Pollution of small lakes and ponds of the Western Balkans - assessment of levels of potentially toxic elements

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Contents

- 1 Introduction
- 2 Small lakes and ponds of the Western Balkans
- 3 Pollution of small lakes and ponds
- 4 Overview of the literature related to pollutants in small lakes and ponds of the Western Balkans
- 5 Creating awareness of the significance of pollution of small lakes and ponds through assessment of levels of potentially toxic elements in small Aleksandrovac Lake in Serbia
 - 5.1 Sampling and methodology
 - 5.2 Potentially toxic elements in fish tissues
- 6 Conclusions

References

Abstract Small lakes and ponds are among the most sensitive ecosystems, vulnerable to changes that have little or no effect on larger water bodies. Therefore this problem should be given more attention around the world. Anthropogenic impacts, which have been extensively researched in larger water bodies, have barely been examined in small lakes and ponds. Considering that most of the studies are related to lakes bigger than 50 ha, a significant knowledge gap was left regarding small lakes and ponds. The same situation is in the Western Balkans, where studies related to pollutants in small lakes and ponds are limited. This chapter presents an overview of studies related to the contamination of small lakes and ponds of the Western Balkans, with special reference to pollution with potentially toxic elements (PTEs) in small lakes and ponds. Since massive fish mortality happened several times, an assessment of PTEs in fish species *Carassius gibelio* from small

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Aleksandrovac Lake in Serbia is presented. The results indicate that fish muscle (meat) was exposed to the lower pressure of PTEs pollution than liver and gills, suggesting that, despite massive fish mortality, there was no risk for human health by fish consumption.

Keywords Western Balkans, pollution, small lake, pond, potentially toxic elements, Aleksandrovac Lake

1 Introduction

Small water bodies, including ponds and small lakes, are freshwater ecosystems of high ecological relevance [1]. They occur in practically all terrestrial environments, from polar deserts to tropical rainforests [2], and, on a global scale, cover a greater area than lakes [3]. In Europe, they constitute a significant part of the continental freshwater resources [4].

Ponds are small standing waters that vary in size from 1 m² to about 2-5 ha in area [5]. The Ramsar Convention adopted a cut-off between ponds and lakes of 8 ha [6]. Additionally, there is no strict definition of what constitutes small or large lake. Given that in Europe the size of lakes is considered significant, according to the Water Framework Directive, 50 hectares provide a de facto cut-off point for small lakes [7]. Their typical characteristics, such as shallow waters and small size, imply a different ecological functioning [8, 9]. Ponds also vary in origin. For example, some natural ponds are the result of glacial activity, and others may be formed as oxbow ponds. There are also many man-made ponds, for example, mill ponds or ponds established for sediment retention or water storage [10].

Small lakes and ponds constitute an important component of freshwater ecosystems. They support higher proportions of biodiversity compared to larger freshwater systems [11] and can be substantially more biologically active than large lakes [12]. It is known that ponds support a disproportionately high species richness and variety of communities given their small size [13]. A wide range of studies have confirmed that, particularly for macrophytes, aquatic micro- and macroinvertebrates, and amphibians, small water bodies are areas of high biodiversity [7]. Also, they support specific and important hydrological, chemical, and biological processes [10].

The fundamental ecological research of ponds and small lakes influenced the growing concern and awareness regarding their abundance, importance for freshwater biodiversity, their role in contributing to ecosystem services, and their sensitivity and vulnerability to anthropogenic disturbances [3, 14]. It has become increasingly clear that there is a growing interest in ponds and small lakes in the early twenty-first century, which is reflected by increasing scientific activity, especially regarding biodiversity [4]. They are ecologically very important and represent powerful model systems for studies in ecology, evolutionary biology, and conservation biology, and can be used as sentinel systems to monitor global change [2].

A number of human activities threaten small lake and pond ecosystems - draining/infilling, eutrophication, contamination, acidification, and invasion of exotic species. Still, they are also threatened by global changes, in particular by increasing temperature and UV radiation [4, 15, 16]. Furthermore, anthropogenic impacts which have been extensively researched in larger waters have barely been examined in small lakes and ponds. Small lakes and ponds are vulnerable to changes that have little or no effect on larger water bodies. Therefore, this problem should be given more attention worldwide.

