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Content of Fe and Mn in waters and zebra mussel (*Dressena polymorpha*) from Ovcharitsa Dam, Stara Zagora region, Bulgaria

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Abstract

Valkova, E., Atanasov, V. & Veleva, P. (2020). Content of Fe and Mn in waters and zebra mussel (*Dressena polymorpha*) from ovcharitsa dam, Stara Zagora region, Bulgaria. *Bulg. J. Agric. Sci., 26 (4)*, 870–876

The main objective of this study is to determine the levels of heavy metals iron (Fe) and manganese (Mn) in the waters and muscles of the "Zebra" mussel (*Dreissena polymorpha*) from Ovcharitsa Dam, Stara Zagora, Bulgaria. Iron concentrations measured in the waters of Ovcharitsa Dam in November, 2018 are 4.5 times higher than the requirement for YAV in accordance with Regulation H-4 of the Bulgarian legislation. Concentrations measured during this period indicate the presence of possible temporary pollution in November, 2018. These values fully correspond to the concentrations of the same element measured in the muscle of the Zebra mussel in January, 2019 of 104.36 mg/kg (16.5 units above the arithmetic mean) due to the passage of iron ions from water into the mussel organism. Higher Fe values are also characterized by specimens of mussels inhabiting the Ovcharitsa Dam in August, 2018. Mn levels measured in the waters of the studied water body in November, 2018 are some times higher (5 times) than the YAV regulated by Regulation H-4 of the Bulgarian legislation. The reported data confirm the existence of possible permanent contamination with Mn during all months of the survey except for March, 2019. The reported amounts of manganese in mussels indicate that *Dreissena polymorpha* mainly accumulates the metal in August, 2018 and March 2019. The concentration of this element in the musculature of the examined mussels is gradually increasing, with the lowest value (5.33 mg/kg) established in November, 2018 and the highest (11.74 mg/kg) in March, 2019. Iron and manganese concentrations found in water can indicate the state of the hydroecosystems at the time of sampling. Aquatic organisms (including *Dreissena polymorpha* mussels) have the ability to accumulate heavy metals over time, making them a more reliable biological indicator of hydroecosystem pollution than conventional water monitoring.

Keywords: zebra mussel; heavy metals; water body; hydroecosystem; musculature
Abbreviations: BSS – Bulgarian State Standard; MAC – maximum allowable concentration; YAV – yearly average value

Introduction

The significant changes in the environment that have occurred in recent decades are reflected in the status of aquatic ecosystems, which is associated with a violation of the ecological balance. Anthropogenic activity is the main factor causing a large number of pollutants of different nature to enter the waters. Changes in the aquatic environment have recently been observed due to the presence of high levels of heavy metals. Most of these elements play an essential role in the cycles of life processes in living cells. The presence of high concentrations of heavy metals has a powerful toxic effect on the hydrobionts, which leads to disturbance of the natural balance and species diversity in the reservoirs

(Vosyliene, et al., 2003; Ashraj, 2005; Vosyliene & Jankaite, 2006; Farombi et al., 2007; Kazlauskienė & Vosylienė, 2008; Vergolyas & Goncharuk, 2016). The main way of intake of these toxicants is in the form of ions, which enter the body of aquatic organisms mainly through gills, skin and ingestion of food and water. The accumulation of heavy metals is localized mainly in the liver, gills and caviar and to a lesser extent in the musculature of different species of mussels and fish (Rambo & Dallinger, 1993; Huang, 2003; Mohamed & Gad, 2005; Sobhanardakani et al., 2018).

Iron (Fe) is absolutely necessary for the normal existence and development of organisms (including aquatic). Practically, the survival of all organisms depends on the presence of iron (Ponka, 1999; Aisen et al., 2001). Its involvement in the composition of enzymes such as cytochromes and catalase and oxygen-binding proteins such as hemoglobin and myoglobin is essential for life.In this regard, Fe is an important element for the growth and development of algae, molluscs, fish and other representatives of flora and fauna (Atanasov et al., 2011; Harangi et al., 2016).

