

Ecological and biochemical aspects of assessment of Cu and Ni pollution of freshwater bodies in the Region of Stara Zagora, Bulgaria – a review

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Abstract. The study aims to make an ecological assessment of Cu (copper) and Ni (nickel) pollution of freshwater bodies in the Stara Zagora industrial Region, Bulgaria. A literature reference has been prepared on the concentrations of Cu and Ni at all levels in the food chain: sediment, aquatic plants and fish in the studied region and worldwide. The results of our own research on the amounts of these metals in the sediment and organisms of aquatic biota from water bodies in the region have also been interpreted. When analyzing the obtained data, a negative correlation is established between the accumulation of Cu in the liver of carps inhabiting the Ovcharitsa and Topolnitsa Dams. A dependence of a similar nature is also observed for the element nickel. When comparing the amounts of copper and nickel within the framework of our study, as well as those from other research worldwide, a similar trend is observed regarding the levels of these metals in the liver and musculature of the tested fish. Studies conducted in the Region of Stara Zagora, Bulgaria, and other countries confirmed the ability of hydrobionts to accumulate heavy metals and to be excellent bioindicators for pollution with these toxicants. Global environmental pollution is a prerequisite for continuing research in this area, with other possible environmental markers.

Key words: assessment, copper, nickel, accumulation, fish, freshwater bodies.

Introduction

Due to the growing problem of pollution of hydroecosystems on a global scale, it is necessary to carry out ecological assessments of the state of aquatic bodies at short intervals, especially in anthropogenically dense areas of the world, including Eastern Europe (Bulgaria).

The Republic of Bulgaria is one of the rapidly developing European countries in terms of industry and agriculture. The toxic substances released from production and daily life have a

strong toxic effect, entering various types of water bodies and from there into aquatic organisms and humans.

The Stara Zagora Region (Fig. 1), located in Southern Bulgaria, belongs to the territory of the South-Central Region and is one of the areas with developed industry. It covers an area of 5,147 square kilometres and has a population of 390,000 inhabitants, distributed across 11 municipalities. The regional center is the city of Stara Zagora with a population of 180,000. The territorial scope of the

region includes the Stara Zagora Plain, parts of the Sredna Gora Mountain, the southern slopes of the Stara Planina Mountain, and the Kazanlak Valley. The entire area is part of Bulgaria's transitional continental climate zone, located mainly between

the Stara Planina and Rhodope Mountain ranges. The climate is temperate continental with Mediterranean influence, with an average annual temperature of 13°C and precipitation of 612 mm/km² (Koprlev et al., 2002; Velev, 2010).



Fig. 1. Map of Stara Zagora Region, Bulgaria.

(Source: <https://www.mapsofindia.com/world-map/bulgaria/stara-zagora/location-map.html>)

The region is characterised by a developed agricultural sector, which is linked to the presence of fertile cinnamon forest soils, combined with resinous and alluvial soils. The surface waters, represented by numerous reservoirs and rivers, are complemented by the valuable hot mineral springs in the village of Starozagorski Mineralni Bani, recognised as a valuable recreation resource since ancient times. The municipality is also rich in groundwater. Most of the rivers and reservoirs located in this part of Bulgaria are connected to industrial activity. Wastewater from production contains many toxic compounds that can enter aquatic organisms, disrupting their life processes (Municipal development plan of Stara Zagora municipality for the period 2014-2020, Velder Consult, 2013).

The coal power plant Mini Maritsa East AD is located within the Stara Zagora Region, which has subjected the area to strong anthropogenic pressure affecting both the soil and the water, sedi-

ments, and all representatives of the flora and fauna found in this territory (Georgieva et al., 2010; Valkova, 2022).

A significant number of waste products and chemicals from households, industry, and agriculture contain heavy metals such as Cu and Ni, which determine a high level of risk of contamination of hydroecosystems with these elements and the resulting metabolic changes in the organisms of hydrobionts and humans. Aquatic organisms can serve as excellent biological indicators of the presence of various pollutants that pose a high risk to animals and humans.

Pollution of aquatic ecosystems

The metabolic reactions of aquatic organisms are directly dependent on the indicators of the waters they inhabit. Nowadays, pollution of water bodies is observed, which leads to a deterioration in water parameters and, consequently, in the vital indicators of the hydrobionts inhabiting them.

The operation of industrial and agricultural enterprises and the production of electricity are the cause of the pollution of water bodies with compounds that are characterised by carcinogenic and mutagenic effects.

The entry and accumulation of toxicants in high doses render the water unsuitable for use as a means of subsistence and a living environment (Valkova, 2022).

The main factor affecting hydroecosystems is human activity. Its impact leads to significant disturbances in the structural and functional state of ecosystems, as well as to a reduction in the diversity of plant and animal species. Pollutants can be of various types, but heavy metal pollution has a powerful toxic effect, which depends not only on the amount and method of introduction into the environment, but also on the total amount of water inflow, the type of water basin, the temperature regime, the flow velocity, etc.

Pollutants can be considered to be all substances contained in the aquatic environment that, above a certain concentration, cause negative effects on the entire system or parts of it. The numerical value of this concentration is referred to as the “threshold” or “threshold level”. In order to correctly determine the permissible threshold limit for content, it is necessary to take into account not only the process of interaction between toxicants but also how environmental factors affect them.

Hydroecosystems can be polluted by various types of sources, such as natural (lakes, rivers, seas, and oceans), waste (industrial and domestic), and rainwater and groundwater.

Pollution with heavy metals such as copper and nickel above their threshold values covers increasingly large areas of the world's oceans, which is already on aquatic organisms and humans. Very often, these elements can be the primary pollutants of water bodies, with sources ranging from metal waste to dyes, pesticides, etc. (Valkova et al., 2022). The degree of toxicity of these elements depends both on their quantity and duration of action, as well as on factors such as temperature, pH of the water, type of water body, etc.

The quantities of these metals found in the air of a given region are of significant importance for the content of heavy elements in water and living systems. High levels of metals in the air and water often cause the onset and development of diseases

resulting from serious disturbances in biochemical reactions. One such disease in humans is Chronic Obstructive Pulmonary Disease (COPD).

Depending on their type and effect on hydroecosystems, heavy metals Cu and Ni are classified as priority pollutants (Fuentes et al., 2007; Gasperi et al., 2008).

To take adequate measures against the pollution of water bodies with copper and nickel, it is necessary to summarise the information at both the national and global levels. This determined the objective of this review, namely, to assess freshwater sites in Bulgaria based on the content of Cu and Ni in sediments and hydrobionts as ecological markers of pollution. The data presented are analysed by comparison with results from other countries and regions.

Standards in Bulgaria and Europe regulating the permissible limits of heavy metals in aquatic ecosystems

At the European Union level, regulatory documents on water include over 25 directives and decisions. Most of these legal provisions, which are in line with the conditions characteristic of Bulgaria, are included in the existing Bulgarian Water Act (WA), which was adopted on 28 January 2000. The application of the WA regulates water management and protection and public health (Valkova, 2022).

On 14 September 2012, Regulation No. H-4 was adopted in Bulgarian legislation for the characterization of surface waters. It is based on European Directive 2008/105/EC on the establishment of environmental and water quality standards. Regulation No. H-4 includes quality standards relating to the content of certain chemical elements and other substances, including the content of heavy metals Cu and Ni.

The Regulation on Environmental Quality Standards for Priority Substances and Certain Other Pollutants of Bulgarian legislation regulates the standards relating to priority pollutants such as heavy metals Pb, Cd, Hg, etc., which have a high degree of negative impact on organisms, including aquatic organisms.

Sediment is an integral part of any hydroecosystem. Its composition can be used to determine the actual state of water bodies, as it provides information about the history of a given water reservoir. This requires their study to include the ana-

lysis of sediment samples. European and Bulgarian legislation contain regulatory documents that limit the amounts of heavy metals in soils. There are no such regulations for sediment. This requires that the standards for arable soils, regulated in

Regulation No. 3 of 1 August 2008 of the Bulgarian legislation on the standards for permissible content of harmful substances in soils, be used when discussing the results obtained (Table 1).

Table 1. Maximum permissible concentration standards for heavy metals Cu and Ni in arable land soils (according to Regulation No. 3 of 1 August 2008 of Bulgarian legislation).

