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CONTAMINATION OF POLLUTANTS IN *ABRAMIS BRAMA* (LINNAEUS, 1758), BIOINDICATION AND ECOLOGICAL RISK ASSESSMENT OF THE WETLAND MANDRA-PODA, BULGARIA

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Abstract

The study presents the data on the contamination of Cd, Cu, Hg, Ni, and Pb in the samples of skin, muscles, and liver of freshwater bream (Abramis brama (Linnaeus, 1758)), as well as in water and sediments samples from the studied ecosystem of the protected wetland Mandra-Poda, Black Sea Region, Bulgaria. Basic ecological indices have been determined. New data for the bioindicator significance of freshwater bream for the accumulation of trace elements in skin, muscles, and liver has been reported. Basic correlation dependencies have been indicated. Discussions on the use of freshwater bream as a food resource have been attached. The risk of pollution to human health and the environment has been assessed.

Key words: *bioindication, chemical status, ecological state, freshwater bream, risk assessment.*

INTRODUCTION

Wetlands play a crucial role in regulating water regimes and supporting unique habitats for diverse plant and animal communities. One such ecologically significant site in Bulgaria is the Mandra-Poda Complex, recognized for its national and international importance. It forms part of the Burgas Wetlands, situated in the Black Sea Basin Region—Ecoregion 12: Pontic Province, Sub-Ecoregion 12-2: Black Sea, in southeastern Bulgaria. The complex is located south of the industrial zone of Burgas, adjacent to the Black Sea coast. The Mandra-Poda Complex is designated as a wetland of international importance under the Ramsar Convention (1971) and is protected by the Habitats Directive (92/43/EEC), the Birds Directive (79/409/EEC), as well as through its status as a Corine Biotope and an Ornithologically Important Site. In accordance with Bulgaria's Law for the Protected Areas (1989), three protected zones have been established within the Complex: "Poda," "Ustie na reka Izvorska," and "Uzungeren," aimed at preserving habitats for endangered and rare bird species. Despite these protections and its recognized ecological value, the area remains under significant anthropogenic pressure. The Lukoil Neftochim Burgas oil refinery uses waters from the Complex Mandra-Poda for industrial needs. Intense anthropogenic pressure from construction activities around the wetland - leading to the destruction of natural habitats (such as wet meadows), eutrophication, pollution from industrial wastewater and household waste, overfishing, poaching, and disturbance of birds and other organisms - poses significant threats to the biodiversity and water resources of the area. Scientific studies examining water pollution indicators and the accumulation of pollutants in freshwater fish species remain limited (Georgieva et al., 2015; Peycheva et al., 2022; Ilieva & Kirin, 2024). The present study aims to provide ecological monitoring data on contamination levels of cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni), and lead (Pb) in the skin, muscle, and liver of *Abramis brama* (Linnaeus, 1758), as well as in water and sediment samples. The findings are intended to support an assessment of the species' potential as a bioindicator of environmental conditions in the Mandra-Poda Complex.

The freshwater bream, *Abramis brama* (Linnaeus, 1758) (family Cyprinidae), was selected as a model species for this study due to its ecological role as a benthopelagic, brackish-water predator. This species feeds primarily on zooplankton, insects, crustaceans, mollusks, aquatic plants, and small fish. According to the International Union for Conservation of Nature (IUCN) Red List, *A. brama* is classified as a species of Least Concern (LC) (Kottelat & Freyhof, 2007; Froese & Pauly, 2024). In Bulgaria, the species is not legally protected (Karapetkova & Zhivkov, 2006). *A. brama* is widely distributed across the country's aquatic ecosystems and is an important species for both sport and commercial fisheries.

MATERIALS AND METHODS

The study was conducted on ten specimens of freshwater bream (*Abramis brama* Linnaeus, 1758) collected from the Mandra-Poda Complex. Sampling was carried out using multi-mesh gillnets in accordance with BSS EN 14757:2015 (*Water quality – Sampling of fish with multi-mesh gillnets*), following approval from the Executive Agency for Fisheries and Aquaculture, Ministry of Agriculture, Bulgaria. The scientific name of the species was verified using the FishBase database (Froese & Pauly, 2020). Specimens were obtained from three locations within the complex: the eastern site (Poda), the northern site (Meden Rudnik), and the western site (Konstantinovo), situated at coordinates 42°24'12.31″N, 27°19'18.05″E, at an altitude of 309 meters (Figure 1).