Good assimilation of iron requires copper (Cu), cobalt (Co) and manganese (Mn). The deficiency of this microelement leads to anemia in mussels and fish. Due to its high distribution and abundance in the soil, Fe is present in all fresh water bodies and often reaches higher concentrations in water and sediments than other metals (Forstner & Wittman, 1979; Dermendzhieva et al., 2018). The divalent and trivalent forms of iron (Fe^{2+} and Fe^{3+}) are essential for the aquatic environment, although other forms may also be present in organic and inorganic waste streams from industrial plants and households.

The paradox of this metal is associated with danger in both situations – deficiency or excess in the body, each of which can have serious or even fatal consequences. Nowadays, it is already clear that iron is an essential element that is involved in many vital processes. However, as it undergoes oxidation and reduction, Fe may be the cause of the initiation and development of free radicals (Bury et al., 2003).

The accumulation of Fe in hydrobionts is mainly from two sources – food and water. The exchange between its two forms (Fe^{2+} and Fe^{3+}) significantly influences the pathways of absorption by organisms. The divalent form of Fe^{2+} is predominant in water, while Fe^{3+} in the form of sludge can significantly contribute to the nutritional concentration of iron in hydrobionts (Vuori, 1995).

In most studies, Fe's toxicity to aquatic organisms has been reported as an inhibitory or asphyxiating effect on gills, eggs, or other body surfaces. These types of toxic effects limit an organism's access to basic resources such as oxygen or food and are considered indirect effects. At pH values of around 5–6, the toxic effect of this metal on the fish organism is enhanced.

Iron, as a significant microelement, also has a positive effect on the human body when it is in the required amounts. Comprising many enzymes and hemoglobin, this metal is vital for metabolism. As the last recipient in most food chains, including fish, mussels and other hydrobionts, humans can absorb high doses of iron in the event of possible contamination of water bodies. This could lead to disruption of normal metabolism and osmoregulation by oxidative cell damage.

Manganese (Mn) is among the twelve most abundant elements on Earth. Its concentration in the oceans exceeds 60% that of the continental. In aqueous systems, manganese concentration is controlled by pH and redox processes, where solubility increases under both acidic and anaerobic conditions (Nàdaskà et al., 2009). In biochemical terms, one of the most important functions of Mn is its involvement as a cofactor in enzymes such as arginase, which is responsible for the production of urea in the liver, superoxide dismutase, a very important antioxidant enzyme that eliminates superoxide radicals in mitochondria, pyruvate carboxylase – a major enzyme in gluconeogenesis (Crossgrov & Zheng, 2004). Manganese boosts the maintenance of the nervous and immune systems and regulates blood sugar. It has a positive effect on the absorption of vitamins B and E and is necessary for the normal growth of bones and the prevention of defects in blood coagulation (Michalik, 2005; Michalik, 2005a; Michalke, 2004).

At high concentrations, like most heavy metals, manganese has a toxic effect on hydro-ecosystems (Atanasov et al., 2013; Dermendzhieva et al., 2019).

Mn found in natural waters is most frequently released from industrial facilities or as an infiltrate from landfills (Hronec et al., 2010).

The accumulation of Mn in aquatic vertebrates, invertebrates and fishes significantly increases with temperature (Miller et al., 1980) and decreases with an increase in pH is present (Rouleau et al., 1996). Its accumulation increases with the decreasing of water salinity (Struck et al., 1997). It turns out that freshwater molluscs and crustaceans are much more sensitive to Mn than marine ones.

In the presence of high doses of manganese, there is a significant decrease in red blood cells, hemoglobin and white blood cells in aquatic invertebrates and fish. The decrease in red blood cells and hematocrit is due to internal hemorrhages, possibly as a result of necrosis of the intestinal mucosa and kidney. It has been suggested that the decrease in white blood cells may be due to increased secretion of corticosteroids as a nonspecific response to environmental stress (Howe et al., 2004).

Man, as the last recipient in the food chain, consuming aquatic invertebrates and fish which accumulated high doses of Mn could become a potential victim of such contamination. The danger Mn accumulation in the human body is much greater than the possibility of it being in short supply, since it is constantly present in the human diet. This metal positively affects the fertility and development of embryos in humans and other mammalian representatives. At very high doses, it can cause damage to the nervous system (Georgiev et al., 2011; O'Neal1 & Zheng, 2015).