Heavy metal	pH (H ₂ O)	Arable land
Copper (Cu)	6.0	150
Nickel (Ni)	6.0 - 7.4	110

Global research shows that the amounts of heavy metals in water reflect the current state of water bodies. It can therefore be argued that the organisms inhabiting the respective hydroecosystems reflect their condition and can be used as bioindicators showing the actual state of the water bodies. Significant amounts of heavy metals accumulate in the bodies of hydrobionts over months and years, which, above certain levels, lead to the disruption of several biochemical processes in these organisms (Valkova, 2022; Valkova et al., 2022; Atanasov et al., 2011).

There are no regulatory documents that clearly define the concentration limits for heavy elements in the bodies of hydrobionts inhabiting different types of water bodies. A more comprehensive regulatory document in Bulgaria was Regulation 31 of 29 July 2004, on the maximum permissible amounts of pollutants in food, which was active until recently and imposed restrictions (MAC) on the content of most highly toxic metals.

In our studies, we used freshwater fish, inhabiting different types of water bodies – dams and rivers in the Stara Zagora Region of Bulgaria. Therefore, Table 2 includes the requirements of Regulation 31 concerning only these organisms.

In order to comply with European requirements, on 9 February 2015, Regulation No. 5 entered into force in Bulgarian legislation and is practically an annex to Regulation (EO) No 1881/2006, amended in 2010, that determines the maximum permissible concentrations of certain contaminants in foods, considering local conditions in Bulgaria. These regulatory documents have been replaced by Regulation (EU) No. 2023/915, which, together with the previous two documents, determines the permissible amounts of contaminants with a view to protecting public health. It is clearly explained there that the regulated MACs apply only to the part intended for consumption of the respective foods, unless otherwise specified in the annex.

Table 2. Maximum permissible levels for certain heavy metals in fish (according to Regulation 31 of 29 July 2004 on the maximum permissible quantities of contaminants in food, which is currently inactive).

Chemical element	Food	Standard (mg.kg ⁻¹ of fresh product)
Copper (Cu)	Freshwater fish	10
Nickel (Ni)	Freshwater fish	0.5

The documents mentioned above do not contain restrictions for the metals copper and nickel in fish analysed in this study, which is why we cannot use them as a benchmark for data analysis. There are only standards for metals of the rank of Pb and Cd. The standards specified in these docu-

ments can only be met if modern and accurate methods are used to analyse the samples from the studied hydrobionts. Atomic absorption is such a method, which is characterised by high selectivity, speed, and sensitivity. The capacity of the apparatus used for the study is 69 elements. The method

is widely used to determine traces of metals and metalloids in food, natural and industrial samples, clinical and pharmaceutical samples, etc.

Hydrobionts as Actual Biological Indicators for Monitoring of the Aquatic Environment.

Variations in the values of the main parameters of the aquatic environment (including the content of heavy metals) lead to a disruption of the biochemical status of hydrobionts (Zaykov, 2008; Valkova et al., 2016; Valkova, 2022). The hydrochemical indicators of water also include the concentration of heavy elements (Valkova, 2022), which, above certain values, have a strong toxic effect on the condition of aquatic organisms. This requires constant monitoring of the levels of these metals, given their significant importance for the state of the environment and the health of animals and humans (Valkova et al. 2015; Valkova et al., 2016). The reason for the high toxicity of heavy metals is their ability to cause the formation of ROS (reactive oxygen species), which are irreversible inhibitors of many enzymes and transport proteins (Flora et al., 2008).

The status of organisms inhabiting different types of water bodies provides a better reflection of the condition of water reservoirs. The algae, as well as many species of higher aquatic plants, form the basis of aquatic food chains. Algae also enrich the water with oxygen and facilitate sedimentation processes, thus facilitating the self-purification of water (Sirakov et al., 2003). Water pepper (*Polygonum hydropiper* L.), which belongs to the higher aquatic plants, has proven to be a good indicator used to assess the condition of freshwater bodies (Atanasov et al., 2011).

Organisms characterised by autotrophic metabolism establish the connection between the water components and the animal elements of food chains. Humans, as final consumers, consume mainly meat from various types of hydrobionts and, to a lesser extent, caviar and other products such as fish oil from certain fish species. In addition to being a key link in food chains, fish serve as excellent bioindicators of water body condition, as they respond sensitively to fluctuations in monitored parameters, particularly heavy metal concentrations (Valkova et al., 2015, 2016, 2021a,b; Schilderman et al., 1999; Arnaudova et al., 2008).

A large number of aquatic organisms are suitable as bioindicators for both passive and ac-

tive biomonitoring of water bodies. A typical representative is the zebra mussel (*Dreissena polymorpha* (Pallas, 1771), which can serve as a biological filter, purifying the water and providing a better environment for the development of other hydrobionts (Sures et al., 1997; Camusso et al., 2001; Matev & Georgiev, 2007; Valkova, 2014). Suitable fish species for monitoring heavy metal content are the common carp (*Cyprinus carpio* L.) and crucian carp (*Carassius* spp.), which are widespread species, resistant to low temperatures, and occupy accessible habitats. For this reason, these species are often used as biological objects in numerous studies aimed at determining the quantities of various toxicants characteristic of hydroecosystems (Valkova et al., 2015; Łuszczek-Trojnar & Nowacki, 2021).

Impact of Cu and Ni accumulation on the life processes characteristic of aquatic organisms and humans

The biogenic elements Cu and Ni, in minimal quantities, are necessary components that participate in a large number of reactions essential for metabolism in both hydrobionts and humans. Heavy metals can be dissolved in the aquatic environment, colloidally dispersed, or suspended. High levels of these types of metals in water lead to their accumulation in sediments and aquatic organisms, which inevitably leads to a significant disruption of the ecological balance in the specific hydroecosystem (Valkova, 2022). This also affects terrestrial ecosystems, affecting all trophic levels. Wastewater from many industries and agriculture can also be a source of these elements.

Heavy metals enter fish and invertebrates in the form of ions, which penetrate through the skin, gills, and through food of various origins (Valkova, 2022).

Metals such as copper and nickel accumulate mainly in organs such as the liver, gonads, roe, and gills, and, to a lesser extent, in the muscles of fish. These elements easily pass into products such as roe and the larvae developing from it (Beattie & Pascoe, 1978; Dave & Xiu, 1991; Brauner & Wood, 2002; Davies et al., 2005; Zhelyazkov et al., 2018).

The impact of heavy metals is mainly expressed in the form of oxidative stress, but most of them (including copper and nickel) also have a mutagenic and carcinogenic effect (Kitchin & Ahmad, 2003). Studies have shown that heavy metals

such as Ni (Hg, Cd, Pb, etc.) affect chemical synaptic transmission in the brain and peripheral systems (Annau & Cuomo, 1988; Casdorff & Walker, 1995; Valkova, 2022).

The interest in copper (Cu) in biology, and more specifically in medicine, is dictated by its global importance for organisms and its toxic effect when it exceeds certain levels. Copper is one of the biogenic elements that play a key role in the normal course of a number of biochemical processes in organisms. In very small quantities, it is necessary for the synthesis and proper functioning of a number of enzymes in the liver, brain, and muscles. This microelement is part of cytochrome oxidase, the terminal enzyme of the respiratory chain in mitochondria, and is therefore necessary for energy generation processes in cells. It also regulates processes such as the metabolism of catecholamines, serotonin, and tyrosine, helps to increase insulin activity, and promotes the complete utilisation of carbohydrates. Copper is also necessary for the production of the pigment melanin, which influences the synthesis of collagen, which is responsible for maintaining healthy bones, cartilage, and skin (Valkova et al., 2013; Valkova, 2022). Copper-containing enzymes (superoxide dismutase) inhibit the production of free radicals or neutralise them (Sevcikova et al., 2011). This metal is also part of other enzymes, such as tyrosinase and lactase. It serves as a catalyst and aids in the assimilation of iron in the body (Atanasov et al., 2011).

Copper accumulates mainly in the tissues of freshwater fish and invertebrates and, to a lesser extent, in marine organisms due to its high complexing ability. It is concentrated mainly in the liver of aquatic organisms. High concentrations of copper have also been recorded in the gills and gonads of fish (Valkova, 2022; Nastova-Gyorgioska et al., 2006).

This element is usually present in potentially harmful concentrations together with other heavy metals (zinc). Copper ions have a highly toxic effect, destroying the blood-forming organs in the gills, which in turn leads to death by asphyxiation. The lethal concentration of Cu in aquatic organisms depends on the species and age of the individuals and the duration of exposure.

Hydrobionts such as fish and mussels are often found in the human diet. These foods are a useful source of many valuable ingredients, but

they may also contain large amounts of heavy metals such as copper. When ingested in high doses, Cu can lead to the development of anaemia, kidney and liver disease (Teschke, 2024).

