



## ACCUMULATION OF MANGANESE IN SELECTED LINKS OF FOOD CHAINS IN AQUATIC ECOSYSTEMS

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### Abstract

The accumulation of elements in biocenoses of aquatic ecosystems depends primarily on the forms of elements in the environment. The bioaccumulation coefficient (BC) is a measure of the intensity of an elements uptake of trace by living organisms. Manganese is an essential element for both plant and animal organisms. However, its excess may cause a toxic effect, i.e. it disturbs the activity of synapses, thus leading to an impaired functioning of the central nervous system. This study focused on the accumulation of manganese in individual links of an aquatic ecosystem food chain under conditions of extensive carp farming. The BC values were computed and the pollution degree of the fish pond was estimated. The investigations were conducted in a fish pond situated in Mydlniki and fed with water from the Rudawa River. Water, bottom sediment, benthic organisms (*Diptera Chironomidae* larvae) and carps were sampled from the pond. Organs most strongly involved in the metal metabolism (gills, gonads, liver and muscles) were prepared from sampled carps. Manganese concentrations were determined in all samples using atomic emission spectroscopy after wet mineralization of samples in a closed system in a microwave mineralizer. The concentrations of manganese in the abiotic elements of pond ecosystems were low and should not pose any threat of its excessive accumulation in living organisms. However, the manganese concentrations in the benthic organisms and in the analyzed carp organs were high. Similarly, other authors found high manganese concentration in fish living in the environments polluted with this element. The value of manganese enrichment coefficient for the bottom sediments in relation to its water concentration was high. The biggest manganese content was assessed in gills, then in the liver and gonads, and the smallest one - in carp muscles. Manganese BCs in the gills of carps in relation to its content in water and bottom sediments were 176.6 and 0.08, respectively. The BC values in relation to the manganese concentration in water were much higher, but lower in comparison to its content in bottom sediments than reported elsewhere. This confirms that the BC for manganese in gills varies depending on the water pollution level.

**Keywords:** manganese, bioaccumulation, food chain, aquaculture.

## INTRODUCTION

Manganese is a crucial element for living organisms, both plants and animals. This element functions as a catalyst of various enzymatic reactions. It is an activator of superoxide dismutase. Moreover, it participates in lipid metabolism and is also an electron carrier in phosphorylation processes. Manganese deficiency in animals leads to bone deformation and inhibited growth, lower fodder uptake, weaker liver detoxication activity and diminished content of this element in muscles. A decreased manganese content in muscles deteriorates their nutritive value (LIN et al. 2008, PAN et al. 2008). The demand for this element differs depending on fish species, but ranges from around 5 to 13 mg kg<sup>-1</sup> of a diet. For yellow catfish, the demand has been estimated at the level of 5.5 mg kg<sup>-1</sup> of a diet (TAN et al. 2012), for tilapia it equals 7.7 mg kg<sup>-1</sup> of a diet (PAN et al. 2008), whereas for carp it reaches 13.5 mg kg<sup>-1</sup> of a diet (LIN et al. 2008). Despite fish's ability to uptake manganese through gills, these organisms are incapable of satisfying the requirement for this element by absorbing it directly from water, even if it contains large quantities of dissolved manganese. Therefore, the manganese uptake with food is of key importance for the metabolism of this element (PAN et al. 2008). Manganese deficiency in fish organisms is rarely observed under natural conditions because its considerable amounts enter the food chain from the bottom sediments. The absorption of this element by fish organisms depends on its supply in the environment, quality parameters of abiotic elements of the environment and the fish species itself, as well as individual conditions (BERVOETS, BLUST 2003).

An excess of this element causes a toxic effect. Large amounts of manganese disturb functions of synapses and impair the central nervous system because manganese ions penetrate the blood-brain barrier. High concentrations of manganese in an aquatic environment cause disturbances in the sodium balance, reduce the absorption of calcium and phosphorus, disturb the metabolism of carbohydrates and impair the immunological functions of fish (PARTRIDGE, LYMBERY 2009, YE et al. 2009).