This chapter represents an overview of studies related to the contamination of small lakes and ponds of the Western Balkans, with special reference to pollution with potentially toxic elements (PTEs). Additionally, an assessment of PTEs in fish species from small Aleksandrovac Lake in Serbia is presented.

2 Small lakes and ponds of the Western Balkans

The precise number of ponds and small lakes in Europe cannot be estimated, nor for the area of the Western Balkans. The available literature data are contradictory. According to Kristensen & Globevnik [10], Europe has at least 600 000 natural lakes, with only around 100 000 having an area greater than 10 ha. Biggs et al. [7] stated that in the whole of Europe, the number of small lakes and ponds is likely between five and ten million. On the other hand, the proportion of loss of the ponds in the European landscapes is estimated higher than 50% [4].

The study of ponds was neglected in the second half of the twentieth century, only gaining momentum in the twenty-first century, particularly in Europe because of the work of the European Pond Conservation Network. The protection of small water bodies and the biodiversity they support is a concern since they are vulnerable to changes that have little effect on larger water bodies [10].

Small water bodies have not been studied systematically in the Western Balkans; nevertheless, specific research topics were related to a specific issue or group of organisms/taxa/species. A survey of ponds and their loss in the Žumberak-Samoborsko gorje Nature Park was conducted by Hutinec & Struna [17], while Sremački et al. [18] conducted environmental monitoring and assessment of protected wetland and lake water quality in Croatia and Serbia. Ranđelović et al. [19] performed phytocenological research of Batušinačke ponds in the vicinity of Niš. Tasevska et al. [20] researched rotifers. The zooplankton communities in the small lakes of the Western Balkans were investigated by Ostojić et al. [21, 22, 23], Špoljar et al. [24, 25], Kuczyńska-Kippen et al. [26], and Mancinelli et al. [27], while Ranković et al. [28] analyzed the phytoplankton community as an indicator of water quality. The following authors studied cyanobacteria: Svirčev et al. [29], Đorđević & Simić [30], and Simić et al. [31]. Temunović et al. [32] explored the diversity of water beetles (Hydradephaga, Coleoptera) in temporary ponds of Lonjsko polje, while Vilenica et al. [33] investigated the suitability of man-made water bodies as habitats for Odonata. The diversity of aquatic insects in small water bodies has been studied by many authors [34, 35, 36, 37, 38, 39], while research regarding fish was conducted by Pavlović et al. [40] and Khanom et al. [41]. Given the size of the Balkan Peninsula's territory, there are still unexplored small water bodies.

3 Pollution of small lakes and ponds

Certainly, shallow lakes and ponds are among the most sensitive ecosystems [42, 43]. Biggs et al. [7] indicated that pollutants might have a higher impact on ponds than larger waters because of ponds' small water volumes and less potential for pollutant dilution and retainment. Additionally, climate change can increase water temperature, decrease water level, and these changes together can increase the effect of pollutants in small water bodies [44].

Small water bodies, small lakes and ponds have not been omitted when it comes to threats by human activities. Those activities have increased nutrient loading, acid rain, invasion of nonnative species, and contamination. Contaminants of small water bodies include agricultural and amenity pesticides, veterinary and human medicines, personal care products, biocides, polyaromatic hydrocarbons (PAHs), and metals (also termed "heavy metals" or "potentially toxic elements").

Among the most important contaminants in small water systems are PTEs, and they are highly susceptible to inputs of even small amounts of these pollutants from their surroundings [7, 45, 46]. A key specificity of metals is that they are present in the earth's geological structures and enter aquatic ecosystems both by natural processes (atmospheric precipitation, geologic weathering, soil, and rock erosion) and through anthropogenic sources (industrial effluents, traffic, mining wastes, and agricultural waste products) as well as by synergistic combinations of the two.

