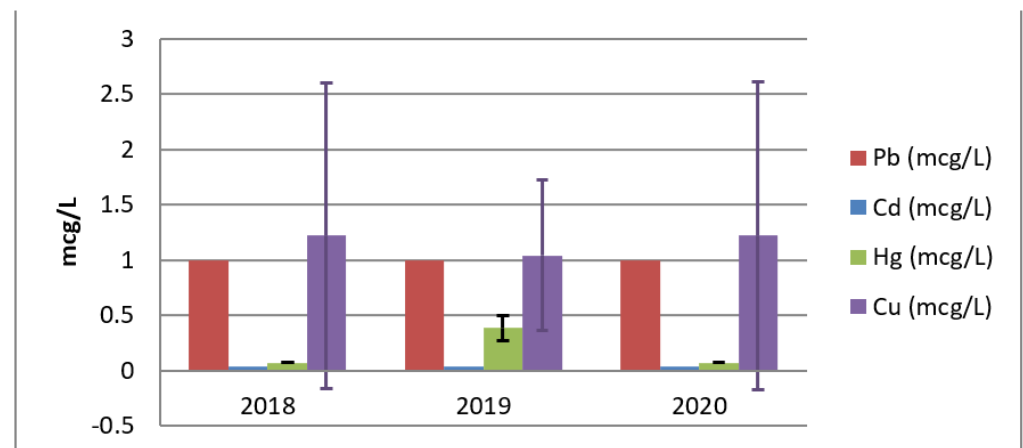
Of all detected pesticides, two (dichlorobenzene, 2,3 dipyridil) have been not approved for use in the EU and in Serbia for years. Metolachlor and nicotinoids were approved in Serbia at the time of sampling, but not in the EU. Of all detected pesticides, one (2,3 dipyridil) was a hormonal disruptor.

There were no pesticides in fish and organs of wild animals in a concentration above LOQ in observed periods. This finding agrees with the detection of pesticides in surface water, where the presence of pesticides was occasional, with no permanent presence.

Table 2. The qualitative detection of pesticide residues in water samples in three observed periods.

| Period | Place of Sampling | | | | | |
|-------------|------------------------------|-----------------|---------------------------------|-------------------------------|---------|---------------|
| | canal 1 | canal 2 | ditch 1 | ditch 2 | ditch 3 | artesian well |
| August 2018 | nicotine | - | nicotine | - | - | - |
| | dichlorobenzene | dichlorobenzene | dichlorobenzene | - | - | - |
| May 2019 | metolachlor | - | - | - | - | - |
| May 2020 | phenylphenol | - | - | - | - | - |
| period | ditch 1 | ditch 2 | ditch 3 | ditch 4 | | |
| August 2018 | dichlorobenzene | nicotine | dichlorobenzene | - | | |
| | metolachlor | - | - | - | | |
| | | 2,3 dipiridil | - | - | | |
| May 2019 | Terbutylazine metolachlor | terbutylazine | Bromophenyliso- cyanate | 4-bromoaniline metolachlor | | |
| May 2020 | - | - | phosphonic acid benzoic acid | - | | |

The results of quantitative determination of selected toxic metals in a surface waters is shown in Scheme 1.

**Scheme 1.** Concentrations of selected toxic metals in a sample of surface water in Backi Petrovac district 2018–2020 ($\mu\text{g/L}$).

