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METAL CONTAMINATION IN THE SYSTEM WATER - SEDIMENTS – *PERCA FLUVIATILIS* LINNAEUS, 1758 AND *EUSTRONGYLIDES EXCISUS* JÄGERSKIÖLD, 1909 LARVAE

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Abstract

The study presents the first data on the accumulation circulation of Al, Ba and Cd in the samples of skin, muscles and liver of the European perch (Perca fluviatilis Linnaeus, 1758), its dominant parasite species Eustrongylides excises Jägerskiöld, 1909 larvae, water and sediments from the freshwater ecosystem of the anthropogenically affected protected area Mandra-Poda, Black Sea Basin, Bulgaria. The potential of European perch and E. Excises for bioaccumulation towards the studied elements has been studied. The bioindicator significance of P. fluviatilis and E. Excises for assessing the pollution of the freshwater ecosystem with Al, Ba, and Cd has been revealed. The risk to human health and living organisms has been evaluated. Measures for the protection of the ecosystem have been indicated.

Key words*: aluminium, barium, cadmium, bioindication, European perch.*

INTRODUCTION

The Mandra-Poda Complex is located south of the industrial zone of Burgas, along the sea coast. It is part of the Burgas Wetlands, within the Black Sea Basin Region and Ecoregion 12: Pontic Province, Sub-Ecoregion 12-2: Black Sea, in southeastern Bulgaria. The Mandra-Poda Complex is recognized as a wetland of international importance under the Ramsar Convention on Wetlands (1971). The site is also protected under the Habitats Directive (Directive 92/43/EEC) and the Birds Directive (Directive 79/409/EEC). Additionally, it is designated as a Corine Biotope (Corine, 1985) and classified as an Important Bird Area by BirdLife International. According to national legislation (Law on Protected Areas, 1989), three protected areas have been designated within the territory of the Mandra-Poda Complex: "Poda" (State Gazette No. 37/1989), "Ustie na reka Izvorska" (State Gazette No. 18/1990), and "Uzungeren" (State Gazette No. 102/2005). These areas were established for the conservation of habitats supporting endangered and rare bird species. The Complex lies along the "Via Pontica" migration route. Four rivers - Izvorska, Fakiyska, Sredetska, and Rusokastrenska - flow into the Complex.
Despite its international and national significance for habitat protection and exceptional biodiversity, the Mandra-Poda Complex is under significant anthropogenic pressure. Nearby, the Lukoil Neftochim Burgas oil refinery utilizes water from the Complex for industrial purposes.
Serious threats to the protection of biodiversity and water resources are the great anthropogenic pressure from the construction around the wetland, associated with the destruction of natural habitats (wet meadows and others), eutrophication of the wetland, pollution with industrial wastewater, household waste, overfishing, poaching, disturbance of birds and other organisms. Studies on parasites and parasite communities of freshwater fish from the Complex Mandra-Poda are scarce (Margaritov, 1959; Ilieva & Kirin, 2024). There were established single scientific studies on the chemical indicators of water pollution and the content of pollutants in the muscles of freshwater fish species (Georgieva et al., 2015; Peycheva et al., 2022), as well as on the contamination of Se in organs and tissues of

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European perch and its parasite *Eustrongylides excisus*, in waters and sediments (Ilieva & Kirin, 2024).

The study aims to present ecohelminthological and monitoring results for contamination with aluminium (Al), barium (Ba), and cadmium (Cd) in tissues and organs of *Perca fluviatilis* Linnaeus, 1758 its dominant helminth species *Eustrongylides excises* Jägerskiöld, 1909 larvae, waters and sediments, as well as an assessment of their bioindicator significance for the condition of the Complex Mandra-Poda.

MATERIALS AND METHODS

The helminthological and environmental monitoring study was conducted on thirty specimens of *Perca fluviatilis* collected from the Mandra-Poda Complex. The perch samples were obtained using multi-mesh gillnets in accordance with BSS EN 14757:2015 - *Water quality: Sampling of fish with multi-mesh gillnets*. Sampling was performed with permission from the Executive Agency for Fisheries and Aquaculture, under the Ministry of Agriculture of Bulgaria.

