AGRICULTURAL UNIVERSITY – PLOVDIV
FACULTY OF PLANT PROTECTION AND AGROECOLOGY
Department of Agroecology and Environmental Protection

Petya Georgieva Zaharieva

HEAVY METALS CONTENT IN FISH AND THEIR PARASITES FROM THE DANUBE RIVER – ECOLOGY AND BIOINDICATION

ABSTRACT
of a dissertation for awarding educational and scientific degree "Doctor"

Scientific specialty:
"Ecology and ecosystems protection"

Supervisor:
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The dissertation is written in 250 pages and includes 42 tables and 106 figures. 349 literature sources were used, of which 28 in Cyrillic and 285 in Latin, 6 regulatory documents, 10 standards (ISO) and 20 online databases.

Research on the dissertation work was carried out in laboratory to the Department of Agroecology and Environmental Protection at the Agricultural University – Plovdiv.

The dissertation was discussed and proposed for defense at the Department Council of the Department of Agroecology and Environmental protection at the Agricultural University – Plovdiv with Protocol No. 2/12.10.2022.

The defense of the dissertation will consist of 21.12.2022 at 11:00 am in 8 auditorium of the Faculty of Plant Protection and Agroecology at the Agricultural University – Plovdiv at a meeting of the Specialized scientific jury, approved by a decision of the Faculty Council of the Faculty of Plant Protection and Agroecology with Protocol No. 26/25.10.2022 and appointed by the Rector of the Agricultural University with Order No. RD-16-1117/31.10.2022, composed of:

Internal members:

1. Assoc. prof. Penka Stancheva Zapryanova-Aleksieva, PhD – Chairman; Reviewer
2. Prof. Vladislav Haralampiev Popov, PhD

External members:

3. Prof. Vasil Kostadinov Atanasov, DSc – Reviewer
4. Prof. Diyan Mihailov Georgiev, PhD
5. Assoc. prof. Gana Minkova Gecheva, PhD

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The materials on the defense are available to those interested in the library of the Agricultural University – Plovdiv, 12 Mendeleev Blvd.

I. INTRODUCTION

The Danube River, with its length of 2,857 km, ranks among the three longest rivers in Europe (together with the Volga River and the Ural River) (Ilie et al., 2016). The Danube River crosses the continent in a west-east direction, passes through four capitals and connects a number of countries until it flows into the Black Sea (Baltălungă, Dumitrescu, 2008; Danalache et al., 2020). Humans with their activities (agricultural, industrial and mining, construction of bridges, dams, power plants, overfishing, etc.) have a strong influence on the quality of water, habitats, species diversity of organisms and others (Schiemer et al., 2004; Juhásová et al., 2019; Pavlović et al., 2019; Frincu et al., 2020; Lenhardt et al., 2020).

Living and feeding in an aquatic environment, **fish** are not protected and are exposed to the harmful effects of various pollutants. They can accept pollutants (including heavy metals) directly from their surrounding or indirectly from other aquatic organisms (smaller fish, invertebrates, aquatic vegetation) (Authman et al. 2015). Fish are at the top of the food chain in aquatic environment (Javed, Usmani, 2017; Jovanović et al., 2017; Jovičić et al., 2018), have long live, accumulate large amounts of pollutants, are easy to sample, and all this makes them suitable bioindicators (Brázová et al., 2012; Petkovšek et al., 2012). Fish and fish products are a recommended and essential part of the human diet (Andreji et al., 2012; Afshan et al., 2014).

Heavy metals are accumulated in all vital organs and tissues of the fish. The highest concentrations of heavy metals are found in the liver, kidneys and gills of the fish, and in some also in the intestines (Javed, Usmani, 2017). Of all fish tissues and organs, the most frequently examined object is the fish muscle, due to its consumption by humans. In the second place, the fish liver is subjected to research. It has a higher potential for heavy metal accumulation than muscles (Jovičić et al., 2018). Heavy metals can damage and disturb the normal functioning of the brain, lungs, kidneys, liver and other important organs (Jaishankar et al., 2014). Toxic heavy metals have a negative impact on the fish, as they can affect their growth, reproduction, mortality (Simionov et al., 2016). The concentrations of toxic elements in different fish tissues and organs can be influenced by factors such as: habitat, physical and chemical properties of water, age, sex, physiological state of fish and others (Andreji et al., 2012). The way fish feed also influences the accumulation of heavy metals in their bodies (Petkovšek et al., 2012; Jovanović et al., 2017).

Research shows that, in addition to fish tissues and organs, high concentrations of heavy metals are also accumulated by fish parasites (Sures et al., 1997a). The uptake and accumulation of heavy metals and metalloids in fish parasites depends on different factors such as: the type of parasites, the stage of development of the parasites (larvae or adults), the localization of the parasites in the host, etc. (Nachev et al., 2013). In some parasites, heavy metals and metalloids accumulate to concentrations that exceed those found in their various fish species hosts is observed (Sures et al., 1997a; Marcogliese, 2005; Sures et al., 2017). In this way, parasites can reduce the levels of heavy metals in their hosts (Nachev et al., 2010).

Few authors investigate the concentrations of heavy metals and metalloids in tissues and organs of fish and their parasites from the Bulgarian section of the Danube River. Most of these studies are focused on the lower section of the river on Bulgarian territory. There is a lack of research on the concentrations of heavy metals in fish, as well as on the circulation of heavy metals in the system fish – parasites – water – sediments in the section immediately after the Danube River enters Bulgaria (near the village of Kudelin). All this motivates the interest and necessary of carrying out the present study.

II. LITERATURE REVIEW

The literature review is based on 193 scientific publications that provide data on the concentrations of heavy metals and metalloids in water and sediments, freshwater fish and their parasites from the Danube River and the Danube River basin. The literature review covers the period of 1975 to 2022.

In the abstract are presented main summaries and conclusions.

1. The research of heavy metals in tissues and organs of freshwater fish species from the Bulgarian section of the Danube River refer mainly to the lower section of the river (the village of Vetren and the town of Silistra). There are also data on concentrations of heavy

metals in fish from the upper section of the Danube River in the area of the towns of Kozloduy and Vidin, but those studies are few.

2. The studies on the levels of heavy metals in water and sediments from the Bulgarian section of the Danube River are again focused on the lower section of the river in the area of the village of Vetren and little data on the area of the town of Silistra. There are also single research on heavy metals in water and sediments from the upper current of the Danube River in the area of the village of Novo selo.
3. There are few studies on *Abr. brama* and *Alb. alburnus* from the Bulgarian section of the Danube River, and those studies are for the lower section of the river in Bulgaria (in the area of the village of Vetren). There are no data on concentrations of heavy metals/ metalloids in tissues and organs of *Ch. nasus* from the Bulgarian section of the Danube River.
4. The literature review shows that there are few research on the concentrations of heavy metals and metalloids in fish parasites from the Bulgarian section of the river. These studies are mainly about parasites in fish from the lower section of the Danube River (the village of Vetren and the town of Silistra), while for the upper sections of the river in Bulgaria there are single data (in the area of the towns of Kozloduy and Vidin, and the village of Archar).
5. The most frequently studied helminths for the accumulation of heavy metals from tissues and organs of fish, water and sediments are the acanthocephalans *P. laevis* (fot the Danube River in other countries and in Bulgaria) and *Ac. lucii* (for the Danube River in Bulgaria and the Danube River basin in other countries), as well as the nematode *E. excisus* (for the Danube River and its basin in Bulgaria).

III. GOAL AND TASKS

The **goal** of the present work is to carry out scientific research on the heavy metals content in fish and their parasites from the freshwater ecosystem of the Danube River.

To realize the set goal, research work was conducted on the following **tasks**:

1. Determination of the content of cooper (Cu), cadmium (Cd) and arsenic (As) in tissues, organs and helminths of *Alburnus alburnus* (Linnaeus, 1758), *Abramis brama* (Linnaeus, 1758) and *Chondrostoma nasus* (Linnaeus, 1758), water and sediments from the Danube River, Kudelin biotope (Vidin District) for the period 2019-2021. Comparative review of the content of Cu, Cd and As in tissues, organs and helminths of *Alburnus alburnus*, *Abramis brama*, *Chondrostoma nasus*, in water and sediments.
2. Determination of the seasonal changes in the content of Cu, Cd and As in tissues and organs of *Alburnus alburnus*, *Abramis brama*, *Chondrostoma nasus*, in water and sediments.
3. Determination of the annual changes in the content of Cu, Cd and As in tissues and organs of *Alburnus alburnus*, *Abramis brama*, *Chondrostoma nasus*, in water and sediments.
4. Tracking the circulation of Cu, Cd and As in the systems water – sediments – fish – dominant helminth species.
   4.1. Tracking the circulation of Cu, Cd and As in the system water – sediments – *Abramis brama* – *Pomphorhynchus laevis*.
   4.2. Tracking the circulation of Cu, Cd and As in the system water – sediments – *Alburnus alburnus* – *Pomphorhynchus laevis*.
   4.3. Tracking the circulation of Cu, Cd and As in the system water – sediments – *Chondrostoma nasus* – *Pomphorhynchus laevis* – *Contracaecum* sp.
5. Seasonal and annual changes in the circulation of Cu, Cd and As in the systems water – sediments – fish.

IV. MATERIALS AND METHODS

IV.1. BRIEF NATURAL AND GEOGRAPHICAL CHARACTERISTICS OF THE DANUBE RIVER, THE DANUBE RIVER BASIN AND THE STUDIED BIOTOPE

IV.1.1. Brief natural and geographical characteristics of the Danube River and the Danube River basin

The Danube River passes through the territory of 10 countries – Germany, Austria, Slovakia, Hungary, Croatia, Serbia, Romania, Bulgaria, Moldova and Ukraine (https://danube-region.eu/wp-content/uploads/2019/08/eusdr_success_stories_bg.pdf), while the Danube River basin extends over the territory of even more countries – 19, including Switzerland, Italy, Slovenia, Czech Republic, Poland, Bosnia and Herzegovina, Albania, North Macedonia, Montenegro (http://www.icpdr.org/). The Danube River, with its length of 2,857 km, ranks second in length in Europe after the Volga River (Yiğiterhan, Murray, 2008). More than 300 rivers flow into the Danube River along its entire course (Gasparotti, 2014). Larger tributaries of the Danube River are: Lech River (at 2,497 river km), Inn River (at 2,225 river km), Morava River (at 1,880 river km), Drava River (at 1,384 river km), Tisza/Tisa River (at 1,215 river km), Sava River (at 1,171 river km), Velika Morava River (at 1,103 river km), Timok River (at 846 uptakeriver km), Jiu River (at 692 river km), Iskar River (at 637 river km), Olt River (at 604 river km) and others (Hock, Kovács, 1987).

IV.1.2. Brief natural and geographical characteristics of studied biotope from the Danube River

The village of Kudelin is located in the Northwestern Bulgaria, along the Danube River and is a part of the Bregovsko-Novoselska Lowland. The village falls within the boundaries of the municipality of Bregovo, which is located in the Vidin District (https://www.strategy.bg/StrategicDocuments/View.aspx?lang=bg-BG&Id=316). The village of Kudelin (44°11′30″ N, 22°40′5″ E; 844 river km) is located about 2 km from the border with the Republic of Serbia, approximately after the confluence of the Timok River with the Danube River. The land of Kudelin is located on the right bank of the Danube River (https://bregovo.net).

IV.1.3. Brief characteristic of the studied heavy metals and metalloid (Cu, Cd and As)

Heavy metals represent a group of chemical elements in the periodic table that have a relative atomic mass greater than 40 and a density over 5 $g.cm^{-3}$. Heavy metals are divided into 3 main groups according to their degree of danger to human health. Cadmium (Cd) and arsenic (As) belong to the first group, and copper (Cu) to the second group. The first group includes the most dangerous among the heavy metals (Georgiev et al., 2011). **Cadmium (Cd).** Cadmium can be found naturally in the environment (air, water, soils, sediments) (Morais et al., 2012). In addition to natural sources (such as volcanic eruptions, erosion, etc.), cadmium also enters the environment through different anthropogenic activities such as mining, burning of household waste, production of fertilizers, and others (Jaishankar et al., 2014). This element can be found in higher concentrations near ore deposits (Blazheva, Bogoeva, 2013). The main source of cadmium in the environment is the burning of fossil fuels (coals, oil), as well as the burning of household waste (Afshan et al., 2014; Levit, 2010). **Copper (Cu).** In rivers, lakes, seas and other bodies of water, copper is present in negligible amounts – approximately 5 $\mu g.l^{-1}$, while in water near mining sites, the copper can be found in more large concentrations (Yablanski, Petkov, 2011). Copper enters the environment from various sources (for example, mining and smelting of copper; enterprises producing copper products such as wire, pipes and sheet iron; burning of fossil fuels and others) (Mahurpawar, 2015). **Arsenic (As).** Arsenic is a metalloid that occurs in various forms (organic and inorganic). Its inorganic forms are more toxic (Bogoeva, Blazheva, 2014). Arsenic enters the environment from both natural and anthropogenic sources. Natural sources include the volcanic eruptions, the soil erosion (Tchounwou et al., 2012), while anthropogenic ones include the mining and processing of ores (Jaishankar et al., 2014), the power plants (Dineva, 2016).

IV.2. FIELD RESEARCH

IV.2.1. Sampling of water, sediments and fish from the Danube River, Kudelin biotope

During the spring (22.03 – 22.06), summer (22.06 – 22.09) and autumn (22.09 – 22.11) seasons of the three research years water, sediments and fish samples were collected to determine the content of heavy metals and metalloid from the Danube River in the vicinity of the village of Kudelin (called the Kudelin biotope), Vidin District (**Fig. 1**).

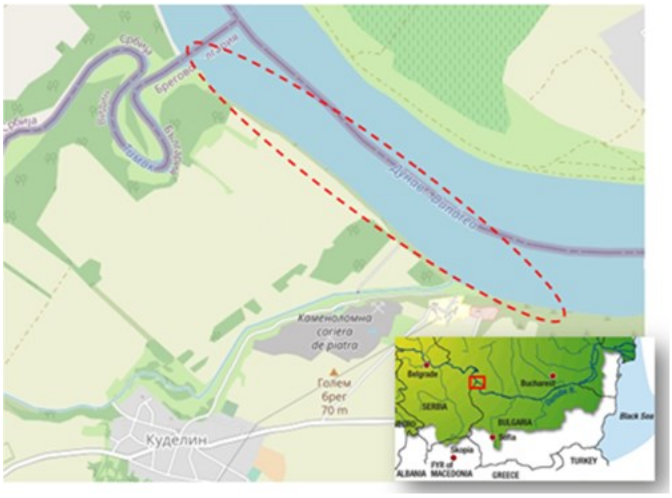


Fig. 1. Location of Kudelin biotope from the Danube River, from which the fish were caught (Caynax Sports Tracker GPS; http://www.icpdr.org/; with changes and additions)

Water samples from the Danube River were collected using a surface water sampling device according to standard ISO 5667-6:2016. Water quality - Sampling - Part 6: Guidance on sampling of rivers and streams; ISO 5667-6:2016/A11:2020. Water quality - Sampling - Part 6: Guidance on sampling of rivers and streams. Sediment samples were collected using an Ekman dipper (range 225 cm^2). Sampling was carried out according to the requirements of the standard ISO 5667-12:2017. Water quality - Sampling - Part 12: Guidance on sampling of bottom sediments from rivers, lakes and estuarine areas. During the studied period 2019-2021, a total of 27 water and sediment samples were taken (3 water and sediment samples per season) from three places along the Danube River from the mouth of the Timok River to the outskirts of the village of Kudelin (**Fig. 2**).

Fig. 2. Coordinates of water and sediment sampling locations along the Danube River (https://maps.google.com)

The fish were caught with different fishing devices in accordance with the requirements of Ministry of Agriculture and the Executive Agency for Fisheries and Aquaculture for research purposes (ISO 14757:2015 Water quality - Sampling of fish with multi-mesh gillnets). The species of caught fish specimens was determined according to “Fish in Bulgaria” by Karapetkova and Zhivkov (2006) and Fröse, Pauly (2022). Dominant fish species for the three studied seasons in the three years were common nase, *Chondrostoma nasus* (Linnaeus, 1758); freshwater bream, *Abramis brama* (Linnaeus, 1758) and bleak, *Alburnus alburnus* (Linnaeus, 1758). The internal organs of each specimen of the studied fish species were fixed in 70% ethyl alcohol for subsequent examination for helminths in laboratory conditions. A total of 810 fish samples were prepared for examination for helminths.

