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CONTENT OF PB IN WATER, SEDIMENT, AQUATIC PLANTS AND MUSCULATURE OF COMMON CARP (*CYPRINUS CARPIO* L.) FROM DIFFERENT WATER BODIES IN STARA ZAGORA REGION, BULGARIA

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Abstract

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Heavy metals in high concentration in aquatic habitat (water bodies) are accumulated in different organisms, damaging their tissues and suppressing metabolic processes. The aim of present study was to survey and assessment of lead (Pb) levels in water, sediment, aquatic plants and musculature of *Cyprinus carpio* from different water bodies in Stara Zagora region, Bulgaria. International standards of ISO for sample preparation of water, sediment, aquatic plants and musculature of common carp analyze were used. The lead levels in collected samples were determined by atomic absorption spectrometry. The studied monitoring points located in a region that is under strong anthropogenic impact. However the levels of lead in the most of the investigated water bodies do not exceed the statutory requirements set by Directive 2008/105/EC and Directive 2013/39/EC. The highest lead concentrations in sediments were measured in Tunja River, Nikolaevo Town (42.96 mg.kg^{-1}), Sazliika River (25.38 mg.kg^{-1}) and Bedechka River (23.88 mg.kg^{-1}). Whit the highest values of this element in the aquatic plants are characterized Sazliika River (9.26 mg.kg^{-1}), Tunja River, Banya Village (8.02 mg.kg^{-1}) and Bedechka River (5.12 mg.kg^{-1}). The highest concentration of lead in the musculature, 5 times exceeding the established norms differ Jrebchevo Dam Lake (1.19 mg.kg^{-1}). These results clearly demonstrate the ability of the sediment, aquatic plants and fish to serve as excellent indicators of lead pollution.

Key words: aquatic plants, assessment, common carp musculature, lead, sediment, water
Abbreviations: MCA – Maximum Allowable Concentration; EC – European Communities; YAV – Yearly Average Value

Introduction

Multifaceted human activity often causes environmental pollution of a different nature. As a result of these processes occurs infraction of normal condition and development of ecosystems. The infraction of the natural balance is often the result of the presence of high concentrations of heavy metals in the environment. This group of elements has a high density (4.5 g.cm^{-3}) and has the ability to exist in the form of cations (Sevcikova et al., 2011). A key factor determining their solubility is the hardness of water. The increase in the hardness leads to solubility decrease of these elements (Long, 2000). Most heavy metals are important microelements and play an essential role in the organisms (Basta et al., 2005).

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To this group belong representatives which did not only play an important role in the biochemical processes, but also have a powerful toxic effect. Infracting the environmental balance these elements change hydrobionts life cycles and reduce biodiversity. Because they have not the ability to decompose they accumulate in sediment and the inhabitants of the water bodies. At presence of high concentrations of pollutants such as heavy metals into the aquatic environment is effected sedimentation of their particles on the bottom of the waterbodies. The sedimentation depends on the rate of water flow and the particle size. This process leads to decrease in the concentrations of various pollutants into the aquatic environment. Passing of heavy metals from sediment to the aquatic plants, aquatic invertebrates and fish are often observed (Abdullah et al., 2007; Sychra et al., 2011). Many species of these hydrobionts are used from human for food purposes and may affect his health (Bhalchandra and Ram, 2013).

● Above a certain concentration, heavy metals cause a wide range of disabilities. To a large extent this applies to elements which have only negative effects on the organisms. Studies globally prove that aquatic plants can be used as bioindicators for registrations the presence of high doses of heavy metals in hydro ecosystems (Aung at al., 2012; Chmielewska and Medved, 2001; Pinto et al., 2003). Due to its wide distribution and its ability to accumulate pollutants, aquatic plants give a real environmental assessment on the state of fresh and marine waters. In this aspect mussels and fishes performs important role of high ranks environment indicators (Wang, 2002). The different type freshwater fish are extremely sensitive to the presence of heavy metals, which is due to their ability to react to temporary deviations of the reference values of this essential hydro chemical indicator. To a highly toxic metals includes and the element lead (Pb). This heavy element is characterized by low chemical mobility and all over distribution.