The scientific names of the studied fish species were verified using the FishBase database (Froese & Pauly, 2024).

Sampling was carried out at three sites located in the eastern (Poda), northern (Meden Rudnik), and western (Konstantinovo) areas of the Mandra-Poda Complex (42°24′12.31″N, 27°19′18.05″E; 309 m; Figure 1).

Figure 1. Studied biotopes from the Complex Mandra-Poda

The helminthological examination of *Perca fluviatilis* was conducted following the methodologies outlined by Zashev and Margaritov (1966), Bauer (Ed.) (1987), and Moravec (2013). Collected helminth specimens were preserved in 70% ethyl alcohol. Dominant nematode specimens were identified using temporary slides, in accordance with Moravec (2013). The predominant species, *Eustrongylides excisus* (Jägerskiöld, 1909), was selected as a model helminth for subsequent monitoring studies.

Tissue samples (skin, muscle, and liver) from *P. fluviatilis*, along with *E. excisus* larvae, were prepared to assess aluminium (Al), barium (Ba), and cadmium (Cd) concentrations, following the protocol of Nachev et al. (2013). Pre-weighed and thawed samples of fish tissues (up to 300 mg wet weight) and helminths (up to 100 mg wet weight) were subjected to acid digestion using aqua regia and microwave heating (Method B: Microwave heating with temperature control at 175 ± 5 °C). Approximately 2 g of each sample were digested in closed vessels under pressure with 6.0 mL HCl and 2.0 mL HNO_3. Elemental concentrations were determined using inductively coupled plasma optical emission spectrometry (ICP-OES), in accordance with EN ISO 16170:2016.

In addition, concentrations of Al, Ba, and Cd in surface water and sediment samples were measured using EN ISO 11885:2009 (*Water quality - Determination of selected elements by ICP-OES*) and ISO 22036:2024 (*Environmental solid matrices - Determination of elements by ICP-OES*).

The Bioconcentration Factors for determining the capacity for accumulation of Al, Ba, and Cd in the muscles, skin, and liver of European perch and *E. excisus* from freshwater environments (Bioconcentration Factor, $BCF=C_{Al,Ba,Cd\_Skin}/C_{Al,Ba,Cd\_Liver}/C_{Al,Ba,Cd\_Muscle}/C_{Al,Ba,Cd\_E.excisus}/C_{Al,Ba,Cd\_Water}/C_{Al,Ba,Cd\_Sediments}$) (Sures et al., 1999), as well as the Bioaccumulation Factor for determining the capacity for accumulation of Al, Ba, and Cd in *E. excisus* from organs and tissues of European perch (Bioaccumulation Factor, $BAF=C_{Al,Ba,Cd\_E.excisus}/C_{Al,Ba,Cd\_fish\_tissues\_organs}$) (Sorensen, 1991; Zaharieva, 2022a) are presented. Spearman's rank correlation coefficient (rs) values were determined to establish the relationship between the concentrations of Al, Ba and Cd in environmental samples (water, sediments), in samples of liver, muscle and skin of host fish and in samples of the nematode *E. excisus* (Sokal & Rohlf, 1981). 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 Al, Ba and Cd in the studied tissues and organs of fish and *E. excisus* (Sokal & Rohlf, 1981). The results are statistically processed using Statistic 10 (Stat Soft Inc., 2011) and MS Excel (Microsoft 2010).

RESULTS AND DISCUSSIONS

Characteristics of the studied fish species

European perch, *Perca fluviatilis* Linnaeus, 1758 (Percidae), was chosen as a model freshwater fish species due to its dominant behavior in the samples and as a predatory species. *P. fluviatilis* is also a demersal and brackish fish species. The perch feeds mainly on zooplankton, zoobenthos and small fish. *Perca fluviatilis* is in the category of Least Concern species according to the International Union for Conservation of Nature Red List (LC; IUCN) (Kottelat & Freyhof, 2007; Froese, & Pauly 2024). The species are not protected in the territory of Bulgaria (Karapetkova & Zhivkov, 2006). The perch inhabits various water bodies in the country and is widely distributed. *P. fluviatilis* is mainly targeted by recreational fishing. Each specimen's maximum length (TL, cm) and weight (B, g) were measured. The maximum length of the studied specimens ranged from 12-22.50 cm (17.77±3.06), and the weight from 21-227 g (75.77±57.09).