IV.3. LABORATORY RESEARCH

Water samples (1 l each) immediately after their collection were fixed in laboratory conditions with nitric acid (5 ml). Sediment samples (1 kg each) were dried in laboratory conditions to dry weight, then prepared in polyethylene zip-lock bags. Fresh and dry weight samples of liver, skin and muscles were prepared in laboratory conditions (5 average samples from each season and each fish species). Tissues and organs samples (liver, skin and muscles) were dried (at 60 ^{0}C) to dry weight. The fish were also subjected to ecologoparasitological studies (Zashev, Margaritov, 1966). The helminths *P. laevis* and *Contracaecum* sp. were selected as model helminth species. Prepared water and sediment samples, organs and tissues of freshwater fish samples, helminth samples were analyzed for Cu, Cd and As content by using an atomic absorption spectrophotometer ICP "OPTIMA 7000" Perkin- Elmer in an accredited laboratory of the Institute of Biodiversity and Ecosystem Research (IBER) at Bulgarian Academy of Sciences (BAS), Sofia.

IV.4. STATISTICAL PROCESSING OF THE DATA

Statistical data processing was performed using software products MS Excel (Microsoft, 2010), BioDiversity Pro (McAleece et al.1997) and Statistica 10 (StatSoft Inc., 2011).

V. RESULTS

V.1. CONTENT OF CU, CD AND AS IN TISSUES AND ORGANS OF *ALBURNUS ALBURNUS*, *ABRAMIS BRAMA* AND *CHONDROSTOMA NASUS* FROM THE DANUBE RIVER, KUDELIN BIOTOPE

In the present section are presented the results of the research on the content of Cu, Cd and As in liver, skin and muscles of dominant fish species from the family Cyprinidae (*Alb. alburnus*, *Abr. brama* and *Ch. nasus*) from the freshwater ecosystem of the Danube River (Kudelin biotope), which differ in their way of life and diet. The following are presented: 1) a brief characterization of the dominant three freshwater fish (by Kottelat, 1997; Karapetkova, Zhivkov, 2006; Bogoev, 2007; Kottelat, Freyhof, 2007; Yankov et al., 2015; Margaritova, 2019; Lepič et al., 2020); 2) Cu, Cd and As content in liver, skin and muscles in examined fish for the period 2019-2021 and 3) exceedances of Cu, Cd and As in tissues and organs of the three fish species in relation to norms in national and international documents.

The presented information is modeled after the example of the Cu, Cd and As content in tissues and organs of *Chondrostoma nasus*.

V.1.3. Content of Cu, Cd and As in tissues and organs of *Chondrostoma nasus*

On the territory of Bulgaria, ***Chondrostoma nasus* (Linnaeus, 1758) (Cyprinidae)** is found in the Danube River and its tributaries. The species reaches a length of up to 50 cm and a weight of up to 1 kg. The common nase lives up to 20 years. The body of the common nase is laterally flattened and elongated. A distinguishing mark of this species is the location and shape of the mouth. It is lower, in the form of a straight slit, located along the entire width of the lower side of the head. The common nase is a freshwater, demersal, gregarious fish. It prefers these sections of the rivers where the water flow is moderate. The species mainly uses algae for food. The common nase is an object of sport and amateur fishing.

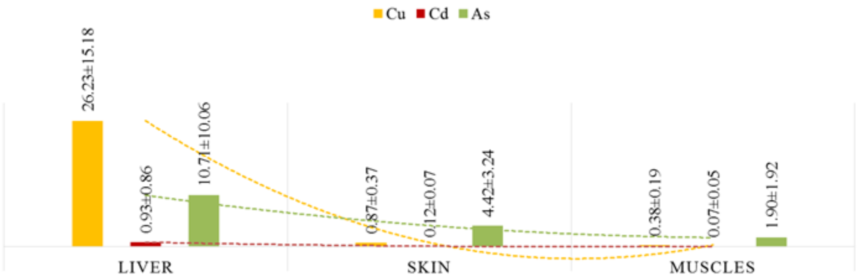
During the period 2019-2021, 270 specimens (90 specimens per year) of *Ch. nasus* from the Danube River, Kudelin were collected and examined for the content of heavy metals and metalloid (Cu, Cd and As). The concentrations of the three studied elements were higher in liver of common nase (wet weight, respectively, C_{Cu}=26.23±15.18 mg.kg^{-1}; C_{Cd}=0.93±0.86 mg.kg^{-1}; C_{As}=10.71±10.06 mg.kg^{-1}), compared to those in skin (wet weight, respectively, C_{Cu}=0.87±0.37 mg.kg^{-1}; C_{Cd}=0.12±0.07 mg.kg^{-1}; C_{As}=4.42±3.24 mg.kg^{-1}) and muscles (wet weight, respectively, C_{Cu}=0.38±0.19 mg.kg^{-1}; C_{Cd}=0.07±0.05 mg.kg^{-1}; C_{As}=1.90±1.92 mg.kg^{-1}). A reliable functional (deterministic) dependence regarding to each of the three investigated elements (Cu, Cd, As) and their content in the corresponding samples of liver, skin and muscles (Spearman correlation coefficient r_s=1.0, n=3-9) was established. The type of the examined samples of tissues and organs of common nase was essential for the content of the analyzed elements (Friedman test F=6.0; p=0.049<0.05). ***The content of copper, cadmium and arsenic in tissues and organs of common nase decreased in the order: liver > skin > muscles. The concentrations of the studied elements in the liver of common nase decreased in following order: Cu > As > Cd, while their concentrations in skin and muscles decreased in the order: As > Cu > Cd*** (**Table 12**).

Table 12. Content of Cu, Cd and As (mg.kg^{-1} wet weight; mg.kg^{-1} dry weight) in tissues and organs of *Chondrostoma nasus*

The Danube River (Kudelin)		Cu		Cd		As	
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Liver	mg.kg^{-1} wet weight	3.03-55.65	26.23±15.18	0.03-4.09	0.93±0.86	2.43-48.70	10.71±10.06
	mg.kg^{-1} dry weight	7.39-426.60	85.61±78.87	0.09-15.91	2.67±3.11	5.09-146.25	30.17±30.02
Skin	mg.kg^{-1} wet weight	0.28-1.95	0.87±0.37	0.03-0.28	0.12±0.07	0.95-14.90	4.42±3.24
	mg.kg^{-1} dry weight	0.64-5.64	2.34±1.07	0.08-2.26	0.50±0.45	2.39-41.62	12.08±9.41
Muscles	mg.kg^{-1} wet weight	0.09-0.82	0.38±0.19	0.01-0.23	0.07±0.05	0.37-9.30	1.90±1.92
	mg.kg^{-1} dry weight	0.42-3.31	1.55±0.73	0.04-0.69	0.24±0.16	1.34-41.19	7.42±8.10

Concentrations of Cu were much higher in liver samples than those in skin (30.15 times) and muscles (69.03 times) samples. Levels of Cd in liver were the highest and were 7.75 and 13.29 times higher than those in skin and muscles, respectively. Values of As were higher in liver, exceeded the arsenic concentrations in skin and muscles by 2.42 and 5.64 times, respectively (**Fig. 11**).

FIG. 11. CONTENT OF CU, CD AND AS (MG.KG-1 WET WEIGHT) IN TISSUES AND ORGANS OF *CHONDROSTOMA NASUS* FROM THE DANUBE RIVER, KUDELIN BIOTOPE (2019-2021)



The content of the examined elements in tissues and organs of common nase from the Danube River were juxtaposed with the MPC in Ordinance No. 31 from 2004 and with the norms given by the FAO and WHO. Cd's concentrations in liver, skin and muscles exceeded 18.6, 2.4 and 1.4 times the MPC in Ordinance No. 31, and those in liver exceeded 4.65 times the norm given by the FAO. The concentrations of As in liver, skin and muscles exceeded only the MPC in Ordinance No. 31 respectively 10.71, 4.42 and 1.9 times. The content of Cu in liver exceeded 2.62 times the MPC in Ordinance No. 31, as well as 1.31 times the norm given by the WHO. ***For the period 2019-2021, higher exceedances of Cd were found in liver of common nase, while higher exceedances of As were found in skin and muscles. Exceedances of Cu were also reported, but they were lower than those of the other two investigated elements in liver*** **(Fig. 12).**

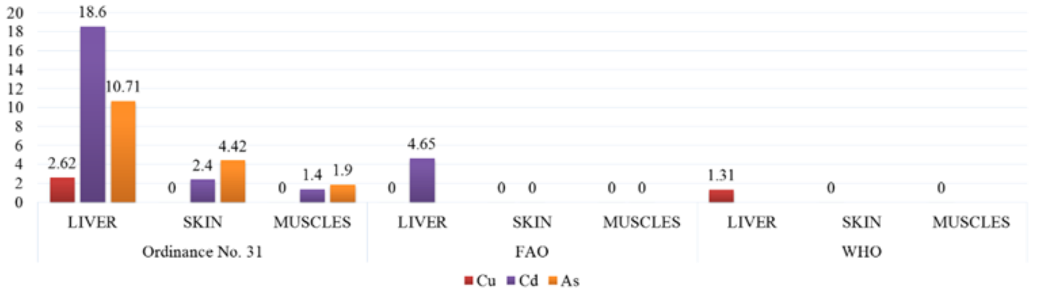


Fig. 12. Exceedances of Cu, Cd and As in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope (2019-2021)

Legend: Biological samples: 1) Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs – MPC (mg/kg wet weight) – **10 mg/kg** for Cu, **0.05 mg/kg** for Cd, **1 mg/kg** for As; 2) FAO – **30 mg/kg** for Cu, **0.2 mg/kg** for Cd; 3) WHO – **20 mg/kg** for Cu.

Discussion

Research on the content of heavy metals/ metalloids in tissues and organs of common nase from the Danube River and its basin are few (Table 13).

Table 13. Research on the content of heavy metals in tissues and organs of *Chondrostoma nasus* from the Danube River and its basin in other countries

***Chondrostoma nasus* from the Danube River in other countries**				
Author	**Location**	**Tissues / organs**	**Elements**	**Values for Cu/ Cd/ As**
Jirsa et al. (2008)	several rivers in Austria, including the Danube River	liver, intestines, muscles, gills	Cd, Pb, Cu, Zn	**for Cu in liver:** 25-333 μ/g dry weight **for Cd in liver:** 1.57 μ/g dry weight (uncontaminated places); 5.58 μ/g dry weight (contaminated places)
Zrnčić et al. (2013)	the Danube River in Croatia	muscles	Hg, Pb, Cd, As	**for Cd in muscles:** 0.005-0.018 μg^{-1} dry weight; 0.008±0.005 μg^{-1} dry weight **for As in muscles:** 0.011-0.045 μg^{-1} dry weight; 0.022±0.098 μg^{-1} dry weight
***Chondrostoma nasus* from the Danube River basin in other countries**				
Stranai, Andreji (2007)	the Nitra River, Slovakia	muscles, liver, kidneys	Co, Ni, Cr, Pb, Cd	**for Cd in liver:** 0.05-0.34 $mg.kg^{-1}$; 0.21±0.14 $mg.kg^{-1}$ **for Cd in muscles:** 0.06-0.10 $mg.kg^{-1}$; 0.08±0.01 $mg.kg^{-1}$
Andreji et al. (2012)	the Nitra River, Slovakia	muscles	Fe, Mn, Zn, Cu, Ni, Pb, Cd	**for Cu:** 15.39-25.97mg/kg wet weight; 21.95±0.84 mg/kg wet weight **for Cd:** 0.00-0.11mg/kg wet weight; 0.01±0.01 mg/kg wet weight
Đikanović et al. (2016a)	Međuvršje Dam (West Morava River basin), Serbia	liver, muscles, gills	As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sr, Zn	**for Cu in liver:** 269.36±128.37 $\mu g.g^{-1}$ dry weight **for Cu in muscles:** 2.29±1.16 $\mu g.g^{-1}$ dry weight **for Cd in liver:** 0.36±0.22 $\mu g.g^{-1}$ dry weight **for Cd in muscles:** 0.03±0.05 $\mu g.g^{-1}$ dry weight **for As in liver:** 4.26±0.95 $\mu g.g^{-1}$ dry weight **for As in muscles:** 3.50±0.66 $\mu g.g^{-1}$ dry weight

Stranai, Andreji (2007) examined muscles and liver of *Ch. nasus* from the Nitra River in Slovakia and reported cadmium concentrations in liver (C_{Cd}=0.21 $mg.kg^{-1}$) that were 4.43 times lower than those found in the present study (C_{Cd}=0.93 $mg.kg^{-1}$), as well as concentrations of Cd in muscles (C_{Cd}=0.08 $mg.kg^{-1}$) that were 1.14 times higher than those found for Kudelin biotope (C_{Cd}=0.07 $mg.kg^{-1}$). Andreji et al. (2012) studied muscles of common nase from the Nitra River in Slovakia for heavy metals and reported the following concentrations for copper and cadmium: C_{Cu}=21.95 mg/kg wet weight and C_{Cd}=0.01 mg/kg wet weight, as the indicated concentrations of Cu exceeded 57.76 times those found for Kudelin biotope (C_{Cu}=0.38 $mg.kg^{-1}$ wet weight), while the concentrations for Cd in muscles of common nase from the Nitra River were 7 times lower than those found in the present study (C_{Cd}=0.07 $mg.kg^{-1}$ wet weight).

There are no studies on the content of heavy metals in tissues and organs of common nase from the Bulgarian section of the Danube River, as well as from the Danube River basin in Bulgaria. The present study provides the first data on the content of Cu, Cd and As in liver, skin and muscles of common nase from the Danube River in Bulgaria.

The obtained results show the liver of Ch. nasus as a good bioindicator for Cd.

V.2. CONTENT OF CU, CD AND AS IN HELMINTHS OF *ALBURNUS ALBURNUS*, *ABRAMIS BRAMA* AND *CHONDROSTOMA NASUS* FROM THE DANUBE RIVER, KUDELIN BIOTOPE

As a result of the ecologoparasitological study, acanthocephalan *P. laevis* is selected as a model species common to *Alb. alburnus*, *Abr. brama* and *Ch. nasus* from the Danube River (Kudelin). *Contracaecum* sp. is selected separately as the only dominant species in the communities of *Ch. nasus*. This species is dominant in the three years and in all seasons at common nase, due to it is separately examine for the presence of heavy metals and metalloid. The dissertation presents a brief characterization of *P. laevis* and *Contracaecum* sp. in terms of taxonomy, distribution, developmental cycle, localization, etc. The presented information is designed based on the example of *Contracaecum* sp. and *P. laevis* of common nase.

The content of Cu, Cd and As ($mg.kg^{-1}$ wet weight) in ***P. laevis*** and ***Contracaecum* sp.** of ***Ch. nasus*** was determined. The highest content of As – C_{As}=370.07±229.35 (in *P. laevis*) and C_{As}=79.10±28.58 (in *Contracaecum* sp.); followed by that of Cu – C_{Cu}=9.25±5.73 (in *P. laevis*) and C_{Cu}=15.07±19 (in *Contracaecum* sp.); and Cd – C_{Cd}=2.31±1.43 (in *P. laevis*) and C_{Cd}=3.51±3.36 (in *Contracaecum* sp.) was found in both parasites. The concentrations of As in *P. laevis* of common nase were 40.01 and 160.2 times higher than those of Cu and Cd, respectively. The levels of As in *Contracaecum* sp. of common nase were 5.25 and 22.54 times higher than those of Cu and Cd, respectively. The concentrations of As in *P. laevis* were 4.68 times higher than those in *Contracaecum* sp., while the concentrations of Cu and Cd in *P. laevis* were respectively 1.63 and 1.52 times lower than those in *Contracaecum* sp. (**Table 16**).

Table 16. Content of Cu, Cd and As ($mg.kg^{-1}$ wet weight; $mg.kg^{-1}$ dry weight) in *Pomphorhynchus laevis* and in *Contracaecum* sp. of *Chondrostoma nasus*

The Danube River (Kudelin)	Cu		Cd		As	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Pomphorhynchus laevis of *Chondrostoma nasus*	3.27-14.69	9.25±5.73	0.82-3.67	2.31±1.43	130.61-587.76	370.07±229.35
***Contracaecum* sp.** of *Chondrostoma nasus*	0.25-51.56	15.07±19	0.43-9.29	3.51±3.36	27.96-111.54	79.10±28.58

The content of the studied elements in P. laevis and Contracaecum sp. of Ch. nasus decreases in the order: As > Cu > Cd. The concentrations of the three elements in skin and muscles of common nase decrease in the same order, while their concentrations in liver of common nase follow the order: Cu > As > Cd.

