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RISK ASSESSMENT OF THE USE OF *RUTILUS RUTILUS* FOR FOOD FROM TWO ANTHROPOGENICALLY INFLUENCED DAMS

Nikolina ILIEVA[1], Viliana PASKALEVA[2], Diana KIRIN[1,*2], Radoslava ZAHARIEVA[2], Petya ZAHARIEVA[2], Georgi PATRONOV[3], Doychin TERZIYSKI[4]

[1]Agricultural University-Plovdiv, Department of Chemistry, Phytopharmacy and Ecology, and Environmental Protection, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria
[2]Bulgarian Academy of Sciences, National Institute of Geophysics, Geodesy and Geography (NIGGG), Hydrology and Water Management Research Center, Acad. G. Bonchev Street, 1113 Sofia, Bulgaria
[3]University of Plovdiv 'Paissii Hilendarski', Department of Chemical Technology, 24 Tsar Assen, 4000, Plovdiv, Bulgaria
[4]Agricultural Academy, Sofia, Institute of Fisheries and Aquaculture, Plovdiv, Bulgaria

Corresponding author email: dianaatanasovakirin@gmail.com

Abstract

The aim of the study is to assess the levels of Cd and Pb contamination in muscle, skin, and liver samples, to assess the potential ecological risk to ecosystems and the health risk associated with using Roach for human food. The studies were performed using Inductively coupled plasma mass spectrometry. The results indicate higher levels of both elements in the tissues and organs of fish from the Topolnitsa dam. In both ecosystems, the highest values of Pb and Cd were observed in sediment samples, and the lowest in water samples. The RI (potential ecological risk) for all indicators shows a low potential ecological risk for Pb and Cd in the Dushantsi dam ecosystem, and a significant potential ecological risk for Cd in the Topolnitsa dam ecosystem. When consuming R. rutilus from both reservoirs, the fish should be thoroughly cleaned of the entrails and skin. No risk to human health is expected when consuming up to 420 g per week of fish muscle from the two dams.

***Key words*:** *cadmium, degree of contamination, health risk assessment, lead, potential ecological risk.*

INTRODUCTION

Dushantsi Dam and Topolnitsa Dam for heavily modified water bodies of the Maritsa River Basin, East Aegean Region, Bulgaria. Dushantsi Dam is of type L13: Medium and small semi-mountainous dams in Ecoregion 7: Eastern Balkans, water body code BG3MA800L180. It is located in the upper reaches of the Topolnitsa River, 2 km from the village of Dushantsi, Sredna Gora. It is a large hydrotechnical facility, determined to be in poor ecological condition, likely related to the region's highly developed industry (River Basin Management Plans (East Aegean Region) 2021-2027). The waters of the dam are mainly used for industrial water supply, fishing, and water sports. No similar studies have been carried out for the freshwater ecosystem of Dushantsi Dam to date. Topolnitsa Dam is located below Dushantsi Dam, towards the middle reaches of the river. It belongs to type L11b: Large deep dams in Ecoregion 7: Eastern Balkans, code of water body BG0001389. The waters of the dam are used for electricity generation, irrigation of large areas of agricultural land, flood control, sport and industrial fishing, tourism, etc. The dam ecosystem is influenced by long-term industrial activities related to mining and mining processing. Fish are one of the main foods for humans. They are sources of high-quality proteins, polyunsaturated fatty acids, vitamins and other essential elements (Stancheva et al., 2012). Heavy metal pollution poses a significant threat to aquatic ecosystems and their biota, especially fish. Fish are sensitive to changes in pollutant concentrations in aquatic environments (Peycheva et al., 2014; Georgieva et al., 2015; Peycheva et al., 2022). They occupy different trophic levels in food chains, but also have a relatively long lifespan, allowing them to accumulate several pollutants in their bodies, including heavy metals, which is why they are sensitive bioindicators of pollution of the aquatic environment (Rashed, 2001; Bervoets &

Blust, 2003; Saeed & Shaker, 2008; Peycheva et al., 2014). The Topolnitsa Dam has been the subject of research by various authors on heavy metal content in fish (Yancheva et al., 2011; Georgieva et al., 2015), but not on *Rutilus rutilus*. The Roach has been studied for heavy metals in other dams (Zhelyazkov et al., 2014), but studies from Bulgarian dams are few. The aim of the study is to assess the degree of anthropogenic loads, the potential ecological risk to ecosystems, and the risk of using the Roach for food based on the levels of Pb and Cd contamination.