It is a well-known fact that PTEs seriously contaminate the environment causing global health problems. Within a lake or pond system, the contamination depends on both the concentration of elements and the processes that occur within the water, sediment, and biota. It was also observed that the intensity of water exchange, which is lower in lotic ecosystems, affects metal bioaccumulation [47]. Contamination with PTEs may have stressful effects on the ecological stability of the recipient, species richness, and diversity and may cause lethal effects to the members of biota [48]. Once released in the aquatic ecosystem, it can be distributed and accumulated in the different parts of the aquatic biota, including flora and fauna. PTEs are one of the greatest threats to aquatic biota due to possible bioaccumulation and biomagnification in food chains [49], toxicity at high concentrations, and neurological impacts. Furthermore, some of them are carcinogenic [50]. Fish are on the top of the food chain and accumulate the highest concentrations of PTEs in the aquatic systems [51], and therefore represents the largest source of mercury in human food.

4 Overview of the literature related to pollutants in small lakes and ponds of the Western Balkans

Oertli et al. [4] indicated that small lakes and ponds receive less scientific attention than other water bodies in general. Research on contamination of small lakes and ponds with different pollutants is less emphasized in recent scientific literature. Brönmark & Hansson [15] predicted that in developing countries, such as Western Balkan countries, threats that derive from pollutants might become more of a problem in the next 25 years. Despite this, in 2021 pollutants in small lakes and ponds in Western Balkan countries are still under-examined.

There is very extensive literature on the effect of anthropogenic impacts resulting from the suite of processes leading to the phenomenon labeled "eutrophication" [52]. It causes turbid and toxic water from cyanobacterial blooms, degradation of lake ecosystems, and biodiversity [53]. Acidification was a major focus in the scientific literature from the middle of the 1980s to the middle of the 1990s [15]. Thereafter, research papers on acidification appeared less frequently, and today acidification is considered mostly a political issue. Also, the problem of nonnative species, as a result of human activities, is reflected in an increasing number of scientific papers published in this area. When it comes to PTEs, research activities are related to larger water bodies (large rivers and lakes).

In the Western Balkans, studies related to pollutants in small lakes and ponds are limited. Most studies deal with pollutants in larger lakes and reservoirs. Numerous studies are dealing with the determination of cyanotoxins and toxicity in small lakes. The largest number of studies of this type is in Serbia — Svirčev et al. [29], Đorđević & Simić [30], Simić et al. [31], Đorđević et al. [54], Svirčev et al. [55], Simić et al. [56], and Drobac et al. [57]. When it comes to persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAH), studies dealing with these pollutants were performed by Sakan et al. [58], Romanić et al. [59], Grba et al. [60], Drvošćak et al. [61], Kljaković-Gašpić et al. [62], Sula et al. [63], etc.

Table I summarizes available literature data on PTEs pollution studies related to lakes and ponds in the Western Balkans. It is important to emphasize that in the Western Balkans, four large lakes (Skadar, Ohrid, Prespa, and Dojran) are shared by two or more countries. Therefore, studies of pollution with PTEs in these countries (i.e., Montenegro, North Macedonia, and Albania) are mainly limited to these lakes.

As seen in Table 1, only the studies Djikanović et al. [48], Svirčev et al. [55], Branković et al. [87], Rašković et al. [93], Đikanović et al. [94, 95], Milošković et al. [96], and Nikolić et al. [97] included small lakes (if we consider 50 ha as the cutoff point for small lakes). Still, not a single study was related to pollution with PTEs in ponds. All these studies are related to the four small lakes in Serbia — Međuvršje, Šumarice, Aleksandrovac, and Bubanj. Study Branković et al. [87] deal with PTEs in water, sediment, and different macrophytes, while studies Svirčev et al. [55], Rašković et al. [93], Đikanović et al. [94], Đikanović et al. [95], Milošković et al. [96], and Nikolić et al. [97] are related to fish contamination with PTEs.

