Impact of fish species on levels of lead accumulation in the meat of common bream (*Abramis brama* L.), white bream (*Blicca bjoerkna* L.) and common bleak (*Alburnus alburnus* L.) from the Vistula River (Poland)

Wpływ gatunku ryby na koncentrację ołowiu w mięsie leszcza (*Abramis brama* L.), krąpia (*Blicca bjoerkna* L.) i uklei (*Alburnus alburnus* L.) odłowionych z rzeki Wisły (Polska)

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Abstract

The aim of this work was to compare the concentration of lead in the meat of common bream (*Abramis brama* L.), white bream (*Blicca bjoerkna* L.) and common bleak (*Alburnus alburnus* L.). The experimental fish were obtained in natural condition from Vistula River, located within Toruń, near wastewater treatment plant. The study involved 60 individuals of freshwater fish caught in autumn. Analyses were carried out on 10 individuals of common bream, 20 white bream and 30 individuals of common bleak. The muscles samples for analyses were taken from the large side muscle of fish body above the lateral line. There were chosen for analyses individuals with similar biometric measurements. Due to a relatively low amounts of meat obtained from white bream and common bleak, the material from individuals of similar body length was combined (about 2-3 pieces). Pb concentrations were determined by atomic absorption spectrophotometer Solaar 939 QZ, ATI Unicam. Analyses of variance (test post hoc -Tukey test) indicated that the mean value of lead was the highest in the meat of common bream (0.086 $\mu g \cdot g^{-1}$ wet weight) and the lowest in the meat of white bream (0.075 $\mu g \cdot g^{-1}$ wet weight). There were no statistical significant differences in the lead content between the analyzed fish species (at $p< 0.05$). Analysis of correlation indicated a negative and statistical significant correlation between the fish body length and Pb concentration.

Keywords: freshwater fish, heavy metals, meat

Streszczenie

Celem pracy było porównanie koncentracji ołowiu w mięsie leszcza (*Abramis brama* L.), krąpia (*Blicca bjoerkna* L.) i uklei (*Alburnus alburnus* L.). Ryby poddane badaniom odłowiono z Rzeki Wisły, warunkach naturalnych na wysokości oczyszczalni ścieków w Toruniu. Badaniami objęto 60 osobników ryb słodkowodnych odłowionych jesienią. Analizy przeprowadzono na 10 osobnikach leszcza, 20 sztuk krąpia i 30 osobnikach uklei. Próby mięsa pobrano z nadosiowej części mięśnia bocznego wielkiego ze środkowej części tułowia. Do badań wybrano osobniki posiadające zbliżone wartości wskaźników biometrycznych. Ze względu na małe rozmiary krąpia, mięso pobrane od tego gatunku łączono od 2-3 osobników posiadających zbliżone długości ciała. Stężenie Pb oznaczono przy pomocy spektrofotometru absorpcji atomowej Solaar 939 QZ, ATI Unicam. Analiza wariancji (test post hoc -Tukey test) wykazała, że średnia zawartość ołowiu była najwyższa w mięsie leszcza (0.086 $\mu g \cdot g^{-1}$ mokrej masy), a najniższe zawartości oznaczono w mięsie krąpia (0.075 $\mu g \cdot g^{-1}$ mokrej masy). Nie stwierdzono statystycznie istotnych różnic w zawartości ołowiu w mięsie pomiędzy analizowanymi gatunkami ryb (przy $p< 0.05$). Analiza korelacji wykazała negatywną i statystycznie istotną zależność pomiędzy długością ciała ryb a zawartością ołowiu w mięsie.