MATERIALS AND METHODS

The objects of the study are thirty specimens of Roach *Rutilus rutilus* (Linnaeus, 1758), each from two freshwater ecosystems: Dushantsi and Topolnitsa dams. The fish were collected with multi-mesh gillnets (BSS EN 14757:2015) and personal permissions from the Executive Agency for Fisheries and Aquaculture, Ministry of Agriculture and Food, Bulgaria. The FishBase database is used to check the scientific and common names of fish (Froese & Pauly, 2020). The fish were collected from the Dushantsi Dam, named as Dushantsi Biotope (42041'49.51''N 24017'27.80''E; 792 m) and from the Topolnitsa Dam, named as Poibrene Biotope (42027'38.88''N 23059'20.81''E; 309 m; Figure 1).

For the ichthyological sample, the following parameters are determined: total number of specimens, total body length (TL, cm), total body width (TH, cm), total body weight (TW, g), biomass in percent (W%), abundance (A%), absolute dominance ($\mathbf{D} = \frac{(A\% + W\%)}{2}$) (Belkinova et al., 2013) and Fulton's condition factor ($\mathbf{K} = \mathbf{100}.\frac{TW}{TL^3}$) (Stavrescu-Bedivan et al., 2023; Zaharieva et al., 2024).

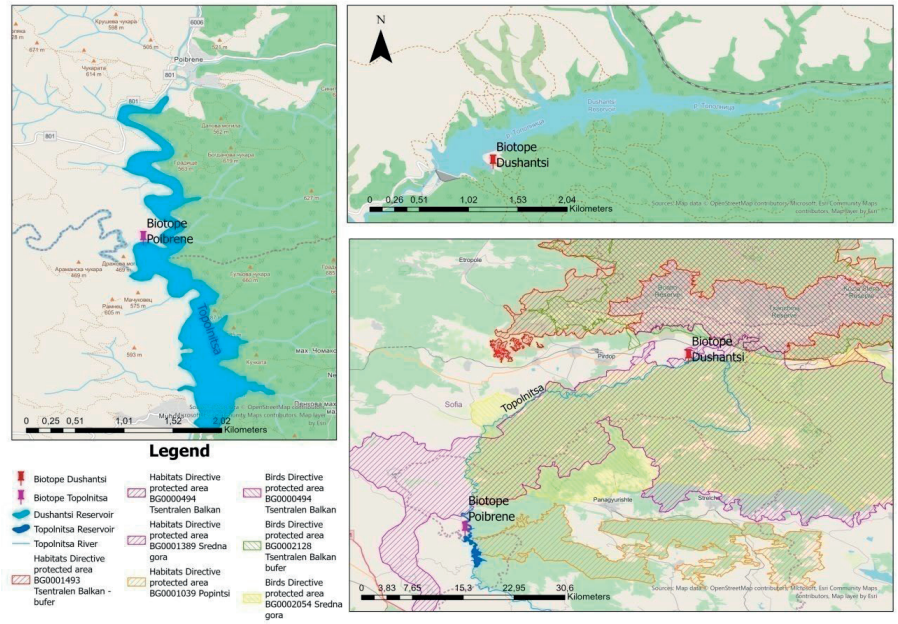


Figure 1. Studied biotopes from the Dushantsi and Topolnitsa dams (Esri Inc. (2023). ArcGIS Pro (Version 3.2.0) https://www.arcgis.com/home/index.html

The requirements of BSS ISO 5667-4:2026 and BSS ISO 5667-12:2017 standards have been used to collect water and sediment samples. BSS EN ISO 5667-3:2024 and BSS EN ISO 5667-15:2009 standards have been applied for the preservation and storage of water and sediment samples, respectively. The standards BSS EN ISO 17294-2:2023 and BSS EN ISO 21424:2020 Milk, milk products, infant formula and adult nutritionals - Determination of minerals and trace elements - Inductively coupled plasma mass spectrometry (ICP-MS)

method (ISO 21424:2018) were used to determine the content of Cd and Pb in samples of water, liver, muscles and skin of the Roach from the two dams, and US EPA Method 3051A for sediment samples. PerkinElmer SCIEX ELAN DRC-e ICP-MS system (MDS Inc., Concord, ON, Canada, Inductively coupled plasma mass spectrometry, Table 1) was used to conduct the elemental analysis.