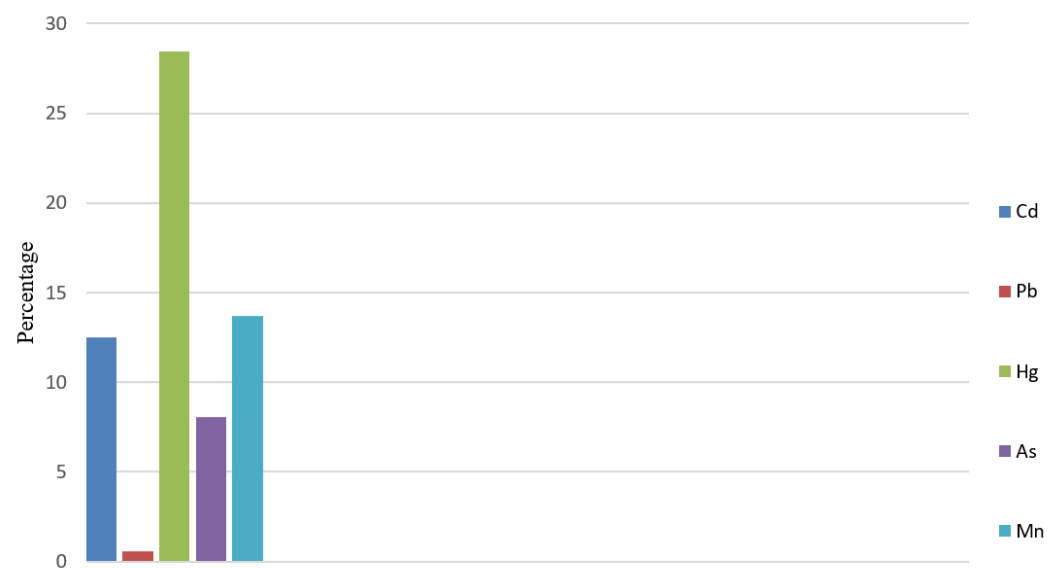
The concentrations of Cd, Pb and Cu in surface water were below the maximum permitted values, with the exception of Hg, for which the concentrations in 2019 exceeded the maximum permitted value of 0.07mcg/L (Scheme 1).

The presence of selected toxic metals in a sample of fish in the investigated period is shown in Table 3. With the exception of cadmium, measured in concentrations above LOQ in only 13.3% of samples, the concentrations of Pb, Hg, As and Mn were detectable in most samples (73.3–86.6%, depending on the metal), but in all cases in concentrations below the maximum permissible concentrations (Scheme 2).

The concentrations of toxic metals in the livers and kidneys of wild animals is shown in Table 4. The concentrations were detectable but under permitted limits in all samples but cadmium, where concentrations above the permitted level were measured in one liver (3.7%) and in four kidney (40%) samples. The concentrations in kidneys were higher than in livers for cadmium, lead, mercury and arsenic, reaching the significance level for cadmium, mercury and arsenic. For manganese, the concentrations were significantly higher in the livers.

Table 3. The average (\bar{x}) and maximum measured concentrations of selected toxic metals in homogenate of fish in Backi Petrovac district in investigated period and percentage of sample with concentrations above LOQ.

| Sample | Cd (ng/g) | Pb (ng/g) | Hg (ng/g) | As (ng/g) | Mn (ng/g) |
|---|--------------|-------------|--------------|---------------|---------------|
| LOQ/MPC | 0.04/50 | 1.0/200 | 0.16/500 | 0.1/2000 | 0.2/20,000 |
| \bar{x} | 0.71 | 4.11 | 33.35 | 56.64 | 1350.71 |
| maximum measured concentrations | 6.25 | 16.3 | 142.2 | 161.3 | 2741.9 |
| % of sample with concentrations above LOQ | 2/15 (13.3%) | 12/15 (80%) | 11/15 (73.3) | 13/15 (86.6%) | 13/15 (86.6%) |



Scheme 2. Ratio of maximum permissible concentrations of toxic metals and maximum measured in fish homogenates (%).

Table 4. The presence of selected toxic metals in the kidneys and livers of wild animals in Backi Petrovac in the investigated period and maximal permitted concentrations (MPC).

| The Concentration of Selected Toxic Metals in Liver and Kidney of Animals (ng/g) and MPC | | | | |
|--|---|--------------------|--------------------|----------------------|
| | Cd | Pb | Hg | As |
| MPC | MPC/WHO liver 500 ng/g kidney 1000 ng/g | MPC/EU 500 ng/g | MPC/EU 100 ng/g | MPC/WHO 2000 ng/g |
| Liver $\bar{x} \pm SD$ | 113.45 \pm 131.375 | 60.656 \pm 68.7 | 11.61 \pm 9.58 | 6.28 \pm 4.26 |
| Liver MIN–MAX | 10.0–631.15 | 2.5–262.75 | 1.5–53.5 | 0.5–18.75 |
| Kidney $\bar{x} \pm SD$ | 1367.99 \pm 1579.29 | 71.47 \pm 65.55 | 26.56 \pm 12.15 | 13.23 \pm 6.06 |
| Kidney MIN–MAX | 106.4–3819.25 | 16.025–204.5 | 13.925–53.175 | 5.47–21 |
| ANOVA | $p < 0.01$ | NS | $p < 0.01$ | $p < 0.05$ |