Considerable amounts of manganese are found in water sediments, where it occurs as MnO<sub>2</sub>. This form of manganese is unavailable to living organisms. Anaerobic conditions occasionally occurring in the bottom zone lead to this compound's transformation into Mn<sup>2+</sup>, a biologically active manganese form. A decreased number of blood cells and poorer immunological activity were detected in Norwegian lobsters exposed to excessive amounts of manganese under conditions of frequently very low levels of dissolved oxygen in the bottom zone. Water animals respond similarly to excessive quantities of manganese in an aquatic environment as to oxygen deficiency (HERNROTH et al. 2004). The effect of manganese toxicity may be observed mainly in organisms inhabiting fresh water bodies, which is due to a much higher concentration of this element in fresh waters, with lower salinity than in seawater.

In seawater, manganese concentrations in water at a level of  $5 \text{ mg dm}^{-3}$  cause negative effects on the growth, development and survivability of fish (PARTRIDGE, LYMBERY 2009). In fresh water, signs of acute toxicity of manganese in silver salmon (*Oncorhynchus kisutch*) were noticed at a concentration of just  $2.4 \text{ mg Mn dm}^{-3}$ , whereas in stinging catfish (*Heteropneustes fossilis*) they became evident at a value of  $3,350 \text{ mg dm}^{-3}$  (VIEIRA et al. 2012). Toxic effects of manganese on fish are reversely proportional to water salinity.

As a result of chemical, physical or biochemical processes connected with water self-purification, manganese accumulates mainly in bottom sediments, owing to which its supply to living organisms becomes limited. A change of the aerobic conditions in the bottom zone of a water body may lead to the mobilization of considerable amounts of this metal (CHANDRA SEK HAR et al. 2003).

The aim of the paper was to determine the accumulation of manganese in selected links of a food chain in a pond ecosystem under conditions of extensive carp farming. On the basis of the research, the manganese bioaccumulation coefficient value in an aquatic ecosystem was computed and the pollution level in the analyzed ecosystem was estimated.

## MATERIAL AND METHODS

The research on manganese cycling was conducted in 2008, in a fish pond which belongs to the Experimental Station of the Department of Ichthyobiology and Fisheries, University of Agriculture in Krakow. The pond lies in the village of Mydlniki ( $50^{\circ}5'18'' \text{ N}$ ;  $19^{\circ}50'58'' \text{ E}$ ) and is fed by the Rudawa River water. The commercial pond covers an area of about 4 ha. The research comprised assessment of the manganese content in water, bottom sediments, benthos and in carp (*Cyprinus carpio* L.) organs.

Manganese concentrations in water were assessed three times during the carp feeding period. Samples were collected at the beginning of the feeding period (in May), during the period of the most intensive fish feeding (in July) and in September, at the end of the fattening period. Water was sampled from six sites in the pond. Bottom sediments were taken from the surface layer of the pond bottom (0-5 cm) after the pond had been emptied. The pond was divided into 8 zones and water samples for laboratory tests were collected from each zone (two samples in the vicinity of the inlet, 4 samples from the central part of the pond and two in the vicinity of the outlet). Bottom sediments were dried, sifted through a sieve with 1 mm mesh and crushed in a mortar. Samples of benthic organisms (larvae of *Diptera* family *Chironomidae*) were collected in the same sites. Manganese concentrations were assessed in carp (*Cyprinus carpio* L.), in 25 randomly selected specimens intended for consumption. The fish gender was determined (organoleptic method), age (according to the production logbook) and weight (gravimetric method). The fish originated from a three-year rearing period, and the

weight of individual specimens fluctuated from 1,500 to 2,200 g. Carps were sacrificed by decapitation and their organs (gills, muscles, livers and gonads) were prepared. Laboratory samples were subjected to wet mineralization in a closed system in a microwave mineralizer. A portion weighed out for analyses was *ca* 0.5 g in conversion to dry mass. Biological material was dissolved in a mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> (5:1, v/v ratio), while bottom sediments were dissolved in *aqua regia*, in the sediment/reagent quantitative ratio equal 1:10.