Table 1 Overview of literature data on PTEs in lakes and ponds of the Western Balkans

Size > 50
ha > 50 ha > 50 ha > 50
> 50 ha > 50 ha > 50
ha > 50 ha > 50
> 50 ha > 50
ha > 50
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ha
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ha
ha
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ha
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ha
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ha
> 50
ha
> 50
ha

(continued)

Table 1 (continu	ed)		
Vrhovnik et	sediment	North Macedonia/Kali-	> 50
al. [77]		manci Lake	ha
Vrhovnik et	sediment	North Macedonia/Kali-	> 50
al. [78]		manci Lake	ha
Tziritis et al.	water	North Macedonia/Micro	> 50
[79]		Prespa Lake	ha
Petrović et al.	sediment, Trapa natans	Montenegro/Skadar Lake	> 50
[80]			ha
Kastratovic et	Phragmites australis	Montenegro/Skadar Lake	> 50
al. [81]			ha
Kastratović et	water, sediment, Phrag-	Montenegro/Skadar Lake	> 50
al. [82]	mites australis, Cera-		ha
	tophyllum demersum,		
	Lemna minor		
Rakočević et	Scardinius knezevici, Al-	Montenegro/Skadar Lake	> 50
al. [83]	burnus scoranza, Cyprinus		ha
	carpio, Rutilus prespensis,		
	Anguilla anguilla, Perca		
T7 1 V1 17	fluviatilis) (G1 1 7 1	
Vukašinović-	Viviparus mamillatus	Montenegro/Skadar Lake	> 50
Pešić et al.			ha
[84]) (C1 1 T 1	. 50
Vukašinović-	macrophytes, molluscs,	Montenegro/Skadar Lake	> 50
Pešić &	fish		ha
Blagojević			
[85]	1	G 1: /C - × I 1-	> 50
Branković et	water, sediment, macro-	Serbia/Gruža Lake	
al. [86]	phytes	C 1. /C - × D-1 .*	ha > 50
Branković et	water, sediment, macro-	Serbia/Gruža, Bubanj*, Šumarice* lakes	
al. [87]	phytes	Sumarice lakes	ha * <
			50
			ha
Milošković et	water, sediment, macro-	Serbia/Gruža	> 50
al. [88]	phytes, fish	SCIUIA/OI UZA	ha
Milošković et	Sander lucioperca, Silurus	Serbia/Bovan Lake	> 50
al. [89]	glanis, Esox lucius	Sciula/Duvail Lake	ha
a1. [07]	giunis, Esox tuctus		1118

(continued)

Table 1 (continued)

Table 1 (continu	(1
Milošković et al. [90]	Sander lucioperca, Silurus glanis, Esox lucius, Carassius gibelio, Abramis brama	Serbia/Bovan Lake	> 50 ha
Sakan et al. [58]	sediment	Serbia/Barje, Ćelije, Vrutci, Garaši, Bojnik, Bovan lakes	> 50 ha
Sunjog et al. [91]	water, sediment, Squalius cephalus	Serbia/Zlatar, Garaši lakes	> 50 ha
Jaćimović et al. [92]	Perca fluviatilis, Ameiurus melas	Serbia/Sava Lake	> 50 ha
Rašković et al. [93]	Squalius cephalus	Serbia/Međuvršje*, Kruščica lakes	> 50 ha * < 50 ha
Dikanović et al. [94])	Chondrostoma nasus, Ruti- lus rutilus, Abramis brama, Barbus barbus, Carassius gibelio, Squalius cephalus, Perca fluviatilis, Silurus glanis, Esox lucius	Serbia/Međuvršje Lake	< 50
Đikanović et al. [95]	Chodrostoma nasus	Serbia/Međuvršje Lake	< 50
Djikanović et al. [48]	sediment, macrophytes, fish	Serbia/Međuvršje Lake	< 50
Svirčev et al. [55]	water, sediment, fish	Serbia/Aleksandrovac Lake	< 50
Milošković et al. [96]	Alburnus spp.	Serbia/Gruža, Međuvršje*, Vlasina, Zavoj lakes Skadar, Ohrid, Prespa, Dojran lakes	> 50 ha * < 50 ha
Nikolić et al. [97]	sediment, Perca fluviatilis	Serbia/Vlasina, Perućac, Zaovine, Međuvršje*, Garaši, Sava lakes	> 50 ha * < 50 ha

5 Creating awareness of the significance of pollution of small lakes and ponds through assessment of levels of PTEs in small Aleksandrovac Lake in Serbia

Looking at the literature overview, it is evident that most of the studies are related to lakes bigger than 50 ha, which leaves a significant gap in knowledge for the small lakes and ponds. Guided by this data, we decided to present the investigation of PTEs in fish species from Aleksandrovac Lake, located in South Serbia.

