Journal of Elementology ISSN 1644-2296

Niemiec M., Szeląg-Sikora A., Sikora J., Cupiał M., Komorowska M. 2018.
Assessment of lead and chromium pollution in the ecosystem of the Dunajec River based on bioindicative methods.
J. Elem., 23(3): 1087-1098. DOI: 10.5601/jelem.2017.22.4.1457

RECEIVED: 8 May 2017
ACCEPTED: 2 March 2018

ORIGINAL PAPER

ASSESSMENT OF LEAD AND CHROMIUM POLLUTION IN THE ECOSYSTEM OF THE DUNAJEC RIVER BASED ON BIOINDICATIVE METHODS*

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Abstract

The Dunajec River is a right-bank tributary of the Vistula River. Main sources of pollution of the river include municipal and industrial sewage as well as domestic sewage discharged from the towns Zakopane, Nowy Targ, Nowy Sącz and Tarnów. The aim of this paper was to assess lead and chromium content in abiotic parts of the Dunajec's ecosystem as well as to determine the bioaccumulation factor for this element in selected organs: muscles, liver, skin, and bones of the bleak fish (*Alburnus alburnus* L.). The samples of water and bottom sediments were collected twice from the Dunajec River, in July and October 2011, in 5 measuring points: Szczepanowice, Janowice, Wróblowice, Lusławice and Zakliczyn. At the same time, bleaks (29 pieces) caught by members of the Polish Angling Association (PZW) in the studied section of the river in the second half of July were used in the research. The concentration of chromium in the solution was determined using atomic emission spectrometry with inductively coupled plasma in an apparatus Optima 7600 DV Perkin Elmer. Lead concentrations were determined on an atomic absorption spectrometer coupled with an electrothermal atomizer. The results indicate that the lead and chromium content in the water exceeded the values characteristic for unpolluted environments. The content of these elements in the sediment was lower than the geochemical background of the water sediments in Poland (10 mg Pb kg^{-1} and 5 mg Cr kg^{-1}). The content of lead in different organs decreased in the following order: liver> skeleton> muscles> skin, while for the content of chromium the sequence was: liver> skeleton> skin> muscle. The values of the bioaccumulation coefficient of lead in the individual organs of the bleak, as compared to the concentration of this element in the water, ranged from 7770 to 18 909, and of chromium – from 1597 to 7895.

Keywords: lead, chromium, Dunajec River, bleak fish, bioaccumulation.

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* This research was funded by the Ministry of Science and Higher Education of the Republic of Poland.

INTRODUCTION

Trace elements are natural components of all aquatic ecosystems. The specificity of metal circulation in these ecosystems is associated with their removal from water by a series of chemical, physical and biological processes. They comprise the process of self-cleaning of water, which maintains the quantities of their water soluble forms at a level that does not endanger living organisms. The disruption of the homeostasis of water bodies, caused by an inflow of large amounts of xenobiotics, a change in the properties of water, or by some disturbance of sediments, can impair the self-purification processes of water and even cause a secondary run of the bottom sediments, associated with toxic elements. The negative impact of heavy metals on living organisms is related to their direct toxicological influence. They also cause mutagenic changes, which could affect populations also outside the direct impact of the pollutants (Russo et al. 2004, Łuczyńska et al. 2016). Lead is a common element in the environment. Due to its qualities, such as low hardness, ductility, toughness, corrosion resistance and ease of production, the anthropogenic effects of the deposition of this element were observed even in antiquity. Also, lead causes abnormal physiological, biochemical and neurological disorders in living organisms. Chromium is a common element in many fields of human activity, which is why it is often found in increased amounts in wastewater, both industrial and municipal. Chromium is widely used in tanning, protective coatings of metal objects, paints and biocides (Ciacci et al. 2012, Qian et al. 2013). The most common content of this element in marine and ocean waters is below 1 μg m^{-3}, while the natural content of this element in water is less than 0.2 μg Cr dm^{-3}. In freshwater, its amount is approximately 10 times greater. Fish are an important part of river ecosystems because of their role in the flow of energy and matter circulation in these ecosystems. Although these are organisms characterized by considerable mobility, they are widely recognized as a good indicator of heavy metal pollution of the aquatic environment. Fish occupy a high position in the trophic chain, hence pollutants from lower links can accumulate in them, depending on the character of pollution in a given ecosystem.

