Bulgarian Journal of Agricultural Science, 25 (Suppl. 3) 2019
Agricultural Academy

Study of the content of copper in waters and Zebra mussel (*Dressena polymorpha*) from Ovcharitsa Dam, Stara Zagora region, Bulgaria

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Abstract

Valkova, E. (2019). Study of the content of copper in waters and Zebra mussel (*Dressena polymorpha*) from Ovcharitsa Dam, Stara Zagora region, Bulgaria. *Bulg. J. Agric. Sci., 25* (Suppl. 3), 182–186

The present study aims to determine the levels of the heavy metal copper (Cu) in the water and muscles of Zebra mussel (*Dreissena polymorpha*) inhabiting Ovcharitsa Dam, Stara Zagora, Bulgaria. The preparation, archiving and storage of samples of water and muscles is conducted in August and November 2015 to January and March 2016. The determination of Cu concentrations is performed using a modern method for measuring macro- and microelements with an atomic absorption spectrometer, which is characterized by high selectivity, speed and sensitivity. Concentrations of copper measured in the waters of Ovcharitsa Dam in August 2015 are three times higher (67.4%) compared to the SCC requirement according to Ordinance H-4 of the Bulgarian legislation. The registered data show the presence of a potential copper pollution of the waters of the studied water body, which has a permanent character during all months of research except for January 2016. The higher Cu levels are characterized by the mussels which inhabited Ovcharitsa Dam in January and March 2013. The content of this heavy metal in all samples tested is far below the MAC as defined in Ordinance 31 (30 mg / kg for molluscs). In the present study, the recorded values of Cu in the Zebra mussel samples, which inhabited Ovcharitsa Dam during the studied period, are several times higher than those observed in the waters of the same water body. Concentrations of copper found in the water can show the state of the hydroecosystem at the time of sampling. However, hydrobionic representatives in the face of Dreissena polymorpha have the ability to accumulate heavy metals over time, making them a more reliable biological indicator of contamination of water bodies.

Keywords: Zebra mussel; copper; water body; hydroecosystem; musculature
Abbreviations: BSS – Bulgarian State Standard, MAC – maximum allowable concentration, YAV – annual average value

Introduction

The state of the aquatic ecosystems over the last decades indicates that there are significant environmental changes leading to a breach of the ecological equilibrium worldwide. The reasons for this can be of varying nature. The main factor is the human activity, which is responsible for the accumulating of compounds of different nature in water bodies. Changes occurring in hydroecosystems are often due to heavy metal contamination. In small quantities, most of these elements are indispensable for the vital activity and reproduction of all living organisms. Above certain values, however, they have disastrous effect on the ecological balance and diversity of hydrobionts (Ashraj, 2005; Vosyliene & Jankaite, 2006; Farombi et al., 2007; Vítek et al., 2007). These elements in the form of ions of a number of organic and inorganic compounds enter the body of aquatic organisms mainly through the gills, skin and food intake. The accumulation of heavy metals is mainly

concentrated in the liver, gills and eggs, and to a lesser extent in the muscles of fish and mussels (Rainbow & Dallinger, 1993; Huang, 2003, Mohamed & Gad, 2005).

The element copper (Cu) is an important biogenic element, playing a key role in the normal course of a number of biochemical processes in organisms and their gametes (Atanasov et al., 2007; Tzanova et al., 2017). The proper functioning of a number of enzymes in the liver, brain and muscles of animals requires very small quantities of copper. This microelement is part of the structure of cytochrome oxidase (cytochrome aa3) – the terminal respiratory enzyme in the mitochondria, and is therefore necessary for the energy generation processes in the cells. It also regulates the metabolism of catecholamines, serotonin, tyrosine, stimulates the increase of insulin activity and improves carbohydrate utilization. Copper-containing enzymes (superoxide dismutase, e.g.) are important antioxidants that inhibit or neutralize free radical production (Sevcikova et al., 2011). Breathing in almost all molluscs and crustaceans is accomplished by the respiratory pigment hemocyanin, which contains copper in its molecule rather than iron.

In many cases, Cu is present in potentially harmful concentrations together with other heavy metals (zinc). Copper ions exhibit highly toxic activity, under which the blooded body in the gills is destroyed, consequently leading to a fatal outcome of asphyxia. The lethal concentration of Cu in shells depends on the hardness of the water, the type and age of the hidrobionts and the exposure time.

The presence of high concentrations of copper ions results in an increase in the activity of the catalase and acid phosphatase enzymes in all mussel cells and in a decrease in the activity of Na-K-ATPase in the gills (Katsumiti et al., 2018). Cu and CuO NPs ions increase the activity of catalase and acid phosphatase in all mussel cells and reduce the activity of Na-K-ATPase in the gills. All Cu-forms cause damage to DNA in haemocytes, whereas in gill cells only Cu and CuO NPs ions are genotoxic.