Significant differences between the concentrations of As in *P. laevis* and *Contracaecum* sp., parasites of *Ch. nasus* (t_{As}=29.45, $p=0.03<0.05$) and Cu (t_{Cu}=21.37, $p=0.04<0.05$) were found. The helminth species is significant for the As and Cu content in the samples.

Exceedance of Cu was reported only in *Contracaecum* sp. of common nase (1.51 times compared to Ordinance No. 31). Regarding the content of Cd, the highest exceedance was again reported in *Contracaecum* sp. of common nase (70.2 times compared to Ordinance No. 31 / 17.55 times compared to the norm pointed by the FAO), followed by *P. laevis* of common nase (46.2 times in comparison with Ordinance No. 31 / 11.55 times compared to the FAO). The exceedances of As were the highest in *P. laevis* of common nase (370.07 times compared to Ordinance No. 31).

There are no studies on the concentrations of Cu, Cd and As in parasites of common nase from the Danube River in Bulgaria.

The obtained results show Contracaecum sp. as a good bioindicator for content of Cd; and P. laevis as a good bioindicator for content of As.

V.3. CONTENT OF CU, CD AND AS IN WATER AND SEDIMENTS FROM THE DANUBE RIVER, KUDELIN BIOTOPE

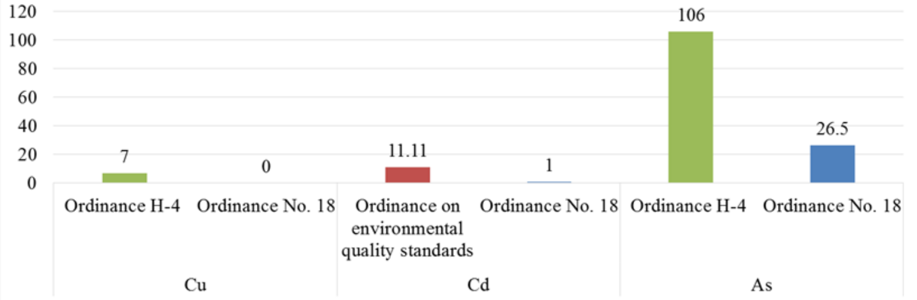
The content of Cu, Cd and As in water ($mg.l^{-1}$) and sediments ($mg.kg^{-1}$ dry weight) from the Danube River (Kudelin biotope) for the period 2019-2021 is examined. The exceedances of the three elements in water and sediments in relation to national and international legislative documents are pointed.

The average values of the studied elements in water from the Danube River (Kudelin) were: $C_{CuWater}$=0.07±0.07 $mg.l^{-1}$; $C_{CdWater}$=0.01±0.02 $mg.l^{-1}$ and $C_{AsWater}$=2.65±2.21 $mg.l^{-1}$. ***The***

concentrations of the three elements in water during the period 2019-2021 decreased as follows: As > Cu > Cd. The concentrations of copper, cadmium and arsenic in sediments from the Danube River were: $C_{CuSediments}$=275.65±184.80 mg.kg^{-1} dry weight; $C_{CdSediments}$=6.08±18.73 mg.kg^{-1} dry weight and $C_{AsSediments}$=423.41±270.48 mg.kg^{-1} mg.kg^{-1} dry weight, respectively. ***The content of the studied heavy metals and metalloid in sediments during 2019-2021 decreased again in the order: As > Cu > Cd***.

The concentrations of Cu in water from the Danube River, Kudelin biotope were compared with the average annual value (AAV) in Ordinance H-4 and with the MPC indicated in Ordinance No. 18 from 2009; the concentrations of Cd – with the values specified in the Ordinance on environmental quality standards and Ordinance No. 18; and the concentrations of As – with the norms specified in Ordinance H-4 and Ordinance No. 18. The highest exceedances were found for As in water compared to Ordinance H-4 and Ordinance No. 18, respectively 106 and 26.5 times. The content of Cd in water exceeded 11.11 times the MPC in the Ordinance on environmental quality standards. The content of Cu in water exceeded 7 times the AAV in Ordinance H-4 and did not exceed the MPC in Ordinance No. 18 (**Fig. 16**).

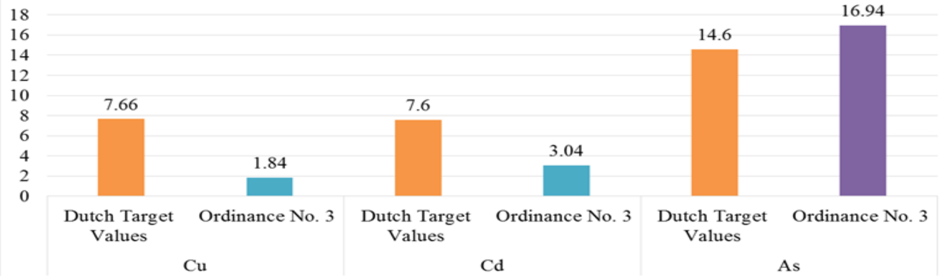
Fig. 16. Exceedance of Cu, Cd and As in water from from the Danube River, Kudelin biotope (2019-2021) in relation to legislative documents



Legend: Water: 1) Ordinance H-4 of 14 September 2012 on the characterization of surface water – AAV (**mg/l**), $CaCO_3$ 100-250 – **0.01 mg/l** for Cu, MPC (mg/l) – **0.025 mg/l** for As; 2) Ordinance on environmental quality standards for priority substances and certain other pollutants – MPC (**mg/l**), class 4 – **0.0009 mg/l** for Cd; 3) Ordinance No. 18 of 27 May 2009 on the quality of water for irrigation of crops – MPC (mg/dm^3) – **0.2 mg/dm^3** for Cu; **0.01 mg/dm^3** for Cd; **0.1 mg/dm^3** for As

The content of copper, cadmium and arsenic in sediments from the Danube River, Kudelin was compared with the Dutch target values and the MPC indicated in Ordinance No. 3. Exceedances were found for all three investigated elements. The exceedance in the content of As in sediments was the highest compared to Ordinance No. 3 – 16.94 times. Regarding the Dutch target values, again the highest was the exceedance of As – 14.6 times. The exceedances of Cu and Cd in relation to the Dutch target values were close, 7.66 and 7.6 times, respectively (**Fig. 17**).

Fig. 17. Exceedance of Cu, Cd and As in sediments from the Danube River, Kudelin biotope (2019-2021) in relation to legislative documents



Legend: Sediments: 1) Dutch Target Values (mg/kg dry weight) – **36 mg/kg** for Cu; **0.8 mg/kg** for Cd; **29 mg/kg** for As; 2) Ordinance No. 3 of 1 August 2008 on the norms for the permissible content of harmful substances in soils: MPC (mg/kg) for agricultural land (pH 6.00-7.4) – **150 mg/kg** for Cu; **2 mg/kg for** Cd; MPC (mg/kg) for agricultural land, independently of pH – **25 mg/kg** for As.

V.4. COMPARATIVE REVIEW OF THE CONTENT OF CU, CD AND AS IN TISSUES AND ORGANS OF FISH, HELMINTHS, WATER AND SEDIMENTS

The average concentrations of Cu, Cd and As for the period 2019-2021 are examined and compared by fish species, their parasites, water and sediments from the Danube River, Kudelin. The comparative review in the dissertation is presented by elements. Summaries of the obtained results are presented in the abstract.

The highest **concentrations of Cu** in liver were found of *Ch. nasus* (C_{Cu}=26.23±15.18 mg.kg^{-1} wet weight), and in skin and muscles of *Alb. alburnus* (C_{Cu}=4.02±2.20 mg.kg^{-1} wet weight and C_{Cu}=1.12±0.48 mg.kg^{-1} wet weight, respectively). The concentrations of Cu in liver of the three fish species decreased in the order: *Ch. nasus* > *Abr. brama* > *Alb. alburnus*, and in skin and muscles follow the order: *Alb. alburnus* > *Ch. nasus* > *Abr. brama*. The Cu content in tissues and organs of the three fish species (4.86 to 374.71 times), as well as in parasites (57.43 to 215.29 times) was higher than that in water. Concentrations of Cu in the tissues and organs of bleak, freshwater bream and common nase (3.22 to 194.12 times), as well as in *Contracaecum* sp. of *Ch. nasus* (4.2. times) were lower than those in sediments (**Figs. 26, 27**).

Fig. 26. Comparative review of the content of Cu in liver, skin and muscles of *Alburnus alburnus*, *Abramis brama* and *Chondrostoma nasus* (mg.kg^{-1} wet weight), in their parasites (mg.kg^{-1} wet weight) and water (mg.l^{-1})

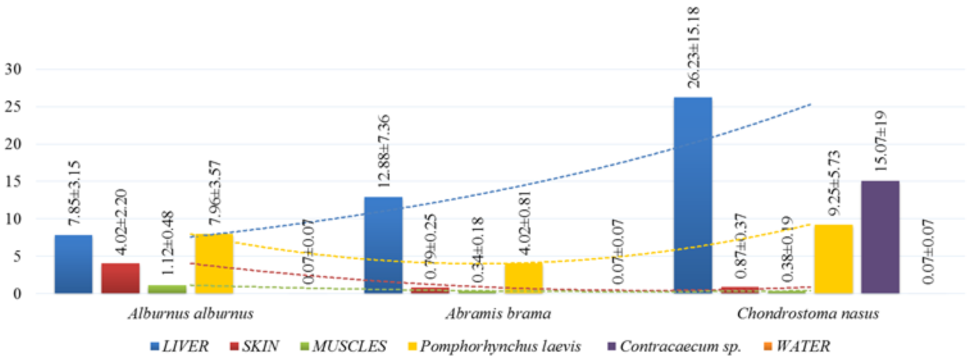
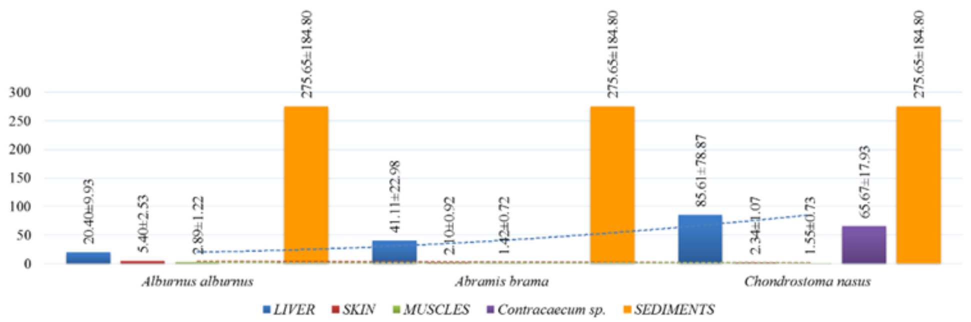


Fig. 27. Comparative review of the content of Cu (mg.kg^{-1} dry weight) in liver, skin and muscles of *Alburnus alburnus*, *Abramis brama* and *Chondrostoma nasus*, in *Contracaecum* sp. and in sediments



The highest **concentrations of Cd** in liver, skin and muscles were established in *Alb. alburnus* (C_{Cd}=1.41±1.24 mg.kg^{-1} wet weight, C_{Cd}=0.61±0.46 mg.kg^{-1} wet weight and C_{Cd}=0.12±0.06 mg.kg^{-1} wet weight, respectively). The concentrations of Cd in liver, skin and muscles decreased in the order: *Alb. alburnus* > *Ch. nasus* > *Abr. brama*. The content of Cd in tissues and organs of bleak, freshwater bream and common nase (5 to 141 times), as well as in parasites (97 to 351 times) was higher than that in water. The Cd content in tissues and organs of bleak, freshwater bream and common nase (1.63 to 27.64 times) was lower, while the Cd content in *Contracaecum* sp. of *Ch. nasus* (1.74 times) was higher than that of sediments (**Figs. 28, 29**).

Fig. 28. Comparative review of the content of Cd in liver, skin and muscles of *Alburnus alburnus*, *Abramis brama* and *Chondrostoma nasus* (mg.kg^{-1} wet weight), in their parasites (mg.kg^{-1} wet weight) and water (mg.l^{-1})

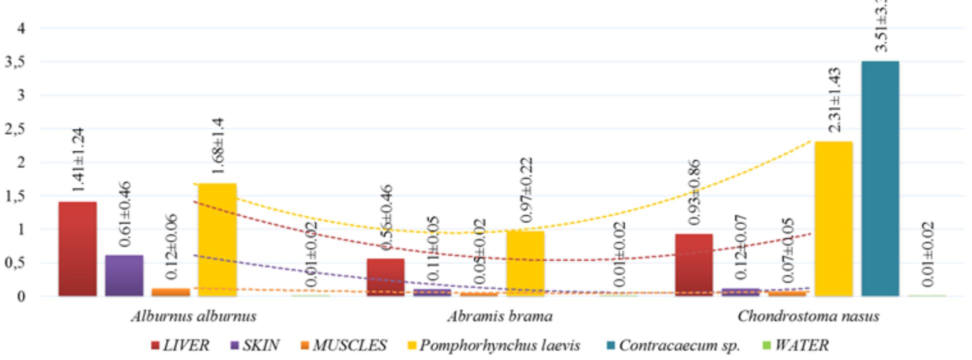
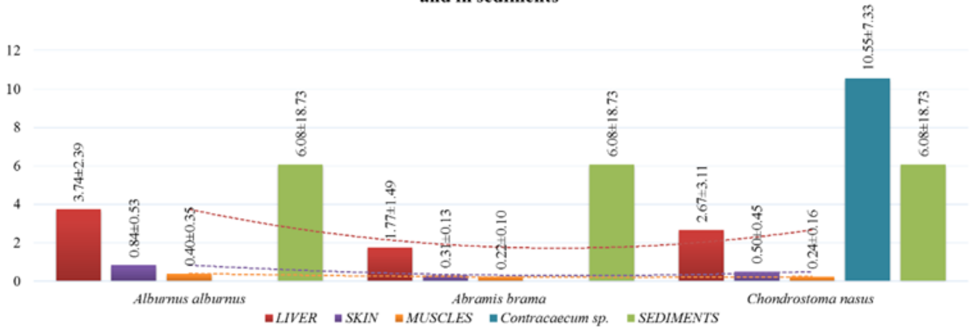
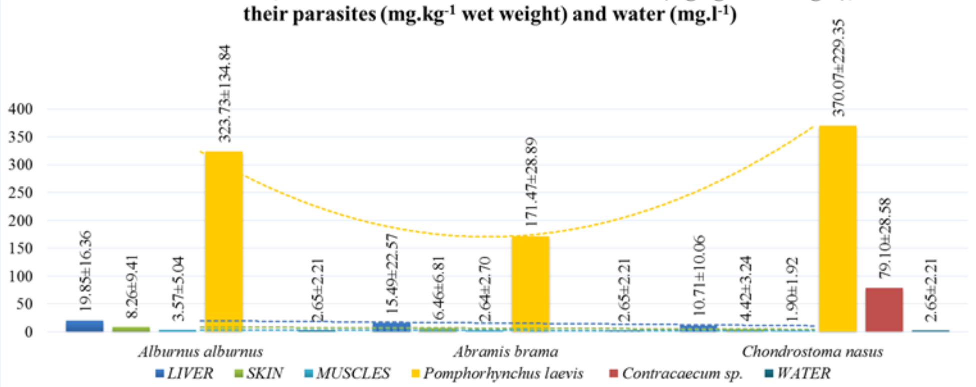


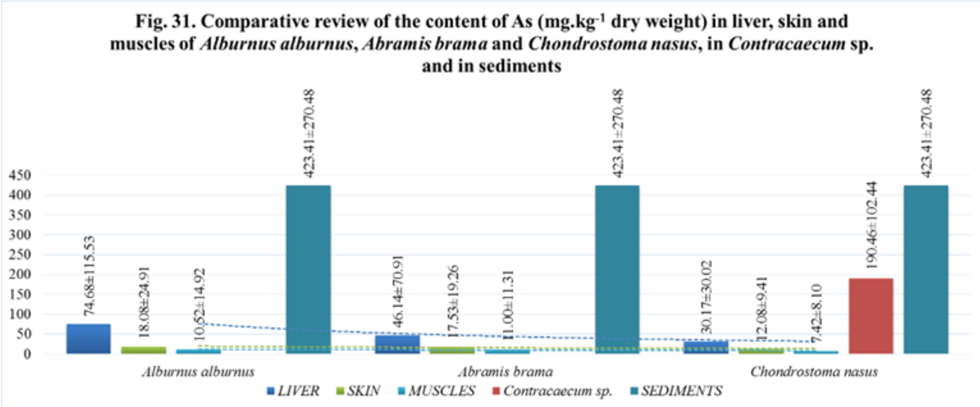
Fig. 29. Comparative review of the content of Cd (mg.kg^{-1} dry weight) in liver, skin and muscles of *Alburnus alburnus*, *Abramis brama* and *Chondrostoma nasus*, in *Contracaecum* sp. and in sediments



The highest **concentrations of As** in the examined tissues and organs were found again in *Alb. alburnus* (C_{As}=19.85±16.36 mg.kg^{-1} wet weight, C_{As}=8.26±9.41 mg.kg^{-1} wet weight and C_{As}=3.57±5.04 mg.kg^{-1} wet weight). The As concentrations in liver, skin and muscles decreased in the order: *Alb. alburnus* > *Abr. brama* > *Ch. nasus*. The levels of As in liver, skin and muscles of *Alb. alburnus* (7.49, 3.12 and 1.35 times, respectively); in liver and skin of *Abr. brama* (5.85 and 2.44 times, respectively); in liver and skin of *Ch. nasus* (4.04 and 1.67 times, respectively); in parasites (29.85 to 139.65 times) were higher than those in water, while concentrations of As in muscles of freshwater bream (1.004 times) and in muscles of common nase (1.39 times) were lower. The content of As in tissues and organs of bleak, freshwater bream and common nase (5.67 to 57.06 times), as well as in *Contracaecum* sp. of *Ch. nasus* (2.22 times) was lower than that in sediments (**Fig. 30, 31**).