● Lead existing in the form of metallic, inorganic ions and salts (Harrison, 2001). In high doses, lead accelerates the process of formation of free radicals and leads to the activation of the glucose-6-phosphate dehydrogenase and catalase in the cells of the organisms.

● In case of Pb involving in to the aquatic plants cells occur membrane damage in particular thylakoids causing poor carbon assimilation seems to be a major contributor to growth rate inhibition (Jabbar, 2010). The aquatic plants biomass possesses a relative high adsorption capacity of 71.10 $mg.g^{-1}$ (for an initial Pb concentration of 702.52 $mg.l^{-1}$), confirming that they can be effectively used as adsorbent (Holan and Volesky, 1994). In the fish its toxic effect is expressed in lysis of the erythrocytes membranes and damage of the brain (Romeo et al., 2000).

In many instances, the absorption of this element increases with the rising in pH and an increment the hardness of the water (Kiyani et al., 2013).

It was postponed predominantly in the liver and kidneys, and less in the bones and muscles of the fishes (Ming-Ho, 2005). Studies of the content of lead in the water, sediment, aquatic plants and fish enable to a better assessment of anthropogenic impacts on aquatic ecosystems. This problem is widespread in southern Bulgaria, where the available water bodies are under high anthropogenic pressure due to the ex-

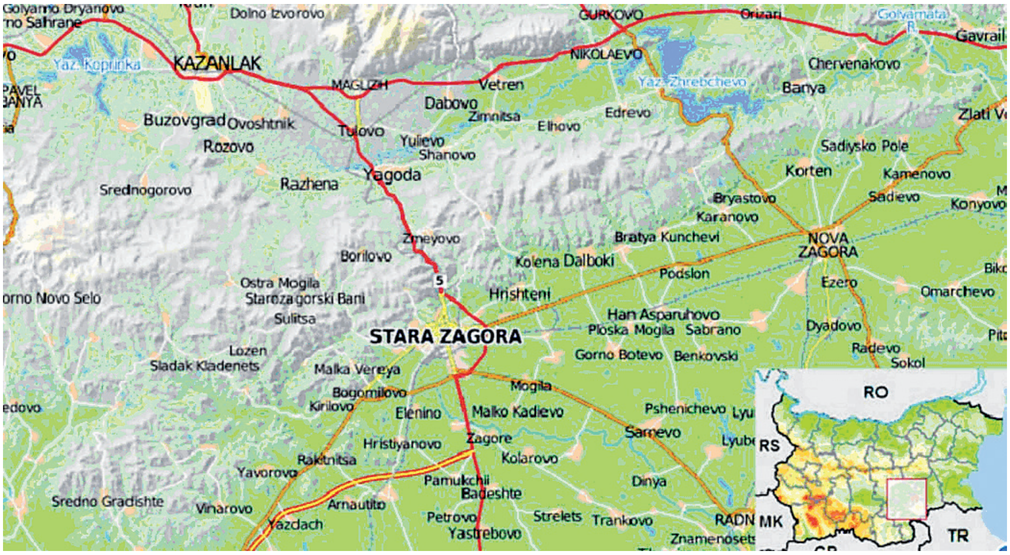

Fig. 1. Map of Stara Zagora region, Bulgaria

istence of large settlements (Stara Zagora, Kazanlak), industrial enterprises and developed agriculture.

The purpose of this study is to investigate and assess the content of the element lead in water, sediment, aquatic plants and musculature of *Cyprinus carpio* in water bodies in Stara Zagora Region, Bulgaria.