Figure 1. Studied biotopes from the Complex Mandra–Poda

Maximum length (TL, cm) and weight (W, g) were measured for each specimen. Samples of skin, muscle, and liver of A. brama were prepared for determination of Cadmium (Cd), Copper (Cu), Mercury (Hg), Nickel (Ni), and Lead (Pb) content. The pre-weighed and thawed samples of liver, muscle and skin of *A. brama* (to 300 mg wet weight) were subjected to acid digestion with aqua regia and microwave heating - Method B: "Microwave heating with temperature control at 175±5°C". Samples of 2 g were used in closed vessels under pressure with 6.0 ml HCl and 2.0 ml HNO_3, and the element was determined using ICP-OES, according to EN ISO 16170:2016. In surface water and sediment samples, concentrations of Cd, Cu, Hg, Ni, and Pb were determined according to EN ISO 11885:2009 Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES) and EN ISO 22036:2024 Environmental solid matrices - Determination of elements using inductively coupled plasma optical emission spectrometry (ICP-OES). The Bioconcentration Factors are presented for determining the capacity for accumulation of Cd, Cu, Hg, Ni, and Pb in the muscles, skin, and liver of European perch, waters and sediments from freshwater ecosystem Mandra-Poda (Bioconcentration Factor, $BCF=C_{Cd,Cu,Hg,Ni,Pb\_Skin}/C_{Cd,Cu,Hg,Ni,PbLiver}/C_{Cd,Cu,Hg,Ni,Pb\_Muscle}/C_{Cd,Cu,Hg,Ni,Pb\_Water}/C_{Cd,Cu,Hg,Ni,Pb\ Sediments}$) (Sorensen, 1991; Zaharieva, 2022). Spearman's rank correlation coefficient (r_s) values are determined to establish the relationship between the concentrations of Cd, Cu, Hg, Ni, and Pb in environmental samples (water, sediments), in samples of liver, muscle and skin of fish. The coefficient of determination (r_s^2) was presented to determine what percentage of the factor variable would cause changes in the output variable. The Friedman test (Friedman's ANOVA test) was calculated to assess the significance of the differences between the contents of Cd, Cu, Hg, Ni, and Pb in the studied tissues and organs of fish (Sokal and Rohlf, 1981). The results are statistically processed using Statistica 10 (StatSoft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Characteristics of the studied fish species

The maximum length of the studied specimens ranged from 8.5-21.0 cm (12.27±3.49), and the weight from 11-203 g (37.27±59.39).

Concentration of Cadmium (Cd), Copper (Cu), Mercury (Hg), Nickel (Ni), and Lead (Pb) in the system Water – Sediments - *A. brama*

Chemical analyses were performed to determine the concentrations of Cd, Cu, Hg, Ni, and Pb in the skin, muscle, and liver of *A. brama*, water, and sediments (Table 1).

Cd is toxic in very low concentrations (Sorensen, 1991; Peycheva et al., 2014), and a non-degradable cumulative pollutant (Ghosh, 2021). The MPC for Cd, according to the Bulgarian Food Codex (Regulation № H-4/2012), is 0.05 mg.kg^{-1} fish; according to FAO, it is 0.5 mg.kg^{-1}. Sources of pollution are industrial wastewater. Sources of Cd in the environment are plant protection products, fertilizers, pesticides, wastewater, and mining activities (Yablanski, Petkov (eds.), 2011). Biomagnification of Cd is observed when the concentration of the element increases from one level to higher levels of the food chain (Ghosh, 2021). Sources of Cu are the mining and ore processing industries. It is contained in insignificant quantities in natural waters. According to national legislation, the norm for Cu content in freshwater fish is 10 mg.kg^{-1} (Regulation № 31/2004). According to FAO, it is 30 mg.kg^{-1}, and according to WHO regulations, it is 20 mg.kg^{-1}. Sources of Hg contamination are organic mercury compounds. Entry into freshwater fish occurs through contaminated food (Ghosh, 2021). According to the Bulgarian Food Authority (Regulation 31/2004), the maximum level permitted of Hg is 0.5 mg.kg^{-1}, and according to FAO/WHO, the permissible weekly intake is 0.3 mg.kg^{-1} for humans (FAO/WHO, 1998; Regulation № 31/2004). Sources of Ni pollution for aquatic organisms are Ni-laden water and food from mining. It is necessary for living organisms as a microelement, but high concentrations are toxic to organisms. According to Regulation № 31/2004 provisions, the norm for Ni content in freshwater fish species is 0.5 mg.kg^{-1}. Sources of Pb are lead ore deposits and industrial wastewater. Pb is a highly toxic element for living organisms, especially aquatic ones. Pb is one of the most persistent toxic elements. The transfer to aquatic organisms occurs with food (Yablanski & Petkov (eds.), 2011; Peycheva et al., 2014; Ghosh, 2021). According to Regulation № 31/2004, the norm for Pb in freshwater fish is 0.2 mg.kg^{-1}.