Fig. 30. Comparative review of the content of As in liver, skin and muscles of *Alburnus alburnus*, *Abramis brama* and *Chondrostoma nasus* (mg.kg^{-1} wet weight), in their parasites (mg.kg^{-1} wet weight) and water (mg.l^{-1})

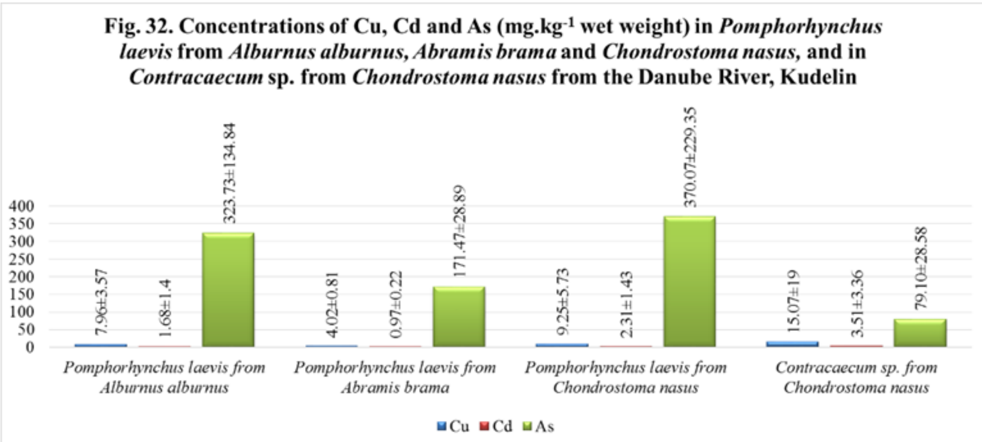




The content of Cu is the highest in liver of Ch. nasus, and the content of Cd and As in liver of Alb. alburnus.

V.4.4. Comparative review of the content of Cu, Cd and As in *Pomphorhynchus laevis* and *Contracaecum* sp.

The content of the studied elements in ***P. laevis*** **and** ***Contracaecum*** **sp.** decreased in the order: As > Cu > Cd. The highest concentration of As (370.07±229.35 mg.kg^{-1} wet weight) was recorded in *P. laevis* of *Ch. nasus*, and the highest concentrations of Cu and Cd in *Contracaecum* sp. of *Ch. nasus* (15.07±19 and 3.51±3.36 mg.kg^{-1} wet weight, respectively). The content of Cu and Cd in parasites decreased in the order: *Contracaecum* sp. of *Ch. nasus* > *P. laevis* of *Ch. nasus* > *P. laevis* of *Alb. alburnus* > *P. laevis* of *Abr. brama*. While the content of As followed the order: *P. laevis* of *Ch. nasus* > *P. laevis* of *Alb. alburnus* > *P. laevis* of *Abr. brama* > *Contracaecum* sp. of *Ch. nasus* (**Fig. 32**).



The content of Cu and Cd is the highest in Contracaecum sp., and the content of As in P. laevis.

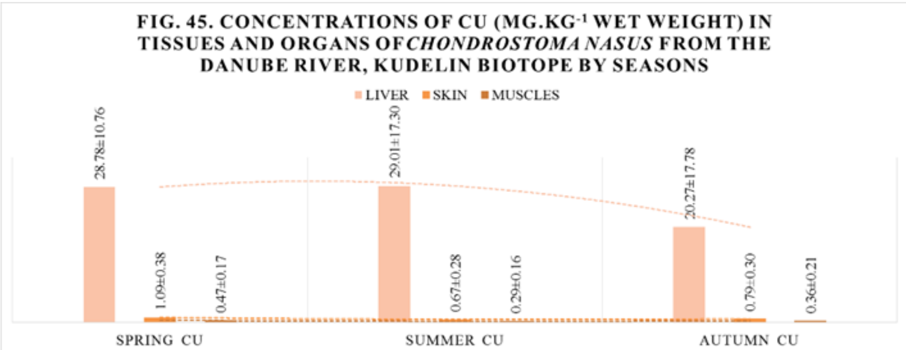
V.5. SEASONAL CHANGES IN THE CU, CD AND AS CONTENT IN TISSUES AND ORGANS OF *ALBURNUS ALBURNUS*, *ABRAMIS BRAMA* AND *CHONDROSTOMA NASUS*, IN WATER AND SEDIMENTS FROM THE DANUBE RIVER, KUDELIN BIOTOPE

The seasonal changes in the content of Cu, Cd and As in tissues and organs of *Alb. alburnus*, *Abr brama* and *Ch. nasus*, in water and sediments are examined. The minimum and maximum concentrations, as well as the average values for the concentrations of copper, cadmium and arsenic (mg.kg^{-1} wet weight; mg.kg^{-1} dry weight) in *Alb. alburnus*, *Abr brama* and *Ch. nasus*; in water (mg.l^{-1}) and sediments (mg.kg^{-1} dry weight) by seasons (spring, summer, autumn) are presented and discussed. The exceedances of the three examined elements in liver, skin and muscles, in water and sediments compared to documents from the national and international legislation by seasons are indicated.

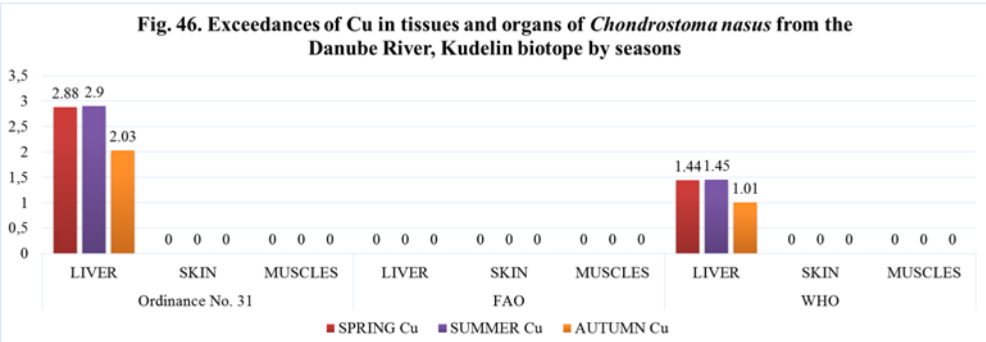
The presented information reflects the seasonal changes in content of Cu, Cd and As in tissues and organs of *Chondrostoma nasus*.

Concentrations of **Cu** in **liver** of common nase were the highest in the summer (C_{Cu}=29.01±17.30 mg.kg^{-1} wet weight; C_{Cu}=75.92±47.95 mg.kg^{-1} dry weight), followed by those in the spring (C_{Cu}=28.78±10.76 mg.kg^{-1} wet weight; C_{Cu}=78.27±26.97 mg.kg^{-1} dry weight). The content of Cu in **skin** and **muscles** was the highest in the spring (C_{Cu}=1.09±0.38 mg.kg^{-1} wet weight, C_{Cu}=2.87±1.24 mg.kg^{-1} dry weight and C_{Cu}=0.47±0.17 mg.kg^{-1} wet weight, C_{Cu}=2.00±0.68 mg.kg^{-1} dry weight, respectively) and lowest in the summer (C_{Cu}=0.67±0.28 mg.kg^{-1} wet weight, C_{Cu}=1.76±0.65 mg.kg^{-1} dry weight and C_{Cu}=0.29±0.16 mg.kg^{-1} wet weight, C_{Cu}=1.22±0.62 mg.kg^{-1} dry weight, respectively).

Cu's concentrations in liver samples of common nase in the summer exceeded 1.01 and 1.43 times those found in the spring and autumn, respectively. The levels of Cu in skin samples in the spring exceeded the levels in the autumn (1.38 times) and the summer (1.63 times). The values of Cu in muscles samples again in the spring exceeded those found in the autumn and summer – 1.31 and 1.62 times, respectively (**Fig. 45**).



The concentrations of Cu in tissues and organs of common nase were compared with the norms in Ordinance No. 31 and those of the FAO and the WHO. Exceedances of Cu were established in all three research seasons, but only in the liver samples. The highest were the exceedances in the summer season, followed by those in the spring season – respectively 2.9 and 2.88 times above the MPC in Ordinance No. 31, and respectively 1.45 and 1.44 times above the value given by the WHO. Exceedances of Cu were not found compared to the norm given by the FAO (**Fig. 46**).



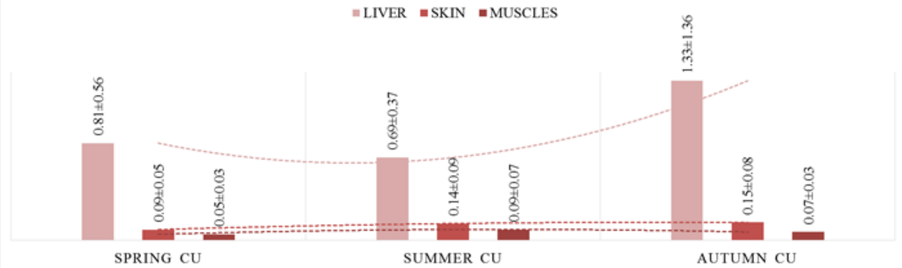
Legend: Biological samples: 1) Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs – MPC (mg/kg wet weight) – **10 mg/kg** for Cu; 2) FAO – **30 mg/kg** for Cu; 3) WHO – **20 mg/kg** for Cu.

The concentrations of **Cd** in **liver** samples of common nase were the highest in autumn season (C_{Cd}=1.33±1.36 mg.kg^{-1} wet weight; C_{Cd}=4.37±5.15 mg.kg^{-1} dry weight) and the lowest in summer season (C_{Cd}=0.69±0.37 mg.kg^{-1} wet weight; C_{Cd}=1.60±0.66 mg.kg^{-1} dry weight). The content of Cd in the **skin** samples was the highest in the autumn (C_{Cd}=0.15±0.08 mg.kg^{-1} wet weight; C_{Cd}=0.71±0.64 mg.kg^{-1} dry weight) and the lowest in the spring (C_{Cd}=0.09±0.05 mg.kg^{-1} wet weight; C_{Cd}=0.30±0.18 mg.kg^{-1} dry weight). The concentrations of Cd in the **muscle** samples were the highest in summer season (C_{Cd}=0.09±0.07 mg.kg^{-1} wet weight; C_{Cd}=0.29±0.19 mg.kg^{-1}

dry weight) and the lowest in spring season (C_{Cd}=0.05±0.03 mg.kg^{-1} wet weight; C_{Cd}=0.21±0.17 mg.kg^{-1} dry weight).

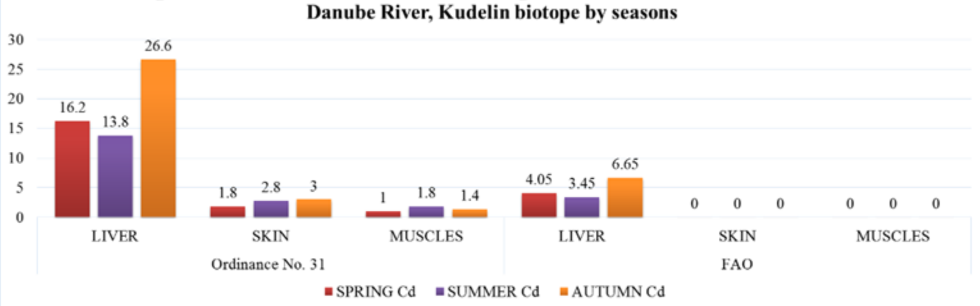
The values of Cd in liver samples of common nase in autumn season exceeded 1.64 times those established in spring season and 1.93 times those found in summer season. The levels of Cd in the skin samples in the autumn were 1.67 times above the spring levels and 1.07 times above the summer levels. The concentrations of Cd in muscle samples in the summer exceeded those in the spring and autumn respectively 1.8 and 1.29 times (**Fig. 47**).

FIG. 47. CONCENTRATIONS OF CD (MG.KG^{-1} WET WEIGHT) IN TISSUES AND ORGANS OF *CHONDROSTOMA NASUS* FROM THE DANUBE RIVER, KUDELIN BIOTOPE BY SEASONS



The levels of Cd in the examined tissues and organs of common nase were compared with the norms in Ordinance No. 31 and of the FAO. The highest exceedances of Cd were found in liver during autumn season – 26.6 times above the MPC in Ordinance No. 31 and 6.65 times compared to the norm given by the FAO. The highest exceedences of Cd in skin were established during the autumn (3 times) and the summer (2.8 times) compared to Ordinance No. 31. While in muscles, the highest exceedences were found during summer season – 1.8 times above the MPC in Ordinance No. 31 (**Fig. 48**).

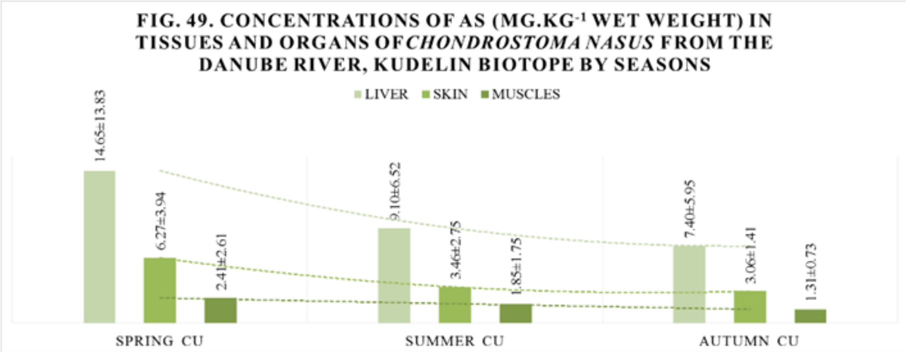
Fig. 48. Exceedances of Cd in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope by seasons



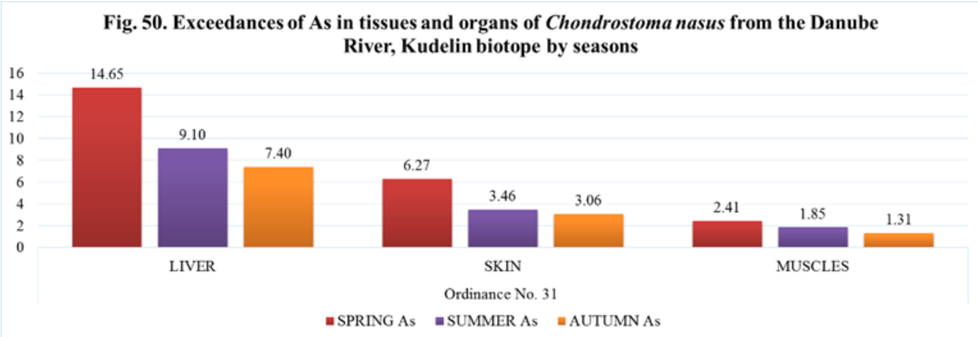
Legend: Biological samples: 1) Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs – MPC (mg/kg wet weight) – **0.05 mg/kg** for Cd; 2) FAO – **0.2 mg/kg** for Cd

The highest levels of As in the liver samples were recorded in spring (C_{As}=14.65±13.83 mg.kg^{-1} wet weight; C_{As}=41.98±42.14 mg.kg^{-1} dry weight), and the lowest in autumn (C_{As}=7.40±5.95 mg.kg^{-1} wet weight; C_{As}=24.02±20.38 mg.kg^{-1} dry weight). In the skin and muscle samples of common nase, the highest concentrations of As were also established in spring season (C_{As}=6.27±3.94 mg.kg^{-1} wet weight, C_{As}=16.51±10.92 mg.kg^{-1} dry weight and C_{As}=2.41±2.61 mg.kg^{-1} wet weight, C_{As}=9.75±11.50 mg.kg^{-1} dry weight, respectively), and the lowest in autumn season (C_{As}=3.06±1.41 mg.kg^{-1} wet weight, C_{As}=9.71±8.36 mg.kg^{-1} dry weight and C_{As}=1.31±0.73 mg.kg^{-1} wet weight, C_{As}=4.75±2.01 mg.kg^{-1} dry weight, respectively).