Materials and Methods

Four waterbodies, located on the territory of Stara Zagora Region, South Bulgaria was studied – the upper reaches of the Tunja River, Bedechka River, Sazliika River and Jrebchevo Dam Lake (Figure 1). The passage of the Tunja River through big settlements, industrial and agricultural regions is a precondition for the pollution of the river basin with toxicants of different nature. Bedechka River and Sazliika River are used mainly for irrigation. Waters of the Jrebchevo Dam Lake are used for electricity production, irrigation and fish farming.

Monitoring points

■ It was set 6 monitoring points for screening purposes in accordance with Regulation No 5/2007 and Regulation No 13/2007, as follows:

■ Monitoring Point 1 (MP-1) – Bedechka River, Stara Zagora Municipality
(N42.27049° E25.37937°);

■ Monitoring Point 2 (MP-2) – Sazliyska River, Stara Zagora Municipality
(N42.26914° E25.29015°);

■ Monitoring Point 3 (MP-3) – Tundzha River at Ygoda village, Maglizh Municipality (N42.32740° E25.3380°);

■ Monitoring Point 4 (MP-4) – Tundzha River at Jrebchevo Dam Lake, Nikolaevo Municipality (N42.38333° E25.49350°);

■ Monitoring Point 5 (MP-5) – Jrebchevo Dam Lake, Nova Zagora Municipality (N42.35346° E25.57020°);

■ Monitoring Point 6 (MP-6) – Tundzha River, Banya Village, Nova Zagora Municipality (N42.36243° E25.59466°).

Investigated indicators

In the water, sediment, aquatic plants and musculature of carp caught by the respective monitoring points, elements Pb has been studied.

Samples collected

Water: Seventy-two water samples of the studied waterbodies were collected from MP each month during one year (from November 23, 2011 to November 23, 2012) in accordance with the requirements of EN ISO 5667 – 1/2007. The water samples were stored in accordance with EN ISO 5667 – 3/2006.

Sediment

Six sediment samples, collected from studied waterbodies, were prepared, archived, stored and analyzed for the period from May to December 2012.

Aquatic plants

Six samples of the studied aquatic plants from water bodies were collected, archived and analyzed from May to December 2012.

Common Carp

Samples of musculature of common carp (18 pieces) were collected, archived and analyzed in the period from May to December 2012.

Methods for analysis

Water, sediment and aquatic plants samples were analyzed in the laboratories of the Scientific – Research Center for Environment at Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

The lead content in water, sediment, aquatic plants and musculature of *Cyprinus carpio* was determined on atomic absorption spectrometer (AAS) "A Analyst 800" – Perkin Elmer.

Analyses for lead in surface water samples were conducted in graphite tube or flame (depending on the concentration of these elements) at a definite wavelength and water preservation in advance of the samples with 5 cm^3 k.HNO_3 of a sample (ISO 8288, BS EN ISO 5667-3/2006). The contents of Pb in water samples were measured in mg.l^{-1}.

Average samples of sediment and aquatic plants are lyophilized to constant weight. Whole amount of dried sediment was milled and sieved repeatedly to fine powder. It was received not less than 20 grams of average representative sample. Decomposition with concentrated hydrochloric and nitric acids of sediment samples was carried out in accordance with ISO 11466. The samples were filtered and diluted to 50 ml with distilled water.

The samples of aquatic plants and musculature of common carp were prepared for the analysis by wet combustion in a microwave oven Perkin Elmer Multiwave 3000. The extracts were extended up to 25 ml with distilled water. The metal concentrations in the acid solutions were amended of AAS in accordance with ISO 11047. The concentrations of the investigated element of sediment, aquatic plants were expressed as mg.kg^{-1} dry weight. The lead content in the samples of common carp musculature was expressed in mg.kg^{-1} wet weight.

The instrument was periodically calibrated with standard chemical solutions prepared from commercially available chemicals (Merck, Germany). An air-acetylene flame and hollow cathode lamp for all samples were used. Calibration curves were prepared using dilutions of stock solutions. The samples (water, sediment and aquatic plants) were measured three times and the mean values were calculated.