Characteristics of the studied endohelminth species

The ecohelminthological study of 30 specimens of European perch, *Perca fluviatilis* Linnaeus, 1758 from the Complex Mandra-Poda shows a clear dominance of the nematode *Eustrongylus excisus* (Jägerskiöld, 1909) (P%=90). The definitive hosts of *Eustrongylus excisus* (Jägerskiöld, 1909) are cormorants (*Phalacrocorax carbo* (Linnaeus, 1758), *Ph. pygmaeus* (Pallas, 1773)). The adult nematodes are localized in the glandular stomach of the host. The life cycle of *E. excisus* occurs with the participation of two intermediate hosts: the first intermediate hosts are aquatic oligochaetes (*Lumbricus variegatus* (Müller, 1774), *Tubifex tubifex* (Müller, 1774), *Limnodrilus* sp.). The first stage of larvae development is localized in the intestine and then in the body cavity. The second stage larva is localized in the abdominal blood vessel, and the third stage larva with the bloodstream passes to the head and tail of the host. Benthic fish species are the second intermediate host (*Ponticola kessleri* (Günther, 1861), *Neogobius melanostomus* (Pallas, 1814), *Rutilus rutilus* (Linnaeus, 1758)). The intermediate hosts and the final host are infected by feeding. In the body of the second intermediate host, the fish and the third-stage larvae are localized in the body cavity or coiled in a circle under the serous coating on the surface of the internal organs. After another moult, they migrate into the muscles of the fish. The fourth stage larva is invasive, coiled and encapsulated. *P. fluviatilis* is a reservoir host of *E. excisus*. Other species, reservoir hosts of *E. excisus* are: *Leuciscus aspius* (Linnaeus, 1758), *Silurus glanis* Linnaeus, 1758, *E. lucius*, *Sander lucioperca* (Linnaeus, 1758), *Sander volgensis* (Gmelin, 1789), *G. cernua*, *Alburnus chalcoides* (Güldenstädt, 1772), *Bentophilus macrocephalus* (Pallas,1787), *Huso huso* (Linnaeus, 1758), *Acipenser ruthenus* Linnaeus, 1758, *A. gueldenstaedtii* Brandt & Ratzeburg, 1833, *Leuciscus idus* (Linnaeus, 1758), *Luciobarbus brachycephalus* (Kessler, 1872). Accidental hosts can also be amphibians (frogs, *Pelophylax ridibundus* (Pallas, 1771)), reptiles (*Natrix tessellata* (Laurenti, 1768), turtles and others), mammals (rabbits, rats, seals, even humans) (Bauer, 1987; Kakacheva-Avramova, 1983; Honcharov et al., 2022). *E. excisus* has been reported in Bulgaria for *P. fluviatilis* from Lake Srebarna (Shukerova, 2010; Shukerova et al., 2010; Hristov, 2013; Kirin et al., 2013a); from the Arda River (Kirin et al., 2013b); from the Danube River (Atanasov, 2012; Kirin et al., 2013a; Zaharieva, 2022b). *E. excisus* has been reported for the Mandra-Poda complex from *S. lucioperca* as a reservoir host and from *Gobius* sp. as an intermediate host (Margaritov, 1959); from

P. fluviatilis (Ilieva & Kirin, 2024 and in the present study).

Concentration of aluminium (Al), barium (Ba) and cadmium (Cd) in the system Water - Sediments - *P. fluviatilis – E. excisus*

Chemical analyses were performed to determine the Al, Ba, and Cd content in the skin, muscle, and liver of *P. fluviatilis*, in samples of *E. excisus*, water, and sediments (Table 1).