Aleksandrovac Lake (Fig. 1) is a man-made lake on the Aleksandrovac River, formed in 1964, at first, for irrigation purposes. It is characterized as oligosaline, shallow lake (maximum depth 4 m, average 2 m) with a surface area of 12 ha. Over time, already highly eutrophic Aleksandrovac Lake suffered almost anaerobic conditions, so it was completely restored and refilled in 2010. Despite this, massive fish mortality happened several times. In December 2012, the mortality of nearly the entire fish population was recorded (more than 1.7 tons), including *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Abramis brama*, *Silurus glanis*, *Carassius gibelio*, *Aspius aspius*, and *Squalius cephalus*.



Fig 1 Map of Aleksandrovac Lake

5.1 Sampling and methodology

Bioindicator species distinguished by its flexibility and tolerance to environmental conditions and pollution, *Carassius gibelio* was sampled immediately after massive fish mortality at Aleksandrovac Lake on 20th December 2012. Twenty specimens were packed in special polyethylene bags and transferred on ice in a hand-held refrigerator to the laboratory. In the laboratory, fish were measured for their total body length (to the nearest cm), weighed (to the nearest g), and subsequently dissected with a decontaminated ceramic knife. The right dorsal muscle, below the dorsal fin,

liver, and gills of each specimen were dissected, weighed using an electronic balance (\pm 0.1 g), and stored at -20 °C before analysis.

The concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, and Zn were analyzed with inductively coupled plasma optical emission spectrometry (ICP-OES), using a Thermo Fisher Scientific iCAP 6500 Duo ICP (Cambridge, United Kingdom). The analytical procedure was described in our earlier research paper Milošković et al. [98]. The mean values and standard deviations were calculated for each group, and element concentrations were expressed as mg kg⁻¹ wet weight (ww).

Although the muscle tissue (meat) of *Carassius gibelio* was used for element analysis, concentrations of the elements were compared with the maximum permitted concentrations (MPCs) in fish meat for utilization in the human diet, established by the European Union [99], FAO [100], and Serbian national legislation [101].

The metal pollution index (MPI), which refers to element concentration in fish tissues, was calculated using the following equation [102]:

$$MPI = (cf1 \times cf2 \times cf3 \times ... \times cfn)^{1/n}$$
 (1)

where cfn = concentration of the element n in the sample.

5.2 Potentially toxic elements in fish tissues

Concentrations of PTEs in three tissues of *Carassius gibelio* are summarized in Table 2. The highest concentrations of Al, As, Cu, Cr, Mn, Ni, Pb, Se, Zn, and Cd in gills, Co, Sn, and Fe in liver, and Hg in muscle were recorded. The distribution pattern was gills - liver - muscle for Al, As, Cu, Cr, Mn, Ni, Pb, Se, and Zn, gills-liver - muscle for Cd, liver - gills - muscle for Sn and Fe, liver - muscle - gills for Co and muscle - gills - liver for Hg.

Table 2 The average PTEs concentrations and standard deviation in the muscle, liver, and gills of *Carassius gibelio* in mg kg⁻¹ww and prescribed MPCs in fish meat

Ele-	Muscle	Liver	Gills	MPC		
ments				MPC	MPC	MPC
				EU [99]	FAO [100]	RS [101]
Al	0.78 ±					
	0.09	ND	3.25 ± 0.8			
As	0.12 ±			0.1-4.0		2.0
	0.05	ND	0.22 ± 0.09			
Cd	0.005 ±	0.007 ±	0.007 ±	0.05	0.05	0.1
	0.001	0.0002	0.0001			

(continued)

Table 2 (continued)