Table 1. Contamination of Cadmium (Cd), Copper (Cu), Mercury (Hg), Nickel (Ni), and Lead (Pb) in tissues and organs of *A. brama* [mg.kg^{-1}], waters [mg.l^{-1}], and sediments [mg.kg^{-1}]

Samples	Min.-Max. Mean±SD	Min.-Max. Mean±SD	Min.-Max. Mean±SD	Min.-Max. Mean±SD	Min.-Max. Mean±SD
Wet weight	**Cd**	**Cu**	**Hg**	**Ni**	**Pb**
Skin (wet weight) *P. fluviatilis*	0.00-0.00 0.00±0.00	2.115-3.663 2.889±0.387	00.00-0.00 0.00±0.00	0.065-0.257 0.161±0.048	0.00-0.00 0.00±0.00
Muscle (wet weight) *P. fluviatilis*	0.00-0.00 0.00±0.00	5.150-8.302 6.726±0.788	0.00-0.00 0.00±0.00	0.29-0.45 0.37±0.042	0.085-0.137 0.111±0.013
Liver (wet weight) *P. fluviatilis*	0.18-0.28 0.232±0.026	12.61-20.34 16.48±1.932	5.466-9.018 7.242±0.888	0.885-1.761 1.323±0.219	0.595-1.123 0.859±0.132
Dry weight	**Cd**	**Cu**	**Hg**	**Ni**	**Pb**
Skin (dry weight) *P. fluviatilis*	0.00-0.00 0.00±0.00	4.146-7.178 5.662±0.758	0.00-0.00 0.00±0.00	0.125-0.505 0.315±0.095	0.00-0.00 0.00±0.00
Muscle (dry weight) *P. fluviatilis*	0.00-0.00 0.00±0.00	9.826-15.834 12.83±1.502	0.00-0.00 0.00±0.00	0.544-0.865 0.705±0.08	0.163-0.259 0.211±0.024
Liver (dry weight) *P. fluviatilis*	0.817-1.293 1.055±0.119	57.35-92.48 74.913±8.782	24.85-40.99 32.92±4.036	4.022-8.006 6.014±0.996	2.706-5.102 3.904±0.599
Water	0.025-0.040 0.031±0.007	0.002-0.004 0.003±0.001	0.016-0.004 0.013±0.008	0.001-0.005 0.003±0.002	0.400-0.467 0.436±0.034
Sediments	0.228-2.755 1.85±1.41	53.94-172.2 99.10±63.88	0.044-0.143 0.079±0.055	4.343-33.66 29.117±22.844	2.592-69.61 0.257±35.004

The standards for the content of Cd, Cu, Hg, Ni, and Pb in water samples are respectively MPC of 0.015 mg.l^{-1} Cd (Regulation № H-4/2012), 0.02 mg.l^{-1} Cu AAC EQS (Average Annual value, Environmental Quality Standarts, Regulation № H-4/2012) and 2 mg.l^{-}