Hydrobionts are often found in human diet. Along with many useful ingredients, these foods can also be a source of large amounts of heavy metals such as copper. When entering the human body in high doses, this metal can lead to anemia, kidney and liver diseases (Georgiev et al., 2011).

Several hydrobionts are used as bioindicators for estimating the state of the environment (Atanasov et al., 1999a,b; 2006; 2017). Both marine and freshwater mussels are known to play biological filters that retain pollutants of a varying nature. These molluscs accumulate a specific pollutant directly by filtering the water during the respiratory process as well as through food intake (Michiel et al., 1992; Gundacker, 1999).

Mussels show their sensitivity even with minor changes in water parameter values, which is why they are often used as bioindicators for assessing the ecological and biochemical state of the water bodies (Valkova, 2015).

Zebra mussel (*Dreissena polymorpha*) has a high metal accumulation potential, which is particularly important for local contamination. Due to these characteristics, the studied hydrobiont is often used in Europe and North America to monitor water bodies, especially for heavy metal pollution (Kwan et al., 2003, Evita, 2005).

Zebra mussel is an invasive species for the Ovcharitsa Dam, which is located near the Maritsa Iztok 2 TPP. The area surrounding this facility is considered to have disastrous effect on the state of the environment. Therefore, in recent years, systematic studies have been carried out on the waters and hydrobiontes inhabiting the water bodies in this region.

The importance of this problem determines the conducting of the present research, which aims to study the levels of the heavy metal copper (Cu) in the water and muscles of Zebra mussel (*Dreissena polymorpha*) inhabiting Ovcharitsa Dam, Stara Zagora.

Material and Methods

Collection of Zebra mussel (Dreissena polymorpha)

The present study was conducted on the territory of Ovcharitsa Dam (Fig. 1), which is located in the Radnevo municipality, Stara Zagora Region, Bulgaria.

The preparation, archiving and storage of samples of water and muscles was conducted in August and November 2015 to January and March 2016.

During the study, the determination of the heavy metal copper (Cu) content in water and muscle samples from Zebra mussel (*Dreissena polymorpha*), taken from the water body, was performed.

Preparation of samples and determination of quantities of heavy metals Cu

Samples of water taken for analysis from the water body are stored by addition of c.HNO_3.

Muscle sample (n = 10) preparation was performed by wet mineralization in a Perkin Elmer 3000 microwave oven. The content of the heavy element copper in the resulting acid solutions was determined by atomic absorption spectrometer (AAS) "A Analyst 800" Perkin Elmer on a cuvette and flame system by using acetylene-oxygen combustion according to BSS ISO 11047.

The statistical analysis and data processing were performed by applying the software packages: MICROSOFT OFICE and STATISTICA 6.0, using the ANOVA test.

Fig. 1. Ovcharica Dam

Results and Discussion

The area where the studied water body is situated – Ovcharitsa Dam is characterized by a high degree of anthropogenic influence. The ecological status of this area as well as the condition of Ovcharitsa Dam is directly dependent on the amounts of toxicants and in praticular heavy metals such as copper.

Despite the important functions they perform, most microelements (including Cu), have a powerful toxic effect on the organisms in high doses (Skibuewska, 2002; Matos et al., 2010; Stern et al., 2010).

Because of the mussel's ability to accumulate this element in it's body, raising copper levels is a clear sign of water pollution. The high sensitivity of these molluscs also determines their rapid reaction even with minimal changes in the amounts of Cu in the water.

Monitoring the status of the aquatic ecosystem of the studied water body includes the determination of Cu concentrations first in the waters and subsequently in the muscles of Zebra mussel.

The results of the analysis of the Cu content in the water and muscles of *Dreissena polymorpha* over the four months of the study are presented in figures 2 and 3.

Content of Cu in waters and Zebra mussel from Ovcharitsa Dam during the 2015 and 2016 surveys.

Concentrations of copper measured in the waters of Ovcharitsa Dam in August 2015 are three times higher (67.4%) compared to the YAV – SEQ SCC requirement according to Regulation H-4 of the Bulgarian legislation.