The content of As in liver samples of common nase in spring season exceeded 1.61 and 1.98 times the content of the element in the summer and autumn seasons, respectively. The levels of As in skin samples again in the spring were 1.81 and 2.05 times higher than those established in the summer and autumn, respectively. In the muscle samples the levels of As during the spring also exceeded those reported in the other two seasons – summer (1.30 times) and autumn (1.84 times) (**Fig. 49**).



The exceedances of As in tissues and organs of common nase were compared with the norm in Ordinance No. 31 from 2004. In all three seasons there were exceedances of As in liver, skin and muscles of common nase. The highest were the exceedances of As in liver, skin and muscles of common nase in the spring – 14.65, 6.27 and 2.41 times above the MPC in Ordinance No. 31 (**Fig. 50**).



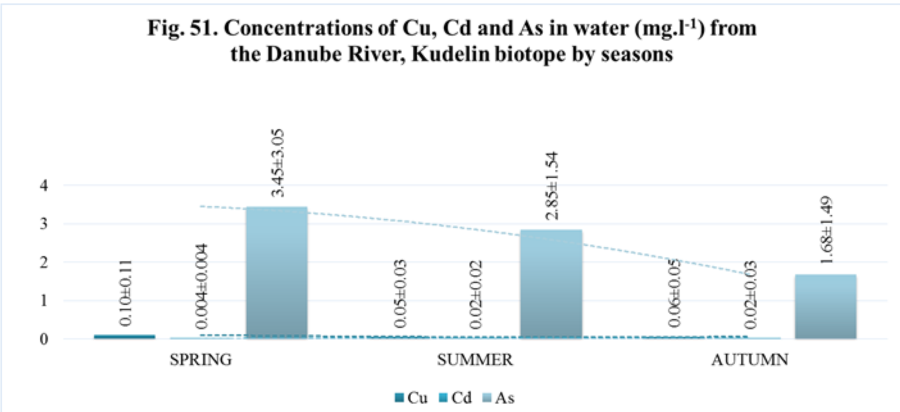
Legend: Biological samples: 1) Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs – MPC (mg/kg wet weight) – **1 mg/kg** for As

The season is determining of the content of Cu in the muscles samples of common nase ($F=6.0$, $p=0.049<0.05$).

V.5.4. Seasonal changes in the Cu, Cd and As content in water and sediments

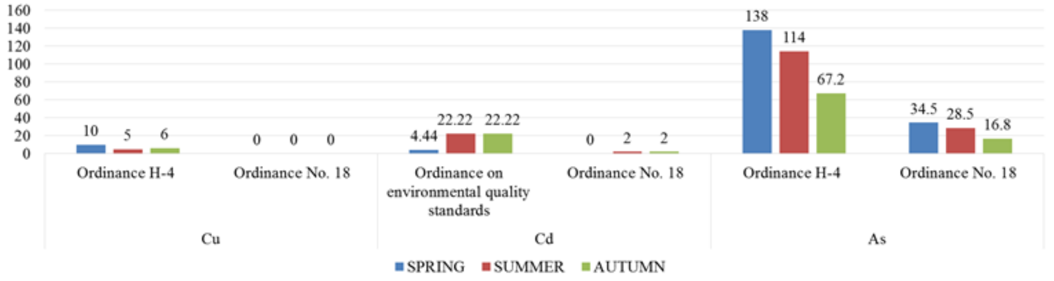
The highest levels of Cu and As in water from the Danube River (Kudelin) were recorded in spring season, respectively $C_{CuWater}=0.10\pm0.11$ mg.l^{-1} and $C_{AsWater}=3.45\pm3.05$ mg.l^{-1}, while the highest and equal concentrations of Cd were found in two of the seasons – summer ($C_{CdWater}=0.02\pm0.02$ mg.l^{-1}) and autumn ($C_{CdWater}=0.02\pm0.03$ mg.l^{-1}). Significant seasonal differences only for the content of Cd in the samples of water in spring and summer seasons ($t=29.61$, $p=0.03<0.05$), spring and autumn seasons ($t=132.57$, $p=0.007<0.05$) were established.

The concentrations of Cu in water in the spring exceeded those in the summer and autumn respectively 2 and 1.67 times. The levels of Cd in water during the summer and autumn exceeded 5 times those reported in the spring. The levels of As in the spring were 1.21 times higher than those in the summer and 2.05 times higher than those in the autumn (**Fig. 51**).



In water from Kudelin, the highest exceedances were found for As. The exceedances of As in the spring were the highest – 138 and 34.5 times above the norms given in Ordinance H-4 from 2010 and Ordinance No. 18 from 2009, respectively. The highest exceedances of Cd in water were established in relation to the Ordinance on environmental quality standards for priority substances and certain other pollutants during the summer and autumn (22.22 times). The highest exceedances of Cu in water were found in the spring – 10 times above the AAV in Ordinance H-4. The content of Cu in water did not exceed the norm in Ordinance No. 18 (**Fig. 52**).

Fig. 52. Exceedances of Cu, Cd and As in water from the Danube River, Kudelin biotope by seasons

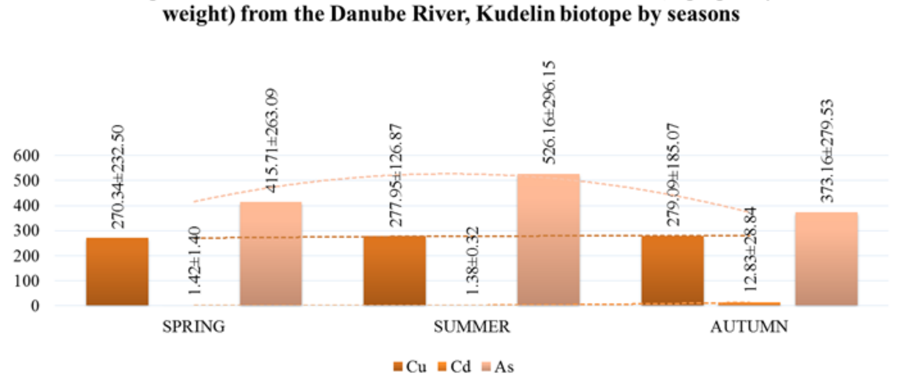


Legend: Water: 1) Ordinance H-4 of 14 September 2012 on the characterization of surface water – AAV (**mg/l**), $CaCO_3$ 100-250 – **0.01 mg/l** for Cu, MPC (mg/l) – **0.025 mg/l** for As; 2) Ordinance on environmental quality standards for priority substances and certain other pollutants – MPC (**mg/l**), class 4 – **0.0009 mg/l** for Cd; 3) Ordinance No. 18 of 27 May 2009 on the quality of water for irrigation of crops – MPC (mg/dm^3) – **0.2** $\mathbf{mg/dm^3}$ for Cu; **0.01** $\mathbf{mg/dm^3}$ for Cd; **0.1** $\mathbf{mg/dm^3}$ for As

The highest values for copper and cadmium in sediments from Kudelin biotope were found in autumn season ($C_{CuSediments}$=279.09±185.07 $mg.kg^{-1}$ dry weight and $C_{CdSediments}$=12.83±28.84 $mg.kg^{-1}$ dry weight), and the highest levels of arsenic in sediments were found in summer season ($C_{AsSediments}$=526.16±296.15 $mg.kg^{-1}$ dry weight). Significant seasonal differences in the content of Cd for the sediments samples in spring and summer ($t=26.15$, $p=0.03<0.05$), spring and autumn ($t=516.02$, $p=0.001<0.05$), summer and autumn ($t=13493.98$, $p=0.0001<0.05$) were found.

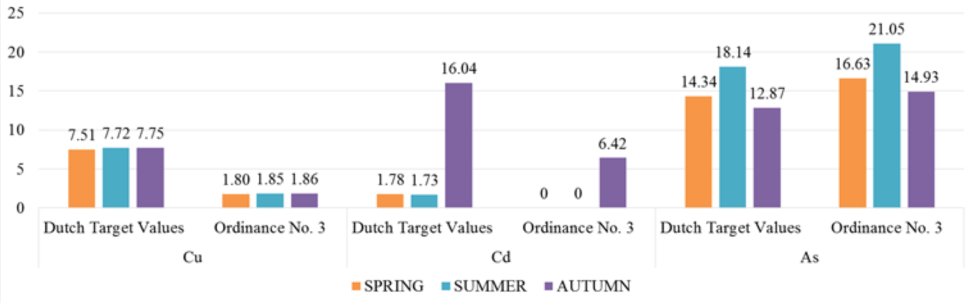
The levels of Cu in sediments from the Danube River in autumn season exceeded 1.03 and 1.004 times those established in the spring and summer seasons, respectively. The concentrations of Cd during the autumn exceeded established concentrations in sediments during the spring (9.04 times) and the summer (9.3 times). The content of As in summer season was 1.27 and 1.41 times higher than that in the spring and autumn seasons, respectively (**Fig. 53**).

Fig. 53. Concentrations of Cu, Cd and As in sediments ($mg.kg^{-1}$ dry weight) from the Danube River, Kudelin biotope by seasons



In sediments, during the three research seasons, the highest exceedances of As were established. The concentrations of As in sediments had the highest exceedances in the summer – 21.05 and 18.14 times above the MPC in Ordinance No. 3 from 2008 and the Dutch target values, respectively. The highest exceedances for Cd were established in the autumn – 16.04 and 6.42 times above the Dutch target values and Ordinance No. 3, respectively. The concentrations of cadmium in sediments from the Danube River during the spring and summer seasons did not exceed the MPC in Ordinance No. 3. The exceedances of Cu in all three research seasons (spring, summer and autumn) were close (**Fig. 54**).

Fig. 54. Exceedances of Cu, Cd and As in sediments from the Danube River, Kudelin biotope by seasons



Legend: Sediments: 1) Dutch Target Values (mg/kg dry weight) – **36 mg/kg** for Cu; **0.8 mg/kg** for Cd; **29 mg/kg** for As; 2) Ordinance No. 3 of 1 August 2008 on the norms for the permissible content of harmful substances in soils: MPC (mg/kg) for agricultural land (pH 6.00-7.4) – **150 mg/kg** for Cu; **2 mg/kg for** Cd; MPC (mg/kg) for agricultural land, independently of pH – **25 mg/kg** for As.

V.6. ANNUAL CHANGES IN THE CU, CD AND AS CONTENT IN TISSUES AND ORGANS OF *ALBURNUS ALBURNUS*, *ABRAMIS BRAMA* AND *CHONDROSTOMA NASUS*, IN WATER AND SEDIMENTS FROM THE DANUBE RIVER, KUDELIN BIOTOPE

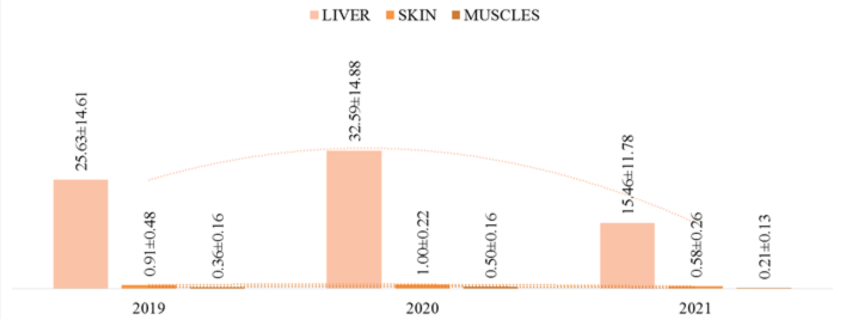
The changes in the levels of Cu, Cd and As in liver, skin and muscles of the three examined fish species, in water and sediments are presented by year. The exceedances of the three elements compared to values specified in both Bulgarian and international legislation are also shown.

The presented information reflects the annual changes in the Cu, Cd and As content in tissues and organs of *Chondrostoma nasus*.

In samples of **liver** (C_{Cd}=32.59±14.88 mg.kg^{-1} wet weight; C_{Cd}=79.69±30.03 mg.kg^{-1} dry weight), **skin** (C_{Cd}=1.00±0.22 mg.kg^{-1} wet weight; C_{Cd}=2.53±0.55 mg.kg^{-1} dry weight) and **muscles** (C_{Cd}=0.50±0.16 mg.kg^{-1} wet weight; C_{Cd}=2.01±0.58 mg.kg^{-1} dry weight), the highest concentrations of **Cu** were found in 2020.

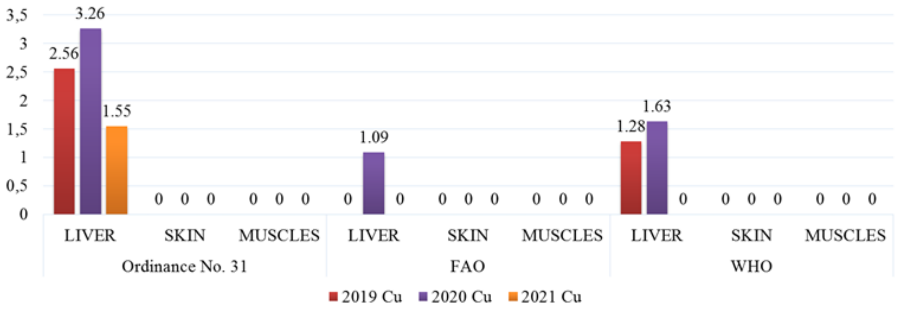
The concentrations of Cu in samples of tissues and organs of common nase in 2020 exceeded the element concentrations in the samples from 2019 and 2021 respectively 1.27 and 2.11 times (for liver), 1.1 and 1.72 times (for skin), and 1.39 and 2.38 times (for muscles) (**Fig. 67**).

Fig. 67. Concentrations of Cu (mg.kg^{-1} wet weight) in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope by years



The content of Cu in liver, skin and muscles of common nase was compared with the norms of Ordinance No. 31 from 2004, the FAO and the WHO. Exceedances of copper were established in all three years of research compared to the MPC in Ordinance No. 31, while the highest exceedence was established in liver samples of common nase in 2020 – 3.26 times. Compared to the value given by the FAO, exceedances were found only in liver samples in 2020, and compared to the value given by the WHO - in liver samples in 2019 (1.28 times) and 2020 (1.63 times) (**Fig. 68**).

Fig. 68. Exceedances of Cu in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope by years

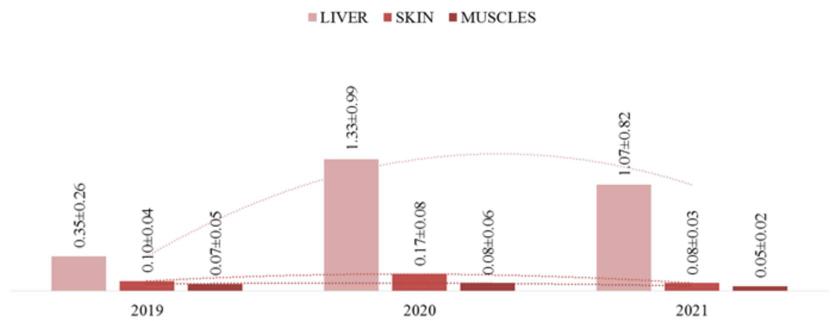


Legend: Biological samples: 1) Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs – MPC (mg/kg wet weight) – **10 mg/kg** for Cu; 2) FAO – **30 mg/kg** for Cu; 3) WHO – **20 mg/kg** for Cu.