Water samples for analyses were condensed ten times. Manganese concentrations in the solutions were assessed by inductively coupled plasma atomic emission spectroscopy (ICP-AES), on a JY 238 ULTRACE Apparatus (Jobin Yvon), at the wavelength 257.608 nm. The manganese determination limit in the applied method was 0.0014 mg Mn dm<sup>-3</sup>. The uncertainty of measurements according to the applied methods was ±4%. The limit of determination for the analyses was 0.042 µg kg<sup>-1</sup> d.m. of biological material and 0.068 µg g<sup>-1</sup> d.m. of sediments. The correctness of manganese assays was verified using certified reference material CRM 16-050. The analytical correctness of the manganese content in the biological material was tested on the basis of internal reference material. Other determinations included the pH and grain-size composition of sediments as well as the organic carbon content (OSTROWSKA et al. 1991). A more detailed description of the research method was presented in an earlier paper (NIEMIEC, WIŚNIEWSKA-KIELIAN 2013).

## RESULTS AND DISCUSSION

The manganese content in the analyzed pond water ranged from 42.11 to 111.7 µg Mn dm<sup>-3</sup>, reaching an average value of 57.92 µg Mn dm<sup>-3</sup> (Table 1).

Table 1  
Statistical parameters of the manganese content in selected compartments of the analyzed aquatic ecosystem

Statistical parameters	Water	Sediment	Benthos	Organs of <i>Cyprinus carpio</i> L.			
				gills	gonads	muscle	liver
	(µg dm <sup>-3</sup> )	(mg kg <sup>-1</sup> d.m.)					
Minimum	42.11	76.13	19.27	2.500	0.803	0.357	1.876
Maximum	111.7	173.6	33.88	16.74	11.88	2.719	10.50
Mean	57.92	128.0	24.51	10.23	2.442	0.876	3.899
Median	49.83	129.3	23.43	10.29	1.631	0.627	3.167
Standard deviation	22.29	36.41	4.901	3.303	2.350	0.630	2.026
Relative standard deviation (%)	38.48	28.44	20.00	32.29	96.23	71.89	51.95

No statistically significant differences were noted between samples collected at the subsequent dates (Figure 1).

Manganese in water originates mainly from the parent rock leaching. An elevated content of this element is often determined in underground waters. Its natural content in unpolluted water reaches  $150 \mu\text{g Mn dm}^{-3}$ . At a higher manganese content in water, fish gills may malfunction due to disturbed ionic exchange, particularly of sodium (GONZALEZ et al. 1990).

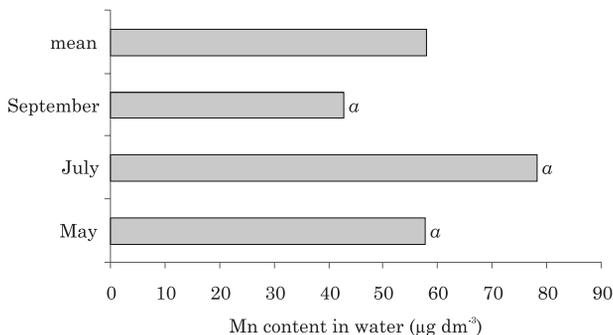


Fig. 1. Concentrations of manganese in water from the pond ( $\mu\text{g Mn dm}^{-3}$ )

Aquaculture animals reared in tanks fed by underground water may experience haematological disturbances, which are caused by an increased manganese amount in the environment (WEPENER et al. 1992, FISH 2009). Manganese toxicity increases as the water hardness and salinity decrease (STUBBLEFIELD et al. 1997).

Water sediments are generally characterized by a high content of manganese, which reflects this element's high affinity to mineral and organic seston particles. A stronger effect of manganese on living organisms is observed in surface water containing little seston than in water full of settling sediment. The availability of manganese to living organisms is more strongly affected by the physicochemical parameters of a biotope, which directly translates into the transformations of manganese dissolved in water and contained in sediments.