Table 1. Contamination with aluminium (Al), barium (Ba), and cadmium (Cd) in tissues and organs of *P. fluviatilis*, its nematode species *E. excisus*, waters and sediments [mg/kg]

Samples	**Min.-Max. Mean±SD**	**Min.-Max. Mean±SD**	**Min.-Max. Mean±SD**
Wet weight	**Al**	**Ba**	**Cd**
Skin *P. fluviatilis*	3.38 – 86.16 58.69±7.54	1.18- 4.07 2.62±0.32	0.00-0.00 0.00±0.00
Muscle *P. fluviatilis*	81.52-133.28 107.40±12.94	0.53-0.93 0.73±0.10	0.00-0.00 0.00±0.00
Liver *P. fluviatilis*	10.49-18.07 14.28±1.89	0.06-0.26 0.16±0.05	0.09-0.15 0.124±0.01
E. excisus	28.51-53.13 40.82±6.16	0.05-0.33 0.19±0.07	0.05-0.08 0.07±0.008
Dry weight	**Al**	**Ba**	**Cd**
Skin *P. fluviatilis*	87.04-147.16 117.1±15.03	3.94-6.54 5.23±0.65	0.00-0.00 0.00±0.00
Muscle *P. fluviatilis*	155.78-258.02 207.9±25.06	1.01-1.83 1.42±0.21	0.00-0.00 0.00±0.00
Liver *P. fluviatilis*	41.75-71.87 56.81±7.53	0.26-1.04 0.65±0.19	0.38-0.60 0.49±0.05
E. excisus	83.92-156.44 120.18±18.13	0.15-1.01 0.68±0.21	0.16-0.24 0.19±0.02
Water	0.01-0.02 0.013±0.003	0.02 – 0.03 0.03±0.005	0.03 – 0.04 0.03±0.008
Sediments	2.65 – 39.97 25.68±20.14	7.08 – 140.1 77.97±66.94	0.23 – 2.75 1.85±1.41
Maximum permissible concentration (MPC)			
Reg. 31/2004	30	-	0.05
FAO/ WHO	7	0.3	0.5
MAFF			0.2

Cd is a major toxic metal until Ba is a minor toxic metal (Mostafa et al., 2022). Human exposure to aluminium can and will result in toxicity (Exley, 2016). Cd causes toxic effects at very low concentrations (Sorensen, 1991; Peycheva et al., 2014). The maximum permissible concentration (MPC) for Cd, according Regulation No. 31/2004), is 0.05 mg/kg fish; according to FAO, it is 0.5 mg/kg, and according to MAFF, it is 0.2 mg/kg. Sources of pollution are industrial wastewater (Yablanski, Petkov (eds.), 2011). In nature, sources of Ba are fossil fuels, igneous rocks, feldspar and mica deposits. The entry of soluble $BaCl_2$, $Ba(NO_3)_2$, or $Ba(OH)_2$ is important for aquatic ecosystems. Ba is quickly released and binds to naturally occurring sulfates or carbonates, forming less soluble compounds - $BaSO_4$ and $BaCO_3$, most found in soils and waters (Verbruggen et al., 2020). Therefore, the toxicity of Ba is closely related to the hardness of the water and the amount of sulfates/carbonates. As the pH decreases, the solubility of Ba increases. This explains the higher concentrations of Ba in sediments (precipitation of poorly soluble barium compounds). A dissolved Ba value of 0.022 $mg \cdot L^{-1}$ has been adopted for human health (Verbruggen et al., 2020).

The highest concentrations of Ba and Cd were found in sediments. The lowest contamination was in water samples for the three elements. The results of the studied tissues and organs of the perch and its parasites showed the highest value of Al in the muscles, followed by those in the skin. The lowest values of Al were found in the liver. The Al content in *E. excisus* was 2.63 times lower than in the muscles. Therefore, the Al content decreased in the order muscles - skin - *E. excisus* - liver - sediments - water. The Al content in the muscles was 7.54 times higher than in the liver and in the parasite - 2.85 times higher than in the liver of the European perch. The highest concentrations of Ba were found in the skin of *P. fluviatilis*. The Ba content decreased in the order sediments - skin - muscles - *E. excisus* - liver - water. The Ba content in the skin was 16.37 times higher than in the liver samples, and the parasite – 1.18 times higher than in the liver samples. No Cd was detected in the skin and muscle samples. The Cd content decreased in the order sediments - liver - *E. excisus* - water. The cadmium (Cd) concentration detected in the liver samples of *Perca fluviatilis* was 1.85 times higher than that found in *Eustrongylides excisus* (Table 1).