MATERIAL AND METHODS

To achieve the research objective, samples of water and sediment were collected from the Dunajec River in 2011. The sampling was performed twice (in July and October), in 5 research points located in Szczepanowice, Janowice, Wróblowice, Lusławice and Zakliczyn. The research points were located over a length of approximately 10 kilometers in the Carpathian part of the Dunajec, in the municipalities of Zakliczyn and Pleśna. The research area includes the stretch of the river downstream off the dam reservoir Rożnów-Czchów. The ecosystem of the river below this water reservoir is more sensitive to contamination having less seston carried by the water below the dam. The water samples were collected from the top layer, at a depth of 10 cm. The sampling points were selected so as to eliminate the risk of sampling stagnant water. The samples were taken at a distance of approximately 2-3 meters from the shore, but always from the full current. Each laboratory sample had a volume of 1 $dm^{3.}$ The samples of sediments were collected at the same locations, from the seston deposition spots on sandbanks. Bulk samples comprised 6-10 primary samples weighing approximately 200 g. The laboratory samples had a mass of about 500 g per dry weight. At the same time, in the second half of July, 29 specimens of fish of the species bleak *(Alburnus alburnus* L.) along the analyzed course of the river. The samples of fish used in the study were provided by members of the Polish Anglers Association, who earmarked the caught fish for consumption. Prior to being transported to the laboratory, the collected water samples were preserved *in situ* by adding nitric acid (V) in the amount of 2 cm^3 per 100 cm^3 of water. The sediments were dried, sieved through a 1 mm mesh and triturated in a mortar. The mass of fish used in the study ranged from 30 to 42 g, and their length was from 15 to 19 cm. The age of the fish was approximately one year. They came from the previous year's spawning and were sexually immature. The fish were dissected, i.e. the skeleton, muscles, liver and skin were removed. The choice of these organs was dictated by their usefulness in the bioindicative assessment method of the environment (Moureaux et al. 2011), and analytical capabilities. The laboratory samples were subjected to wet digestion in a closed system with the use of microwave energy. The digestion was carried out using a microwave system Anton Paar Multivawe 3000. The analytical sample weight was approximately 0.5 g per dry matter. The biological material was dissolved in a mixture of HNO_3 and H_2O_2 at a ratio of 5:1, v/v, and the sediments in *aqua regia*, in a 1:10 ratio of the sediment to the reactants.

The concentration of chromium in the solution was determined using atomic emission spectrometry with inductively coupled plasma in an apparatus Optima 7600 DV Perkin Elmer. Lead concentrations was determined on an atomic absorption spectrometer coupled with an electrothermal atomizer. The wavelengths used in the analysis, as well as the quality parameters of the analytical methods are shown in Table 1. To ensure the accuracy of the analysis certified reference materials IAEA-407 and CRM 16-050 were used (Table 1). The results of the mean element content were compared using Student's *t*-test at the significance level of $p = 0.01$. Based on the results, the bioaccumulation factors of individual elements were calculated by dividing the concentration of a given element in the dry matter of fish by the content of the element in the water and bottom sediments

$$\textit{bioaccumulation coefficient} = \frac{\textit{metal concentration in sediment or fish organs}\ (\mathrm{mg\ kg^{-1}})}{\textit{metal concentration in water}\ (\mathrm{mg\ dm^{-3}})}$$

Table 1

Parameters of the analytical method

Specification	Pb	Cr
Wavelenghts (nm)	217.0	267.707
Detection limit (μg dm^{-3})	0.042	7.1
Limit of quantification for biological material (mg kg^{-1})	0.0021	0.355
Limit of quantification for the bottom sediments (mg kg^{-1})	0.0042	0.71
Limit of quantification for water (μg dm^{-3})	0.042	0.71
Content in the certificated material IAEA-407 (mg kg^{-1})	0.12	0.73
Measured (mg kg^{-1})	0.126	0.71
Recovery (%)	104.59	97.26

The concentration of metals is expressed in μg dm^{-3} in water and in mg kg^{-1} DM (dry matter) in sediments and fish organs.