[1] according to WHO, 0.07 $mg.l^{-1}$ Hg (Regulation № H-4/2012), MPC 0.034 $mg.l^{-1}$ Ni (Regulation № H-4/2012), AAC EQS 0.012 $mg.l^{-1}$ and MPC 0.014 $mg.l^{-1}$ Pb and according to WHO 0.01 $mg.l^{-1}$. The standards for Cd, Cu, Hg, Ni, and Pb content in sediment samples are 3 $mg.kg^{-1}$, 150 $mg.kg^{-1}$, 1.5 $mg.kg^{-1}$, 110 $mg.kg^{-1}$, 100 $mg.kg^{-1}$. The liver samples found the highest concentrations for the five studied elements. The average values in the bream liver show excesses compared to Regulation № 31/2004 norms for the samples from the three studied biotopes. The most considerable excess compared to the norms in Regulation № 31/2004 was registered in terms of the content of Hg (14.44 times), followed by that of Cu and Pb (4.64 and 4.29 times, respectively), of Ni (2.65 times), of Cu (1.65 times). Regarding the samples from the three biotopes, the excesses in the concentrations of Cd compared to the norm under the same regulation are 3.6-5.6 times; of Cu – from 0.98-1.58 times; of Hg – from 49.7-81.98 times; of 8.04-16.01 times; of Pb – from 13.53-25.51 times. The highest exceedances of Cd, Hg and Pb are recorded in the “Konstantinovo” Biotope and the lowest in the “Poda” biotope. The highest excesses of Cu were recorded in the biotope "Meden Rudnik", followed by the biotope Konstantinovo and Poda. According to the FAO norm (0.2 $mg.kg^{-1}$ Cd in food), the excess in the liver is, on average, 1.16 times for the three biotopes and 1.4 times for the biotope “Konstantinovo”. According to the WHO human health norm (20 mg Cu/kg fresh weight), the excess in the liver samples is, on average, 1.65 times for the three biotopes and 0.04 times for the biotope “Meden Rudnik”. Muscles accumulate more significant amounts of Cu and Ni than the skin (on average, 2.33 and 2.3 times, respectively). Compared to the liver samples, the content in Cu, Ni, and Pb muscle was 2.45, 3.57, and 7.74 times lower, respectively. No excesses of Cu and Ni were detected in skin and muscle samples, and no excesses of Pb were detected in muscle samples. No Cd, Hd, and Pb were detected in skin samples of *A. brama*, and no Cd, Hg were detected in muscle samples (Table 1). In the water samples, exceedances of the MPC for Cd (0.015 $mg.l^{-1}$) were found, respectively 1.67 times, 1.87 times and 2.67 times in the Poda, Meden Rudnik and Konstantinovo biotopes; of the MIC for Pb (0.014 $mg.l^{-1}$) - 28.57 times in the Poda biotope, 33.36 times in the Meden Rudnik biotope, 31.5 times in the Konstantinovo biotope or on average for the three biotopes - 31.14 times, regulated in the Regulation on Environmental Quality Standards for Priority Substances and Certain Other Pollutants/2010 under national legislation (Table 1). The analyses of sediment samples show excesses in the Cd content in the Poda biotopes (2.567 $mg.kg^{-1}$ dry matter – 1.28 times) and Meden Rudnik (2.755 $mg.kg^{-1}$ dry matter – 1.39 times), as well as in the Cu content in the Poda biotope (172.2 $mg.kg^{-1}$ dry matter – 1.15 times) compared to the norms regulated in Regulation No. 3/2008 of the national legislation. According to the Effects Range-Low (ERL), developed by the US Environmental Protection Agency (US ERA) to assess the ecological significance of concentrations in sediments, the excesses of Cd concentrations (norm 1.2 $mg.kg^{-1}$ dry weight) are 2.14 times in the Poda biotope, 2.3 times in the Meden Rudnik biotope or an average of 1.54 times for the studied freshwater ecosystem; excesses of Cd are not reported only for the Konstantinovo biotope. According to the same document, the excesses of Cu concentrations (norm 34 $mg.kg^{-1}$ dry weight) were obtained for all three studied biotopes – 5.07 times, 23.73 times, 1.59 times, respectively. Regarding the content of Ni (ERL=21 $mg.kg^{-1}$ dry weight) in the sediments, excesses were found in the biotopes Poda and Meden Rudnik (1.60 and 2.35 times, respectively), as well as about Pb (ERL=47 $mg.kg^{-1}$ dry weight) for both biotopes (1.48 times and 0.40 times, respectively). Exceedances of the norms for Ni and Pb were not found in the samples from the biotope Konstantinovo. Adverse effects on organisms are expected at values of the elements below the ERL (Assessment criteria for contaminants in sediment) (Table 1). Therefore, Cd concentrations decrease in the order sediments – liver – waters – skin – muscles; of Cu and Ni: sediments – liver – muscles – skin – waters; of Hg: liver – sediments – waters – skin – muscles; of Pb: liver – sediments – waters – muscles – skin. The bioconcentration factors (BCF) and bioaccumulation factors (BAF) are presented. The highest BCF were obtained for

