Marijana KRIVOKAPIĆ

RISK ASSESSMENT OF TOXIC ELEMENTS (Pb, Cd, Hg, As) IN WATER, SEDIMENT AND IN THINLIP GREY MULLET (BOJANA RIVER)

SUMMARY

The Bojana River is outflow of the Skadar Lake. River is shared by Albania and Montenegro, it forms part of their border before flowing into Adriatic Sea. During most of the year the water quality of the Bojana River is acceptable, but pollution increases the most frequently in summer period.

For this study, research of toxic elements (Pb, Cd, Hg, As) in the water and sediment of the Bojana River, as well as in the muscle tissue and digestive tract tissue of thinlip grey mullet (Chelon ramada) was done at the end of the summer season. This research of toxic elements in muscle tissue and digestive tract tissue of thinlip gray mullet is done for the first time for this species, as well as bioconcentration factor (BCF), biota sediment accumulation factor (BSAF) and Spearman correlation between the matrices. The grade of metals concentrations decreased in the subsequent order: As>Pb>Cd=Hg in water and As>Pb>Cd>Hg in sediment. The order of decreasing concentration for metals in muscle tissue is as follows: Hg>Pb=Cd=As and in digestive tract tissue of thinlip grey mullet: Pb>Hg>Cd=As. The highest values of BCF in muscle tissue of fish have been established for Hg and Cd and in digestive tract for Pb and Hg. The values of biota sediment accumulation factor (BSAF) decreased in the following order: Hg>Cd>Pb=As for muscle tissue and Hg>Pb>Cd>As. for digestive tract tissue.

Key words: Bojana River, thinlip grey mullet, toxic elements, BCF, BSAF

INTRODUCTION

Bojana is a large river (5.187 km²) and is located in the 5th Ecoregion, i.e the Dinaric Western Balkans region and its length of river flow belonging to Montenegro (27.5 km). It is proposed to be one water body and belongs to type 9 - large area basin, valley (altitude <200 m), geological characteristics limestone-organic. The hydrological characteristics of the Bojana River depend on the hydrological characteristics of Skadar Lake and its tributaries, and especially of the River Drim, which flows into Bojana. Drin River (Drinisa) with the Lake

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Skadar outflow (Bojana-Buna) the total catchment comprises about 19.600 km$^2$ and the average discharge is 672 m$^3$/s (Schneider-Jacoby, 2006, Buric et al., 2019). The Bojana is the border River between Montenegro and Albania. The bottom of the riverbed is between 2 to 5 meters below sea level. Although the course of the Bojana River is relatively short, the hydrological regime of this river is extremely complex. The Skadar (Shkoder) Lake and Bojana (Buna) River system, with its delta area on the Adriatic Sea, contains important ecosystems with fresh and brackish water. The Bojana Buna River mouth represents a rare example of a natural delta on the East Adriatic coast (Fanelli et al., 2015).

Thinlip grey mullet, *Chelon ramada* (Risso, 1827) is frequently observed in large shoals throughout coastal areas, brackish water and entering also freshwater. According to the IUCN red list of threatened species is classified as least concern. The wide distribution and success of this species is attributed to their high euryhaline capacity (Turan, 2015; Freyhof and Kottelat, 2018).

Many factors influence speciation of elements in freshwaters. Evaluation of elements speciation in waters is a complex task. The more soluble forms of “heavy” metals are considered as more bioavailable and toxic (De Paiva Magalhães et al., 2015). With the assumption that heaviness and toxicity are inter-related, term “heavy metals” also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure (Tchounwou et al., 2012). Big impact on the water quality of the Bojana River has waste water from Skadar (Albania) and Ulcinj (Montenegro), as well as unsolved contamination of Port Milena (Ulcinj). The Bojana River links marine and freshwater ecosystems being a fish migration corridor, moving from the Adriatic Sea to Skadar/ Shkoder Lake (Fanelli et al., 2015).

The aim of this research is to analyse four toxic elements (Pb, Cd, Hg, As) in Bojana River water and sediment, as well as, in muscle tissue and in digestive tract tissue of thinlip grey mulet, with BCF and BSAF assessment.