Nickel (Ni), even in small quantities, has a strong effect on various types of aquatic plants and animals (Barceloux, 1999; Phipps et al., 2002). High concentrations of this metal pose a real threat to aquatic ecosystems due to its persistence and bioaccumulation (Valkova, 2022). They are the reason for the negative impact of copper on the growth, reproduction, and behaviour of aquatic organisms, as well as for the occurrence of diseases in animals and humans (Valkova, 2022; Regulation No. 31, 2004).

Exposure to Ni in the environment occurs through inhalation, ingestion, and skin contact. Humans are in constant contact with high levels of this metal, mainly due to its widespread distribution in the air, water, food, and products used in everyday life and practice (Faroon et al., 2012). Most of the nickel in aquatic ecosystems is absorbed mainly by unicellular organisms (bacteria, algae, etc.) and invertebrates. Aquatic invertebrates are a major food source for fish and, therefore, an important link in the Ni transport chain to fish. The toxic effects of this metal on fish have been established and described (Pane et al., 2003a,b, 2004a,b, 2005; Brix et al., 2004), while information on invertebrates is rather limited (Sreedevi et al., 1992; Martinez-Tabche et al., 1999; Rathore & Khangarot, 2002).

According to a number of studies, the toxicity of Ni²⁺ is achieved through inhibition of Na⁺ - Ca²⁺ exchange. Through ion mimicry, nickel ions (Ni²⁺) compete with Mg²⁺ and Ca²⁺ in the cell membrane, thereby prolonging the period of cell repolarisation and subsequently causing various functional disorders (O'neill et al., 1988; Rainbow & Dallinger, 1993; Bridges & Zalups, 2005; Pane et al., 2006). High nickel levels cause oxidative damage to DNA and proteins, which can lead to the inhibition of cellular antioxidants (Eisler, 1998). Aquatic organisms are more sensitive to Ni²⁺ during their embryonic development than adult individuals. High concentrations of nickel ions have a negative effect on the hatchability of eggs and the development of larvae during the yolk sac resorption phase in aquatic organisms (Hunt et al., 2002).

The immediate effects of Ni in humans are respiratory tract irritation and neurological effects

such as headache, nausea, and diarrhoea. Nickel also has a proven carcinogenic effect (Nickel: Toxicological Overview, 2009).

A wide range of heavy metal ions (including copper and nickel) is present in water bodies, which in higher concentrations have a complex negative impact on aquatic organisms. Of greater scientific importance are the antagonistic interactions between these elements. Studies have found that the intake of larger amounts of trace elements such as Cu, Ni, Zn, etc. leads to a reduction in the negative effects caused by metals such as Pb and Cd. From a scientific point of view, the influence of zinc, which reduces the effect of heavy metals such as lead and copper in membrane structures, is interesting (Valkova, 2022).

The content of toxicants such as heavy metal ions in water is indicative of the current state of water bodies. Hydrobionts have the ability to accumulate these metals over time, which is why they are more reliable bioindicators showing the actual status of the water bodies studied. Different organs and tissues in aquatic organisms have different abilities to accumulate these elements due to the presence of specific mechanisms for accumulation at the biochemical level. The transfer of heavy metals in aquatic food chains starts from the water, passes through the sediment, and reaches the plant and animal representatives of the hydrobionts.

Cu and Ni content in the sediments of water bodies

Historically, the composition of sediment is extremely indicative of the ecological and biochemical status of a given water basin. Sediments deposited at the bottom could even illustrate temporary pollution with short and rare peak concentrations. Sediment, acting as soil, continues the food chain in hydroecosystems. In this regard, it is common practice in aquaculture to periodically drain freshwater bodies and plough and fertilise their bottoms. As a result of these activities, a significant increase in hydrobiont yields per unit of water area is observed after stocking.

Heavy elements are present in the aquatic environment in the form of organic and inorganic structures. They cannot be eliminated and, when present in high concentrations, accumulate in the sediments of water bodies. The sedimentation process is directly dependent on the speed of the

water flow and the size of the sedimenting particles. As a result, a decrease in the concentrations of various pollutants (including heavy metals) in the water is observed.

Sorption and ion exchange are opposite processes that affect the composition of heavy metals in sediments.

The study by Algül & Beyhan (2020) found that a large proportion of heavy metals (including nickel, copper, cadmium, etc.), regardless of their origin (natural or from dyes and fuels used in fishing boats, phosphate fertilisers and pesticides used in agricultural areas, or domestic wastewater), are concentrated and accumulated in the sediment of the freshwater lake Bafa (Turkey). This study recorded high levels of Cu and Ni in the sediment. During the analysis, it was found that in autumn, when the water flow is greater due to rainfall, the concentration of these elements also increases, which confirms the importance of the flow rate for the sedimentation of metals in the soil (sediment) (Valkova, 2022).

In their study, Chițescu et al. (2021) track seasonal changes in the amounts of organic and inorganic pollutants (including heavy metals such as Cu, Cd, Zn, Pb, etc.). Their analysis shows the accumulation of higher doses of the metals studied in the sediment of the Danube River at the delta before the Black Sea, which proves the role of sediment as a natural filter for heavy metal pollution, retaining them through ion exchange and adsorption processes (Chitescu et al., 2021).

Zimovets & Ovsepyan (2021) conclude in their study that in winter, there is a high degree of self-purification of water for the elements Cu (54%), Pb (25%), Cd (67%), Cr (82%), and for bottom sediments – Zn (42%) and Ni (56%). In summer, a high degree of self-purification of river water was found for Zn (44%) and Ni (50%), and for bottom sediments – Cu (37%), Pb (90%), and Cd (75%). The results recorded in this study show that high levels of self-purification of river water are characteristic of Cu during both the cold and warm seasons, that even though the concentration of this metal in the river water at the mouth of the Northern Dvina (located in the northern part of the European territory of Russia) exceeds the maximum permissible concentrations in the river throughout the year. In this regard, it can be concluded that sediments contribute significantly to the self-purification of water bodies (rivers).

The results obtained from these studies conducted in Europe show the need for continuous monitoring of heavy metal levels in sediments and water bodies located in the Stara Zagora Region, Bulgaria. In our own studies, we monitored the levels of certain heavy metals (including copper and nickel) in the sediments of Zagorka Lake, the Ovcharitsa Dam, Opan Dam, Pastren Dam, Gita Dam, Zmeevo MicroDam, Sazliyka River, Tundzha River, Yagoda Village, Zhrebchevo Dam, the Nikolaevo checkpoint, the Zhrebchevo Dam wall, and the Tundzha River after the Zhrebchevo Dam, all located in the Stara Zagora Region, Bulgaria.

Content of Cu in the sediments of freshwater basin on the territory of Stara Zagora Region, Bulgaria

During the biochemical study, fluctuations in copper content were found in sediment samples taken from sampling points in the Stara Zagora Region, Bulgaria. It was found that higher levels of this element were observed in the sediments of Zagorka Lake and the Sazliyka River (44.019 mg.kg⁻¹ and 47.158 mg.kg⁻¹, respectively), while the highest levels were recorded in samples from Zhrebchevo Dam, Nikolaevo checkpoint (86.16 mg.kg⁻¹), and Ovcharitsa Dam (86 mg.kg⁻¹). The lowest copper values were recorded in the sediments taken at the wall of the Zhrebchevo Dam (8.78 mg.kg⁻¹) and the water body located near the landfill in the Zmeevo Village (7.06 mg.kg⁻¹). The reported Cu values in the analysed water bodies are significantly lower than the regulated MAC for grasslands in the regulatory documents of Bulgarian legislation (140 mg.kg⁻¹). The quantities reported in the sediments of the Ovcharitsa and Zhrebchevo Dams, Nikolaevo checkpoint, accumulated in plant organisms over time, can certainly have toxic effects on other participants in food chains (Valkova, 2022).

Content of Ni in the sediments of freshwater bodies on the territory of Stara Zagora Region, Bulgaria

Analysis of sediment samples taken from the above-mentioned water bodies shows that the detected Ni levels do not exceed the regulatory requirements of Bulgarian legislation regarding the content of metals in soil fractions (Regulation No. 3, 2008).