4. Discussion

When it comes to environmental pollution in agricultural areas, most attention is paid to pesticides. In Backi Petrovac district, the total number of pesticide residues identified

in two canals was only four (including dichlorobenzene), which is comparable to other findings from Serbia (in two research projects on the Danube and Tisza rivers only five pesticides were detected) [11]. In the DTD canal Backi Petrovac-Karavukovo in 2018 and 2019, the Serbian Environmental Protection Agency detected only six and five pesticides, respectively [10]. In other areas in Serbia the findings did not differ significantly. In our research in shallow water all together eight pesticides were identified. Of pesticides identified, dichlorobenzene and 2,3 dipiridil (a precursor of paraquat) were not approved for use in Serbia or in the EU. We found dichlorobenzene in 2018 in both canals (the running water) as well as in three out of four ditches connected to canals, and in one sample taken from the canal in 2019. Dichlorobenzene is a banned pesticide in the EU market since 2004 as well as in Serbia. The dichlorobenzenes (DCBs) are widely used in industry and in domestic products such as odour-masking agents, chemical dyes and pigments, and insecticides [17,18]. The dichlorobenzene's origin might be the wastewater from upstream factories, where the purification systems are still not adequate, despite the efforts and investments of the government in this area, as well as the laws that define limits very clearly [19]. However, as it was present in a marked amount in 2018, less in 2019 and absent in 2020, another possibility is that it was released during an ecological accident. Other identified pesticides were detected in different localities, each only once. Having in mind the fact that the Vojvodina region is a region with intense agricultural production, pollution with pesticides, with only 33% positive samples, was among the lowest, comparing to other agrarian regions.

In the Netherlands, Sjerpsab et al. [20] identified pesticides in 82% of their 23 surface water samples. In Spain, in the river Ebro, in 2011, more than 40 pesticides were detected. Some of them, such as organophosphate, chlorpyrifos, triazine, azole and others were present in high concentrations [21]. In 2016, 47 pesticides were identified, mostly insecticides [21] and herbicides [22]. Of them, 70% of pesticides did not have a marketing authorization in the EU and Spain at the time of the study. Pesticides that have not been used for a long time were still present in the waters of the rivers; 26 of the 47 pesticides were known or suspected endocrine disruptors [22]. In Greece, atrazine, simazine, alachlor, metolachlor and trifluralin were detected in rivers in 2006, and diazinon, parathion methyl from insecticides and lindane, and endosulfan and aldrin from organochlorine pesticides, which had not been used for years. Later, a limited number of herbicides used to protect corn, cotton and rice as well as organochlorine compounds, a residue from the past, were detected. Concentrations were only a small fraction of levels that, according to environmental risk assessment analysis, are not harmful to the aquatic world [23,24]. In Vietnam, Chau et al. [25] detected pesticides in all surface water samples, including drinking water. At the same time, the use of pesticides was so great that the concentrations, apart from exceeding the allowed ones in the EU, did not fall even during the off-season period.

In our research we did not detect pesticides in sample from fish and animals. This finding supports the incidental and only occasional presence of pesticides in water, with still no permanent negative consequences for animals. The absence of the pesticide residue in animal and fish tissue as an indicator of environmental pollution shows that the management of pesticides in the agricultural environment is still satisfactory and environmentally friendly.

Heavy-toxic metals are well-known environmental pollutants due to their toxicity and possibility of bioaccumulation. The As, Cd, Pb and Hg are considered most toxic to humans, animals, fish and the environment. Excessive concentrations of heavy metals are detrimental. They destabilize ecosystems because of their bioaccumulation in organisms, and toxic effects on biota and can cause death in most living beings [26]. In our research, the concentrations of toxic metals in samples taken from canals and shallow water were below MPC with the exception of Hg, for which concentrations in 2019 exceeded maximum permitted concentrations. Similar results were obtained by the Serbian environmental agency for 2019. The increased concentrations of 0.25 µg/L of total and soluble Hg were measured in Karavukovo Backi Petrovac canal. The reason for this rise in Hg concentration

is not known. However, upstream, the concentrations of soluble Hg were also increased up to 0.13 µg/L in July and to 0.46 in December 2019.