**MATERIAL AND METHODS**

**Study area.** All samples were collected from Bojana River (Fig.1). The sampling sites were near to the Bojana River delta area (19°20’58.61”E, 41°52’12.00”N). The water samples (of 1 l), were bottled and saved in hand held refrigerator and transferred to the laboratory. The sediment samples were taken by Ekman dredge, from the depth of 0–20 cm, packed in plastic boxes and transported to the laboratory. The samples of fish were taken from local professional fishermen, packed in plastic bags and transported to the laboratory by hand held refrigerator.

The analysis of water samples of the Bojana River (Pb, Cd, Hg, As), as well as muscle and digestive tissue samples of thinlip gray mulet was done in the Chemical Laboratory of the Institute of Public Health, while the sediment analysis at the Eco-Toxicological Center (CETI).

The content of elements in water samples (previously acidified to pH <2) were directly determined using the ICP-OES technique (induced coupled plasma-
optical emission spectrometer, Spectro ARCOS). Standard solutions of Pb, Cd, Hg and As concentration 1000 mg/l (BT Baker) were used for preparation of calibration solutions. Operating wavelengths and PQL values for the analysed elements are given in Tab.1

The analysis of the elements content in the muscle and digestive tissue of fish was determined in the wet tissue of fish. In parallel, the fish tissue was dried at 105°C, and then treated in the same way as the wet tissue of the fish, in order to determine the normalized content of elements in them. Preparation of samples for analysis of metal content in fish tissue was done in accordance with the standard method (Analytical Method Perkin Elmer of AAS).

Table 1. Operating wavelengths and practical limits of quantification

<table>
<thead>
<tr>
<th>Metals</th>
<th>Working wavelength</th>
<th>PQL (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>283.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Cd</td>
<td>228.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Hg</td>
<td>225.6</td>
<td>0.0005</td>
</tr>
<tr>
<td>As</td>
<td>224.5</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Figure 1. Bojana River outflow of Skadar Lake, inflow Adriatic Sea

Regarding the sediment analysis, 0.3-0.5 g of the sample was taken and transfer to a digestion vessel. Then, 9 ml of concentrated nitric acid and 3 ml of concentrated hydrochloric acid were added. The vessel was closed and placed in
the microwave oven for digestion. After the digestion, the cooled solution from the vessels (with filtration) was transferred to the volumetric flask (25 ml) and made up to the mark with deionized water. Following methods and instrumental techniques were used: Pb by AOAC 985.01 method, instrumental technique AAS; Cd by AOAC 982.23 method, AAS; Hg by CETI 1 method, Hg analyser; As by EPA 3051A* (accredited).

The concentrations of elements in water are given in mg/l, while in sediment and in fish muscle tissue and digestive tract tissue are given in mg/kg.

**Statistical analyses**

Bioconcentration factor (BCF). Several terms are used to define the concentration of elements in aquatic biota. BCF is defined as the net result of the absorption, distribution, and elimination of a particular substance in biota that has entered through water (Mc Geer et al., 2003). Bioconcentration factor is defined as the uptake of contaminants from the dissolved phase. It can be calculated by the following equation: $\text{BCF} = \frac{C_B}{C_W}$, where $C_B$ represents the average concentration of the element in the biota i.e a certain tissue (μg/g of most mass), and $C_w$ concentration of the element in water (μg/ml) (Mc Geer et al., 2003; Mackay and Fraser, 2000; Pollman and Axelrad, 2014; USEPA, 2015).

Biota sediment accumulation factor (BSAF) is defined as the concentration of contaminant uptake by biota from sediment: $\text{BSAF} = \frac{C_B}{C_S}$, where $C_B$ represents the average concentration of the element in the biota i.e a certain tissue (μg/g) and $C_S$ refers to the concentration of the element in the sediment (μg/g) (Mackay and Fraser, 2000; Pollman and Axelrad, 2014; USEPA, 2015).