In the examined tissues and organs, the highest levels of **Cd** were found in 2020, namely: C_{Cd}=1.33±0.99 mg.kg^{-1} wet weight; C_{Cd}=3.81±4.13 mg.kg^{-1} dry weight (for **liver**); C_{Cd}=0.17±0.08 mg.kg^{-1} wet weight; C_{Cd}=0.53±0.35 mg.kg^{-1} dry weight (for **skin**) and C_{Cd}=0.08±0.06 mg.kg^{-1} wet weight; C_{Cd}=0.26±0.18 mg.kg^{-1} dry weight (for **muscles**).

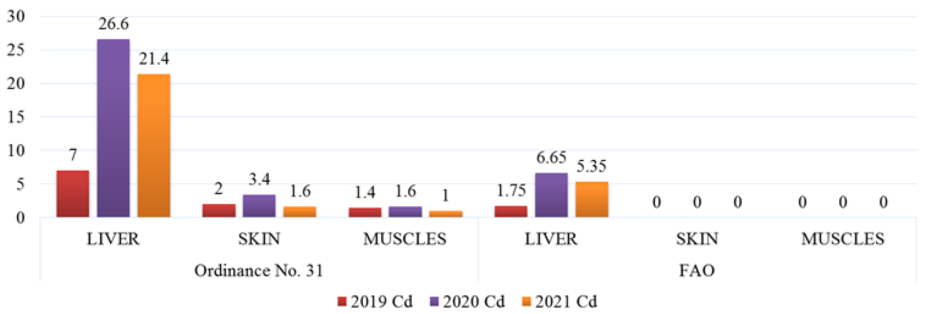
The levels of Cd in samples of the examined tissues and organs of *Ch. nasus* from 2020 exceeded the levels of Cd in the examined samples from 2019 and 2021, namely: for liver: 3.8 and 1.24 times, for skin: 1.7 and 2.13 times and for muscles: 1.14 and 1.6 times (**Fig. 69**).

Fig. 69. Concentrations of Cd (mg.kg^{-1} wet weight) in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope by years



The concentrations of cadmium in tissues and organs of common nase were compared with the values specified in Ordinance No. 31 and the FAO. During all three years of research, exceedances in liver, skin and muscle samples of common nase compared to the MPC in Ordinance No. 31 were established. The highest exceedances of Cd in liver during 2020 and 2021 were found – respectively 26.6 and 21.4 times above the value in Ordinance No. 31, and respectively 6.65 and 5.35 times above the value given by the FAO. In skin and muscle samples the highest exceedances were found in 2020 – 3.4 and 1.6 times above the MPC in Ordinance No. 31, respectively. Exceedances were not found in the samples of skin and muscles compared to the value given by the FAO (**Fig. 70**).

Fig. 70. Exceedances of Cd in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope by years

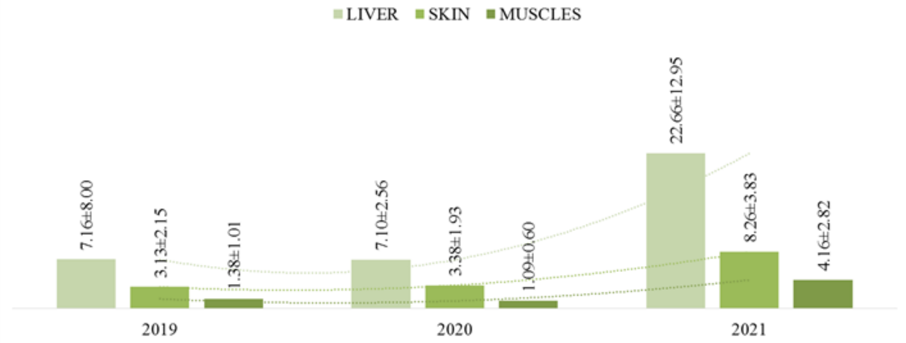


Legend: Biological samples: 1) Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs – MPC (mg/kg wet weight) – **0.05 mg/kg** for Cd; 2) FAO – **0.2 mg/kg** for Cd

The highest content of As was found during 2021, namely: for **liver**: C_{As}=22.66±12.95 mg.kg^{-1} wet weight and C_{As}=65.08±40.31 mg.kg^{-1} dry weight; for **skin**: C_{As}=8.26±3.83 mg.kg^{-1} wet weight and C_{As}=24.39±11.12 mg.kg^{-1} dry weight; for **muscles**: C_{As}=4.16±2.82 mg.kg^{-1} wet weight and C_{As}=16.39±13.29 mg.kg^{-1} dry weight.

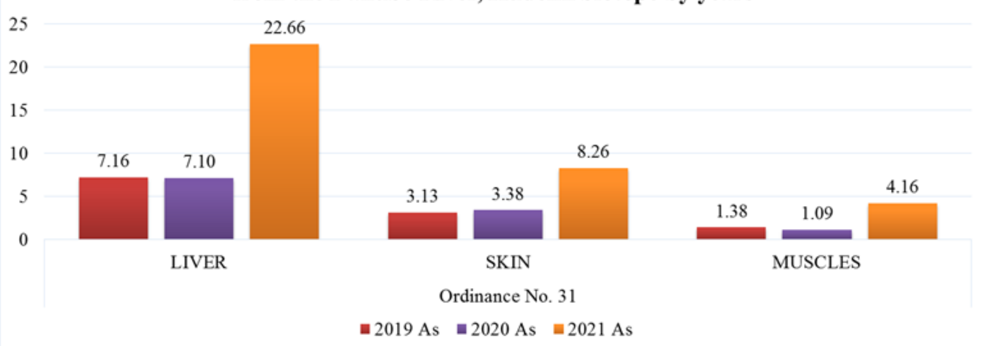
The As content in tissues and organs of common nase from 2021 exceeded that from 2019 and 2020 respectively 3.17 and 3.19 times (for liver), 2.64 and 2.44 times (for muscles), and 3.02 and 3.82 times (for skin) (**Fig. 71**).

Fig. 71. Concentrations of As (mg.kg^{-1} wet weight) in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope by years



Exceedances of As were found in liver, skin and muscles of common nase in all three years. The highest exceedances of As in liver, skin and muscles were found in 2021, respectively 22.66, 8.26 and 4.16 times above the MPC specified in Ordinance No. 31 (**Fig. 72**).

Fig. 72. Exceedances of As in tissues and organs of *Chondrostoma nasus* from the Danube River, Kudelin biotope by years



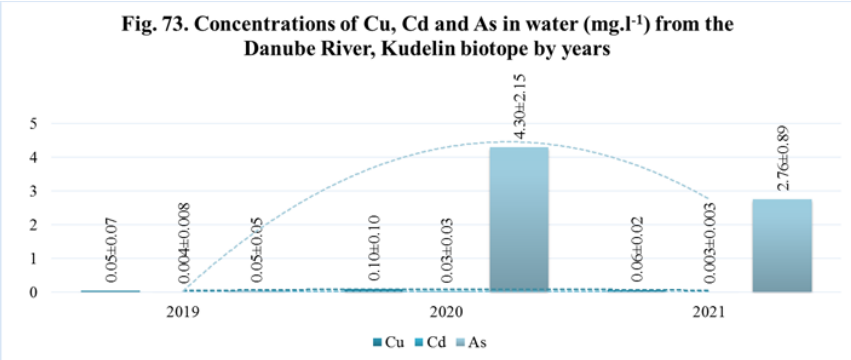
Legend: Biological samples: 1) Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs – MPC (mg/kg wet weight) – **1 mg/kg** for As

Differences in habitat conditions during the three years of the studied period were a determining factor in the content of Cu (F=6.0, p=0.049<0.05), Cd (F=6.0, p=0.049<0.05) and As (F=6.0, p=0.049<0.05) in the liver samples of *Ch. nasus*. Significant differences for the content of Cd in liver for the samples from 2019 and 2020 (t=26.6, p=0.04<0.05) and in muscles for the samples from 2020 and 2021 (t=18.47, p=0.05) were found. Differences in the content of As between the liver and muscle samples from 2020 and 2021 were also significant (t=21.84, p=0.044<0.05; t=16.06, p=0.05, respectively).

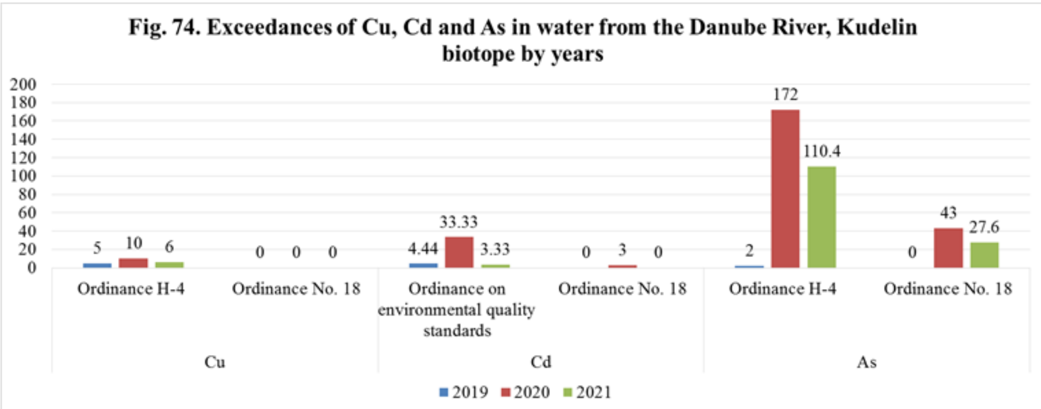
V.6.4. Annual changes in the Cu, Cd and As content in water and sediments

The highest concentrations of Cu, Cd and As in water from Kudelin biotope were found during 2020 ($C_{CuWater}$=0.10±0.10 mg.l^{-1}; $C_{CdWater}$=0.03±0.03 mg.l^{-1} and $C_{AsWater}$=4.30±2.15 mg.l^{-1}). Significant annual differences were established for the content of Cu between water samples from 2020 and 2021 (t=25.86, p=0.03<0.05); for the content of Cd between the samples from 2019 and 2020 (t=23.80, p=0.04<0.05), 2020 and 2021 (t=115.99, p=0.008<0.05), as well as for the content of As between the samples from 2019 and 2020 (t=2512.75, p=0.0004<0.05), 2019 and 2021 (t=328.94, p=0.003<0.05).

The established concentrations of Cu in water from Kudelin biotope during 2020 exceeded 2 and 1.67 times those reported during 2019 and 2021, respectively. The levels of Cd in water in 2020 were higher than those in 2019 (7.5 times) and 2021 (10 times). The concentrations of As in water in 2020 exceeded those found in 2019 (86 times) and less than those in 2021 (1.56 times) (**Fig. 73**).



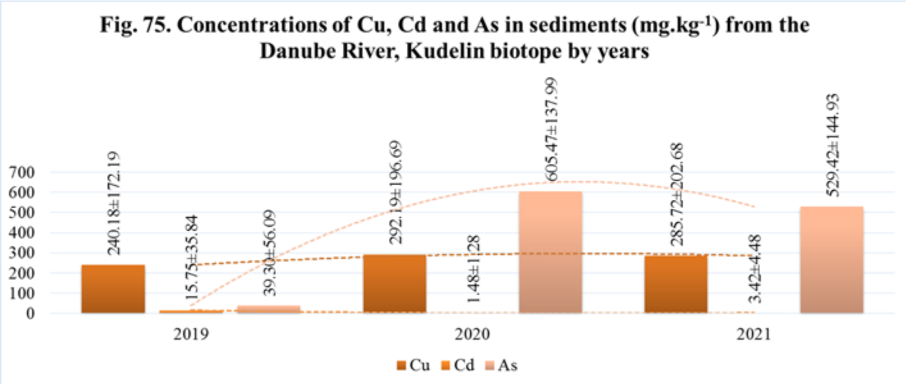
The highest exceedances in water from Kudelin biotope were found for As in 2020 – 172 times above the MPC in Ordinance H-4 and 43 times above the MPC in Ordinance No. 18, followed by those in 2021. Only in 2019 the concentrations of As in water did not exceed the norm in Ordinance No. 18. The highest exceedances of Cd in water were found in 2020 – 33.3 times above the MPC in the Ordinance on environmental quality standards and 3 times the MPC in Ordinance No. 18. Exceedances of cadmium in 2019 and 2021 compared to Ordinance No. 18 were not established. The highest exceedances of Cu in water were reported in 2020 – 10 times above AAV in Ordinance H-4. The concentrations of Cu in water did not exceed the norm in Ordinance No. 18 (**Fig. 74**).



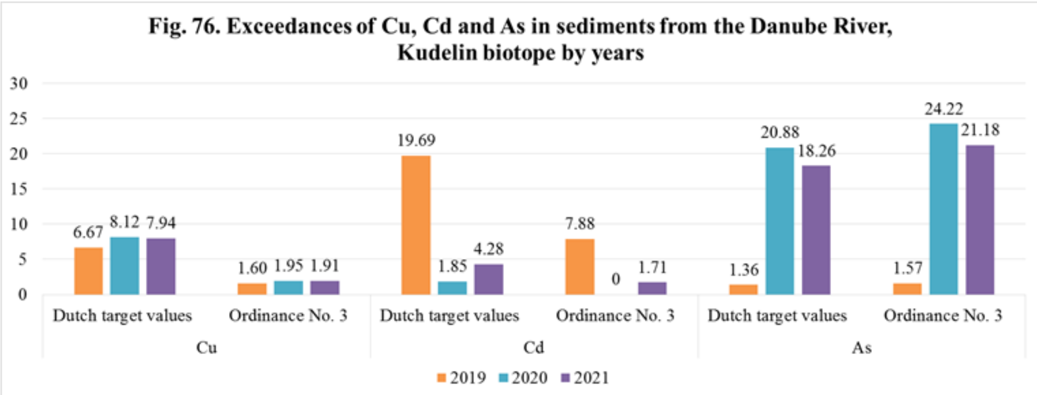
Legend: Water: 1) Ordinance H-4 of 14 September 2012 on the characterization of surface water – AAV (**mg/l**), $CaCO_3$ 100-250 – **0.01 mg/l** for Cu, MPC (mg/l) – **0.025 mg/l** for As; 2) Ordinance on environmental quality standards for priority substances and certain other pollutants – MPC (**mg/l**), class 4 – **0.0009 mg/l** for Cd; 3) Ordinance No. 18 of 27 May 2009 on the quality of water for irrigation of crops – MPC (mg/dm^3) – **0.2 mg/dm^3** for Cu; **0.01 mg/dm^3** for Cd; **0.1 mg/dm^3** for As

The highest concentrations of copper and arsenic in sediments were found in 2020 ($C_{CuSediments}$=292.19±196.69 $mg.kg^{-1}$ dry weight and $C_{AsSediments}$=605.47±137.99 $mg.kg^{-1}$ dry weight). The highest values for cadmium in sediments were found in 2019 ($C_{CdSediments}$=15.75±35.84 $mg.kg^{-1}$ dry weight). Significant annual exceedances for the content of Cd between the samples of sediments from 2019 and 2020 (t=508.85. $p=0.002<0.05$), the samples from 2019 and 2021 (t=47.21, $p=0.02<0.05$) and between the samples from 2020 and 2021 (t=10.78, p=0.05) were found.

The content of Cu in sediments from the Danube River (Kudelin biotope) in 2020 exceeded 1.22 and 1.02 times that in 2019 and 2021, respectively. The concentrations of Cd in sediments in 2019 were 10.64 times higher than in 2020 and 4.61 times higher than in 2021. The levels of As in 2020 were much higher than those in 2019 (15.41 times) and slightly higher than those in 2021 (1.14 times) (**Fig. 75**).



In sediments from Kudelin biotope exceedances for all three investigated elements in all three years were found. The highest exceedances of As in sediments were found in 2020 – 24.22 and 20.88 times compared to the MPC in Ordinance No. 3 and the Dutch target values, respectively, followed by those in 2021. The content of Cd was the highest in 2019 and exceeded 19.69 and 7.88 times the Dutch target values and Ordinance No. 3, respectively. The exceedances of Cu in sediments during the three years of the research were close, but the highest in 2020 – 8.12 times above the Dutch target values and 1.95 times above the MPC in Ordinance No. 3 (**Fig. 76**).



Legend: Sediments: 1) Dutch Target Values (mg/kg dry weight) – **36 mg/kg** for Cu; **0.8 mg/kg** for Cd; **29 mg/kg** for As; 2) Ordinance No. 3 of 1 August 2008 on the norms for the permissible content of harmful substances in soils: MPC (mg/kg) for agricultural land (pH 6.00-7.4) – **150 mg/kg** for Cu; **2 mg/kg for** Cd; MPC (mg/kg) for agricultural land, independently of pH – **25 mg/kg** for As.