Although the concentrations of toxic metals, including the Hg, in fish, were under the LOQ, the situation in animals was different. In all samples, the concentration of toxic metals was above LOQ, with concentrations in kidney samples being significantly higher than in the liver samples for all metals except manganese, where the situation was the opposite, in accordance with reports of other authors (3.110 mg/kg liver and 1.190 mg/kg kidney in cattle) [27]. Concentration of cadmium was in some animals above maximum permitted values. Cadmium (Cd) is a heavy metal that does not have a physiological function and is often considered a toxicant. It accumulates in plants and animals with a long half-life of about 25–30 years [28]. Cadmium exposure has been established to induce cancer in various tissues and possess a genotoxic effect. Furthermore, cadmium exposure at relatively low doses induces circulatory diseases in laboratory animals [29]. In rabbits, in *ex vivo* studies, cadmium is toxic to spermatozoa [30]. Based on *in vivo* data, it causes subsequently possible decreased reproductive functions in environmentally polluted areas [31]. Other authors also reported increased concentrations of cadmium in tissue of and animals of carnivorous species. In wild boar, kidney concentrations of Cd (mean 4.16 mg/kg wet weight (w.w.), range 0.16–12.8) were higher than the permissible level for human consumption in 99.9% of the samples [32]. Cadmium absorption after dietary exposure in humans is relatively low (3–5%) but cadmium is efficiently retained and accumulates in the kidney and liver in the human body, with a very long biological half-life ranging from 10 to 30 years [33]. Therefore, the livers and kidneys of wild animals should not be eaten. Although the food-producing animals may act as an effective filter of Cd in the case of an environmental increase of this metal, consumption of visceral organs from such animals may pose a hazard [34].

Although the status of surface water in the investigated region is good considering pesticides and heavy metals, the rivers and shallow waters in Serbia are generally polluted, primarily with uncleaned wastewater from the domestic, industrial and agricultural sectors. According to Eco-Bilten, published by the Republic's Institute for Statistics, in 2019, the total amount of released wastewater was 1109 million cubic meters. Only 8.5% of this was purified, and that the total amount of wastewater discharged in the public sewerage system in 2019 increased by more than 3.5% in relation to 2018 [35]. Organic pollution, caused by discharges of wastewater and wastewater treatment plants, industrial influence and agricultural runoff, may lead to rapid deoxygenation of river water, a high concentration of ammonia and the disappearance of fish and aquatic invertebrates [36]. The situation is aggravated by water heating during recent years, which is inversely proportional to the solubility of oxygen in water. Oxygen solubility in water is one of the most common indicators according to which waters in Serbia are classified in category 5 [10]. This type of pollution, together with the accidental release of chemical waste, threatens to pollute rivers in such a way as to seriously endanger flora and fauna.

5. Conclusions

In this paper, we examined the presence of pesticides and heavy metals in surface waters, fish and wild animal samples in an agricultural environment unburdened by nearby industry in the northern part of Serbia. We detected pesticides in surface water, but it was a sporadic finding, considering the place and time. No POPs were found. Only one organochlorine pesticide, banned in Serbia and the EU but used in industry, was found in running water. No pesticides were found in samples of fish and wild animals. This supports the rational management of pesticides in the examined area. We encountered heavy metals in all the tested samples, but concentrations were within the allowable limits in waters with the exception of Hg in 2019. Concentrations of heavy metals in fish samples were below the maximal permitted values, while for cadmium they were increased in a small percentage of wild animal kidney samples. Therefore, consumption of organs like kidneys should be avoided in wild animals. The presence of heavy metals supports the

need for regular control of their existence and identification of pollution sources. This is extremely important as heavy metals stay in living organisms for a long time and are practically indestructible.

Our findings indicate that the situation in the agricultural area we investigated is still satisfactory from the aspect of chemical pollution. If the existing laws, fully harmonized with the EU, are applied, there is a real chance that agricultural areas like Backi Petrovac municipality will be protected from significant chemical pollution.

This kind of research is of great importance precisely because of the possibility of preserving the devastation of agricultural resources, that can provide both healthy food and a sustainable environment.

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