Among the sediment samples from the studied water bodies with the highest concentrations

of nickel (Ni), the sampling points at the Zhrebchevo Dam – Nikolaevo checkpoint (104 mg.kg⁻¹) and the Ovcharitsa Dam (35.5 mg.kg⁻¹) stand out. According to this indicator, only the Zhrebchevo Dam – Nikolaevo checkpoint approaches the MAC in arable land, with a concentration lower than the standard by 6 units. Relatively high concentrations of Ni were also recorded in the sediments of the Opan and Gita Dams. Again, a downward gradation in the levels of this metal is observed in the direction of the Zhrebchevo Dam – Nikolaevo checkpoint (104 mg.kg⁻¹), the Zhrebchevo Dam – wall (16.1 mg.kg⁻¹), and the Tundzha River after the Zhrebchevo Dam (5.6 mg.kg⁻¹) (Valkova, 2022). The lowest nickel values were recorded in the sediment of the water body near Zmeevo (4.6 mg.kg⁻¹).

Cu and Ni content in algae and higher aquatic plants, which act as biological indicators in freshwater bodies

Freshwater bodies (rivers, dams, etc.), which are habitats for aquatic organisms, are often used for industrial purposes. The resulting wastewater re-enters the water bodies, together with the heavy metals it contains. The presence of excessive levels of metals in the water leads to their entry into the organisms of hydrobionts, mainly through feeding and water intake. Heavy elements completely change their life cycle, leading to a series of pathological changes.

Algae are the hydrobionts that are the primary producers of organic matter in water bodies. They are at the base of the food chain, as they serve as food for many invertebrates and vertebrates. This group of organisms includes phytoplankton, which are microalgae that live suspended in the water and belong to different taxonomic groups. As autotrophic organisms, microalgae often initiate food chains in water bodies, serving as food for many species of oysters, mussels, fish, etc. (Velichkova, 2014; Valkova, 2022).

Both phytoplankton and other algae are extremely sensitive to pollutants of various kinds, which is why they are an excellent indicator of the ecological status of water bodies. The importance of higher aquatic plants (e.g., water buttercup) is not limited to their role as a food source. These organisms are equally sensitive to heavy metals and other types of pollutants and can serve as biological markers for water pollution.

Cu content in algae and higher aquatic plants inhabiting freshwater bodies in the Stara Zagora Region, Bulgaria

The microelement copper (Cu) plays a key role in plant cells, being part of specific enzymes such as cytochrome oxidase and others. In high doses, this metal has a toxic effect, causing the formation of free radicals and subsequent oxidation. By examining samples of algae and water pepper in the present study, we found high levels of copper in the Opan Dam (85.8 mg.kg⁻¹) and Ovcharitsa Dam (68 mg.kg⁻¹) as well as in Zagorka Lake (50.5 mg.kg⁻¹). The recorded levels exceed multiple times those from the samples taken at the wall of the Zhrebchevo Dam (8.32 mg.kg⁻¹) and Tundzha River after the Zhrebchevo Dam (10.62 mg.kg⁻¹). There is a trend towards high levels of heavy metals in the first three reservoirs and significantly lower levels in the samples taken from the Zhrebchevo Dam and Tundzha River after the Zhrebchevo Dam. Interestingly, the copper levels measured in the water pepper (23 mg.kg⁻¹) are lower than most of the values measured in algae. Most likely, in higher aquatic plants, copper as a microelement is needed in larger quantities for their vital functions, and its accumulation there is to a lesser extent (Sirakov & Velichkova, 2014; Velichkova et al., 2016; Valkova, 2022).

Ni content in algae and higher aquatic plants inhabiting freshwater bodies in Stara Zagora Region, Bulgaria

Nickel (Ni), as a biogenic element, even in low doses, aids oxygen transport, stimulates metabolism, and plays a key role in many enzyme systems typical of plant and animal organisms. In addition, it participates in the transmission of the genetic code and is present in the enzymes that carry out carbohydrate metabolism. Nickel is essential for biochemical reactions in plants, animals, and humans (Zambelli & Ciurli, 2013).

During our study, the highest concentrations of this metal were found in algae samples from the Ovcharitsa Dam – 4.39 mg.kg⁻¹, where toxic effects are possible due to the high levels present. To a lesser extent, this is also possible in samples from the Tundzha River at the Nikolaevo checkpoint (3.10 mg.kg⁻¹) and Tundzha River after the Zhrebchevo Dam (3.15 mg.kg⁻¹). High nickel values were also measured in the water pepper samples (2.85

mg.kg⁻¹). Likely the biological mechanisms of accumulation in algae and higher aquatic plants are similar and that these plants could be used to purify river beds and water bodies from nickel (Sirakov & Velichkova, 2014; Velichkova et al., 2016).

Cu and Ni content in carp liver (*Cyprinus carpio* L.) from freshwater bodies

The liver is an organ with a very high metabolic load, which allows the passage of significant amounts of blood and lymph, delivering components of exogenous and endogenous origin. In this respect, this organ could be used as a reliable biochemical indicator in the studied hydroecosystems due to its ability to bioaccumulate various substances, including heavy metals. This necessitates an analysis of the content of certain heavy metals in liver samples from cyprinid fish.

Zhang et al. (2014), tracking the amounts of certain heavy metals in fish organs and tissues such as the bladder, intestines, liver, gills, and muscles, reemphasize the retention role of the liver, thanks to which the concentrations of metals in other parts of the body are lower. Specifically, in this study, Cu levels in the liver (2.79 mg.kg⁻¹) are found 3-5 times higher than those in other organs. Cadmium and chromium are also concentrated in the liver of freshwater fish. Here, the difference in metal content between the liver and other organs and tissues of fish is significant.

Dulich et al. (2018) confirm this thesis by performing an analysis aimed at determining the amounts of about 20 elements, including the heavy metals Cu, Zn, Pb, Cd, Ni, Fe, Mn, and Cr in the liver and musculature of *Cyprinus carpio*, and find significantly higher levels of these elements in the liver compared to the muscles. Their results clearly show the accumulative capacity of metabolically active organs such as the liver. Apparently, metal accumulation is activated in spring and summer, and according to Dulić et al. (2018), accumulation decreases during the colder months, but the high content of heavy metals in the liver remains.

Yancheva et al. (2014) conducted studies in 2012 in the Topolnitsa Dam, Bulgaria, and their analysis of the data showed high concentrations of heavy metals in the organs they examined. The results concerning the liver of cyprinids in the spring, where the recorded values of Cu and Ni were 16 mg.kg⁻¹ and 14 mg.kg⁻¹, respectively, are

impressive. The results concerning the liver of cyprinid fish in spring show recorded values of Cu and Ni being 16 mg.kg⁻¹ and 1.7 mg.kg⁻¹, respectively. This can be explained by the fact that during this season, the rate of metabolic reactions is high and the accumulation of these metals is maximal. The microelement copper is part of the composition of several enzymes and transport proteins, and, despite its necessity for metabolism in spring, it has clearly not been consumed, and the reported levels are much higher than the norms for Cu content in fish in Bulgarian legislation at that time – 10 mg.kg⁻¹. The high metal values measured in the liver of these fish prove the ability of this organ to act as a biomarker for pollution, as well as its accumulative capabilities. Subsequently, Georgieva et al. (2016) confirmed the presence of high levels of the heavy metals copper and nickel in liver samples from carp inhabiting the Topolnitsa Dam during the study period. During the spring season, the following concentrations of these elements were recorded: Cu–15.7 mg.kg⁻¹; Ni–1.3 mg.kg⁻¹. The results obtained in this study prove that the liver is the main depot for the bioaccumulation of heavy metals (Shinn et al., 2009; Poleksic et al., 2010).

The high concentration of metals in the liver of fish may be related to the processes of haematopoiesis and detoxification, as well as to the antioxidant defense system and the excretion of metals from the body (Siscar et al., 2014).

The metals copper and nickel, which are also essential elements, were also found in high levels, but lower than those of lead and zinc, which is entirely understandable given their role in the structure of enzyme systems that are important for metabolism.

Cu content in the liver of carp from the studied water bodies in Stara Zagora Region, Bulgaria

The microelement copper (Cu) is essential for hydrobionts due to its participation in the composition of proteins with transport (cytosol) and antioxidant functions (the transport pigment haemocyanin and the enzymes peroxidase, catalase, etc.). Higher levels of this metal in the liver of fish pose a potential risk of large quantities entering the bodies of secondary and tertiary consumers (certain animal species and humans).

During the study period (2010), the highest values were recorded in liver samples from carp delivered from the Zagorka, Zetovo, Ovcharitsa, and Chirpan Dams. The measured values exceed or are close to the MAC regulated in Regulation 31 of Bulgarian legislation, which was in force at the time of the study. On 9 February 2015, Regulation No. 5 came into force, which is an application of Commission Regulation (EU) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Regulation 1881 was subsequently replaced by Commission Regulation (EU) No 2023/915 (2023) on maximum levels for certain contaminants in foods.