Statistical analyses are applied in the study, as well as Spearman's (rho) coefficient of correlation. Spearman's rho rank correlation coefficient is denoted by the symbol $r_s$ for the sample data and the $p_s$ symbol by the base set data. This correlation coefficient is the simple linear correlation coefficient between ranks.

There is no strictly standard rule for assessing the strength of a relationship, but most often in use is: weak: 0 - 0.25, moderate: 0.25 to 0.60 and strong: 0.60 to 1. The smaller sample, the higher the coefficient required to achieve statistical significance. It is important to note that the degree of correlation between two variables is not static, but can swing over a wide range from inverse to positive and vice versa, over the time (Dodge, 2010; *Myers et al.*, 2003). Statistical analysis has been done in R statistical computing software, Version 3.5.3. for Windows.

**RESULTS AND DISCUSSION**

Heavy metals are well-known environmental pollutants due to their toxicity, persistence in the environment, and bioaccumulative nature. Metal pollution from multivarious sources has adverse effects on aquatic ecosystems (Ali, 2019; Perera et al., 2015).

Pollution of aquatic ecosystems with toxic elements is an environmental problem of public health concern. Being persistent, pollutants accumulate in the environment and consequently contaminate the food chains. Elements that pose
the highest risks for human health are: mercury, cadmium, lead and arsenic, which cause important complications. Trophic transfer of these elements in aquatic food chains, has important implications for wildlife and human health (USEPA, 2015). Among a wide range of toxic substances that contaminate fish, three heavy metals; cadmium, lead and mercury are the only heavy metals included in the European Union regulations for hazardous metals (Perera et al., 2015).

**Pb in water and sediment of Bojana River and in thinlip grey mullet**

Lead is the most important toxic heavy element in the environment. According to its physico-chemical properties, its use can be retraced to historical times. Globally it is an abundantly distributed, important yet dangerous environmental chemical. Its important properties like softness, malleability, ductility, poor conductivity and resistance to corrosion seem to make difficult to give up its use. Accordingly to its non-biodegradable nature and continuous use, its concentration accumulates in the environment with increasing hazards (Wani et al., 2015). Lead gets to the aquatic system due to the ground superficial erosion and atmospheric deposition. Environmental levels have increased over 1,000 times in the last three centuries as result of human activity (Rodriguez et al., 2015). Human exposure to lead and its compounds occurs mostly from various sources: industrial processes such as smelting of lead and its combustion, pottery, boat building, lead based painting, lead containing pipes, battery recycling, grids, arm industry, pigments, printing of books, etc (Wani et al., 2015). Most of the toxic metal pollutants are adsorbed by the suspended particles in water. These adsorbed metals undergo complex migration and transformation processes in the water–sediment–organism, such as adsorption, desorption, precipitation, biological absorption, and other reactions (Yi, 2020).

By analysing the concentration of toxic elements in the water of Bojana River, lead is at the second place (Fig. 2). The order of decreasing concentration of toxic elements in water of Bojana River is as follows: As>Pb>Cd=Hg, as well as in sediment: As>Pb>Cd>Hg. The determined lead values in sediment were 7±1 mg/kg. In digestive tract tissue of thinlip gray mullet determined values were 0.01, while in the digestive tract have been established value of 2.54 mg/kg (Fig. 2). In accordance with the Regulation on the maximum level for certain contaminants in food (ILA, 2018, Annex, Part III Metals), the allowable MPC (mg/kg wet weight) in fish muscle tissue is 0.30. The correlation coefficient between water and sediment is 0.949 (p =0.051) as shown in Fig.5.

In the aquatic environment lead is primarily bound in sediment, under the low pH and along with number of other factors becomes more bioavailable. In freshwater and marine ecosystems, the accumulation of lead is insignificant and considered as non-dangerous for humans by using fish in the diet, except in cases of large and extraordinary pollution. According to the sediment quality guidelines (SQGs) of USEPA concentrations of metals is mostly lower than the possible effect level (PEL), (Yi, 2020; USEPA, 2000, 2007). Consequently, sediment quality has emerged as an important and critical consideration for protection of
benthic ecosystem health, fisheries conservation, and protection of surface water quality in both freshwater and marine environments (Babut et al., 2005; Wenning et al., 2005, Kvok et al., 2014).