Table 2 (co	minucu)					
Co	0.001 ±	0.02 ±				
	0.002	0.005	ND			
Cr	$0.33 \pm$					
	0.18	0.3 ± 0.1	0.39 ± 0.1			
Cu	0.17 ±			30		30*
	0.08	0.46 ± 0.1	0.56 ± 0.32			
Fe	3.03 ± 1.4	87 ± 16.25	36 ± 9.81			30*
Hg	0.10 ±		0.0005 ±	0.5	0.5	0.5
	0.02	ND	0.0001			
Mn	0.30 ±					
	0.21	0.17 ± 0.06	8.64 ± 2.44			
Ni	0.006 ±	0.002 ±				
	0.006	0.001	0.04 ± 0.003			
Pb	0.22 ±			0.3	0.5	0.4
	0.05	0.13 ± 0.06	0.43 ± 0.31			
Se	0.21 ±					
	0.07	0.12 ± 0.04	0.32 ± 0.13			
Sn	$0.0007 \pm$					
	0.001	0.14 ± 0.05	0.12 ± 0.05			
Zn	12.7 ±				100	100*
	0.64	8.61 ± 1.9	75.13 ± 15.16			

ND - not detected, *MPCs in canned products

Since it was introduced in European freshwaters in the 1970s, *Carassius gibelio* has been economically important for fisheries in Serbia [103]. The negative impact of PTEs on human health was analyzed by comparing the concentrations of these elements with the appropriate MPCs [99, 100, 101]. Concentrations of As and Fe in gills were above MPC prescribed by Serbian legislation, while Pb in gills was above MPC prescribed by Serbian legislation and EU. There were no PTEs in fish muscle (fish meat) above MPCs, suggesting no risk for humans consuming fish despite massive fish mortality.

The MPI is more reliable for expressing the pollution status of fish than accumulation patterns since it considers all metals synchronously. The concentrations of PTEs affected the MPI values (Fig. 2), which points out that the muscle was exposed to the lower pressure of PTEs pollution than liver and gills. The reason for higher values of MPI for gills may be the fact that gills are the primary site of element uptake from water, especially if elements are bound to particulate matters. It is recommended that gills should be removed before using fish in the human diet due to elevated concentrations of the most toxic elements — As and Pb.

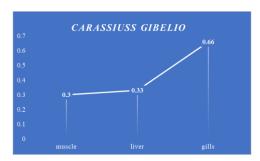


Fig. 2 Metal pollution index (MPI) values of the total element accumulation levels in the muscle, liver, and gills tissues in *Carassius gibelio*

Svirčev et al. [55] collected data a month before massive fish mortality happened in Aleksandrovac Lake. Their results represent concentrations of PTEs in muscle, liver, and gills of undetermined fish species, so we cannot compare the concentrations of PTEs in our study with their published results. We can solely emphasize that values of PTEs were higher in the study Svirčev et al. [55]. Svirčev et al. [55] excluded the possibility of metal poisoning and assumed that toxic metabolites produced by cyanobacterium *Cylindrospermopsis raciborskii* caused the fish mortality in Aleksandrovac Lake.

Aleksandrovac Lake came into the public spotlight after over 1.7 tons of fish were killed. Before fish mortality, the problems in this lake were solved occasionally and insufficiently. Recognizing in time the importance of small lakes and ponds is the basis for continuous long-term monitoring and legal regulations which can provide protection of small water bodies. This is a global problem that needs to be solved in the future in order to preserve small water bodies.

6 Conclusions

This chapter provides an insight into the pollution status of lakes and ponds in the Western Balkan countries. Analysis of available literature shows that most studies are related to lakes bigger than 50 ha, which leaves a significant knowledge gap for the small lakes and ponds. Presented results of the investigation of PTEs in fish species from small Aleksandrovac Lake in Southern Serbia indicate that fish muscle (meat) was exposed to the lower pressure of PTEs pollution than liver and gills. That suggests, despite massive fish mortality, there was no risk for human health by fish consumption. In the future, more comprehensive studies need to be done globally to conserve small water bodies, especially shallow lakes and ponds.

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