It is common knowledge that gametes, and especially those of hydrobionts, are often used as biological markers to assess the state of the aquatic environment (Atanasov et al., 1999; Atanasov et al., 2007). Both marine and freshwater mussels are themselves biological filters that retain pollutants of various nature. In the feeding and breathing processes, these molluscs accumulate contaminants of different nature directly through water filtration (Michiel et al., 1992; Gundacker, 1999).

Mussels are characterized by a high degree of sensitivity to water parameters and also respond even to a slight change in their values. This fact determines their frequent use as bioindicator species for assessing the ecological and biochemical status of water bodies (Valkova, 2015; Valkova et al., 2015, 2016).

Dreissena polymorpha (Zebra mussel), as a representative of molluscs, is characterized by excellent heavy metal accumulation capabilities. These mussels accumulate heavy elements over a short period of time, especially in the case of contaminated ponds of this type. These facts determine the frequent use of zebra mussels in determining the level of local heavy metal pollution in North America and Europe (Kwan et al., 2003; Evita, 2005). The studied mussel species is invasive to the Ovcharitsa Dam, which is located in the area of TPP Maritza-Iztok 2. The area around this enterprise is considered of high risk in terms of the state of the environment. Therefore, it is necessary to carry out systematic studies of the waters and hydrobionts inhabiting the water bodies in the region.

The importance of the problem requires the conduct of this study, which aims to monitor the levels of heavy metals iron (Fe) and manganese (Mn) in the waters and muscles of the Zebra mussel (*Dreissena polymorpha*) from Ovcharitsa Dam, Stara Zagora, Bulgaria.

Material and Method

Collection of Zebra mussel (Dreissena polymorpha)

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

Fig. 1. Ovcharica Dam, Radnevo Municipality, Stara Zagora Region, Bulgaria

During the study, samples were taken of water and muscle of the Zebra mussel (*Dreissena polymorpha*) inhabiting the studied reservoir. The preparation, archiving and storage of water and muscle samples were performed from August and November, 2018 until January and March, 2019.

Preparation of samples and determination of quantities of heavy metals Fe and Mn

Water samples taken for analysis from Ovcharica Dam were stored by adding c. HNO_3. Muscle samples were processed by wet mineralization in a Perkin Elmer 3000 microwave. The content of heavy metals iron (Fe) and manganese (Mn) in the obtained acid solutions was determined by a Perkin Elmer Atomic Absorption Spectrometer 800 (AAS) in a cuvette and a flame system by mixing acetylene-oxygen combustion according to BSS ISO 11047.

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

Results and Discussion

Fe content in water and musculature of the Zebra mussel (Dreissena polymorpha) from Ovcharitsa dam during the studied months of 2018 and 2019

Iron concentrations measured in the waters of Ovcharitsa Dam in November, 2018 are 4.5 times higher (77.8%) compared to the requirement for YAV in accordance with Regulation H-4 of the Bulgarian legislation.

The iron levels indicated in Figure 2 show the presence of possible temporary pollution in November, 2018. The reasons for this can be various, with high levels of iron at this time possibly due to the presence of high concentrations of

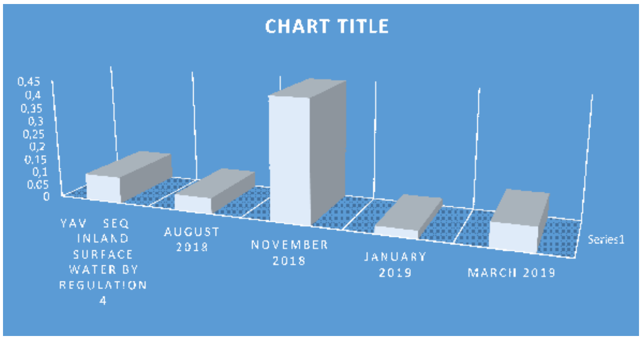

Fig. 2. Ferrum content in water from the Ovcharitsa Dam in the study months of 2018 and 2019