The regulations in force from 2015 to date do not contain restrictions for copper (Cu). Analysis of the data shows that the highest Cu values are found in the livers of carp from Zagorka Lake (an increase of 26.5% compared to the MAC in Regulation 31, active during this period), while the lowest values are found in samples from Lake Pastren (90.2 % below the MAC in the same regulation). The liver samples taken from Zagorka Lake and Zetovo Dam exceeded the regulatory requirements at that time by 2.65 and 2.63 units, respectively.

Nachev & Sures (2016), in their study, found similar and higher copper values in the liver of the representative of the Cyprinidae family, *Barbus barbus*, in the range of 11.76 mg.kg⁻¹ and 16.72 mg.kg⁻¹.

Despite the lack of standards set in current legislation, the higher copper levels suggest possible pollution of these hydroecosystems during the study period in 2010 (Fig. 2). The minimal changes in the aquatic environment are again reflected in the liver due to its proven accumulation capacity.

In 2011, the highest concentrations were recorded in samples from the Ovcharitsa Dam (Fig. 2). Compliance with the regulatory requirements in force at the time was initially established in the study conducted in 2010, when the concentration recorded in the samples from the Ovcharitsa Dam was almost equal to the MAC. The amounts of copper measured during these periods clearly indicate the presence of high levels of this metal in the studied ecosystem and possible pollution at a given moment.

The determination of the actual ecological and biochemical status of the Ovcharitsa Dam required a separate investigation, and its realisation

in 2014 refuted the hypothesis of permanent Cu pollution of Ovcharitsa Dam and the presence of higher levels of the same metal in the fish livers. The study also found that the copper concentrations were twice lower than the then standards in Bulgarian legislation. The study found a twofold

decrease in the concentrations of this metal compared to the then standards in Bulgarian legislation. The gradual decrease in Cu concentrations from 2010 to 2014 indicates the probable presence of higher levels of this metal in the Ovcharitsa Dam in the past.

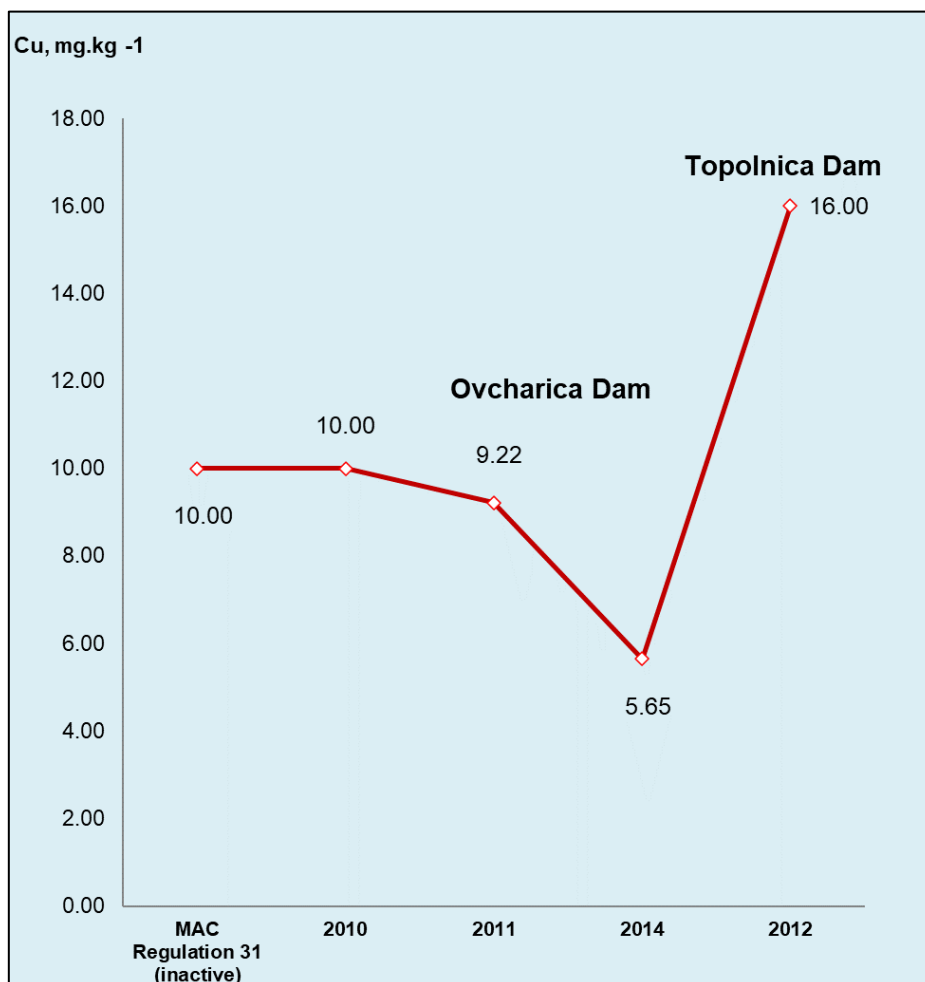


Fig. 2. Cu content in the liver of carp inhabiting the Ovcharitsa Dam (2010-2014) and the Topolnitsa Dam (2012).

In the study conducted by Yancheva et al. (2014), the reported Cu levels (16 mg.kg⁻¹) are relatively high compared to the maximum values measured in the liver of fish from the Ovcharitsa Dam (10 mg.kg⁻¹; 2010). The amounts of Cu in the samples from the Ovcharitsa Dam correspond to those recorded in the liver of carp from the Topolnitsa Dam regarding the accumulation of the studied metal copper.

The results obtained in this study, as well as those from other studies, provide clear evidence that the main depot for the accumulation of copper is the liver of hydrobionts (Peyghan et al.,

2003; Salami et al., 2008; Karayakar et al., 2010; Papagiannis et al., 2004; Malhotra et al., 2020). It is no coincidence that this organ is called the "biochemical laboratory of the body", as it detects even the smallest deviations in the amounts of heavy metals in water bodies (Valkova et al., 2022).

Ni content in the liver of Common Carp from the studied water bodies in Stara Zagora Region, Bulgaria.

When ingested in low doses, nickel (Ni) is sufficient to meet metabolic needs, which is why it is classified as an essential element. This metal aids

in the production of blood cells, but in large doses it has a powerful toxic effect. High levels of Ni are a likely cause of health problems such as severe weight loss, heart and lung damage, and skin irritation (Singh & Sharma, 2024; Sharma et al., 2025).

Nickel is widely distributed in nature and is often present in elevated concentrations in hydroecosystems. The reasons for this are complex and related to industrial activity and environmental factors (Eisler, 1998; Muysen et al., 2004; Haque et al., 2024). Ni concentrations are typically below 10 µg/l in clean waters, but can reach up to 1000 µg/l in heavily polluted waters (Eisler, 1998; Reza & Singh, 2010). In general, nickel is considered responsible for the high species diversity in animals, but its importance for hydrobionts has not been fully established (Muysen et al., 2004). Global studies report Ni-related depressive states of the immune system in both vertebrates and invertebrates (Eisler, 1998; Harkin et al., 2003; Vijayavel et al., 2009; Sun et al., 2011). It has been established that in *Scylla serrata* (Forsskål, 1775) (Crustacea: Malacostraca), Ni modulates the haemocyte defense system (Vijayavel et al., 2009). According to other studies, Ni has a suppressive effect on immune responses in fish, reducing the number of lymphocytes (Zelikoff et al., 1996; Min et al., 2015). In mammals (including humans), Ni also has suppressive effects on the immune system (Zhang et al., 2008; Guo et al., 2020).

During our study in 2010, the highest Ni values were recorded in liver samples originating from the Zagorka, Chirpan, and Zetovo water bodies. None of the samples reached the MACs regulated by Regulation No. 31, which was in force at the time. The highest value was recorded in specimens from Zagorka Lake, and the lowest in those from Koprinka Dam (46% and 97% below the permissible value, respectively). The Ni concentration recorded in the livers of fish from Zagorka Lake was significantly lower than the MAC regulated at that time, which indicates the absence of toxic levels of this metal during the above-mentioned period (2010). Zagorka Lake is significantly lower than the MAC regulated at that time, which indicates the absence of toxic levels of this metal during the above-mentioned period (2010), which could potentially lead to a disturbance of the ecological balance in the studied hydroecosystem (Valkova, 2022).

During the 2011 study period, we found that higher concentrations of nickel were present in samples from the Ovcharitsa and Pastren water bodies. All recorded values are well below the regulatory requirements of Regulation No. 31, which was in force at the time. The highest value was recorded at the Ovcharitsa Dam, and the lowest at the Opan Dam (86% and 92% below the MAC, respectively).