According to Regulation No. 31/2004, the MPC for Al in freshwater fish is 30 mg/kg wet weight. Exceedance of the norm was not found only in liver samples. The Al content in muscle samples is 3.58 times higher than the approved MPC, in the skin - 1.95 times, and in the parasite - 1.36 times. According to the same regulation, the MPC for Cd is 0.05 mg/kg wet weight. The exceedances are respectively 2.48 times in the liver and 1.32 times in the nematode. The Al content in water samples is within the norm and has been approved according to Regulation No. H-4/2012. No exceedances of the Cd content were found according to the approved norms by FAO and WHO for food (FAO/WHO (1998)) (0.2 and 20 mg/kg wet weight, respectively), according to Regulation No. 3/2008 on the standards for permitted content of Harmful Substances in soil, and Regulation on environmental quality standards for priority substances and certain other pollutants. Exceedances of these norms were found in liver and *E. excisus* samples.

The obtained results show excesses in the content of Al in skin (1.96 times), in muscles (3.58 times), and in *E. excisus* (1.36 times) according to the national legislation (Regulation No. 31/2004) for the content of Al in freshwater fish species (MPC 30 mg/kg); excesses in water samples (0.005 $mg.l^{-1}$ norm according to Regulation No. 18/2002) when using surface waters for irrigation. The content of Ba was exceeded in skin samples (119.09 times) and muscles (33.18 times). Cd concentrations were exceeded in liver samples (1.8 times) and *E. excisus* (1.4 times), according Regulation No. 31/2004. Cd concentrations were exceeded in water samples (3000 times, Regulation No. 18/2002). Cd concentrations were exceeded in sediment samples on average 2.31 times (Regulation No. 18/2002) (Table 1).

The bioconcentration factors (BCF) and bioaccumulation factors (BAF) are presented. The highest BCF and BAF were obtained for Al and the lowest for Cd (Table 2).

Very high correlations (r_s) were found between all the results obtained for Al, Ba and Cd content in water, sediments, skin, muscles and liver of *P. fluviatilis* and the dominant parasite species *E. excisus* (Table 2).

Significant differences were found between contaminations with Al and Ba in *E. excisus* and the samples of skin, muscles, liver of European perch, waters and sediments, as well as between contaminations with Cd in *E. excisus* and in the samples of 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 with Al, Ba, and Cd in the waters and sediments of the Complex Mandra-Poda and the specific habitats of *P. fluviatilis* will lead to changes in the concentrations of these elements in the skin, muscles, and liver of the fish and the dominant nematode *E. excisus* in them.

Table 2. Bioconcentration factor (BCF), Bioaccumulation factor (BAF), and Spearman correlation coefficient (r_s) between the content of Al, Ba, and Cd in water, sediments, *P. fluviatilis* and *E. excisus*

Fish - Parasite - Water (wet weight)	**Al BCF**	**Ba BCF**	**Cd BCF**
Skin - Water	4514.62****	109.17****	0.00
Muscle - Water	8261.54****	30.42****	0.00
Liver - Water	1098.46****	6.67****	4.14****
E. excisus - Water	3140.00****	7.92****	2.20****
Fish - Parasite - Sediments (dry weight)	**Al BCF**	**Ba BCF**	**Cd BCF**
Skin - Sediments	4.56****	0.07****	0.00
Muscle - Sediments	36.60****	0.02****	0.00
Liver - Sediments	2.21****	0.008****	0.26****
E. excisus - Sediments	4.68****	0.009****	0.10****
***E. excisus* - Fish** (wet weight)	**Al BAF**	**Ba BAF**	**Cd BAF**
E. excisus- Skin	0.69****	0.07****	0.00
E. excisus - Muscle	0.38****	0.26****	0.00
E. excisus - Liver	2.85****	1.19****	0.53****
***E. excisus* - Fish** (dry weight)	**Al BAF**	**Ba BAF**	**Cd BAF**
E. excisus - Skin	1.03****	0.13****	0.00
E. excisus - Muscle	0.58****	0.47****	0.00
E. excisus - Liver	2.12****	1.05****	0.38****

r_s **** - very significant correlation, p = 0.001.