Table 1. Instrumental parameters and monitored isotopes used for ICP-MS analysis

Parameter	Value
Ar plasma flow rate	15 L min^{-1}
Auxiliary Ar flow rate	1.20 L min^{-1}
Nebulizer gas flow rate	0.90 L min^{-1}
Lens voltage	6.00 V
ICP RF power	1100 W
Integration time	2000 ms
Dwell time	50 ms
Measurement mode	Peak hopping
Sample uptake rate	2 mL min^{-1}
Rinse time between samples	180 s
Rinse solution	3% HNO_3
Monitored isotopes	^{27}Al, ^{54}Fe, ^{56}Fe, ^{57}Fe, ^{55}Mn, ^{75}As, ^{112}Cd, ^{114}Cd, ^{52}Cr, ^{63}Cu, ^{65}Cu, ^{202}Hg, ^{60}Ni, ^{62}Ni, ^{207}Pb, ^{208}Pb, ^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se, ^{66}Zn

HNO_3 (67-69%, Fisher Chemicals, Ultra Trace Metal Grade, Loughborough, UK), HCl (37%, Fisher Chemicals, Ultra Trace Metal Grade, Loughborough, UK) and H_2O_2 (30%, Fisher Chemicals, Ultra Trace Analysis Grade, Loughborough, UK) were used for sample preparation and analysis. All solutions were prepared with double-deionised water. Working standard solutions were prepared by diluting the ICP-MS multi-element calibration standard solution IMS-102 (Ultra Scientific, Agilent, Santa Clara, CA, USA), which contains, among others, Cd and Pb with an initial concentration of 10 mg/L. After collection, water samples were stored at 4°C in dark glass bottles before transport to the laboratory. For ICP-MS analysis, 50 mL of each water sample was filtered through a 25 mm sterile PES syringe filter with a 0.45 µm pore size. The filtrates were acidified with 0.05 mL concentrated nitric acid to pH 2 and subsequently analysed. Biological samples were digested with concentrated HNO_3 and then diluted before analysis. A total of 10 tissue, organ, water and sediment samples were examined for each of the two dams, collected during the three seasons (spring, summer, and autumn) in 2025. Skin, muscle and liver samples were weighed twice, once as wet weight and once as dry weight. The degree of accumulation of lead and cadmium in the studied tissues and organs of *R. rutilus* relative to their content in water and sediment samples was estimated using the Bioconcentration factor (BCF): $\mathrm{BCF} = \frac{C}{Cw}$ and $\mathrm{BCF} = \frac{C}{Cs}$, where C is the concentration of the metal in the muscles, skin or liver of the Roach in µg/kg, respectively; C_w is the concentration of the metal in the water samples (µg/L), and C_s is their concentration in the sediments (µg/kg). The calculations are for each organ or tissue and for each metal separately. BCF is an important indicator for assessing ecological risk. The relationship between the concentrations of Pb and Cd in samples of water and sediments and samples of Liver, Muscle, and Skin was presented through Spearman's rank correlation coefficient (r_s). The ecological risk ($\mathrm{RI} = \sum E r^i = \sum \mathrm{Tr}^i . \mathrm{Cf}^i$) is defined as the sum of the products of the toxic response factor (specific for each element) and the contamination factor for each of the trace elements. The contamination factor (Cf^i) is defined as the ratio of the actual to the reference concentration of each trace element. The sum of all contamination factor values indicates the contamination level (Cl) (Hakanson, 1980; Tang et al., 2023; Younis et al., 2024). The USEPA (1989) methodology was used to assess the health risk of consuming roach from both dams. A Target Hazard Quotient (THQ) (THQ) was calculated using the formula: $THQ = \frac{(EFr.ED.FIR.C)}{(BWa.AT.RfD)} . 10^{-3}$, where EFr is the frequency of exposure, ED is the average life expectancy for men and women or the duration of exposure, FIR is the average daily intake of fish consumed, C is the concentration of the elements tested in muscle samples (µg.g^{-1}, ww), BWa is the average body weight for women and men, AT is the average time of exposure to non-carcinogens, RfD is the reference oral dose (0.001 for Cd and 0,004 for Pb, according USEPA, 2011). The Hazard index (HI) is calculated using the formula: HI = ∑THQ, calculated for each of the two dams in terms of Pb and Cd content. The results are statistically

processed by MS Excel (Microsoft, 2011) with relevant mathematical formulas.