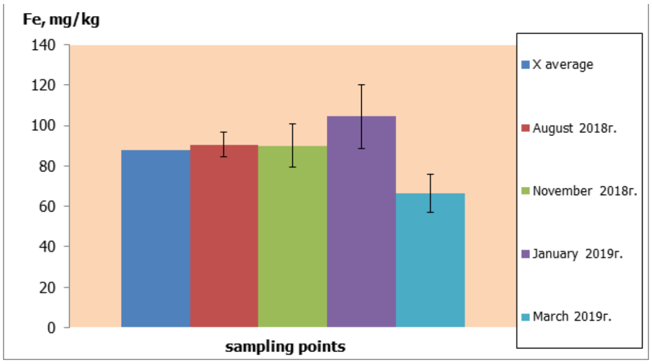

Fig. 3. Ferrum content in musculature of Zebra mussel from the Ovcharitsa Dam in the study months of 2018 and 2019

the element in the soil near the water, due to the autumn fertilization of organic origin. High concentrations approaching YAV in the regulatory documents were also registered in March, 2019. In Regulation H-4 on the characterization of surface water of 14.09.2012. (Regulation No 4-H of 14/09/2012) no standards for Fe maximum concentrations (MAC) in waters of this type are specified.

The measured values of heavy metals in the waters of a given reservoir characterize only the current state of the hydroecosystem. In order to get a complete picture, it is necessary to determine the content of the studied elements in the body of the hydrobionts inhibiting these waters.

Iron is an element thought to be at the boundary between micro- and macroelements. Therefore, to have a toxic effect, its values must reach significantly high levels. Although, according to some authors, iron and its compounds do not have a pronounced toxic effect on hydrobionts and humans, the interest around it is aroused by its essential role as an integral part of many enzymatic and transport proteins, as well as by the fact that the mechanisms that regulate its absorption are activated in diseases and long-term exposure to high levels of heavy metals (Brucka-Jastrzębska et al., 2009; Siemianowska et al., 2016).

The regulatory documents of the Bulgarian and European legislation lack a regulated MRL for the iron content in the mussel musculature, which is why the discussion of the obtained results was done on the basis of the arithmetic mean for the whole study period.

Figure 3 shows that higher concentrations of Fe are characteristic of the muscle samples of *D. polymorpha* inhibiting the Ovcharitsa Dam in August, 2018, and especially in January, 2019, whose value exceeds the arithmetic average by 16.5 units. The lowest levels were reported in March, 2019 (18. 8% below average).

Iron is one of the most important biogenic elements that make up a number of transport and enzyme proteins. Because of its great importance for metabolism in living organisms, some authors believe that its presence in larger quantities in aquatic organisms does not lead to toxic effects (Zverkova, 2009). Metals that are actively involved in metabolic processes (Fe, Mn, Zn, Cu, etc.) have been found to accumulate more in molluscs than highly toxic elements such as Pb and Cd. Of course, in very large doses, iron ions exert their toxic effect on aquatic inhabitants. From a biochemical point of view, since the values obtained are not extremely high, such an effect on the mussels during this study period is not possible.

Mn content in the waters and musculature of the Zebra mussel (Dreissena polymorpha) from Ovcharitsa Dam during the studied months of 2018 and 2019

Mn levels reported in November 2018 (Figure 4) are significantly higher than the YAV, regulated in Regulation H-4 of the Bulgarian legislation (Regulation No 4-H from 14/09/2012). The concentration of this metal in this case is 5 times (84.5%) higher than the determined value of this indicator.

Data on Mn concentrations in the waters of Ovcharitsa Dam during the reported period (Figure 4) confirm the presence of possible permanent contamination with this metal during all months of the study, except for March, 2019. The reasons for this can be various, for example, the inflow of fresh water in spring (March) as a result of snowmelt and rainfall. While in the months of August, November and January, the flow of the dam is lower, resulting in a higher concentration of manganese. Another reason may be the increased rate of accumulation of this heavy metal in the mussels in the spring as a result of seasonal activation of their metabolism (Figure 5). Regulation H-4 of the Bulgarian legislation (Regulation No 4-H of 14/09/2012) does not specify

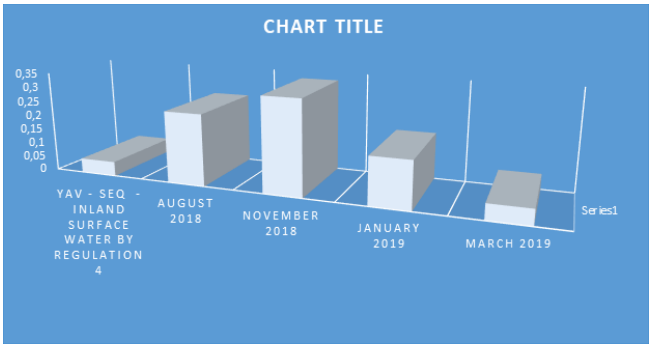

Fig. 4. Manganese content in water from the Ovcharitsa Dam in the studymonths of 2018 and 2019