Assessment of the Pb levels in the investigated components

Quality assessment of surface water by the respective monitoring points was carried out compared to average annual concentration values of lead in Directive 2008/105/EC and MCA in Directive 2013/39/EC.

The results from analysis of sediment samples from investigated water bodies are interpreted in accordance with Regulation № 3 from 01.08.2008 for norms of permissible content of harmful substances in the soil due to lack of documents relating to the sediments.

The assessment of Pb content in aquatic plants samples was not done, because regulated norms for this element are not available in Bulgaria.

Samples of common carp musculature are discussed according to Regulation № 31 – July of 29th 2004 on the maximum permissible quantities for contaminants in food and Commission regulation (EC) № 1881/2006.

Statistical analysis

Statistical processing of the results was computed by the program STATISTICA 6 using ANOVA test.

Result and Discussion

To realization of an overall monitoring of hydro ecosystem is necessary the tracking of the examined parameters both in the water and in the organism of hydrobionts (Bulgariu et al., 2010). The adsorption of heavy metals from the sediment on the bottom of the fresh water bodies leads to reduce their concentration in the water. This fact determines

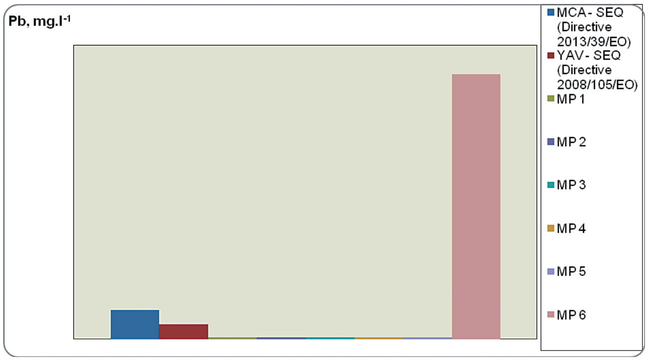

Fig. 2 Content of Pb in water of water bodies from Stara Zagora Region, Bulgaria

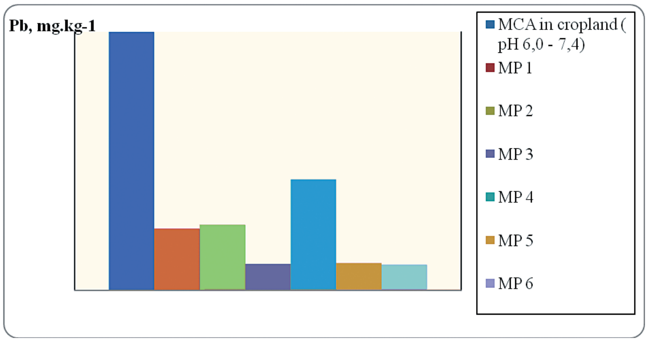

Fig. 3 Content of Pb in sediment of waterbodies from Stara Zagora Region, Bulgaria

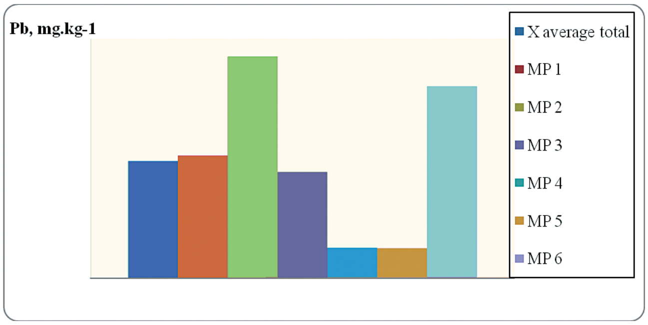

Fig. 4 Content of Pb in aquatic plants of waterbodies from Stara Zagora Region, Bulgaria