Additionally, using a non-parametric test, values of the linear correlation were calculated between the concentration of the analyzed elements in individual organs of fish.

RESULTS AND DISCUSSION

The average lead content in the tested water samples was 0.120 μg Pb dm^{-3}, and ranged from 0.080 to 0.180 μg Pb dm^{-3}. The chromium content in the tested water samples ranged from 2.856 to 4.521 μg Cr dm^{-3}, and its mean value was 3.338 μg Cr dm^{-3}. For either of the elements, there was no significant difference in their concentrations in water samples collected in summer and autumn; however, differences were observed in the content of these elements in water from individual sampling points. The highest content of lead and chromium was found in water sampled in Zakliczyn. The lowest content of chromium occurred in the water taken from the Dunajec in Szczepanowice, while the least of lead was present in the samples taken in Szczepanowice and Wróblowice. The content of the two elements in the water from the Dunajec was not high and did not indicate anthropogenic enrichment. The average content of chromium in the water from fish ponds located in the vicinity of Krakow was 7.761 μg Cr dm^{-3} (Niemiec et al. 2013). Lead content in the water of the Ripol River in Spain, which is subject to anthropogenic pollution, was between 109 and 150 μg dm^{-3} (Maceda-Veiga et al. 2013). The lead and chromium content in the water of the Dunajec, found in our study, was similar to the results obtained by Wiśniowska-Kielian and Niemiec (2004), but the cited authors found greater differences in the content of these elements in water samples taken on different dates.

The average content of lead in sediments was 16.70 mg kg^{-1}, and ranged from 11.88 to 26.38 mg Pb kg^{-1}. Slightly more of this element was found in the samples collected in autumn than in summer. The average chromium content in the studied sediment was 13.294 mg kg^{-1}, and ranged widely from 5.828 to 21.263 mg kg^{-1} DM (Table 2). Most of this element was found in the

Table 2

Statistic parameters of lead content in the elements of the aquatic ecosystem

Statistic parameters	Water	Sediments	Fish organs			
			skin	skeleton	muscle	liver
	(μg Pb dm^{-3})	(mg Pb kg^{-1} DM)				
Minimum	0.080	11.88	0.157	0.650	0.380	0.686
Maximum	0.180	26.38	1.936	4.731	3.617	4.375
Mean	0.120	16.70	0.932*a*	2.114*c*	1.831*b*	2.269*c*
Relative standard deviation (%)	25.46	26.70	52.1	47.8	44.0	44.7
	(μg Cr dm^{-3})	(mg Cr kg^{-1} DM)				
Minimum	2.856	5.828	3.431	4.384	2.937	4.578
Maximum	4.521	21.263	12.478	11.03	12.40	12.203
Mean	3.338	13.294	6.422*a*	8.299*b*	6.174*a*	8.574*b*
Relative standard deviation (%)	16.57	35.66	30.96	20.47	34.47	23.02

Different letters beside the mean values indicate statistically significant differences among the samples from different sampling sites at a significance level of $p = 0.01$.