According to MPC of toxic substances in sediment due to Dutch and Canadian recommendations, lead values obtained in this study (7±1) are much lower (Fig.2) than the quality standards and recommendations for sediment, according to Dutch methodology, target value (85 mg/l), Netherlands Government Gazette, 2000; and from Canadian legislation (theoretically possible impact value (35 mg/kg), Canadian sediment quality guidelines, 2001.

Fish among the group of aquatic organisms represent the largest and most diverse group of vertebrates. A number of characteristics make them experimental models for toxicological research, especially for the contaminants which are likely exert their impact on aquatic system.

Fig 2. Concentration of Pb, Cd, Hg and As in water, sediment, muscle and digestive tissue

Since not all chemical forms of pollutants are equally bioavailable and some pollutants can be accumulated in living organisms to a greater extent than others, we need to study the levels of pollutants in the organisms to be able to predict the environmental risk (Yancheva et al., 2015).

The concentration of lead in the digestive tissue of thinlip gray mullet is 2.54 mg/kg (Fig.2), while the value of bioconcentration factor in digestive tract
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tissue is 508 (Fig. 3). Biota sediment accumulation factor shows lower values (Fig. 4).

**Cd in water and sediment of Bojana River and in thinlip grey mullet**

Cadmium and its compounds are very toxic, although their toxicity was established only in the first half of the last century. Cd is persistent in freshwater ecosystems for many years, while in the oceans it is assumed that it can remain for thousands of years. It ranks seventh on the list of 20 most important toxins (ATSDR, 2007). Cadmium is not subject of biomethylation processes, but it accumulates to a large extent (Agbaba, unpublished article). It has a long biological half-life.

![Figure 3. BCF of Pb, Cd, Hg and As in thinlip grey mullet (muscle and digestive tract tissue)](image)

The concentration of cadmium in the sediment of the Bojana River is at the third place between the toxic elements (Fig. 2): As>Pb>Cd=Hg. The presence of cadmium in the water of the Bojana River is in accordance with the concentration as mercury. The value obtained in this study (0.2 mg/kg Cd) are much lower than the quality standards and recommendations for sediment, according to Dutch
methodology and Canadian legislation, Dutch methodology-reference target value is 0.8 mg/kg, and intervention value:12 mg/kg; Canadian recommendations ISQG (0.6 mg/kg), PEL (3.5), ICPDR (1.2 mg/kg). According to the EU directive 466/200 and Regulation EC, No 333/2007, the permitted cadmium (Cd) value in fish meat is 0.1 mg/kg. The values determined by this study are lower (Fig.2).

Authors Neziri and Gössler, 2006 had analysed Bojana River water and established Cd concentration value of <0.1, while in the sediment they determined Cd value of 27.3 mg/kg. These values are higher than the values determined in this research (Fig. 2).

The established value of BCF for cadmium in muscle tissue, as well as in the digestive tract of thinlip grey mullet is 20 (Fig.3). Biota sediment accumulation factor shows lower values (Fig.4). Deviations in BSAF values (biota, sediment, accumulation factor) predicted with fission models may be due in part to differences in the bioavailability of chemicals, which may lead to marked variation of specific sites, in the bioaccumulative profiles of certain pollutants (Van der Oost et al., 2003).

**Hg in water and sediment of Bojana River and in thinlip grey mullet**

Mercury can arise in the environment from natural sources such as volcanic eruptions, soil erosion and bacterial decomposition of organic mercury compounds and anthropogenic sources such as: municipal waste incinerators, fossil fuel, combustion factory, electrolysis factory (here mercury is used as an electrode). Elemental mercury and its salts in the environment, mainly are result due to direct emissions from industry, while organo-metallic mercury which can also occur in the environment, is most often the result of pesticides (e.g. mercury-based fungicides), The basic problem of mercury pollution is that its organometallic compounds can accumulate and metabolize in the biosphere (Sofilic, 2014).