V.7. CIRCULATION OF CU, CD AND AS IN WATER AND SEDIMENTS, TISSUES AND ORGANS OF FISH AND THEIR HELMINTHS FROM THE FRESHWATER ECOSYSTEM OF THE DANUBE RIVER, KUDELIN BIOTOPE

The values of the bioconcentration factor for Cu, Cd and As in tissues and organs of the three fish species in relation to water and sediments from the Danube River, Kudelin biotope for the period 2019-2021 are presented. The bioconcentration factors for Cu, Cd and As in parasites in comparison with water and sediments, as well as the bioaccumulation factors in parasites in comparison with tissues and organs of the three fish species, are calculated.

The bioconcentration factors for Cu, Cd and As in tissues and organs of the three fish species in relation to water and sediments are modeled after the example of *Ch. nasus* and its parasites *P. laevis* and *Contracaecum* sp., and the bioaccumulation factors after the example of *Contracaecum* sp.

The highest value of the bioconcentration factor was found for Cu in liver of common nase (BCF_{Cu}=374.71). The liver of *Ch. nasus* bioaccumulated 92.75 times more copper from water than arsenic and 4.03 times more copper than the accumulation rate of cadmium. The lowest value of the factor was reported for As in muscles (BCF_{As}=0.72). ***The values of bioconcentration factors for copper, cadmium and arsenic in liver and skin of common nase in relation to water decreased in the order: $BCF_{Cu} > BCF_{Cd} > BCF_{As}$, while in muscles they***

decreased as follows: BCF_{Cd} > BCF_{Cu} > BCF_{As}. The bioconcentration factors in *P. laevis* of *Ch. nasus* in relation to water were the highest for Cd (BCF_{Cd}=231), and the lowest for Cu (BCF_{Cu}=132.14). *P. laevis* bioaccumulated 10.63 times more Cu from the water than the skin and 24.34 times more Cu from water in comparison with the muscles, but 2.84 times less Cu from water compared to the liver of its host *Ch. nasus*. *P. laevis* bioaccumulated 2.48 to 33 times more Cd from water and 34.57 to 193.96 times more As from water compared to the accumulation in the tissues and organs of its host. ***The values of bioconcentration factor for Cu, Cd and As in P. laevis of Ch. nasus in regard to water decreased in the order: BCF_{Cd} > BCF_{As} > BCF_{Cu}.*** *Contracaecum* sp. bioaccumulated 17.32 times more Cu from water than the skin and 39.65 times more Cu from water than the muscles, but 1.74 times less Cu from water compared to the liver of its host *Ch. nasus*. *Contracaecum* sp. bioaccumulated 3.77 to 50.14 times more Cd from water and 7.39 to 41.46 times more As from water compared to the accumulation in the tissues and organs of its host. ***The values of bioconcentration factor for Cu, Cd and As in Contracaecum sp. of Ch. nasus in regard to water decreased in the order: BCF_{Cd} > BCF_{Cu} > BCF_{As}*** **(Table 38)**.

Table 38. Bioconcentration factors (BCF) for Cu, Cd and As in tissues and organs/ parasites of *Chondrostoma nasus* compared to water ($BCF=[C_{tissues\ and\ organs/parasites}]/[C_{Water}]$)

Chondrostoma nasus/ Water	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C_{LIVER} / C_{WATER}	374.71	93	4.04
C_{SKIN} / C_{WATER}	12.43	12	1.67
C_{MUSLES} / C_{WATER}	5.43	7	0.72
$C_{P. LAEVIS}$ / C_{WATER}	132.14	231	139.65
$C_{CONTRACAECUM\ SP.}$ / C_{WATER}	215.29	351	29.85

The value of bioconcentration factor in liver of *Ch. nasus* in relation to sediments was the highest for Cd (BCF_{Cd}=0.44), followed by Cu (BCF_{Cu}=0.31) and As (BCF_{As}=0.07). In the skin and muscles, the highest values of bioconcentration factor were again found for Cd, but followed by As and Cu. The liver bioaccumulated 6.29 times more Cd from the sediments than the accumulation of As and 1.42 times more than the accumulation rate of Cu. The bioconcentration factor had the lowest value for copper (BCF_{Cu}=0.01) in skin and muscles. ***The values of bioconcentration factor for Cu, Cd and As in liver of common nase in relation to sediments decreased as follows: BCF_{Cd} > BCF_{Cu} > BCF_{As} and in skin and muscles in the order: BCF_{Cd} > BCF_{As} > BCF_{Cu}.*** *Contracaecum* sp. bioaccumulated 24 times more Cu from the sediments than the skin and muscles, but 1.29 times less Cu from the sediments than liver of its host *Ch. nasus*. *Contracaecum* sp. bioaccumulated 3.96 to 43.5 times more Cd from the sediments and 6.43 to 22.5 times more As from the sediments compared to the accumulation in the tissues and organs of its host. ***The values of biconcentration factor for copper, cadmium and arsenic in Contracaecum sp. of common nase compared to sediments decreased as follows: BCF_{Cd} > BCF_{As} > BCF_{Cu}*** **(Table 39)**.

Table 39. Bioconcentration factors (BCF) for Cu, Cd and As in tissues and organs/ parasites of *Chondrostoma nasus* compared to sediments ($BCF=[C_{tissues\ and\ organs/parasites}]/[C_{Sediments}]$)

Chondrostoma nasus/ Sediments	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C_{LIVER} / $C_{SEDIMENTS}$	0.31	0.44	0.07
C_{SKIN} / $C_{SEDIMENTS}$	0.01	0.08	0.03
C_{MUSLES} / $C_{SEDIMENTS}$	0.01	0.04	0.02
$C_{CONTRACAECUM\ SP.}$ / $C_{SEDIMENTS}$	0.24	1.74	0.45

The bioconcentration factors for Cu, Cd and As in liver, skin and muscles of Ch. nasus compared to water and sediments from the Danube River in Bulgaria have not been investigated.

In the dissertation a comparative review of the bioconcentration factors for Cu, Cd and As in tissues and organs of the three fish species, as well as in *P. laevis* and *Contracaecum* sp. in relation to water and sediments; the bioaccumulation factor for Cu, Cd and As in the parasites of the three fish species and the Spearman correlation coefficient (r_s) are presented. Summaries of the obtained results are presented in the abstract.

The **BCF** values for **Cu** in tissues and organs of the three examined fish species compared to water and sediments from the Danube River, Kudelin biotope were the highest in liver of *Ch. nasus* ($BCF_{Cu\ Liver/Water}$=374.71; $BCF_{Cu\ Liver/Sediments}$=0.31); for **Cd** and **As** – in liver of *Alb. alburnus* ($BCF_{Cd\ Liver/Water}$=141, $BCF_{Cd\ Liver/Sediments}$=0.62; $BCF_{As\ Liver/Water}$=7.49, $BCF_{As\ Liver/Sediments}$=0.18, respectively).

The values of the bioaccumulation factor (**BAF**) decreased in the order: $\mathbf{BAF_{As} > BAF_{Cd} > BAF_{Cu}}$ (*P. laevis*/ liver, skin and muscles of bleak, freshwater bream and common nase; *Contracaecum* sp./ liver of common nase); in the order: $\mathbf{BAF_{Cd} > BAF_{As} > BAF_{Cu}}$ (*Contracaecum* sp./ skin and muscles of common nase). The BAF values for Cu, Cd and As in parasites compared to the tissues and organs of their hosts decreased in the order: muscles > skin > liver.

For Cu and Cd, there was **a very strong correlation dependence** between the tissues and organs of the three fish species, their parasites, the water and sediments, but **a strong correlation** between the concentrations of Cd in *P. laevis* of bleak and common nase in relation to the water (r_s=0.87-0.89, $p<0.01$). Regarding the bioconcentration of As, there was **no significant correlation dependence** between tissues and organs of fish, parasites and water, while **very strong correlations** between tissues and organs of fish, parasites and sediments (except for **the strong dependence** between *P. laevis* and liver of freshwater bream (r_s=0.89, $p<0.01$), and between *Contracaecum* sp. and liver and muscles of common nase (r_s=0.88, $p<0.01$)) were established. There was no significant correlation between the content of Cu, Cd and As in sediments and that in the samples of *Contracaecum* sp. ($p>0.05$). The accumulation of Cu and Cd in the three fish species is influenced by the water, sediments and food, while the accumulation of As is mainly by sediments and food.

The values of bioconcentration factor in parasites in relation to water and sediments were the highest for Cd. *P. laevis* of bleak, freshwater bream and common nase had the highest values of bioaccumulation factor for As and *Contracaecum* sp. of *Ch. nasus* – for Cd.

V.8. CIRCULATION OF CU, CD AND AS IN WATER AND SEDIMENTS, TISSUES ANDORGANS OF FISH BY SEASONS

In the dissertation, the circulation is presented by elements. A summary of the results is presented in the abstract.

The values of **BCF** for **Cu** in the spring, summer, and autumn were the highest in **liver of *Ch. nasus*** ($BCF_{Cu\ Liver/Water}$=287.80, $BCF_{Cu\ Liver/Sediments}$=0.29; $BCF_{Cu\ Liver/Water}$=580.20, $BCF_{Cu\ Liver/Sediments}$=0.27; $BCF_{Cu\ Liver/Water}$=337.83, $BCF_{Cu\ Liver/Sediments}$=0.37, respectively); **for Cd** – in the spring and summer in **liver of *Alb. alburnus*** ($BCF_{Cd\ Liver/Water}$=430, $BCF_{Cd\ Liver/Sediments}$=2.59; $BCF_{Cd\ Liver/Water}$=78, $BCF_{Cd\ Liver/Sediments}$=3.75, respectively), and in the autumn – in **liver of *Ch. nasus*** ($BCF_{Cd\ Liver/Water}$=66.50, $BCF_{Cd\ Liver/Sediments}$=0.34); **for As** – in the spring, summer and autumn – in **liver of *Alb. alburnus*** ($BCF_{As\ Liver/Water}$=7.93, $BCF_{As\ Liver/Sediments}$=0.28; $BCF_{As\ Liver/Water}$=6.58, $BCF_{As\ Liver/Sediments}$=0.11; $BCF_{As\ Liver/Water}$=6.53, $BCF_{As\ Liver/Sediments}$=0.10, respectively).

V.9. CIRCULATION OF CU, CD AND AS IN WATER AND SEDIMENTS, TISSUES ANDORGANS OF FISH BY YEARS

In the dissertation, the circulation is presented by elements. A summary of the results is presented in the abstract.

The values of **BCF** for **Cu** in 2019, 2020 and 2021 were the highest in liver of *Ch. nasus* ($BCF_{Cu\ Liver/Water}$=512.60, $BCF_{Cu\ Liver/Sediments}$=0.32; $BCF_{Cu\ Liver/Water}$=325.90, $BCF_{Cu\ Liver/Sediments}$=0.27; $BCF_{Cu\ Liver/Water}$=257.67, $BCF_{Cu\ Liver/Sediments}$=0.39, respectively); for **Cd** in 2019, 2020 and 2021 were the highest in liver of *Alb. alburnus* ($BCF_{Cd\ Liver/Water}$=342.50, $BCF_{Cd\ Liver/Sediments}$=0.18; $BCF_{Cd\ Liver/Water}$=50, $BCF_{Cd\ Liver/Sediments}$=2.99; $BCF_{Cd\ Liver/Water}$=446.67, $BCF_{Cd\ Liver/Sediments}$=1.15, respectively); for **As** in 2019 and 2020 were the highest in liver of *Alb. alburnus* ($BCF_{As\ Liver/Water}$=395, $BCF_{As\ Liver/Sediments}$=2.64; $BCF_{As\ Liver/Water}$=2.33, $BCF_{As\ Liver/Sediments}$=0.05, respectively), and in 2021 – in liver of *Abr. brama* ($BCF_{As\ Liver/Water}$=14.44, $BCF_{As\ Liver/Sediments}$=0.22).

SUMMARY AND CONCLUSIONS

For the period 2019-2021, three fish species from the Cyprinidae family – *Alburnus alburnus* (Linnaeus, 1758), *Abramis brama* (Linnaeus, 1758) and *Chondrostoma nasus* (Linnaeus, 1758) from the Danube River, Kudelin biotope were studied. Samples of tissues and organs (liver, skin and muscles) of the three fish species; samples of their parasites and samples of water and sediments were examined for the content of copper (Cu), cadmium (Cd) and arsenic (As).

Based on the conducted research and the obtained results, the following summaries and conclusions can be made:

1. The content of the studied elements in **tissues and organs of bleak, freshwater bream and common nase** decreased in the order: **As > Cu > Cd**, except for that in **liver of common nase**, which decreased in the order: **Cu > As > Cd**. The content of copper, cadmium and arsenic ($mg.kg^{-1}$ wet weight) in *Alb. alburnus*, *Abr. brama* and *Ch. nasus* decreased in the order **liver > skin > muscles**. The highest exceedances of Cd in **liver** of bleak and common nase (28.2 times compared to Ordinance No. 31 / 7.05 times compared to the FAO and 18.6 times compared to Ordinance No. 31 / 4.65 times compared to the FAO, respectively), and of As in liver of freshwater bream (15.49 times compared to Ordinance No. 31) were found. The highest excedances of Cd and As respectively in **skin** of bleak (12.2 times compared to Ordinance No. 31/ 3.05 times compared to the FAO), and in the skin of freshwater bream and common nase (6.46 and 4.42 times compared to Ordinance No. 31, respectively) were established. The highest exceedances of As in **muscles** of bleak, freshwater bream and common nase (respectively 3.57, 2.64 and 1.9 times compared to Ordinance No. 31) were established. Exceedances of Cu were found only in liver of freshwater bream (1.29 times compared to Ordinance No. 31) and common nase (2.62 times compared to Ordinance No. 31 and 1.31 times compared to the WHO). The nature of the examined tissue and organ samples and the fish species are determinant of the content of Cu, Cd and As (Friedman test F=6.0; $p=0.049<0.05$). The liver of *Ch. nasus* and *Alb. alburnus* can be used as a good bioindicator for Cd, and the liver of *Abr. brama* for As.
2. The content of the investigated elements in **parasites** of bleak, freshwater bream and common nase decreased in the order: **As > Cu > Cd**. The highest concentrations of As were found in *P. laevis* of *Ch. nasus* ($C_{As}=370.07\pm229.35$ $mg.kg^{-1}$ wet weight); of Cu and Cd – in *Contracaecum* sp. of *Ch. nasus* ($C_{Cu}=15.07\pm19$ $mg.kg^{-1}$ wet weight and $C_{Cd}=3.51\pm3.36$ $mg.kg^{-1}$ wet weight, respectively). The host species is significant for the content of As in *P. laevis* ($\chi^2_{Pl/Abr}/_{Pl/Alb}=15.66$, $p=0.0004<0.05$; $\chi^2_{Pl/Abr}/_{Pl/Ch}=66.07$, $p=0.0000$; $\chi^2_{Pl/Alb}/_{Pl/Ch}=22.69$, $p=0.0000$). The helminth species in common nase is significant for the content of As and Cu in the samples ($t_{As}=29.45$, $p=0.03<0.05$;$t_{Cu}=21.37$, $p=0.04<0.05$). An exceedance of Cu was reported only in *Contracaecum* sp. of common nase (1.51 times compared to Ordinance No. 31). The highest exceedance of Cd was reported in *Contracaecum* sp. of common nase (70.2 times compared to Ordinance No. 31); of As – in *P. laevis* of common nase (370.07 times compared to Ordinance No. 31). A good bioindicator for content of Cd is the nematode *Contracaecum* sp., and for As is *P. laevis*.
3. The content of the three elements in **water and sediments** decreased in the order: **As > Cu > Cd**, as excesses were found for all of them. The highest exceedances for As (106 times above the MPC in Ordinance H-4 from 2012 / 26.5 above the MPC in Ordinance No. 18 for water; 16.94 times compared to Ordinanace No. 3 / 14.6 times compared to Dutch target values for sediments) were established.
4. **The concentrations of Cu** in liver decreased in the order: *Ch. nasus* > *Abr. brama* > *Alb. alburnus*. **The concentrations of Cu** in skin and muscles, as well as of **Cd** in liver, skin and muscles decreased in the order: *Alb. alburnus* > *Ch. nasus* > *Abr. brama*. **The concentrations of As** in liver, skin and muscles decreased as follows: *Alb. alburnus* > *Abr. brama* > *Ch. nasus*. The content of Cu and Cd in the examined tissues and organs of bleak, freshwater bream and common nase; of As in the tissues and organs of bleak; of As

in liver and skin of freshwater bream and common nase; of Cu, Cd and As in their parasites was higher than that in water. The content of Cd in *Contracaecum* sp. of *Ch. nasus* was higher than that in sediments. The content of As in muscles of freshwater bream and common nase was lower than that in water. The content of Cu, Cd and As in tissues and organs of bleak, freshwater bream and common nase; of Cu and As in *Contracaecum* sp. of *Ch. nasus* was lower than that in sediments. The content of Cu was the highest in liver of *Ch. nasus*, and of Cd and As – in liver of *Alb. alburnus*. The content of Cu and Cd was the highest in *Contracaecum* sp., and of As was the highest in *P. laevis*.