RESULTS AND DISCUSSIONS

Characteristics of the studied fish species

Roach, *Rutilus rutilus* (Linnaeus, 1758) (Cyprinidae) is the dominant species in the collected fish samples from both dams. It is a freshwater, benthopelagic, and brackish fish species. Staple food for the Roach are crustaceans, worms, insect larvae, algae, and higher aquatic vegetation (Karapetkova & Zhivkov, 2006; Kottelat & Freyhof, 2007). *R. rutilus* is included in the International Union for Conservation of Nature Red List as a Least Concern species (LC; IUCN) (Kottelat & Freyhof, 2007). The species is widely distributed in various habitats in the country. *R. rutilus* is an object of sport and industrial fishing. In both dams, the Roach is an eudominant species ($D_{Dushantsi}$=47.85; $D_{Topolnitsa}$=22.65). Fulton's condition factor (K > 1 for both dams) indicates that the fish are fatty, well-nourished, with high fat content and developed gonads. The condition factor suggested a favourable body condition (Table 2).

Table 2. Metric data, dominance and status of *Rutilus rutilus* from Dushantsi and Topolnitsa reservoirs

Fish species *Rutilus rutilus* (Linnaeus, 1758)	Dushantsi	Topolnitsa
Total length, TL cm	9.98±2.53 (7.40-21.00)	16.34±2.52 (9.1-19.5)
Total width, TH см	2.39±0.77 (1.70-5.70)	4.44±0.94 (2.20-5.90)
Weight, W г; W %	12.50±20.48 (4.00-117.00) 53.92%	53.24±23.91 (7.00-98,00); 13.30%
Abundance, A %	47.62	32.00
Absolute dominance, D	47.85	22.65
Fulton's condition factor, K	1.26	1.22

Concentration of Lead (Pb) and Cadmium (Cd) in Roach and the Environment

The content of Pb and Cd in samples of Skin, Muscle and Liver of R. rutilus, water and sediments are presented in Table 3. Lead (Pb) and cadmium (Cd) are major toxic metals (Liu et al., 2013). Lead is not a natural element in natural waters. It enters natural water bodies from lead ores and industrial wastes, from mines and smelters used for lead processing, and from all production activities that use lead. Lead is a highly toxic metal. Studies have shown that the accumulation of lead by aquatic organisms increases with increasing pH and decreasing water hardness. Lead is transported through the body's bloodstream and accumulates mainly in soft tissues, the liver, and the kidneys. It is permanently deposited in bones. High concentrations of oil in aquatic ecosystems stimulate biodiversity, stimulate fish development and cause other effects (Yablanski & Petkov, 2011). The degree of toxic effects of lead is influenced by the stage of fish development, pH, temperature, water hardness, the presence of dissolved organic matter and other factors. (Hellawel, 1896; Peycheva et al., 2014). The content of Pb and Cd in water samples was assessed in accordance with the requirements of Regulation H-4/2012 and the Regulation on environmental quality standards for priority substances and certain other pollutants of the national legislation. In Bulgaria and the European Union, standards for heavy metals in sediments have not been established; therefore, those approved by the US Environmental Protection Agency (US EPA) were used (Effect Range-Low - ERL) (O'Connor, 2004; Cholakova, 2021). The content of these two elements in fish skin, muscle and liver samples was assessed according to Regulation (EU) 2023/915, FAO (1983), MAFF (1995). For waters, the maximum permissible concentration is 1.20 µg/L (Regulation on EQS, on priority substances and certain other pollutants). For sediments, ERL for Pb is 46.7, and for Cd – 1.20 mg/kg dry weight. According to national legislation, the maximum permissible content (MPC) of Pb in fish muscles is 0.2 mg/kg (Regulation 31/2004), according to FAO (1983), it is 0.5 mg/kg, and according to Regulation (EU) 2023/915 - 0.30 mg/kg wet weight. According to Regulation 31/2004, the MPC for Cd is 0.05 mg/kg, according FAO and MAFF - 0.5 mg/kg and 0.2 mg/kg respectively, and according to Regulation (EU) 2023/915 – 0.05 mg/kg wet weight. Industrial wastewater has been identified as a major source of Cd pollution (Yablanski & Petkov, 2011).