Similar to non-metals and in contrast to most other metals, mercury (Hg) forms organic compounds that are very stable in the environment. All forms of mercury are potentially toxic but the levels of toxicity vary considerably (Agbaba, unpublished article).

The intake of metals through food in comparison with the direct absorption from the solution is of fundamental importance in heterotrophic aquatic organisms, because in the polluted aquatic environment the intake of dietary excess i.e, trophic levels is higher than the intake through water, due to richer metal content in sediment, particles and detritus (Sharmeen et al., 2014). The concentration value of mercury in the water of the Bojana River is the same as for cadmium (Fig. 1).

Authors Neziri and Gössler (2006), analysing mercury in the water of Bojana River determined value of <0.1 (µg/l), while in sediment <0.5 mg/kg. The stated value for sediment is higher than the value determined in this study, where determinate value is in the concentration of 0.024 mg/kg (Fig. 2).
Contamination of sediments is mostly dangerous because of how it “travels up” the food chain, ultimately becoming harmful to animals and humans (USEPA, 2000; EPA, 2007).

In relation to quality standards and recommendations for sediment, according to Dutch methodology, Canadian legislation, as well as ICPDR, Dutch methodology, reference value is 0.3 mg/kg, Canadian recommendations PEL value is 0.486, ICPDR: 0.8 mg/kg, and there values are higher than ISQG value: of 0.17 mg/kg (Babut et al., 2003; Canadian sediment quality guidelines, 2001; Kvok et al, 2014). The values obtained by this study are much lower in relation to quality standards and sediment recommendations, according to Dutch methodology, Canadian legislation, as well as ICPDR.

Figure 4. Biota sediment accumulation factor (BSAF of Pb, Cd, Hg and As in thhilnip grey mullet)
The established concentration of mercury in the tissue of the thinlip gray mullet digestive tract was 0.056 mg/kg (Fig. 1), value of bioconcentration factor 112 and the determined values of BCF in muscle tissue of thinlip gray mullet 22. (Fig. 3). Consistent with the bioconcentration of toxic metals in this study, mercury was found to be in the first place in terms of bioconcentration in muscle tissue of thinlip gray mullet (Hg>Cd>Pb>As). BSAF values show much lower values, although the established dominance of biota sediment accumulation factor were also observed both in muscle tissue and in the digestive tract: Hg>Cd>Pb>As, (Fig. 4).

The concentration of toxic elements: Pb, Cd, Hg and As in water of Bojana River are less than the limit, established by the EU directive 75/440 EEC (European Environment Agency, EPA, 2007).

Elements Pb, Hg, Cd are the most important from the ecotoxicological aspect since they are not essential (they do not have a known metabolic function) and their presence in the body is exclusively a consequence of contamination. They have a high ratio of anthropogenic and natural intake in the body. Pb, Hg, Cd are endocrine disruptors and immunosuppressants (Agbaba, unpublished article). Fish have been found to be good indicators of water contamination in aquatic systems because they occupy different trophic levels; they are of different sizes and ages and in comparison with invertebrates are also more sensitive to many toxicants (Yancheva et al., 2015).

**As in water and sediment of Bojana River and in thinlip grey mullet**

Arsenic is often found in ores of silver, tin, lead, cobalt, copper and nickel. The ores are not mined for arsenic, but it is obtained as a by-product in the isolation of ores of other metals such as copper, lead, cobalt and gold. Oxide ores of which the most important is arsenolite \((\text{As}_4\text{O}_6)\) are much rarer (Petrak and Pavlovic, 2015).