samples taken in Zakliczyn, approximately thrice as much as in deposits sampled in Szczepanowice, both in summer and autumn. A significant influence of the date of sampling for chromium content in bottom sediments was reported. The mean for all samples collected in the summer amounted to 11.15 mg kg^{-1}, while the mean chromium content for samples collected in the autumn was 15.43 mg kg^{-1}. Differences in the content of elements may be due to changes in water flow and temperature. According to Bojakowska and Sokołowska (1998), the value of the geochemical background of the Polish water deposits is 10 mg Pb kg^{-1} and 5 mg Cr kg^{-1}. Skorbiłowicz and Skorbiłowicz (2008) found a similar chromium content in the sediments of several tributaries of the Supraśl River to that obtained in the present study. The higher concentration of this element was observed by Niemiec et. al (2012) in sediment from anthropogenic tanks collecting water from roads. The authors found large differences in the accumulation of this element in sediments collected in the summer and early spring. Similar content of chromium and nickel was reported by Skorbiłowicz (2014) in the sediments of the River Bug. The content of lead and chromium in the sediment of the Yangtze River in areas impacted by anthropogenic pollution was 37 mg Pb kg^{-1} and 76 mg Cr kg^{-1}, at the content of these elements in water being 2 and 1.3 μg dm^{-3} (Yi et al. 2011). In the studied synovial ecosystem, approximately 2,000 times more chromium was determined in the sediment than in water. Oyoo-Okoth et al. (2012) obtained the values of the coefficient of enrichment of sediments with chromium at about 1500 for Lake Victoria. The lead and chromium content determined in the abiotic components of the analyzed river ecosystem are not high and do not indicate a threat to living organisms. The literature data, however, indicate the threat of excessive bioaccumulation of trace elements, even with their small amounts found in the water or the bottom sediments (Tao et al. 2012). The effects of xenobiotics on aquatic organisms and their level of bioaccumulation depend not only on the amount of such elements in the environment, but on numerous factors related to the physical, chemical and physicochemical properties of the water environment (Jia et al. 2013). It is therefore appropriate to evaluate the content of trace elements in those compartments of biocenosis in which both plants and animals from various levels of the trophic chain are used (Cacador et al. 2012). When using fish in biomonitoring research, it is important to choose an organ in which the level of bioaccumulation of trace elements will be assessed. The literature reports the usefulness of various organs of fish, such as the gills, liver, intestines, skin, bone, scales, brain and eye (Greig et al. 2010, Pereira et al. 2013 Tao et al. 2012). Heavy metals are absorbed by fish from food through the digestive system and from water through the gills and skin, which are responsible for breathing and for the ion exchange between a fish and the environment. Thus, these organs are most often used in biomonitoring research.

The average lead content in the skin of the studied fish was 0.932 mg kg^{-1}, and ranged widely from 0.157 to 1.963 mg kg^{-1} DM. The value of the relative standard deviation of the content of the element in the skin was 52.1%. The content of chromium in this organ ranged in a lesser extent, from 3.431 to 21.263 mg kg^{-1} DM, and its mean value was 6.422 mg kg^{-1} DM (Table 2). The content of trace elements in the bones of fish is a valuable source of information on the environmental threats on the part of trace elements, as pointed out by other authors (Feldlite et al. 2008, Pereira et al. 2013). The level of these elements in the bones is related to both the habitat of fish, and the species. The content of chromium in bones of various species of marine fish caught in the coastal areas of Norway ranged from 2.4 to 16.9 mg Cr kg^{-1} DM, while the determined amount of lead ranged from trace to 0.26 mg Pb kg^{-1} DM (Toppe et al. 2007). The region of the research was characterized by a low level of human pressure.

The mean content of lead and chromium in the bones of the studied fish amounted to 2.114 and 8.229 mg kg^{-1} DM. The content of lead in these organs ranged from 0.650 to 4.731 mg Pb kg^{-1} DM, and of chromium – from 4.384 to 11.03 (Table 2). Fish muscles are not considered a good indicator of the pollution of the water environment with trace elements, but the content of xenobiotics in these organs is a criterion of the suitability of aquatic organisms for human consumption, and determines the risk of including harmful elements in the human food chain. They are an important source of both micronutrients and harmful elements for the populations consuming large amounts of seafood and aquaculture products.

The lead content in the muscles of the examined fish ranged between 0.380 and 3.617 mg Pb kg^{-1} DM, and of chromium – between 2.937 to 12.40 mg Cr kg^{-1} DM. The average content of these elements in the muscles was, respectively, 1.831 and 6.174 mg Cr kg^{-1} DM. The maximum content of lead allowed in fish suitable for human consumption is 0.3 mg Pb kg^{-1} DM (Commission Regulation 2015). In our study, 20 samples were found to exceed the critical values for the fish for human consumption, as per fresh weight.