Oxidation of  $\text{Mn}^{2+}$  without participation of microorganisms occurs rapidly in alkaline reaction,  $\text{pH} > 9$ , whereas at  $\text{pH}$  value  $< 7.5$ , oxidation of this element takes place mainly with the contribution of microorganisms (MOORE, PATRICK 1989). Reduction of  $\text{Mn}^{7+}$  manganese appears when the oxidative-reductive potential is low enough to create conditions for anaerobic microorganisms to develop (JAUREGUI, REISENAUER 1982). ADHIKARI et al. (2009) report that fish pond sediments contained  $298.5 \text{ mg Mn kg}^{-1}$ ; simultaneously the water concentration of manganese was about  $66 \mu\text{g dm}^{-3}$ . The coefficient of sediment enrichment in manganese in the above experiment was about 4,522. The cited authors also stated that the content of this element in ses-

ton reached  $7.2 \text{ mg kg}^{-1} \text{ d.m.}$ , although its amounts accumulated in fish were smaller than in the currently presented analyses.

In our investigations, the manganese content in the sediments was almost 2,210 times higher than its water concentration. FAIRA et al. (2008) detected nearly ten-fold smaller quantities of manganese in the sediments from streams at the manganese content in water approximately the same as obtained in our research. Such a low coefficient of sediments enrichment in manganese was caused by the acid reaction of water in the analyzed streams. ALAM et al. (2001) reported that the value of the coefficient of enrichment with this element in sediments of commercial fishponds was about 5, whereas the value for natural reservoirs under similar conditions exceeded 20. The coefficient of the sediment enrichment in manganese determined in the current study was less than half the value determined by ADHIKARI et al. (2009). An average content of manganese in the sediments from the analyzed pond was  $128.0 \text{ mg kg}^{-1} \text{ d.m.}$ , fluctuating from 76.13 to  $173.6 \text{ mg kg}^{-1} \text{ d.m.}$  (Table 1). The manganese concentrations determined in the sediments were close to the values presented in literature (VYMAZAL, ŠVEHLA 2012). The analyzed sediments are usually silt deposits with between 33 and 54% floatable particles and the content of organic matter between 13.9 and  $50.1 \text{ g kg}^{-1} \text{ d.m.}$  The analyzed bottom sediments reveal physical and physicochemical properties typical for sediments from fishponds (YUVANATEMIYA, BOYD 2006).

The most important representatives of benthic invertebrates in the investigated ecosystem are larvae of *Diptera* family *Chironomidae*. These organisms are common in eutrophic water bodies in the northern hemisphere, at temperate latitudes, and may be found both in lenitic and lotic environments. Owing to their ecological functions connected with the decomposition of detritus, and the key role in a diet of benthic-eating fish, larvae of the *Diptera* family *Chironomidae* are a frequent object of investigations conducted in fresh waters because they constitute the major part of bottom associations (GARCIA-BERTHOUS 1999). *Chironomidae* are an important group of organisms used for biomonitoring of the environment pollution (FARIA et al. 2008). The manganese concentrations in the benthic organisms of the analyzed ecosystem revealed low variability and fluctuated from 19.27 to  $33.88 \text{ mg Mn kg}^{-1} \text{ d.m.}$ , on average  $24.51 \text{ mg Mn kg}^{-1} \text{ d.m.}$  (Table 1). Benthic organisms are especially sensitive to the accumulation of manganese, which is much higher in water sediments. The coefficient of manganese bioaccumulation in the analyzed *Chironomidae* larvae was high, reaching 423 in relation to its water concentration and 0.191 in relation to the content in bottom sediments. FOXALL et al. (2000) reported a very high manganese content in snails from Tanganyika Lake, namely between 5,200 and  $7,890 \text{ mg Mn kg}^{-1} \text{ d.m.}$  depending on a sampling location. The content of this element in benthic organisms sampled in coastal waters of the south eastern part of the Mediterranean Sea ranged from 2.77 to  $6.27 \text{ mg Mn kg}^{-1} \text{ f.m.}$  (KRESS et al. 1998), which in conversion to dry mass gives slightly higher results than obtained in our research. Very high manganese concentrations in benthic organisms result

from the high accumulation of this element in sediments. The cited authors pointed to the specific character of sediments in tropical waters, which are characterized by a very high manganese content.