With the production of protective chemicals for wood, then herbicides, insecticides, the USA stood out as the largest consumer of As until 2003, after which the use decreased. Herbicides with As are allowed in the USA, while EU regulations recommend avoiding chemicals that contain As, and pesticides based on As are prohibited. High concentrations of arsenic (As) have been reported for water samples in several parts of the world and over 200 million persons globally are at risk of arsenic exposure at levels of concern for human health. The United States Environmental Protection Agency (USA EPA) sets limits for arsenic concentration in freshwaters to protect aquatic organisms from arsenic. It has established criteria maximum concentrations (CMC) and criteria continuous concentrations (CCC) for most inland surface waters. According to the US EPA, the CMC for acute arsenic exposure is 340 μg As/l, while the CCC for chronic arsenic exposure is 150 μg As/l (Barral Fraga, 2020).

Arsenic distribution between the water column and the sediment is controlled by several physico-chemical and biological processes, such as precipitation, solubilisation, adsorption, desorption, oxidation, reduction,
incorporation in the crystal structure of minerals, and biological exchanges. Arsenic is ranked the first of all hazardous substances by the Agency for Toxic Substances and Disease Registry (Barral-Fraga, 2020). As is found in waters such as seawater, warm springs, groundwater, rivers, and lakes. In aquatic environments, As occurs as a mixture of arsenate and arsenite, with arsenate usually predominating.

The concentration of arsenic in water, as well as in the sediment is in the dominant first position (Fig. 2): As>Pb>Cd=Hg. Arsenic levels in Pampean surface waters have been attributed to the hydrogeology of the streams, fed by an aquifer with high levels of arsenic (0.6 to 4.9 mg/l) originated from quaternary loess sediments (Barral Fraga, 2020). Geochemical investigations showed that most arsenic in the sediments of the Anllóns River is associated with low-mobility phases specifically as bound to Fe-oxide forms and in the residual phase. Arsenic levels in natural systems are often well below those that cause mortality in higher organisms such as fish, but even low concentrations may impede normal functioning. Arsenic is toxic in all four oxidation states (+V, +III, 0, -III), and trivalent and pentavalent arsenic are the most common found in ecosystems (Rahman et al., 2014).
The concentration of arsenic of 9.6 mg/kg in the sediment of Bojana River was determined by the authors Neziri and Gössler (2006). The stated value for sediment are higher than the value determined in this research, which is 8±1 mg/kg (Fig. 2). In this research, in terms of bioconcentration, arsenic is at the fourth place Hg>Cd>Pb>As.

Continuous exposure of freshwater organisms including fish to low concentrations of As results in bioaccumulation, notably in liver and kidney. As a consequence induces hyperglycemia, depletion of enzymatic activities, various acute and chronic toxicity and immune system dysfunction (Kumari et al., 2016).

Arsenic is persistent in the environment and risk for biota and human health. This metalloid enters the biota in primary consumers and then biomagnifies to higher order organisms (Alvarado-Flores et al., 2019).

Concentrations of total arsenic in uncontaminated nearshore marine and estuarine sediments usually fall in the range of 5 to 15 μg/g dry weight. Concentrations of total arsenic in whole or muscle tissues of marine organisms worldwide range from below the detection limit of the analytical method, usually 0.01–0.6 μg/g dry weight (depending on the method). Pollution of aquatic ecosystems with toxic elements is a worldwide problem because of the environmental persistence and biomagnifications in food chain (Ali et al., 2019; Flores et al., 2019).

CONCLUSION

The influence of toxic elements and the associated risk assessment is in accordance with the physico-chemical and biological conditions in the aquatic ecosystem. The concentration of toxic elements: Pb, Cd, Hg and As, decreased in the subsequent order: As> Pb> Cd=Hg in the water of the Bojana River and As>Pb>Cd>Hg in sediment. The order of decreasing concentration for metals in muscle tissue is as follows: Hg>Pb=Cd=As and in digestive tract of thinlip grey mullet: Pb>Hg>Cd=As. The highest values of BCF in muscle tissue of thinlip grey mullet has been for Hg and Cd, while in digestive tissue for Hg and Pb. The values of BCF decreased in the following order: Hg>Cd>Pb>As in muscle tissue, as well as in digestive tract.

Transfer through the food chain of these metals could have consequences for aquatic biota and for human health and it is of great importance to assess and monitor the concentrations of these potentially toxic elements and their bioavailability.

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