In this study, the liver accumulated higher concentrations of Cd than the parasite. Similar results have been obtained by other authors regarding the content of the studied elements (Al, Ba and Cd) in the liver of freshwater fish species, followed by that in the intestine and muscle (Leite et al., 2019; Mostafa et al., 2022). This is explained by Cd's affinity for proteins rich in the liver (Dallinger et al., 1987). In contrast to these results for Ba in the liver, the skin accumulated the highest concentrations of Ba in this study. According to other studies (Mazhar et al., 2014), Al is one of the few metals that accumulate in the highest concentrations in the muscles, which was also revealed by the present study. The highest content of Al was found in the samples from *E. excisus*, followed by that of Ba and Cd. The L4 larvae (fourth-stage larvae) were studied, localized in the body cavity, where they feed on blood and tissues before being encapsulated, which is associated with higher levels of Al (Nachev et al., 2013; Honcharov, 2022). Al, Ba and Cd contents are much higher in sediment samples than in water samples. This has also been found in other studies and is explained by the fact that metals attach to suspended particles or are adsorbed to organic matter and thus precipitate and accumulate at the bottom of reservoirs (Mostafa et al., 2022). Only about 1% dissolves in water during the hydrological cycle (Salomons & Stigliani, 1995).

According to the results obtained in this study, it could be assumed that the main source of Al, Ba and Cd for *P. fluviatilis* is food and, to a lesser extent, water.

CONCLUSIONS

As a result of the study it was found that the highest concentrations of Al accumulate in the muscles, the highest concentrations of Ba in the skin, and the highest concentrations of Cd in the liver. The main sources of Al, Ba and Cd for *P. fluviatilis* are food and to a lesser extent water. *E. excisus* can be used as a sensitive bioindicator for Al pollution of freshwater ecosystems. Sediment samples contain much higher concentrations of Al, Ba and Cd than water samples. Very significant correlations were found between the content of the observed metals (Al, Ba and Cd) and their amounts in water, sediments, organs and tissues of *P. fluviatilis* and its dominant nematode species *E. excisus*.

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REFERENCES

Anonymous (1991). *Empfehlungen für die Nährstoffzufuhr*. Deutsche Gesellschaft für Ernährung, Germany, 72-75.

Atanasov, G. (2012). Fauna, morphology and biology on the endohelminths of fish from Bulgarian part of the Danube River. *PhD these, Sofia.*

Bauer, O. N. (1987). *Key to the Parasites of Freshwater Fishes of the USSR*. Leningrad, RU: Nauka (in Russian).

BSS EN 14757:2015 Water quality. Sampling of fish with multi-mesh gillnets.

Dallinger, R., Prosi, F., Segner, H., & Back, H. (1987). Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia*, *73*, 91-98.

Directive 79/409/EC of 2 April 1979 on the conservation of the wild birds. http://www.central.eu

Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora. http://ec.europa.eu

EN ISO 11885:2009 Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES)

EN ISO 16170:2016 In situ test methods for high efficiency filter systems in industrial facilities

FAO (Food and Agriculture Organization) (1983). FAO Fishery Circular No. 464,5-10. Food and Agriculture. The results obtained for toxic and trace elements in analyzed fish. Organization of the United Nations, Rome.

FAO/WHO (1998) *Preparation and use of food-based dietary guidelines.* Report of a joint FAO/WHO consultation. Geneva, World Health Organization (WHO Technical Report Series, No. 880).

Exley, C. (2016). The toxicity of aluminium in humans. *Morphologie*, *100*(329), 51-55.