Cu in liver and the samples of water and the lowest for Pb in muscles, as well as highest BCF for Hg in liver and lowest for Ni in skin and in the samples of sediments. Very significant positive correlations were established between contamination of Cd in liver and water; of Cu in skin, muscle, liver and in waters and sediments; of Hg in liver and sediments; of Ni in skin, muscle, liver and waters and sediments; of Pb in muscle, liver and water and sediments. A significant but negative correlation ($-r_s$) was determined only between contamination of Hg in the liver and in water samples (Table 2).

Table 2. Bioconcentration factor (BCF), and Spearman correlation coefficient (r_s) between the content of Cd, Cu, Hg, Ni, and Pb in water, sediments, *Abramis brama*

Fish – Water (wet weight)	**Cd BCF**	**Cu BCF**	**Hg BCF**	**Ni BCF**	**Pb BCF**
Skin – Water	0.00	963.00****	0.00	53.67****	0.00
Muscle – Water	0.00	2242.00****	0.00	123.34****	0.25****
Liver – Water	7.48****	5494.00****	557.00-****	441.00****	1.97****
Fish – Sediments (dry weight)	**Cd BCF**	**Cu BCF**	**Hg BCF**	**Ni BCF**	**Pb BCF**
Skin – Sediments	0.00	0.057****	0.00	0.01****	0.00
Muscle – Sediments	0.00	0.129****	0.00	0.02****	0.82****
Liver – Sediments	0.125****	0.756****	416.71****	0.21****	15.19****

r_s **** - very significant correlation, p=0,001.

Significant differences were found between contaminations of Cu and Ni in the samples of skin, muscles, and liver of Freshwater bream, waters and sediments, between contaminations of Cd and Hg in water and sediments and the samples of liver, as well as between contaminations of Pb in water and sediments and in the samples of muscles and liver (Friedman's ANOVA, $p<0.05$). The coefficient of determination (r_s^2) in all cases is 100%. This gives reason to believe that all changes in the concentrations of Cd, Cu, Hg, Ni, and Pb in the waters and sediments of the Complex Mandra-Poda and the specific habitats of *A. brama* will lead to changes in the concentrations of these elements in the skin, muscles, and liver of the fish. The content of heavy metals in *A. brama* from the Mandra-Poda complex has been studied only by Peycheva et al., 2022. The authors studied the content of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in muscle samples. They found much lower values for Cu (0.12 and 6.73 $mg.kg^{-1}$ fresh weight, respectively) and Ni (0.06 and 0.37 $mg.kg^{-1}$ fresh weight, respectively) than in this study and slightly higher values for Pb content (0.15 and 0.11 $mg.kg^{-1}$ fresh weight, respectively).

Cd, Hg, Ni, and Pb are major toxic metals, and Cu is in the group of essential metals with potentially toxic effects (Yablanski & Petkov, 2011; Mostafa et al., 2022). According to Peycheva et al. (2014a), even at low concentrations, Hg is a very toxic element in metabolically active tissues and is associated with an ecological risk for aquatic organisms. They point out that Hg concentrations increase towards higher levels in the food chain, which is why predatory fish species can be suitable bioindicators. Conversely, for Cd, no such increase was found along the food chain (Peycheva et al., 2014b). According to studies by several authors, the liver accumulates Cd, Cu, Hg, Ni, and Pb in much higher concentrations than other tissues and organs of fish, which was also found in this study (Leite et al., 2017; Mostafa et al., 2022).

The content of the studied elements is higher in sediments than in water samples, as also obtained in other studies, which is explained by the fact that they are adsorbed by organic particles and precipitate and accumulate at the bottom, with only 1% of the pollutants participating in the hydrological cycle (Salomons & Stigliani, 1995; Mostafa et al., 2022).

CONCLUSIONS

As a result of the ecological monitoring study of liver, skin and muscle samples of *A. brama* from the Mandra-Poda complex, the highest content of heavy metals was found in the liver samples. The liver of the Freshwater bream appears as a sensitive bioindicator for the Cd, Cu, Hg, Ni, and Pb content.

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