5. In the three studied fish species, the highest **exceedances of copper, cadmium and arsenic** were found in samples of liver. In ***Alb. alburnus*** and ***Abr. brama***, the highest exceedances of **Cu** were established in the autumn, and of **Cd** and **As** in the spring; in ***Ch. nasus***, the highest exceedances of **Cu** were found in the summer, of **Cd** – in the autumn, and of **As** – in the spring. The season is a determinant factor of the content of Cu, Cd and As in liver, skin and muscles of *Alb. alburnus* (Friedman test, $F=48.37$, $p=0.003<0.05$); for the content of Cu and Cd in the samples of muscles of *Abr. brama* (Friedman test, $F=6.0$, $p=0.049<0.05$), as well as for the content of Cu in the samples of muscles of *Ch. nasus* ($F=6.0$, $p=0.049<0.05$). The seasonal differences in the content of Cu, Cd and As in the examined tissues and organs of bleak, freshwater bream and common nase are related, in addition to the content of these elements in water and sediments in different seasons, and to differences in the diet of the fish and their way of feeding.
6. The highest **exceedances of As in water and sediments** were reported in the spring and summer, respectively; of **Cd** – in the summer and autumn in water and in the autumn in sediments; and of **Cu** - in the spring, and in sediments – in the autumn. Significant seasonal differences were found for the content of Cd in water and sediments in the seasons spring and summer ($t=29.61$, $p=0.03<0.05$ and $t=26.15$, $p=0.03<0.05$, respectively), spring and autumn ($t=132.57$, $p=0.007<0.05$ and $t=516.02$, $p=0.001<0.05$, respectively); of Cd in sediments and in the seasons summer and autumn ($t=13493.98$, $p=0.0001<0.05$).
7. In the three studied fish species, the highest **exceedances of copper, cadmium and arsenic** were found in samples of liver. In ***Abr. brama*** and ***Ch. nasus***, the highest exceedances of **Cu** were established in 2019 and 2020, respectively, and in ***Alb. alburnus*** there were no exceedances; in ***Alb. alburnus*** and ***Ch. nasus*** the highest exceedances of **Cd** were established in 2020, and at ***Abr. brama*** in 2019. In all three fish species the highest exceedances of **As** were found in 2021. Differences in the habitat conditions in the three years of the studied period were a determining factor ($F=6.0$, $p=0.049<0.05$) for the content of: Cu in the samples of liver and for the content of Cd and As in the samples of muscles of *Alb. alburnus*; of Cu in the samples of skin and for the content of As in the samples of liver, skin and muscles of *Abr. brama*; of Cu, Cd and As in the samples of liver of *Ch. nasus*. The annual differences in the content of Cu, Cd and As in liver, skin and muscles of the three examined fish species are due to the different content of the examined elements in water, sediments and in the food used by the fish.
8. The highest **exceedances** of **Cu** and **As** in **water** and **sediments** were found in 2020; of **Cd** in water – in 2020, and in sediments – in 2019.
9. The values of **BCF** for Cu, Cd and As in liver and skin of ***Alb. alburnus*** in relation to **water**, in muscles of ***Abr. brama*** and ***Ch. nasus*** in relation to water; in ***P. laevis*** and ***Contracaecum* sp.** in relation to **water**; in liver, skin and muscles of ***Alb. alburnus*, *Abr. brama* and *Ch. nasus*** in relation to **sediments**; in ***Contracaecum* sp.** in relation to sediments were the highest for **Cd**. The values of **BCF** for Cu, Cd and As in muscles of ***Alb. alburnus*** in relation to **water**, as well as in liver and skin of ***Abr. brama*** and ***Ch. nasus*** in relation to **water** were the highest for **Cu**. The values of **BCF for Cu** in tissues and organs of the three fish species in relation to water and sediments were the highest in liver of *Ch. nasus* ($BCF_{Cu\ Liver/}$

$_{\text{Water}}$=374.71; $BCF_{\text{Cu Liver/Sediments}}$=0.31); **for Cd and As** – in liver of *Alb. alburnus* ($BCF_{\text{Cd Liver/Water}}$=141, $BCF_{\text{Cd Liver/Sediments}}$=0.62; $BCF_{\text{As Liver/Water}}$=7.49, $BCF_{\text{As Liver/Sediments}}$=0.18).

10. The values of **BCF** for the three elements in ***P. laevis* of *Alb. alburnus*, *Abr. brama* and *Ch. nasus*** in relation to water, as well as in ***Contracaecum* sp.** of *Ch. nasus* in relation to **water** and **sediments** were the highest for **Cd**. ***Contracaecum* sp.** of *Ch. nasus* in relation to **water** had the highest value of BCF (BCF_{Cd}=351). The highest value of the bioconcentration factor in ***Contracaecum* sp.** in relation to **sediments** was also found for Cd (BCF_{Cd}=1.74). The values of the bioaccumulation factors (**BAF**) for Cu, Cd and As in parasites in relation to the examined tissues and organs decreased in the order: **muscles > skin > liver**. The value of BAF in ***P. laevis*** in relation to liver, skin and muscles of its hosts, as well as in ***Contracaecum* sp.** in relation to liver of common nase were the highest for **As**, and in ***Contracaecum* sp.** in relation to skin and muscles of common nase were the highest for **Cd**. The highest values of BAF in ***P. laevis*** were found in relation to the muscles of the three fish species, as the highest were in relation to muscles of ***Ch. nasus*** for As (BAF_{As}=194.77), and in ***Contracaecum* sp.** in relation to muscles of ***Ch. nasus*** for Cd (BAF_{Cd}=50.14). The values of bioconcentration factor for Cu in fish tissues and organs of fish in relation to water and sediments were the highest in liver of common nase, and for Cd and As were the highest in liver of bleak. The bioconcentration factors in parasites in relation to water and sediments were the highest for Cd. *P. laevis* of bleak, freshwater bream and common nase had the highest values of bioaccumulation factor for As, and *Contracaecum* sp. of common nase had the highest values of bioaccumulation factor for Cd.
11. For Cu and Cd, there was **a very strong correlation dependence** between the tissues and organs of the three fish species, their parasites, the water and sediments, but **a strong correlation** between the concentrations of Cd in *P. laevis* of bleak and common nase in relation to water (r_s=0.87-0.89, p<0.01). For As, there was **very strong correlations** between tissues and organs of fish and sedimens, as well as between parasites, tissues and organs of their hosts (except for the **strong dependence** between *P. laevis* and liver of freshwater bream (r_s=0.89, p<0.01), and between *Contracaecum* sp., and liver and muscles of common nase (r_s=0.88, p<0.01)) were established. For As, there was **no significant correlation dependence** between tissues and organs of fish, parasites and water. The accumulation of Cu and Cd in the bleak, freshwater bream and common nase is influenced by the food, sediments and water, while the accumulation of As is mainly influenced by sediments and food.
12. The **concentrations** of As and Cd in liver, skin and muscles of *Alb. alburnus* (24.29 to 393.33 times and 2.61 to 22.74 times, respectively); of As in liver, skin and muscles of *Abr. brama* (3.69 to 39.72 times); of As in parasites of fish (5.63 to 12.76 times); of Cu, Cd and As in water/sediments (2.98 to 13.46 times for Cu in water; 3.27 times for Cu in sediments; 1.41 times for Cd in sediments; 132.5 to 1472.22 times for As in water; 73.77 to 97.34 times for As in sediments) were higher for Kudelin biotope than those in previous studies for the lower section of the Danube River in Bulgaria. **The values of BCF** for Cd in tissues and organs of *Alb. alburnus* in relation to water and sediments; for As in tissues and organs of *Alb. alburnus* in relation to sediments (in most cases) from Kudelin biotope **were higher** than those found for the lower section of the river (Vetren biotope). **The values of BAF** for As in *P. laevis* in relation to liver, skin and muscles of bleak and freshwater bream from Kudelin biotope **were lower** than those found for Vetren biotope, except for the bioaccumulation factor for As in *P. laevis* in relation to muscles of freshwater bream from Kudelin biotope, which **was higher** ($BAF_{\textit{P. laevis}\ /\ \text{Muscles}\ \textit{Abr. brama}}$=64.95).

SCIENTIFIC AND SCIENTIFIC-APPLIED CONTRIBUTIONS

1. New data for the content of Cu, Cd and As in liver, skin and muscles of *Ch. nasus* and its parasites *P. laevis* and *Contracaecum* sp. from Bulgaria and the Bulgarian section of the Danube River are presented.
2. New data for the content of Cu in liver, skin and muscles of *Alb. alburnus;* of Cu and Cd in *P. laevis* of *Alb. alburnus*; of Cd in *P. laevis* of *Abr. brama* from the Danube River in Bulgaria; of Cd in liver, skin and muscles of *Abr. brama* from the Danube River and its basin in Bulgaria are presented.
3. Data for the content of Cd and As in liver, skin and muscles of *Alb. alburnus*; of Cu and As in liver, skin and muscles of *Abr. brama*; of As in *P. laevis* of *Alb. alburnus* and *Abr. brama*; of Cu in *P. laevis* of *Abr. brama*; of Cu, Cd and As in water and sediments from the Bulgarian section of the Danube River are updated with the results from Kudelin biotope.
4. Comparative review of the content of Cu, Cd and As in liver, skin and muscles of *Alb. alburnus*, *Abr. brama* and *Ch. nasus* is presented for the first time. The seasonal changes in the content of Cu and Cd in liver, skin and muscles of *Alb. alburnus* and *Abr. brama*; the annual changes in the content of Cu, Cd and As in liver, skin and muscles of *Alb. alburnus* and *Abr. brama*; the seasonal and annual changes in the content of Cu, Cd and As in liver, skin and muscles of *Ch. nasus* from the Bulgarian section of the Danube River are considered for the first time.
5. Data for the seasonal changes in the content of As in liver, skin and muscles of *Alb. alburnus* and *Abr. brama* from the Bulgarian section of the Danube River are updated with the results from Kudelin biotope.
6. The scientific literature on research on the content of Cu, Cd and As in tissues and organs of *Alb. alburnus*, *Abr. brama* and *Ch. nasus*, parasites (*P. laevis*, *Contracaecum* sp), as well as in water and sediments from the freshwater ecosystem of the Danube River is enriched.
7. Excesses of Cu, Cd and As in liver, skin and muscles of *Alb. alburnus*, *Abr. brama* and *Ch. nasus* (except for Cu in liver of *Alb. alburnus*) from the Danube River, Kudelin biotope are established.
8. The circulation of Cu and Cd in liver, skin and muscles of *Alb. alburnus* and *Abr. brama*, as well as the circulation of Cu, Cd and As in liver, skin and muscles of *Ch. nasus* their parasites, water and sediments from the Danube River in Bulgaria are considered for the first time.
9. The circulation of As in liver, skin and muscles of *Alb. alburnus*, *Abr. brama*, their parasites, water and sediments from the Bulgarian section of the Danube River are updated with the results from Kudelin biotope.
10. Data for the values of bioconcentration factor for Cu in liver, skin and muscles of *Alb. alburnus* in relation to water and sediments; for Cd in liver, skin and muscles of *Abr. brama* in relation to water and sediments; for Cu in liver, skin and muscles of *Abr. brama* in relation to water; for Cu, Cd and As in liver, skin and muscles of *Ch. nasus* in relation to water and sediments; for Cu and Cd in *P. laevis* of bleak and freshwater bream in relation to water; for Cu, Cd and As in *P. laevis* of common nase in relation to water; for Cu, Cd and As in *Contracaecum* sp. of common nase in relation to water and sediments; as well as for the values of bioaccumulation factor for Cu and Cd in *P. laevis* of bleak; for Cd in *P. laevis* of freshwater bream; for Cu, Cd and As in *P. laevis* and *Contracaecum* sp. of common nase from the Danube River in Bulgaria are reported for the first time.
11. Data for the values of bioconcentration factor for Cd and As in liver, skin and muscles of *Alb. alburnus* in relation to water and sediments; for As in liver, skin and muscles of *Abr. brama* in relation to water and sediments; for Cu in liver, skin and muscles of *Abr. brama* in relation to sediments; for As in *P. laevis* of bleak and freshwater bream in relation to water; as well as for the values of bioaccumulation factor for Cu in *P. laevis* of freshwater

bream; for As in *P. laevis* of bleak and freshwater bream from the Danube River in Bulgaria are updated with the results from Kudelin biotope.

12. As bioindicators are revealed: liver of common nase and bleak for the content of Cd; liver of freshwater bream for the content of As; *Contracaecum* sp. for the content of Cd; *P. laevis* for the content of As.

RECOMMENDATIONS

1. Removal of the skin of fish before consumption is recommended due to the fact that from the examined tissues and organs, the skin of all three examined fish species during all examined period ranked second in terms of content of Cd and As, which exceeded the norms in Ordinance No. 31.
2. Restricting consumption of the three examined fish species (bleak, freshwater bream and common nase) is recommended due to the reported exceedances in concentrations of Cd and As in samples of muscles in comparison to the norms in Ordinance No. 31.
3. Removal of the internal organs of the small fish such as bleak is recomanteded due to the established high concentrations and exceedances of the three examined elements in the samples of liver.
4. Permanent research (Ministry of Environment and Water, Executive Environment Agency, Ministry of Agriculture, Bulgarian Food Safety Agency) of the content of heavy metals, metalloid in tissues, organs and parasites of fish, water and sediments of the examined section of the Danube River is recommended.
5. Using the liver of *Ch. nasus* and *Alb. alburnus* as a bioindicator for Cd and the liver of *Abr. brama* as a bioindicator for As is recommended.
6. *Contracaecum* sp. to be included in the biomonitoring systems (Ministry of Environment and Water, Executive Environment Agency) as a successful bioindicator for the content of Cd and *P. laevis* as a bioindicator for the content of As is recommended.
7. Strict control (Ministry of Environment and Water, Executive Environment Agency, Regional Environment and Water Inspection) over the quality of water and sediments in the examined section of the Danube River is recommended due to the inflated concentrations of Cu, Cd and As.
8. Other recommendations include optimizing cross-border collaboration in relation to restriction of the negative impact of industry and agriculture; improving the condition of the freshwater ecosystem and preserving the diversity of species.

LIST WITH SCIENTIFIC PAPERS IN RELATION TO THE DISSERTATION

1. **Zaharieva, P., Kirin, D., 2020.** A contribution to the studies on the content of Cu, Cd and As in *Alburnus alburnus* (Linnaeus, 1758) from the Danube River. Scientific Papers. Series D. Animal Science, LXIII (2), 405-412, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750
2. **Zaharieva, P., Kirin, D., 2020.** Content of copper, cadmium and arsenic in *Chondrostoma nasus* (Linnaeus, 1758) from the Danube River. Scientific Papers. Series D. Animal Science, LXIII (1), 481-488, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750.

PARTICIPATION IN INTERNATIONAL SCIENTIFIC CONFERENCES

1. Zaharieva P., Zaharieva R. 2020. Ecologohelminthological investigations and circulation of arsenic in the system Water – Sediments – *Chondrostoma nasus* – *Contracaecum* sp., larvae from the Danube River. Book of Abstracts of 16th International May Conference on Strategic Management - IMCSM20, 29. (EBSCOHost Database; Web of Science) http://media.sjm06.com/2020/09/IMCSM20-Book-of-Abstracts.pdf
2. Zaharieva P., Zaharieva R., 2021. Parasite communities and a content of copper in *Chondrostoma nasus* and *Alburnus alburnus* from the Danube River, Bulgaria. Book of Abstracts of 17th International May Conference on Strategic Management - IMCSM21, 23. (EBSCOHost Database; Web of Science) ISBN 978-86-6305-114-0 https://drive.google.com/file/d/1dm8vGyrgYJosbIYfQvZ_EkRgZZximrk9/view
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