The liver is the organ of fish often used in biomonitoring of the pollution with trace elements due to its function related to detoxification and accumulation of this element (Maceda-Veiga et al. 2013). The average lead content in the liver of the studied fish was 2.269 mg Pb kg^{-1}, and the chromium content equalled 8.574 mg Cr kg^{-1} DM. The lead content in the liver of the bleak ranged from 0.686 to 4.375 mg Pb kg^{-1}, while chromium content varied from 4.578 to 12.203 mg Cr kg^{-1} (Table 2). The highest concentrations of lead and chromium were found in the liver and bones, which suggests that these organs may be the most useful ones in biomonitoring studies. The lead and chromium content found in our study was high, despite low concentrations of these elements in the abiotic components of the studied river ecosystem. Zhao et al. (2012) report the content of lead and chromium in the skin, muscle and liver of several fish species caught in the estuary of the Yangtze River at a level similar to the results obtained in the present study. The content of these elements in sediments from this area of research was significantly less than the data obtained in the present study. The chromium content in the muscle of fish of various species of *Sparidae* from the estuary of the Tagus River in Portugal averaged approximately 0.4 mg kg^{-1}, and the mean lead content was approximately 12 mg kg^{-1} (Cacador et al. 2012). Qian et al. (2013) indicate the chromium content in the muscles of several species of coarse fish from several fish farms in northeastern China at the level from 0.3 to 1 mg Cr kg^{-1} DM, approximately. On the other hand, the chromium content in different species of fish from the Yangtze River was 1.04 to 5.06 mg Cr kg$^{-1,}$ while the level of accumulation of lead in these fish ranged from 3.35 to 27.18 mg kg$^{-1.}$ The authors found large concentrations of these metals in bottom-dwelling fish, as compared to the pelagic fish. The chromium content in the sediment ranged from approximately 30 to more than 100 mg kg^{-1}, while the lead content in the sediment ranged from 33.55 to 400 mg kg^{-1} (Fu et al. 2013). The concentration of lead in the bones of three freshwater fish species from the aquacultures exposed to the element was from 0.5 to 4 mg Pb kg^{-1} DM (Feldlite et al. 2008). These authors found that a greater accumulation of lead is found in the bones of fish exposed to the element. The lead content in the bones was positively correlated with the content of this element in the feed and in bones. The level of bioaccumulation of trace elements in fish depends on many factors, of which the method of food ingestion is very important. Generally, benthos-feeding fish accumulate much more metal as than pelagic fish. YI et al (2011) report that the chromium and lead content in the benthos-feeding fish from the middle course of the Yangtze River was twice as high as in the pelagic fish caught in the same place. The bioconcentration factor of trace elements in aquatic organisms is an important indicator of environmental pollution. This is due to the lack of a linear relationship between the content of trace elements in abiotic components of aquatic ecosystems and their amount accumulated by living organisms (PEREIRA et al. 2013). In this paper, values of the bioaccumulation of the two elements determined in different organs of fish *(Alburnus alburnus* L.) were calculated, and compared to the content of these elements in the water and sediments. The level of bioaccumulation of trace elements in fish depends on many factors, of which the method of feeding and the environment of the fish are very important. Pelagic fish consume more trace elements directly from the water through the gills and skin. For planktivorous organisms inhabiting the littoral zone, the supply of trace elements from bottom sediments is of less importance in comparison with the benthos-feeding fish, whose diet consists of insect larvae living in the sediments (YI et al. 2011).

The coefficient of bioaccumulation of lead in the skin, bones, muscles and liver, as compared to the concentration of this element in the water was, 7770, 17619, 15261 and 18909, respectively (Table 3) The average value of the coefficient of bioaccumulation for lead in the respective organs of the fish, in relation to the content of this element in the sediment, was 0.056 for the skin, 0.127 for the bones and 0.110 for the muscles and liver (Table 3). The coefficient of bioaccumulation of chromium in individual organs, as compared to the content of this element in the water, ranged from 1597 to 7895 (Table 3). The highest value of this parameter was observed in the liver and the lowest one - in the skin and muscle. The value of the coefficient of bioaccumulation of chromium in the individual organs, as compared to the content of this element in the sediments, was approximately 0.5 for the muscles and skin, and above 0.6 for liver and bones (Table 3). The organs of fish used in the present study are often used in biomonitoring research of aquatic eco-