The analysis of the nickel data from 2014 shows that the amounts recorded in the livers of the carp from the Ovcharitsa Dam are three times lower than the standards specified in Regulation 31, which was in force at the time (70% below MAC). The levels measured in 2014, compared to the data from 2011, show an increase of 114%, which is most likely due to an increase in the levels of this metal in the studied ecosystem (Valkova, 2022).

When comparing Ni values for the period 2010–2011, a gradual decrease in the levels of this heavy element is observed in samples from the Ovcharitsa, Opan, and Pastren water bodies in 2011 compared to 2010. The observed changes are most likely based on the ability of different individuals to accumulate different amounts of heavy metals depending on their own metabolism, as well as on changes in environmental conditions. In 2014, an increase in Ni concentrations was reported, which is most likely related to a slight increase in its content during this period and in the waters of the Ovcharitsa Dam. The results of the study conducted in 2010, 2011, and 2014, with the highest value being 0.3 mg.kg⁻¹; clearly show that there is no potential risk to human health from consuming such fish, as the standards set out in Regulation No. 31, which was in force at the time, have not been exceeded. Furthermore, these are the levels reported in the liver of carp, not in the musculature that is consumed (Valkova, 2022).

It is known that the current national and European documents do not impose restrictions on the Ni content in fish liver. However, values of 0.3 mg.kg⁻¹ do not pose a risk to the organisms studied, as the liver, as a detoxification organ, can cope with such small amounts of nickel (Fig. 3). The high degree of accumulation of Ni and other heavy elements, accompanied by high concentrations in the liver, can inevitably affect a significant number of biochemical parameters in this organ, which in turn leads to disruption of the processes in the organism of the aquatic organisms as a whole (Vinodhini & Narayanan, 2008).

In a study conducted in 2012 by Yancheva et al. (2014), Ni values of 1.7 mg.kg⁻¹ were recorded in the Topolnitsa Dam, which exceed the current Regulation No. 31 by 1.2 mg.kg⁻¹ (70.6%) and 1.55 mg.kg⁻¹ (91.2%) higher than the maximum average value of 0.15 mg.kg⁻¹ measured by us in 2014 in liver samples from the Ovcharitsa Dam (Fig. 3).

The current regulatory documents for Europe (Regulation (EU) No. 2023/915) do not impose any restrictions on the Ni content in freshwater fish. The high levels of nickel in the liver are indicative of the amounts in the body as a whole, including in the edible muscle tissue of the studied hydrobionts.

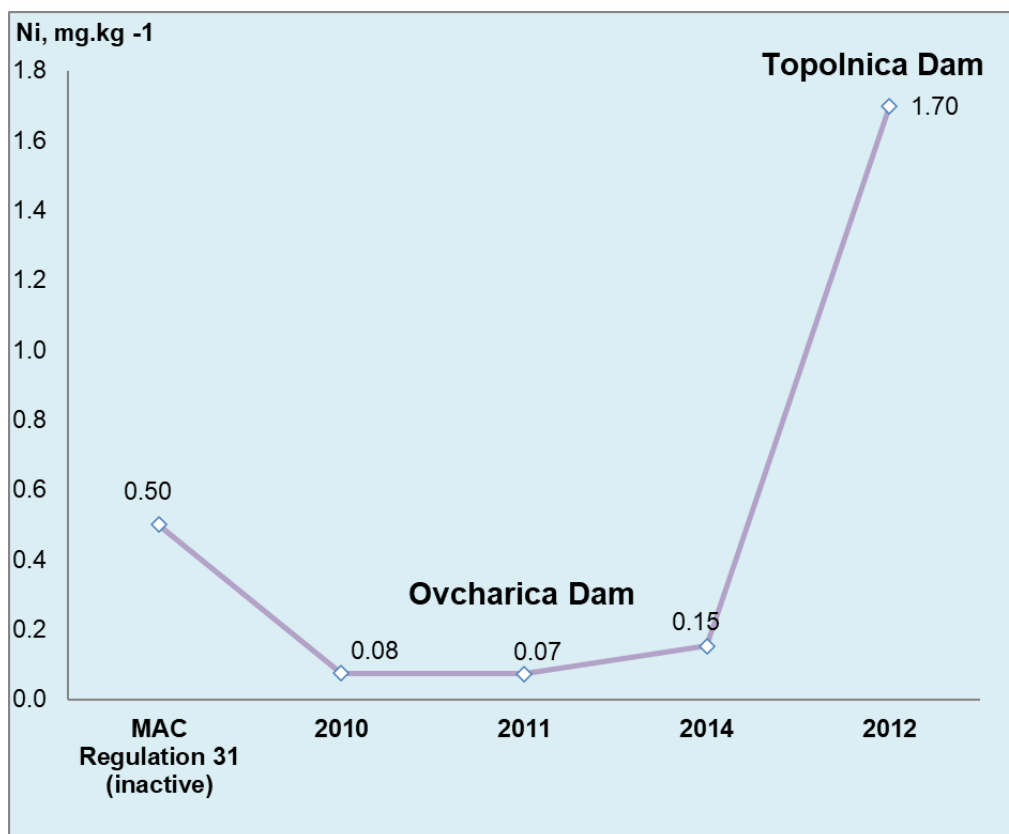


Fig. 3. Ni content in the liver of carp inhabiting the Ovcharitsa Dam (2010-2014) and the Topolnitsa Dam (2012).

Cu and Ni content in the musculature of Common Carp (*Cyprinus carpio* L.) from freshwater bodies

The liver, the body's biochemical laboratory, is where most of the metabolic and detoxification reactions take place, both in hydrobionts and in other organisms. In contrast, the musculature of aquatic organisms is more important for locomotion and physiology. Mammals and humans occupy the top of the food chain and, as such, consume more muscle than hydrobionts and much less caviar, fish oil and liver.

The former Regulation No. 31 of Bulgarian legislation regulated the maximum permissible levels for most of the metals studied in the period 2010-2014 that have a high degree of toxicity, such

as Cu, Zn, Ni, Pb, Cd, etc. Some of these metals (such as copper and nickel) are absolutely necessary for metabolism in low doses, but in larger quantities they act as typical heavy elements, exerting a strong toxic effect.

Regulation (EU) 2023/915 of the European Parliament (in force since 25 April 2023) on the maximum permissible levels of certain contaminants in foods only contains restrictions on typical heavy metals such as Pb, Cd, Hg, etc. No MACs indicating the degree of toxicity are specified for Zn and Ni. Until recently, the existing regulatory documents (Regulation No. 5 of 9 February 2015 of Bulgarian legislation and Regulation (EO) No 1881/2006 setting maximum levels contaminants in foods) also lack legal restrictions on the metals

copper and nickel studied. The lack of restrictions on these metals limits the accuracy of the interpretation of the results obtained, due to the fact that in high doses these elements exhibit significant toxicity to organisms (aquatic and terrestrial).

The amounts of heavy metals in the waters of the studied water bodies provide only instantaneous data on their condition, and it is necessary to monitor the levels of these elements not only in the liver but also in the musculature of the cyprinid fish inhabiting the water bodies during this period.

Jaber et al. (2021) studied the musculature of carp of the species *Cyprinus carpio*, found low levels of the essential elements Zn, Cu, and Fe in the musculature of the fish, given that these metals are consumed by the body due to their participation as cofactors in of protein systems. In the case of samples from the Tiger River, located in northern Iraq, a higher concentration of toxicants such as lead (1.27 mg.kg^{-1}) was found. In the current regulatory documents relating to Pb levels in fish musculature, the regulated MAC is 0.3 mg.kg^{-1} . The presence of levels of around 1.27 mg.kg^{-1} of lead inevitably leads to the formation of oxygen radicals, causing damage to important respiratory enzyme and transport proteins, lipids, and nucleic acids.

In an analysis of *Cyprinus carpio* meat, Sahiti et al. (2020) recorded significantly lower levels of Cd, Pb, Ni, and Cu compared to those in metabolically active gills and liver. Studies such as this one, as well as several others in which analyse various organs and tissues, including musculature, show that this is not a place for the accumulation of heavy metals (Jayakumar & Paul, 2006; Vinodhini, & Narayanan, 2008; EL-Moselhy et al., 2014; Jithesh & Radhakrishnan, 2017). From this point of view, musculature is covered on the outside by skin, which prevents direct contact with the environment. According to EL-Moselhy et al. (2014), another significant reason why musculature is not an active site for metal accumulation metals, is that musculature do not play a role in the detoxification process. The lowest level of accumulation in all organs and tissues is found in blood plasma. In fact, blood serves as a carrier of heavy metals to other tissues and organs.