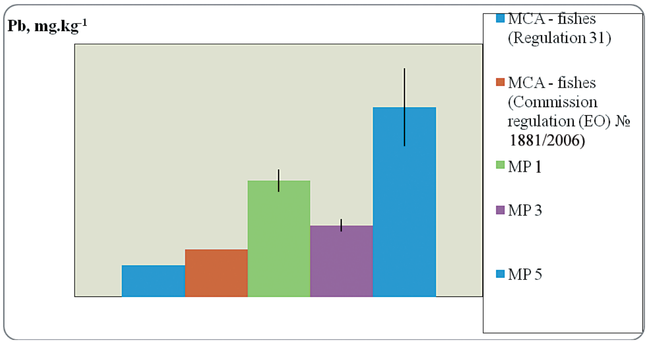

Fig. 5. Content of Pb in musculature of *Cyprinus carpio* of waterbodies from Stara Zagora Region, Bulgaria

the need to be measured the quantities of lead in water first and then in the sediment, aquatic plants and fish. Sediments postponed in the time may indicate the existence of even the temporary contaminations of the water body.

The sediment "seals" the past of aquatic ecosystems, "recording" the existence and concentration of pollutants. It is suitable habitat, which put it at the beginning of aquatic food chains (Pierzynski, 1998; Mc Laughlin, 2002). The last units of these chains represent hydrobionts of higher rank (omnivores and carnivorous fish). The results for the concentration of lead in water, sediment, aquatic plants and musculature of *Cyprinus carpio* from studied water bodies are shown on the following figures (Figures 2, 3, 4, 5).

The concentration of lead in the water of most of the investigated monitoring points is 0.001 mg.l^{-1} (Figure 2). Only in MP6 was measured value of 0.126 mg.l^{-1} which exceeds MCA is regulated by Directive 2013 with 0.112 mg.l^{-1} and YAV (Yearly Average Value) in Directive 2008 with 0.119 mg.l^{-1}. High concentrations of lead in the MP6 can are explains by the fact, that in this section of the river are flowing wastewater from households and agricultural activities.

Because globally and in Bulgarian legislation missing clear certain limits on the lead content in the sediments of waterbodies of a different nature interpretation the received data is extremely difficult. MCA, stipulated in Regulation № 3 of 01.08.2008 for norms for acceptable content of harmful substances in the soil for lead (Pb) in cropland at a pH of 6 to 7.4 is with a value 100 mg.kg^{-1}. All tested samples of sediment do not exceed this limit (Figure 3). However, the highest value of Pb was observed in the sediment taken from the MP4 (42.96 mg.kg^{-1}). The post represents the mouth of the Tunja River, by which it flows into Jrebchevo Dam Lake. Moreover, this zone of the river is near the Nikolaevo Town whereat it has been subjected to strong anthropogenic influence. Relatively high concentration is registered and in samples of Bedechka River (23.88 mg.kg^{-1}) and Sazliyka River (25.38 mg.kg^{-1}). Sediment taken from MP3 (Tunja River, Yagoda Village) is characterized by low concentrations of lead (10.3 mg.kg^{-1}), due to the high speed of aquatic currents. It was observed higher value in MP4 and the gradual decrease in the levels in MP5 and MP6.

The data of the lead content in water and sediments of the studied water bodies are quite contradictory. In water samples taken from monitoring points from 1 to 5 are observed only lead traces. At the same time the Pb concentrations registered in the sediment samples from MP1, MP2 and MP4 are comparatively high.

Levels recorded in the sediment of the Tunja River, Banya Village (MP6) are the lowest, while the value in water samples from the same point is highest. The results obtained are not sufficient to make conclusions regarding the adsorption processes of lead from water in sediment of water bodies (Rainbow, 2006).