The highest concentrations of Pb and Cd were found in sediments, and the lowest contamination was in water samples for the two elements and dams. The results for the Roach tissues and organs showed the highest values for the two elements in liver samples, followed by

skin samples. The lowest content of Pb and Cd is in the muscle samples. This trend is established for both elements and for the Roach from both dams, in both fresh- and dry-weight samples. The differences in the two dams are in the concentrations of the two elements and the exceedances compared to the established norms. In the Dushantsi Dam, the exceedances in Pb and Cd concentrations are as follows: 9.2 times for Liver>6.7Skin>5.0Muscle for Pb and 3.9 times for Liver>1.40Skin>1.20Muscle for Cd. For the Topolnitsa Dam, the exceedances are: 28.95 times for Liver>7.60Skin>6.90Muscle for Pb. and 11 times for Liver>10.80Skin>1.80Muscle for Cd.

In the Topolnitsa Dam, the exceedances for Pb compared to those in Dushantsi Dam are 3.15 times higher in Liver samples, 1.13 times higher in Skin samples and 1.38 times higher in Muscle samples.

Regarding the exceedances for Cd, which are also higher in Topolnitsa Dam samples: 2.82 times higher for Liver samples, 7.71 times higher in Skin and 1.50 times higher in Muscle. We assume that the higher concentrations of Pb and Cd in the Topolnitsa reservoir are due to river flows passing through areas with intensive mining and ore processing.

The content of Pb and Cd in the studied muscle samples of fish from the Dushantsi Dam is respectively 3.34 and 1.2 times higher, and for the Topolinitsa Dam, 4.6 and 1.8 times higher, compared to the maximum permissible levels of Pb and Cd in fish set by Regulation (EU) 2023/915.

Table 3. Content of Lead (**Pb**) and Cadmium (**Cd**) in tissues and organs of *R. rutilus,* waters and sediments

Samples Dushantsi	**Min-Max Mean±SD**	**Min-Max Mean±SD**
Topolnitsa	**Mean±SD**	**Mean±SD**
Wet weight	**Pb**	**Cd**
Skin *R. rutilus*	1.52±0.42 (1.22-1.81)	0.54±0.25 (0.37-0.77)
Muscle *R. rutilus*	1.38±0.06 (1.33-1.42)	0.09±0.03 (0.07-0.11)
Liver *R. rutilus*	5.79±2.43 (3.86-7.29)	0.55±0.17 (0.43-0.67)
Water	0.02±0.01 (0.01-0.03)	0.007±0.002 (0.005-0.009)
Dry weight	**Pb**	**Cd**
Skin *R. rutilus*	1.96±0.54 (1.58-2.35)	0.88±0.48 (0.54-1.22)
Muscle *R. rutilus*	1.94±0.09 (1.87-2.00)	0.12±0.04 (0.09-0.14)
Liver *R. rutilus*	9.20±2.32 (7.56-10.84)	1.60±0.56 (0.52-2.08)
Sediments	28.26±1.35 (27,30-29,21)	1.39±0.30 (1,17-1,60)

The bioconcentration factors (BCF) are presented. The highest BCF$_s$ were obtained for Pb and Cd in Liver samples (Table 4).

Table 4. Bioconcentration factor (BCF) and Spearman correlation coefficient (r_s) between the content of Pb and Cd in water, sediments and *R. rutilus*

Dushantsi				
Fish – Water (wet weight)	**Pb BCF**	**Cd BCF**	**rs Pb**	**rs Cd**
Skin – Water	6700.00	350.00	1****	1****
Muscle – Water	5015.00	300.00	1****	1****
Liver – Water	9200.00	900.00	0,999****	1****
Fish – Sediments (dry weight)				
Skin – Sediments	0.11	0.18	1****	-0.82***
Muscle – Sediments	0.06	0.13	1****	1****
Liver – Sediments	0.13	0.41	1****	1****
Topolnitsa				
Fish – Water (wet weight)	**Pb BCF**	**Cd BCF**	**rs Pb**	**rs Cd**
Skin – Water	0.07	0.75	0,999****	0,996****
Muscle – Water	0.06	0.13	0,997****	1****
Liver – Water	0.26	0.77	0,997****	1****
Fish – Sediments (dry weight)				
Skin – Sediments	0.12	0.24	0,999****	0,999****
Muscle – Sediments	0.03	0.03	0,999****	0,994****
Liver – Sediments	2.54	0.43	0,999****	0,529^{ns}

Legend: r_s **** - very significant correlation, p≤0,001; *** - strong correlation, p≤0,01; ^{ns} – insignificant correlation

Results of the study show that Cd accumulates at higher concentrations in the liver than in fish tissues. Higher Cd content in liver samples of freshwater fish species compared to skin and muscle has been reported in other studies (Georgieva et al., 2015; Peycheva et al., 2022; Ilieva & Kirin, 2025). Dallinger et al. (1987) indicated that this is related to the higher protein content in the liver. In the study, sediments had a higher Cd content than in water samples. Salomons & Stigliani (1995) explained this result by the fact that heavy metals bind to suspended particles or adsorb to organic matter, leading to their precipitation.