Valkova (2022) examined a large number of samples of carp musculature, delivered from the water bodies observed in the period 2010-2011, in order to determine the amounts of heavy metals

in those parts of the fish that are used for consumption not only by many animal species but also by humans. It is no coincidence that the Ovcharitsa Dam was among the observed water bodies, characterised by a strong anthropogenic influence due to its proximity to the Maritsa East 2 Thermal Power Plant. In order to obtain a realistic picture of the changes in the quantities of the heavy metals studied and the subsequent changes in the condition of this reservoir over time, an analysis was carried out on the musculature of carp inhabiting the water body in 2014.

Cu content in the musculature of Carp from the studied water bodies in Stara Zagora Region, Bulgaria

Despite its significant importance for metabolism, above certain levels, the trace element Cu is highly toxic to both fish and humans as consumers. The data presented in Fig. 4 show that its content in the 2010 samples is much lower than the MAC for concentrations in fish regulated by the normative documents at that time.

The results obtained from this year's study contradict the established trend regarding the amounts of this heavy metal in the liver of hydrobionts from the same period, where high values were reported in samples delivered from the Zagorka, Zetovo, Ovcharitsa and Chirpan water bodies. The concentrations recorded in the livers of these fish exceed or are close to the MAC included in Regulation 31, which was in force at the time. The current Regulation (EU) No. 2023/915 of the European Parliament does not set limits on the levels of this metal in the musculature of the aquatic organisms studied.

High concentrations of copper were found in the musculature of carp delivered from the Koprinka Dam in the period May-December 2010. The lowest samples were from the Tundzha River in the Yagoda Village - 92.7% and 96.6% below the MAC in Regulation No. 31 at the time.

In 2011, the trend of Cu concentrations in the musculature, much lower than the MAC in the then current Regulation No. 31, compared to fish, was maintained. The highest values, well below the then regulated standards, were found in the Ovcharitsa Dam (94% below the MAC). The minimum concentration was measured in the muscles of carp inhabiting the Pastren Dam (97.3% below the standard). Due to the fluctuations in the amounts

of copper accumulated in the musculature of carp from the Ovcharitsa Dam, it was necessary to analyse samples and determine the average concentration of copper in the musculature of carp from the Ovcharitsa Dam.

Due to the fluctuations in the amounts of copper accumulated in the muscles of carp from the Ovcharitsa Dam, it was necessary to analyse samples from 2014. The amounts recorded during this period did not differ significantly from those recorded in 2011 (a decrease of 3.4%). The levels found are much lower than the standards regulated at that time (a decrease of 94.2% compared to the MAC in Regulation 31 in force at the time). The studies conducted in the periods of 2010, 2011 and

2014 on Cu levels confirm the presence of Cu in the water and sediment of the studied reservoirs. The studies conducted in the periods of 2010, 2011, and 2014 on Cu levels confirm a slight decrease, confirming the low levels of this metal in the muscles of carp from the Ovcharitsa Dam. The concentrations recorded during this period do not differ significantly from those established in 2011 (a decrease of 3.4%). The concentrations recorded during this period do not differ significantly from those established in 2011 (a decrease of 3.4%). The established levels are much lower than the standards regulated at that time (a decrease of 94.2% compared to the MAC in Regulation No. 31 in force at that time).

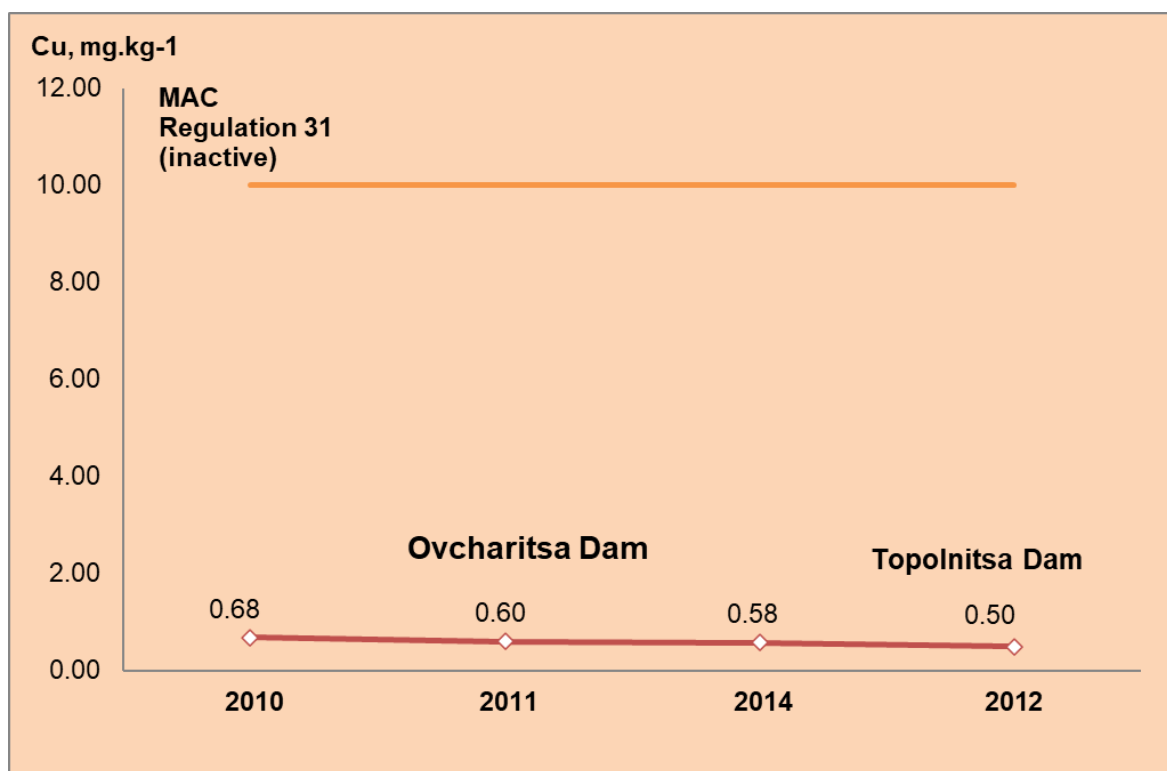


Fig. 4. Cu content in the muscles of carp inhabiting the Ovcharitsa Dam (2010-2014) and the Topolnitsa Dam (2012).

As can be seen in Fig. 4, the copper levels recorded in the waters of the Topolnitsa Dam in the study by Yancheva et al. (2014) (0.5 mg.kg⁻¹) are five to seven tenths lower than those found by us in the muscles of carp from the Ovcharitsa Dam in the period 2010–2014 (from 0.58 to 0.68 mg.kg⁻¹).

All concentrations discussed (including those recorded by Yancheva et al. (2014) are approximately 10 times lower than the limits set in Regula-

tion 31 on contaminants in food (10 mg.kg⁻¹), which was in force at the time.

Ni content in the musculature of Carp from the studied water bodies in Stara Zagora Region, Bulgaria.

The need for the trace element nickel in connection with the normal course of most biochemical processes in organisms has long been known. High doses of Ni can be harmful to both aquatic organisms and humans. This necessitated the

study of its content not only in the liver but also in the muscles of the above-mentioned fish species.

The amounts of Ni recorded in the muscles of the hydrobionts from the above-mentioned water bodies during the period May-December 2010 in the Stara Zagora Region do not pose a problem, as they are well below the permissible requirements regulated in the normative documents in force at the time. Although the measured levels are much lower than the standards, the samples from the Chirpan Dam have the highest concentrations (91% below the then permitted MAC). The lowest recorded value is for the Tundzha River in the Yagoda Village (96% below the standard). The results from May-August 2011 regarding the levels of Ni found in the musculature of fish are characterised by much lower values compared to the levels found in the muscles of the above-mentioned fish species.

The results from the period May-August 2011 regarding the levels of Ni found in fish muscle tissue are characterised by much lower values compared to the MAC according to Regulation 31, which was in force at the time. The highest values were measured in musculature samples from the Opan Dam (0.1 mg.kg^{-1}). The lowest concentration was recorded in samples from the Ovcharitsa Dam (0.02 mg.kg^{-1}).