Froese, R. and D. Pauly. Editors. 2024. FishBase.
World Wide Web electronic publication. www.fishbase.org, (10/2024)

Georgieva, G., Stancheva, M., Makedonski, L. (2015). Persistent Organochlorine Compouds (PcBs, DDTs, HCB &HBDE) in Wils Fishfrom the Lake Burgasand the Lake Mandra, Bulgaria. *Ecology & Safety*, 9, 515-523.

Honcharov, S. L., Soroka, N. M., Galat, M. V., Zhurenko, O. V., Dubovyi, A. I., & Dzhmil, V. I. (2022). Eustrongylides (Nematoda: Dioctophymatidae): epizootology and special characteristics of the development biology. *Helminthologia*, *59*(2), 127.

Hristov, S. (2010). Circulation of some heavy metals in the freshwater ecosystem of the Srebarna Biosphere Reserve. *Journal of Ecology&Safety*, *4*(2), 204-213.

https://lex.bg/laws/ldoc/2134445060

Ilieva, N., & Kirin, D. (2024). Helminth biodiversity and heavy metal contamination of *Perca fluviatilis* (Linnaeus, 1758) and *Eustrongylides excisus* (Jägerskiöld, 1909) larvae from the wetland Mandra-Poda. *Scientific Papers. Series D. Animal Science*, *67*(2)

ISO 22036:2024 Environmental solid matrices — Determination of elements using inductively coupled plasma optical emission spectrometry (ICP-OES).

IUCN. 2022. *The IUCN Red List of Threatened Species. Version 2022-2*. https://www.iucnredlist.org

Kakacheva-Avramova, D. (1983). *Helminths of freshwater fishes in Bulgaria*. Sofia, Bulgarian Academy of Sciences.

Karapetkova, M., Zhivkov, M. (2006). *Fishes in Bulgaria*. Publ. house Gea-Libris, Sofia (in Bulgarian).

Kirin, D., Hanzelová, V., Shukerova S., Hristov S., Turčeková, L., Spakulova, M., Barciová T.(2013a). Biodiversity and ecological appraisal of the freshwater ecosystem of the Arda River, Bulgaria. *Scientific Papers. Series D. Animal Science. LVI,* 341-348.

Kirin, D., Hanzelová, V., Shukerova S., Hristov S., Turčeková, L., Spakulova, M. (2013b). Helminth communities of Fishes from the River Danube and Lake Srebarna, Bulgaria. *Scientific Papers. Series D. Animal Science, LVI*, 333-340.

Kottelat, M. &Freyhof, J. (2007). Handbook of European freshwater fishes. *Publications Kottelat, Cornol and Freyhof, Berlin*. 646.

Law for the Protected Areas. State Gazette, issue 133 of November 11, 1998.

Leite, L.A., Januário, F.F., Padilha, P.M., do Livramento, E.T., de Azevedo, R.K., Abdallah, V.D. (2019) Heavy Metal Accumulation in the Intestinal Tapeworm *Proteocephalus macrophallus* Infecting the Butterfly Peacock Bass (*Cichlao cellaris*), from South-eastern Brazil. *Bulletin of Environmental Contamination and Toxicology* 103, 670– 675.

MAFF (1995). Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1993. *Aquatic Environment Monitoring. Report No. 44. Direcorate of Fisheries Research, Lowestoft*

Margaritov, N. (1959). *Parasites of some fresh water fishes.* Varna, BG: Publishing House NIRRP (in Bulgarian).

Mazhar, R., Shazili, N. A., & Harrison, F. S. (2014). Comparative study of the metal accumulation in *Hysterothalycium reliquens* (nematode) and *Paraphilometroides nemipteri* (nematode) as compared with their doubly infected host, *Nemipterus peronii* (Notched threadfin bream). *Parasitology Research*, *113*, 3737-3743.

Moravec, F. (2013). Parasitic nematodes of freshwater fishes of Europe. *Academia, Prague.* 601 pp.

Mostafa, O., Hanfi, T., Abd-eltwab, D., Al-Shehri M., Moustafa M., Al-Emam A., Alhamdi, H., Nigm, A. (2022). Fish Parasites as Biological Indicators of Metals Pollution in the Aquatic Environments. *Research Square*, 1-17.