Table 3

Mean value of bioaccumulation coefficient of lead and chromium

Element of ecosystem	Element	Skin	Bones	Muscle	Liver
Water	Pb	7770	17619	15261	18909
	Cr	1924	2486	1849	2568
Sediment	Pb	0.056	0.127	0.110	0.136
	Cr	0.483	0.624	0.464	0.645

systems (Feldlite et al. 2008, Maceda-Veiga et al. 2013). The calculated values of the coefficient of bioaccumulation of cadmium and chromium were higher than those reported by other authors for environments exposed to anthropogenic pollution. The value of the coefficient of bioaccumulation of chromium in the muscles of fish of different species from the estuary of the Tagus River was approximately 0.1 in relation to the content of this element in sediments (Cacador et al. 2012). In turn, Yi et al. (2011) reported values of the coefficient of bioaccumulation of lead and chromium in the muscles of pelagic fish of the Yangtze at 1300 and 500, respectively, as compared to the content of these elements in the water, and 0.065 and 0.08, respectively, as compared to the content of lead and chromium in the sediments. The value of the coefficient of bioaccumulation of lead in the muscles of different species of fish from the estuary of the Tagus River was approximately 0.18 in relation to the content of this element in sediments (Cacador et al. 2012). The value of the coefficient of bioaccumulation of lead in the muscles of the bleak from the Liobregat River in Spain, as compared to the content of the element in water, was 2144, which is several times lower than the results obtained in our study. On the other hand, as compared to the quantity of this element in sediments, the value of this parameter was 0.429 and was several times higher than the coefficient of bioaccumulation of lead in the bleak from Dunajec (Merciai et al. 2014). The coefficient of bioaccumulation of chromium in fish of the genus *Characidae* from the Sinos River in Brazil, with the water content of the element at 5.5 and 12.1, was approximately 1700 and 700, respectively, in relation to that of water. Under these conditions, the sediment contained 15.1 and 8.5 mg kg^{-1} DM of chromium The coefficient of bioaccumulation of chromium and lead in fish of the genus *Characidae* in the rivers of Brazil, compared to the content of the element in sediment, was approximately 0.720 and 0.950 (Weber et al. 2013). The lead content in the water of Lake Tititaca in areas with different levels of pollution ranged from 2.57 to over 32 μg dm^{-3}, and in the sediments, from 2 to 25 mg kg^{-1} DM. In such conditions, the content of this element in the muscle and liver of the fish was below 0.1 mg kg^{-1} DM (Monroy et al. 2014).

Based on these results, the coefficients of correlation were calculated between the content of the analyzed elements in different organs of fish. A statistically significant correlation, at p = 0.01, was determined between the lead content in individual organs of the fish, with the exception of the relationship between the amount of this element in bone and liver, which was statistically significant at a significance level of $p = 0.05$ (Table 4). The highest rate was observed for the lead content in the skin and liver ($r = 0.677$). In the case of chromium, a statistically significant correlation at $p = 0.01$ was found between the content of this element in the skin and bones and in muscles and liver. The coefficient of correlation between the content of chromium in the skin, muscles and bones was statistically significant at $p = 0.05$ (Table 4).

Table 4

Value of the correlation coefficient for lead and chromium

Item	Bones	Muscle	Liver
Lead			
Skin	0.443*	0.552**	0.677**
Skeleton		0.559**	0.534**
Muscle			0.496**
Chromium			
Skin	0.616**	0.359*	0.203
Skeleton		0.424*	0.159
Muscle			0.590**

** correlation coefficient significant at $p = 0.01$
* correlation coefficient significant at $p = 0.05$

CONCLUSIONS

1. The content of lead and chromium in the water of the Dunajec River does not indicate anthropogenic enrichment. The content of these elements in the sediment was lower than the geochemical background of the water sediments in Poland.

2. The content of lead in different organs of fish decreased in the following order: liver> skeleton> muscles> skin, while for the content of chromium the sequence was: liver> skeleton> skin> muscles.

3. In approximately 58% of the samples of muscle, the levels of lead exceeded the limits in fish intended for human consumption (0.3 mg Pb kg^{-1} DM).

4. The values of the bioaccumulation coefficient of lead in the individual organs of the bleak, as compared to the concentration of this element in the water, ranged from 7770 to 18 909, and of chromium – from 1597 to 7895.

5. The values of the bioaccumulation coefficient of lead in the individual organs of the bleak, as compared to the concentration of this element in the sediment, ranged from 7770 to 18 909, and of chromium – from 0.483 to 0.645.

6. The values of the coefficient of bioaccumulation of lead and chromium were found higher in relation to the concentration of these elements in the water and sediment than those reported in the literature.

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