A study conducted in 2014 found a slight increase of 69.7% in Ni concentrations in the musculature of carp inhabiting the Ovcharitsa Dam during that period compared to the results from 2011. During the last year of the study, a slight increase in nickel levels was observed in the muscles of the carp studied, but these results are 92.6% below the regulatory limits. In the last year of the study, a slight increase in nickel levels was observed in the muscles of the carp studied, but these results are 92.6% below the regulatory requirements of 0.5 mg.kg^{-1} active during this period. Our study (in the period 2010-2014), as well as a large number of studies worldwide (Nussey et al., 2000; Akan et al., 2012; Sow et al., 2019; Ghosn et al., 2020; Hussain et al., 2021), show that the nickel concentrations reported in the muscle tissue of fish are extremely low compared to the high levels in the liver of the same hydrobionts. Once again, the ability of the liver to accumulate most of the heavy metals entering the body and prevent their accumulation in the muscle tissue of aquatic organisms has been proven. This statement is confirmed by the study of Dulich et al. (2018), who also found higher levels of this element in the liver compared to the muscles.

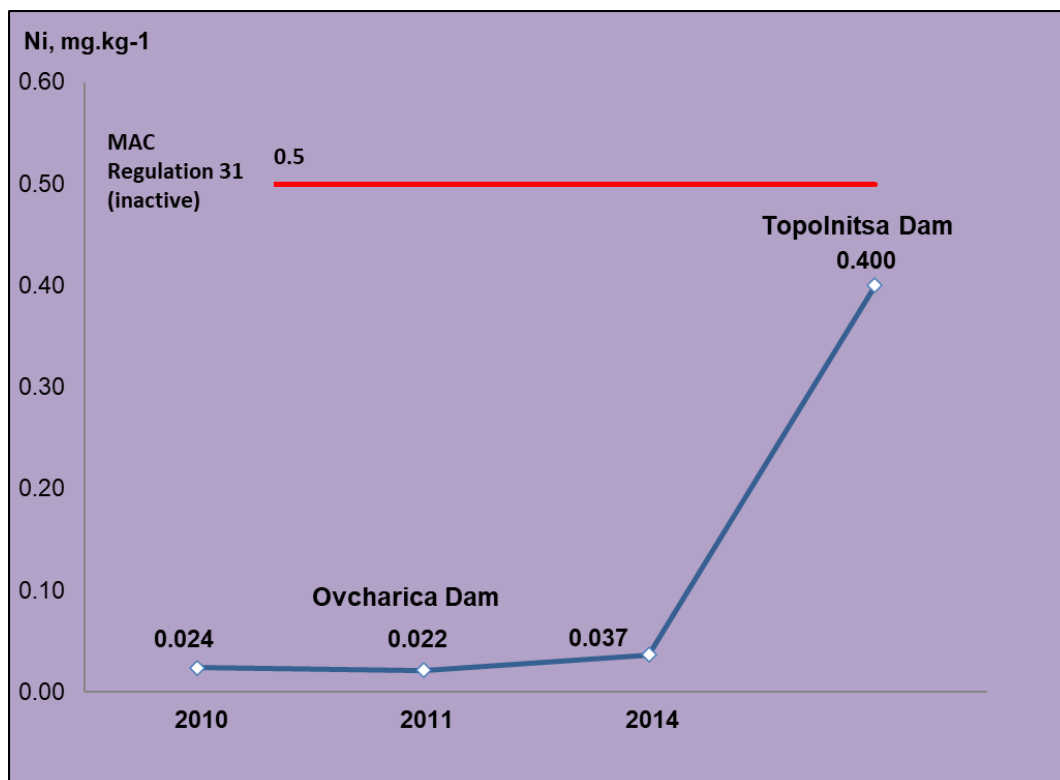


Fig. 5. Ni content in the musculature of carp inhabiting the Ovcharitsa Dam (2010-2014) and the Topolnitsa Dam (2012).

The nickel concentrations measured in the study by Yancheva et al. (2014) of 0.4 mg.kg^{-1} in the musculature of carp from the Topolnitsa Dam are ten times higher than the values we found in the period 2010-2014 in musculature samples from the Ovcharitsa Dam (the highest value in 2014 is 0.037 mg.kg^{-1}) (Fig. 5).

All representatives of hydrobionts are exposed to the action of most heavy metals present in water in ionic form, and the accumulation of one metal is often accompanied by that of another metal. It is also possible that the accumulation of one element is associated with the inhibition of the accumulation of another. Our study is a typical example of the existence of such positive and negative dependencies regarding the uptake and accumulation of some metals relative to others (Valkova, 2022). It is for this reason that a significant number of positive and negative dependencies between individual metals have been found in the musculature of the studied hydrobionts.

When conducting a correlation analysis of the results regarding the amounts of heavy elements in the musculature of carp from 2010, a number of significant relationships between the studied metals were found. The results show that there is a positive correlation between the elements Cu and Pb, Cd and Ni, Zn and Cd; Pb with Cd and Ni; Cd with Zn and N, which means that the increase in manganese is accompanied by an increase in the above-mentioned metals. Negative correlations between Fe and Mn, Cu, Pb, Cd, and Ni have also been proven, i.e., the accumulation of these metals was directly related to a decrease in the accumulation of the listed elements.

The correlation matrix of the data compiled in 2011 shows statistically significant positive correlations between Mn and Cu, Pb, Ni, and Cd; Cu with Pb, Ni, and Cd; Pb with Ni and Cd; Ni and Cd. During the study period in 2011, there were no significant negative correlations. There are correlations with a high degree of reliability between Fe and the elements Zn, Ni and Cd, but they are not statistically proven.

In 2014, no statistically proven positive correlations were reported between the metals Mn and Cr, and Cd and Ni. The analysis also shows high-degree positive correlations between Fe and the elements Mn and Cr, as well as between Cu and

Pb, but these are not statistically proven. The negative correlations between the metals studied during this period are not reliable. However, a strong inverse correlation between Ni and Cd with Fe, Mn, Cr and Zn is observed.

Conclusions

Based on the established positive and negative correlations regarding the content of the heavy metals copper and nickel in the studied water bodies and the hydrobionts inhabiting them, the following conclusions can be drawn:

The reported values of Cu and Ni in the analysed sediment samples are significantly lower than the regulated MAC for metal content in soil fractions (Regulation No. 3 of 1 August 2008 on the standards for permissible content of harmful substances in soils), with the highest values being recorded at the points Zhrebchevo Dam – the Nikolaevo checkpoint and Ovcharitsa Dam. The values of Ni in the analysed sediment samples are significantly lower than the MAC for Ni content in soil fractions.

Symmetry has been established in terms of Cu and Ni levels in sediments, with a gradual decrease observed in the direction from the Zhrebchevo Dam – the Nikolaevo checkpoint to the Zhrebchevo Dam – Tundzha River point, which suggests at least two facts:

- There was probably heavy metal pollution in the section between the Yagoda Village and the town of Nikolaevo, due to ore mining in the Tvarditsa area.
- The Zhrebchevo Dam acts as a sedimentation basin for heavy metals (and other toxicants), preventing them from entering the Tundzha River.

In our study to determine the amounts of metals in algae and higher aquatic plants, a correlation was found when comparing the high values, among which the algae samples from the Opan Dam (85.8 mg.kg^{-1} Cu), the Ovcharitsa Dam (68 mg.kg^{-1} Cu), Zagorka Lake (50.5 mg.kg^{-1} Cu) and the water pepper from the Tundzha River after the Zhrebchevo Dam (23 mg.kg^{-1} Cu).

A correlation was also established between the higher nickel concentrations in the algae samples from the Ovcharitsa Dam (4.39 mg.kg^{-1} Ni), the Tundzha River at the Nikolaevo point (3.10 mg.kg^{-1} Ni), the Tundzha River after the Zhreb-

chevo Dam (3.15 mg.kg⁻¹ Ni) and the water pepper from the Tundzha River after the Zhrebchevo Dam (2.75 mg.kg⁻¹ Ni).

Analysis of the musculature and liver of the fish studied showed an inversely proportional relationship in the accumulation of nickel and copper, with this process being more intense in the liver.

The results of our studies and others conducted worldwide confirm the thesis that sediments from water bodies can serve as good ecological markers for heavy metal pollution.

Studies conducted in the Stara Zagora Region - Bulgaria and other countries have confirmed the ability of hydrobionts to accumulate heavy metals and serve as excellent bioindicators of pollution with these toxicants.

Global environmental pollution is a prerequisite for continuing research in this area with other potential ecological markers. The ecological status of water bodies, determined on the basis of the content of a number of toxic metals (such as cadmium, mercury, zinc, nickel, lead, etc.) in hydroecosystems and the hydrobionts that inhabit them, is sufficient grounds for conducting future research.

Acknowledgments

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