Table 2

Bioaccumulation coefficients of manganese in benthos and in individual organs of *Ciprinus carpio* L. in the analyzed ecosystem

Element of ecosystem	Larvae	Gills	Gonads	Muscles	Liver
Water	423.0	176.6	33.21	15.12	67.30
Sediment	0.191	0.080	0.015	0.007	0.030
Larvae	-	0.417	0.079	0.036	0.159

As an element, manganese undergoes very strong bioconcentration, particularly in aquatic organisms situated lower in a food chain (OWESON, HERNROTH 2009). The value of this element's bioaccumulation coefficient in fish may even exceed 900 (VIEIRA et al. 2012). Manganese is most easily absorbed by the skin, gills and intestinal epithelium. It is accumulated mainly in the liver and cranial bones (NYBERG et al. 1995). There are no data in literature to confirm the biomagnification of this element.

Manganese concentrations in carp muscles were highly varied, fluctuating from 0.357-2.719 mg Mn kg<sup>-1</sup> d.m., with an average value of 0.876 mg Mn kg<sup>-1</sup> d.m. (Table 1). UYSAL et al. (2008) estimated the manganese content in muscles of various fish species migrating from the Beymelek Lagoon in Turkey within the range from 0.09 to 0.28 mg kg<sup>-1</sup> f.m., which in conversion to dry matter gives values approximately the same as ours. KORKMAZ et al. (2012) reported manganese concentrations in muscles of sea fish from the Black Sea caught near the Turkish shore ranging from 0.56 to 0.93 Mn kg<sup>-1</sup> d.m., depending on a fish species. On the other hand, COOPER et al. (2006) detected a very high content of manganese in *Danio rerio* fish carcasses, within the range of 20 to over 25 mg Mn kg<sup>-1</sup> d.m., depending on the amount of this element in feed. The same authors point to a significant effect of a feed's richness in manganese on this element's level in fish organisms. HENRY et al. (2004) reported manganese concentrations in muscles of several fish species caught in the North Sea, such as between 0.87 and 2.4 mg Mn kg<sup>-1</sup> d.m., thus similar to the ones determined herein. FOXHALL et al. (2000) stated that manganese concentrations in muscles of fish from Tanganica Lake were between 0.6 and 0.8 mg Mn kg<sup>-1</sup> d.m. No effect of a sampling point location on the amount of manganese accumulated in fish organisms was confirmed. AL-YOUSUF et al. (2000) noted a manganese content of ca 0.1 mg Mn kg<sup>-1</sup> f.m. in muscles of *Lethrinidae* fish caught in the Persian Gulf, in locations exposed to strong anthropopressure, which in conversion to dry matter was about 0.5 mg Mn kg<sup>-1</sup>. GLADYSHEV et al. (2009), who, in 2005-2007, studied seasonal dynamics of manganese concentrations in muscle tissue of Siberian grayling (*Thymallus arcticus* Pallas) taken from the Yenisei River upstream of Kra-