The registered data (Figure 2) show the presence of a potential copper pollution of the waters of the studied water

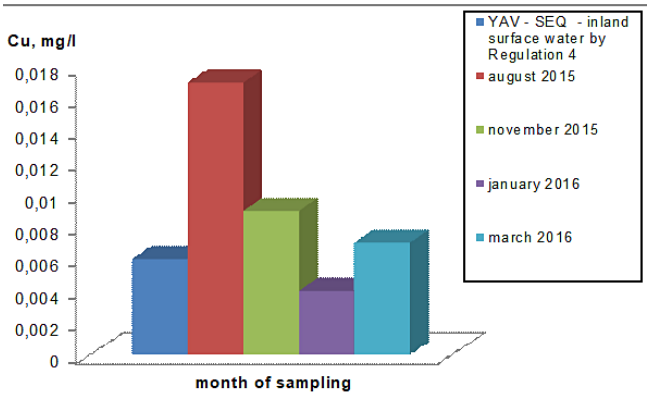

Fig. 2. Copper content in water from the Ovcharitsa Dam in the study months of 2015 and 2016.

body, which has a permanent character during all months of research except for January 2016. Reasons for this may be of varyingly different nature, but most likely they are due to high concentrations of copper salts in the soils around the water body and due to the use of fertilizers of such composition. In Regulation H-4 for characterization of surface water from 14.09.2012. no MACs for Cu in this type of water body are available.

It has been found that the values of heavy metals in the waters of a given water body show the current state of the given hydroecosystem. Due to this fact, the content of Cu in the organism of the inhabiting hydrobiontes is of particular importance for the condition of the studied water basin. Copper, like other microelements, is indispensable for the normal course of biochemical processes in these organisms. This element is an important component of a number of enzymes such as cytochromeoxidase, NADH dehydrogenase and tyrosinase found in the cytoplasmic membrane, and is also a major contributor to the respiration of molluscs by its presence in the pigment hemocyanin. The need for Cu also arises from its involvement in innumerable biological processes, including antioxidant protection, neuropeptide synthesis and immune function (Bonham et al., 2002, Uriu-Adams & Keen, 2005).

Over a certain threshold, the metal under analysis has a powerful toxic effect on mussels and other aquatic organisms, blocking important protective mechanisms. High doses of copper disturb the immune defense of mussels, reducing the number of white blood cells with peroxidase and phenoxidase activity. The number of eosinophils decreases at the expense of the number of basophils (Pipe et al., 1999).

The results, shown in Fig. 3 show that higher Cu levels are characterized by the mussels which inhabited Ovcharitsa Dam in January and March 2016. The content of this heavy

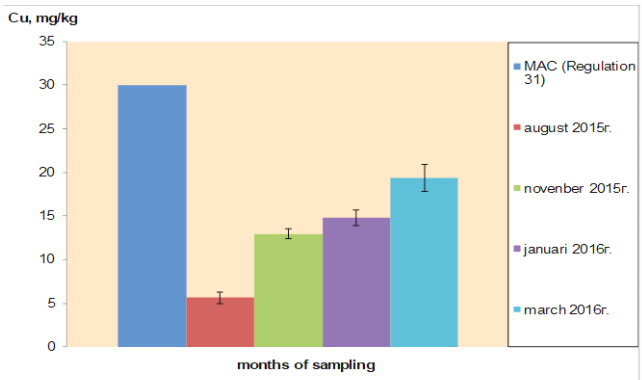

Fig. 3. Copper content in musculature of mussel „Zebra“ from the Ovcharitsa Dam in the study months of 2015 and 2016

metal in all samples tested is far below the MAC as defined in Regulation 31 (30 mg / kg for molluscs) (Regulation № 31, 2004). The copper concentration in March 2016, which represents the highest reported value, is 35.5% below the established regulatory requirements. Contrary to expectations, the lowest value in Zebra mussel muscles was measured in the samples from August 2015. (81.2% below the maximum admissible value). Although the amount of Cu in the environment was relatively high in the summer, the metal does not accumulate intensively in the mussels. On the contrary, during the autumn and winter the accumulation of copper is at its highest rates. Probably this is due to temperature, active water reaction and other hydrochemical parameters. Moreover, during this season the water temperature remains relatively high because it is a cooling lake of the Maritsa East 2 TPP. Optimal parameters cause a higher rate of metabolism, which implies a high-speed intergation of this biogenic element into copper – protein complexes such as hemocyanin, azurine and placocyanin. Typically, doses administered in an amount of 0.2 ppm result in the activation of the immune system of the hydrobionts and in an increase in white blood cell levels. This is quite understandable, as the body acts defensively, striving to eliminate toxins. Levels of 0.5 ppm and above have the effect of decreasing white blood cell count, make the breathing process difficult, water filtration and growth of the stressed organisms (Hanna et al., 2014, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5304694/).

Conclusion

In the present study, the recorded values of Cu in the Zebra mussel samples, which inhabited Ovcharitsa Dam during the studied period, are several times higher than those observed in the waters of the same water body. Concentrations of copper found in the water can show the state of the hydroecosystem at the time of sampling. However, hydrobionic representatives in the face of *Dreissena polymorpha* have the ability to accumulate heavy metals over time, making them a more reliable biological indicator of contamination of water bodies.

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