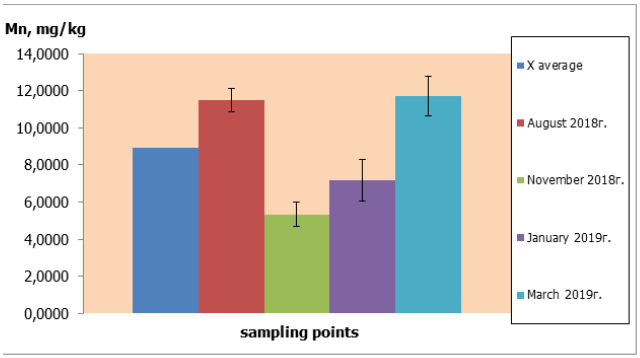

Fig. 5. Manganese content in musculature of Zebra mussel from the Ovcharitsa Dam in the study months of 2018 and 2019

values for maximum permissible concentration of Mn in this type of water. The only regulated value for this metal is a YAV of 0.05 mg/l.

Greater awareness of the environmental status of the Ovcharitsa Dam is obtained when determining the manganese content not only in the studied waters but also in the organism of the zebra mussel inhabiting this reservoir.

The manganese element is usually found in traces in the composition of surface waters (Nàdaskà et al., 2009). Its importance for living organisms (including hydrobionts) is mainly determined by its involvement as a cofactor in a number of enzymes, as well as in the maintenance of nerve and immune functions (Crossgrove & Zheng, 2004). It is an antagonist of iron (Fe) and can replace magnesium (Mg) in some enzymes. Due to its similar ionic radius, it can interfere with calcium (Ca) metabolism (O'Neal & Zheng, 2015). High levels of Mn are toxic to aquatic inhabitants.

Difficulties in interpreting the obtained data on the content of mussel muscles are also observed in the manganese element (Mn) again due to the lack of requirements for maximum concentrations in Regulation No 31 of July 29, 2004 on maximum levels of contaminants in food (Regulation No. 31 of July 29, 2004) and in fish liver.

Reference values are also not mentioned in Commission Regulation (EC) No 1881/2006. For this reason, the comparison of the results obtained was made with the mean calculated and the Mn quantities by years of study.

In Figure 5 we can see that the results imply that *Dreissena polymorpha* mussel accumulates manganese mainly in August, 2018 and March, 2019, which values exceed the average by 28.7% and 31.3% respectively. The lowest is the concentration measured in the samples from November, 2018. (40.3% below average). In our view, the activation of biochemical processes in the spring and summer and the direct involvement of Mn in them have influenced the levels of this element in the mussel during these seasons.

Usually, as a result of the change in abiotic environmental factors with the onset of the spring season, in the summer and early autumn acceleration of metabolic processes in aquatic organisms (including mussels) is observed. As a result of this phenomenon, and due to the fact that manganese is an active participant in metabolism, an increase in its values in mussels is often found during this part of the year. A similar result was obtained in a study conducted by Salman (2011), who also found higher levels of Mn during the months mentioned above.

Conclusion

This study showed the values of Fe and Mn recorded in the muscles samples of the Zebra mussel that inhabited the Ovcharitsa Dam in the studied months of 2018 (August and November) and 2019 (January and March) are several times higher than the ones observed in the waters of the same water body. The highest levels of iron in the water samples (0.45 mg/l) taken in November, 2018 were recorded, and as a consequence the highest concentrations in mussels were recorded in January, 2019. For Mn, the highest reported values in water (0.33 mg/l) are also in November, 2018, and in the mussel musculature there is a gradual increase in the levels with the highest value – March, 2019. The levels of heavy metals in mussels are highest in the spring and summer season due to the activation of specific biochemical accumulation processes. The iron and manganese concentrations found in the waters can indicate the state of the hydroecosystems at the time of sampling. Aquatic organisms (including *Dreissena polymorpha* mussels) have the ability to accumulate heavy metals over time, making them a more reliable biological indicator of water body contamination.

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