The contamination factor is very high (Cf) for Pb in the muscles of the Roach from both dams and moderate for Cd (Table 3). The contamination level (Cl) is 1.50 times higher in the muscles of *R. rutilus* from Topolnitsa dam than in Dushantsi dam. The risk of Pb and Cd loading in the muscles is 1.49 times and 1.5 times, respectively, higher for *R. rutilus* from Topolnitsa dam than for those from Dushantsi dam. The RI (potential ecological risk) for all indicators indicates a low potential ecological risk for Pb and Cd in the Dushantsi dam ecosystem, and a significant potential ecological risk for Cd in the Topolnitsa dam ecosystem (Table 5). When consuming *R. rutilus* from both reservoirs, the fish should be thoroughly cleaned of the entrails and skin.

Table 5. Risk assessment and degree of contaminations

Factors	Tissues/Sediments	Pb Dushantsi	Cd Dushantsi	Pb Topolnitsa	Cd Topolnitsa
Cf	Skin	10.65 very high	2.20 moderate	9.80 very high	17.60 very high
	Muscle	6.50 very high	1.60 moderate	9.70 very high	2.40 moderate
	Liver	12.95 very high	5.00 considerable	46.00 very high	32.00 very high
	Sediments	0.43 low	0.51 low	1.62 moderate	3.12 considerable
Cl	Skin	12.85 moderate		41.70 very high	
	Muscle	8.10 moderate		12.20 moderate	
	Liver	17.95 considerable		63.60 very high	
	Sediments	0.94 low		4.74 low	
Er	Skin	53.25 moderate	66.00 moderate	49.00 moderate	528.00 very high
	Muscle	32.50 low	48.00 moderate	48.50 moderate	72.00 moderate
	Liver	64.75 moderate	150.00 considerable	230.00 high	960.00 very high
	Sediments	2.15 low	15.30 low	8.10 low	93.60 considerable
RI	Skin	119.25 low		758.00 very high	
	Muscle	80.50 low		121.00 low	
	Liver	214.75 moderate		1008.50 very high	
	Sediments	17.45 low		101.70 low	

The health risk assessment was made assuming equal intake and adsorbed doses of pollutants, an average fish food intake rate of 60 grams per day (FIR), an assumption of an average body weight (BWa) of 65 kg for women and 85 kg, an average lifetime (ED) of 79 for women and 72 for men, reference oral dose for Pb 0.004 and 0.001 ($mg.kg^{-1}.day^{-1}$) for Cd, the Target Hazard Quotient (THQ) is as follows (Table 6):

Table 6. THQ and HI of Dushntsi (Dishantsi Dam) and Poibrene biotopes (Topolnitsa Dam)

THQ	Dushnatsi Biotope		Poibrene Biotope	
	Pb	Cu	Pb	Cu
Women	0.230	0.055	0.318	0.083
Men	0.176	0.067	0.243	0.063
Total	0.406	0.122	0.561	0.146
HI	0.528		0.707	

The obtained values for the HI are below 1. Therefore, according to USEPA (2011) no risk to human health is expected when consuming up to 420 g per week of fish muscles from the two dams.

CONCLUSIONS

The studied *R. rutilus* specimens from both dams are healthy. Fulton's condition factor (K) indicates favourable conditions in both habitats. The chemical analyses showed that Pb levels were 3.15 times higher in liver samples, 1.13 times higher in skin samples, and 1.38 times higher in muscle samples in the Topolnitsa dam than in the Dushantsi dam. The excesses for Cd are also higher in the samples from the Topolnitsa dam: 2.82 times higher in liver, 7.71 times higher in skin and 1.50 times higher in muscle. The use of Roach no risk to human health is expected when consuming up to 420 g per week of this species of fish from the two dams.

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