snoyarsk (Siberia, Russia) found significant irregular variations of its content ranging from 0.2 to 1.0 mg Mn kg<sup>-1</sup> f.m. Manganese concentrations in the muscles of Indian carps (*Cirrhana mrigala*) kept in water with a manganese content similar to the one in the carp pond chosen for our study ranged from 0.11 to 0.14 mg Mn kg<sup>-1</sup> d.m. (ADHIKARI et al.2009). PARTRIDGE and LYMBERY (2009) determined the manganese content in muscles of *Argyrosomus regius* on the level of 2.8 mg Mn kg<sup>-1</sup> d.m. Keeping fish in water with 5 mg Mn dm<sup>-3</sup> concentration caused an increase in the manganese content in their muscles to the level of 9.4 mg Mn kg<sup>-1</sup> d.m. In the light of references, it may be suggested that the analyzed fish were characterized by a high manganese content. The value of the manganese bioaccumulation coefficient in muscles of the carp fish was 15.12 and 0.007 in relation to its concentrations in water and bottom sediments, respectively, whereas in relation to the manganese concentration in the benthos, this parameter equalled 0.036. ADHIKARI et al. (2009) obtained much lower manganese bioaccumulation coefficients in muscles of Indian carps (*Cirrhana mrigala*) kept in polluted environments, i.e. 2.121 in relation to the Mn concentration in water and 0.00046 in relation to its content in bottom sediments.

Manganese accumulation in fish gills is connected with its excretion and uptake from water, which is of crucial importance for this element's cycling. On the other hand, absorption of this metal from feed is less important. Generally, its high amounts are found in fish gills irrespective of a manganese level in abiotic elements of the environment, which is particularly visible in species inhabiting the bottom zones (HERNROTH et al. 2004). In acidic water, manganese becomes mineralized and its toxic effect on fish is exacerbated, especially in water with a low eutrophication level. Such an effect is observed in streams inhabited by *Salmonidae* fish (NYBERG et al. 1995). At a low water pH, the precipitation of manganese compounds into bottom sediments is limited (FARIA et al. 2008). In our experiment, the mean manganese concentration in the gills of carp fish was 10.23 mg kg<sup>-1</sup> d.m., ranging widely from 2.500 to 16.74 mg kg<sup>-1</sup> d.m. (Table 1). UYSAL et al. (2008) demonstrated a manganese content in gills of various fish species, migrating from the Beymelek Lagoon in Turkey, within the range from 1.40 to 12.81 mg kg<sup>-1</sup> f.m. PARTRIDGE and LYMBERY (2009) noted the manganese content in gills of *Argyrosomus regius* at 28 mg Mn kg<sup>-1</sup> d.m. Keeping fish in water with 5 mg Mn dm<sup>-3</sup> caused an increase in the content of this metal in fish gills to 255 mg Mn kg<sup>-1</sup> d.m. Values of the manganese bioaccumulation coefficient in gills were 176.6 and 0.080 in relation to its content in water and sediments, respectively, whereas the coefficient of manganese bioaccumulation in relation to its content in *Diptera* larvae was 0.417. ADHIKARI et al. (2009) reported the coefficient of manganese accumulation in the gills of Indian carps (*Cirrhana mrigala*) kept in polluted environment as 5.151 in relation to the Mn concentration in water and 0.00114 in relation to its content in the bottom sediments. Manganese concentration in fish gills is the best indicator of an environmental hazard posed by this element (VIEIRA et al. 2012). Primary

manganese accumulation occurs in these organs. A sudden increase in an Mn water concentration rapidly results in its elevated content in fish gills (PEREIRA et al. 2010).

The fish liver is an organ distinguished by high capability of accumulating manganese, which is why it is frequently used for the biomonitoring of manganese pollution. The mean content of manganese in the liver of carp fish was 3.899 mg Mn kg<sup>-1</sup> d.m. and ranged from 1.876 to 10.50 mg Mn kg<sup>-1</sup> d.m. (Table 1). The manganese content in the liver of several fish species from coastal waters of the North Sea in northern France fluctuated from 2.9 to 11.0 mg kg<sup>-1</sup> d.m. (HENRY et al. 2004). PARTRIDGE and LYMBERY (2009) determined 4.4 mg Mn kg<sup>-1</sup> d.m. in the liver of *Argyrosomus regius*. Keeping fish in water with 5 mg Mn dm<sup>-3</sup> caused a nearly fourfold increase in the manganese content in the liver, to the level of 19.5 mg Mn kg<sup>-1</sup> d.m. AL-YOUSUF et al. (2000) conducted research on manganese bioaccumulation in livers of the *Lethrinidae* fish caught in the Persian Gulf, in sites with strong anthropopressure. Their results showed that an average manganese concentration in females' livers was 1.35 and in males – 0.97 mg kg<sup>-1</sup> f.m. The authors emphasize the effect of a fish gender on manganese concentrations in their livers. In our research, no statistically significant differences were assessed in manganese concentrations in male or female livers (Figure 2).