To obtain a comparatively an accurate picture of potential contamination for a certain past period was necessary to analyze and samples of aquatic plants from the same points. The data exhibited in Figure 4 shows that the highest value of Pb is characterized MP2 (Sazliyka River) – 9.3 mg.kg^{-1}. Aquatic plants taken from Tunja River, Banya Village also contain high levels of Pb (8,02 mg.kg^{-1}). In point MP5 (Jrebchevo Dam Lake) – 1.25 mg.l^{-1} the lowest value was measured. The diversity of data received in analyzing of aquatic plants from different water bodies clearly shows the influence of specific factors for the deposition of Pb in each individual water body.

The trends for lead accumulation in aquatic plants and sediment of the studied reservoirs are radically different. Quantities of Pb registered in sediment from MP1 and MP4 are bigger than aquatic plants from the same locations. In MP6 is observed feedback – a higher content of Pb in aquatic plants compared to that in the sediment. Obviously, are necessary additional scientific researches which provide grounds to seek a direct relationship between the content of Pb in sediment and aquatic plants (Wang et al., 2010; Aly et al., 2012).

The availability in MP6 of high concentrations of Pb in waters, low concentrations in sediment and high in aquatic plants indicates that the passage of this element has proceeded from water to aquatic plants. By researching the heavy metals in water, sediment and aquatic plants Kamala-Kannan et al. (2008) have established that the concentration of lead in the aquatic plants is higher than that in the sediment and the water.

Table 1
Concentration ratio of Pb sediment/water, aquatic plants /water and carp/water in the monitoring points of investigated water bodies

Points	Coefficient of Pb concentration (sediment/water), in times more	Coefficient of Pb concentration (aquatic plants /water), in times more	Coefficient of Pb concentration (musculature of carp/water), in times more
MP 1	23 883	5120	736
MP 2	25 380	9260	–
MP 3	10 200	4440	453
MP 4	42 964	1270	–
MP 5	10 568	1250	1199
MP 6	78,6349	8020	–

The presented on Figure 5 results show that with the highest recorded value is distinguished MP5 (Jrebchevo Dam Lake) – 1.2 mg.kg^{-1}. This concentration is five times higher compared to the statutory requirements in Bulgarian and European legislation.

Significantly higher levels of Pb exceeding the limits are observed in MP1 and MP3. Lowest registered value in the musculature of common carp from the Tunja River, Jagoda Village (MP3) correspond with the levels of Pb in the sediment, aquatic plants and water taken from the same section of the river. The variety of data on the content of Pb in water, sediment, aquatic plants and musculature of common carp of the studied water bodies is a challenge to determine the ratio of this element between these components (Table 1).

Ratios obtained between Pb concentrations in sediment and water have shown that this heavy metal accumulates in the sediment from 78.6 (Tunja River, Banya Village) to 42964 (Tunja River, Nikolaevo Town) times more than the water. The results of the ratio aquatic plants/water show that lead reaches from 1250 (Zhrebchevo Dam Lake) to 8020 (the river Tunja Banya) times in aquatic plants compared to water. The proportion of common carp musculature/water leads to the conclusion that this metal accumulates 736 (Bedechka River) to 1199 (Jrebchevo Dam Lake) times more in musculature compared to water.

Conclusions

The highest value of Pb (0.126 mg.l^{-1}) was observed in the Tunja River locating near Banya Village, Bulgaria – an area with high human impact. The concentrations established in the water by all other monitoring points are significantly lower than MCA for this water type. With highest Pb value are characterized sediments taken from Tunja River – Nikolaevo Town, Bedechka River and Sazliyka River (Bulgaria). The ability of sediment to illustrate even temporary pollution in the hydro ecosystem makes it a good indicator showing the pollution degree of each specific water body. With the highest levels of Pb are distinguished aquatic plants from Sazliyka River, Tunja River – Banya Village and Bedechka River (Bulgaria). The common carp musculature from Jrebchevo Dam Lake has a Pb value 1.2 mg.kg^{-1}, which several times exceed the requirements of the Bulgarian and European legislation. These results clearly demonstrate the ability of the sediment, aquatic plants and fish to serve as excellent indicators of lead pollution.

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