Nachev, M., Schertzinger, G., Sures, B. (2013). Comparison of the metal accumulation capacity between the acanthocephalan *Pomphorhynchus laevis* and larval nematodes of the genus *Eustrongylides* sp. infected barbel (*Barbus barbus*). *Parasites & Vectors*, *6*(21), 1-8.

Peycheva, K., Panayotova, V., Makedonski, M., Stancheva, R. (2014). Toxic and essential concentration of freshwater fishes from *Pyasachnik* Dam, Bulgaria. *Agricultural Science and Technology*, 6(3), 364-369.

Peycheva, K., Panayotova, V., Stancheva, R., Makedonski, L., Merdzhanova, A., Parrino, V., Nava, V., Cicero, N., Fazio, F. (2022). Risk Assessment of Essential and Toxic Elements in Freshwater Fish Species from Lakes near Black Sea, Bulgaria. *Toxics*, *10*(11), 675.

Ramsar Convention of Wetlands (1971). www.ramsar.org

Regulation No. 18 of 27.05.2009 on the quality of water for irrigation of agricultural crops Issued by the Minister of Environment and Water and the Minister of Agriculture and Food. *State Gazette*, 43 of 09.06.2009.

Regulation no. 3 of August 1, 2008 on the standards for permitted content of Harmful Substances in soils. *State Gazette*, 71 of 12.08.2008.

Regulation No. 31 of 29 July 2004 on the Maximum Permitted Quantities of contaminants in foodstuffs. *State Gazette*, 88 of 08.10.2004.

Regulation No. H-4 of September 14, 2012 on characterization of Surface Waters. *State Gazette*, 22 of 05.03.2013.

Regulation on environmental quality standards for priority substances and certain other pollutants. *State Gazette*, 88 of 09.11.2010.

Salomons W, Stigliani W (1995) Biogeodynamics of Pollutants in Soils and Sediments. *Springer Verlag, Heidelberg*, pp. 352

Shukerova, S. (2010). Helminths and helminth communities of fishes from Biosphere Reserve Srebarna. *PhD thesis*

Shukerova, S., Kirin, D., Hanzelova V. (2010). Endohelminth communities of the perch, *Perca fluviatilis* (Perciformes, Percidae) from Srebarna Biosphere Reserve, Bulgaria. *Helminthologia, 47*(2), 99-104.

Sokal, R. R. (85). RohlfFJ (1981) Biometry. The principles and practice of statistics in biological research. *WR Freeman and Company, New York*, 429-450.

Sorensen, E.M.B. (1991) Metal Poisoning in Fish. *CRC Press, Boca Raton.*

State Newspaper 102, 2005. Order No. РД-1152 of 23.11.2005. Protected area Uzungeren.

State Newspaper 18, 1990. Order No. 170 of 16.02.1990. Protected area "Ustie na reka Izvorska".

State Newspaper 37, 1989. Order No. 443 of 20.04.1989. Protected area Poda.

Statsoft Inc. (2011) (n.d.). STATISTICA, version 10. Retrieved from www.statsoft.com.

Sures, B., Siddall, R., & Taraschewski, H. (1999). Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today*, *15*(1), 16-21.

Verbruggen, E.M.J., Smit, C.E., Vlaardinger, P.L.A. (2020). Environmental quality standards for barium in surface water. *Published by: National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport, RIVM,* The Netherlands.

Yablanski, Z., Perkov, G. (Eds.), 2011. Manual Book of Applied Ecology. Alfamarket, Stara Zagora.

Zaharieva, P. (2022a). Content of heavy metals in Fishes and their Parasites from the Danube River - Ecology and Bioindication. *PhD thesis,* Plovdiv (in Bulgarian).

Zaharieva, R. (2022b). Parasites and Parasite Communities on Fishes from the Danube River - Ecology and Biodiversity. *PhD thesis,* Plovdiv (in Bulgarian).

Zashev, G., Margaritov, N. (1966). *Diseases of fish.* Sofia, BG: Naukaiizkustvo (in Bulgarian).