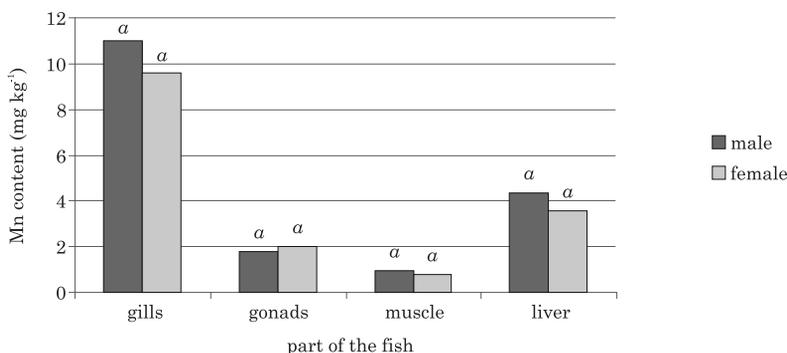


Fig. 2. Content of manganese in organs of *Ciprinus carpio* L. depending on a sex (mg Mn kg<sup>-1</sup>)

The coefficient of manganese bioaccumulation in the livers of carps was 67.3 and 0.030 in relation to its content in water and sediments, respectively, whereas in relation to its content in benthic organisms it equaled 0.159 (Table 2). ADHIKARI et al. (2009) reported much lower values of Mn bioaccumulation coefficients in livers of Indian carp (*Cirrhana mrigala*) kept in polluted environments, i.e. 8.03 in relation to the manganese concentration in water and 0.00178 to its content in bottom sediments. The authors notice that the coefficient of metal accumulation in organisms of farmed fish is invariably lower than the same parameter computed for wild fish.

The mean content of manganese in the analyzed fish gonads was 1.924 and fluctuated from 0.803 to 4.71 mg Mn kg<sup>-1</sup> d.m. The bioaccumulation coefficient of the metal in gonads in relation to its concentrations in water and sediments was 33.21 and 0.015, respectively, whereas in relation to the manganese content in benthic organisms it assumed the value of 0.079 (Table 2).

The highest mean content of manganese was assessed in gills, then in the liver and gonads, and the lowest one in carp muscles (Figure 3).

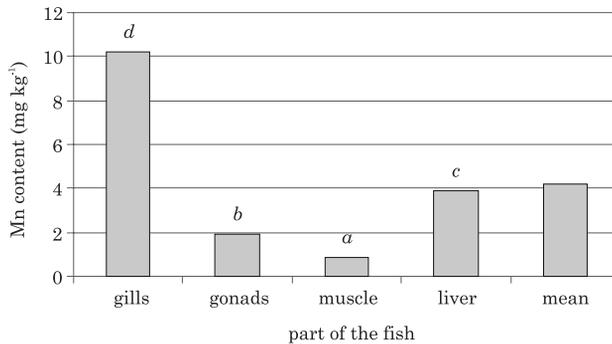


Fig. 3. The mean content of manganese in organs of *Ciprinus carpio* L.

## CONCLUSIONS

1. The manganese concentration in water and bottom sediments of the fish pond was not high and did not pose any potential threat to living organisms.

2. The manganese content in individual carp organs diminished in the following order: gills > liver > gonads > muscles.

3. The manganese content in the individual organs of fish was high, comparable to the one assessed in fish kept in polluted waters.

4. Considerably higher values of the manganese bioaccumulation coefficient were determined for its content in water, bottom sediments and benthic organisms than reported in literature.

5. A high content of manganese in the analyzed ecosystem's abiotic elements was reflected by the high bioaccumulation of this element.

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