Słowa kluczowe: metale ciężkie, mięso, ryby słodkowodne

Streszczenie szczegółowe

Celem pracy było oznaczenie zawartości ołowiu w mięsie leszcza (*Abramis brama* L.), krąpia (*Blicca bjoerkna* L.) i uklei (*Alburnus alburnus* L.) odłowionych z rzeki Wisły. Badaniami objęto 10 osobników okonia, 20 sztuk krąpia i 30 sztuk uklei. Na każdym osobniku dokonano pomiarów biometrycznych: masy ciała (BW) (± 0,01g) i długości ciała (Lc) (± 0,1cm). Próbki mięsa pobierano z mięśnia bocznego wielkiego powyżej linii bocznej ciała. Do badań wybrano osobniki posiadające zbliżone wartości wskaźników biometrycznych. Ze względu na małe rozmiary krąpia, mięso pobrane od tego gatunku łączono od 2-3 osobników posiadających zbliżone długości ciała. Uzyskane próbki mięsa były zamrożone bezpośrednio po pobraniu i zliofilizowane przez dokonaniem analizy. Wszystkie próbki mięsa zliofilizowanao w liofilizatorze firmy Finn-Aqua Lyovac GT2 przy parametrach: temperatura -40 °C, ciśnienie 6 10^{-2} mbar. Tak przygotowane próbki mięsa zmineralizowano przy pomocy mineralizatora mikrofalowego Ethos Plus, Milestone. Stężenie ołowiu oznaczono za pomocą spektrofotometru absorpcji atomowej Solaar 939 QZ, ATI Unicam. Stężenie ołowiu oznaczone w mięsie zostało wyrażone w $\mu g \cdot g^{-1}$ masy mokrej ($\mu g \cdot g^{-1}$ mm). Dokładność analizy była kontrolowana przy pomocy roztworów wzorcowych (Recipe Chemicals + Instrument GmbH, Germany). Analiza wariancji (test post hoc -Tukey test) wykazała, że średnia zawartość ołowiu była najwyższa w mięsie leszcza (0.086 $\mu g \cdot g^{-1}$ masy mokrej) a najniższe wartości oznaczono w mięsie krąpia (0.075 $\mu g \cdot g^{-1}$ masy mokrej). Nie wykazano statystycznie istotnych różnic w zawartości ołowiu pomiędzy analizowanymi gatunkami ryb (przy $p< 0.05$). Analiza korelacji wykazała ujemną i statystycznie istotną zależność pomiędzy długością ciał analizowanych ryb i stężeniem Pb w mięsie.

Słowa kluczowe: metale ciężkie, mięso, ryby słodkowodne

Introduction

Fish are located at the end of the aquatic food chain and may accumulate toxins which can pass to human organism through food. Accumulation of metals in fish depends on concentrations of metals in water and food organism, on physiochemical factors and exposure duration. Heavy metals pollution in fish has become an important worldwide concern due to the health risk associated with fish consumption (Al-Busaidi, et al., 2011).
Lead is a non-essential element which may cause neurotoxicity, nephrotoxicity and many others adverse health effects (Rahman, et al., 2012). An important biochemical effects of lead on the hematopoietic system is its inhibition of the biosynthesis of heme. Lead binds strongly to the sulfhydryl functional group in δ-aminolevulinic acid dehydrase (ALAD) which is a key enzyme in the formation of heme. Inhibition of ALAD occurs at blood lead concentration of 10-20 $\mu g \cdot 100 cm^{-3}$ (Al-Busaidi, et al., 2011). Lead is a toxic metal that can affect humans when ingested or inhaled in high doses. In fish, it can cause deficits or decreases in survival, growth rates, development and metabolism (Yilmaz, et al., 2010).
The distribution of metals varies between fish species, depending on age, season of the catch, development status, the position of particular fish species in a food chain and other physiological factors (Mendil, et al., 2010). In fish, metals accumulate mainly in kidney, liver and intestine epithelium. Fish muscles contain low levels of metals, but are often examined for metal content due to their use for human consumption (Lidwin-Kaźmierkiewicz, 2009). As Allen-Gil and Martynov (1995) indicated species differences in muscle content of Pb may reflect the proportion of the diet made up of crustaceans. UE acceptable limit for lead is 0.3 $mg \cdot kg^{-1}$ and the range of international standards for fish is 0.5-1.0 $\mu g \cdot g^{-1}$ (Al-Busaidi, et al., 2011).
The aim of this work was to compare the concentration of lead in the meat of three species of fish from the Vistula River and to estimate the influence of fish species on specific metabolic of lead. The aim of analyses was to assess the potential human health risk due to the consumption fish from study area.

Materials and Methods

The study involved 60 individuals of freshwater fish caught in autumn. The experimental fish were obtained in natural condition from Vistula River, located within Toruń, near wastewater treatment plant (figure 1).
Analyses were carried out on 10 individuals of common bream (*Abramis brama* L.) (n=10), 20 white bream (*Blicca bjoerkna* L.)(n=10) and 30 individuals of common bleak (*Alburnus alburnus* L.)((n=10) – three species belonging to the *Cyprinidae* family – benthic species (common bream and white bream) and pelagic species (common bleak). Measurements of the mass of the fish body (BW) (± 0,01g) and body length (Lc) (± 0,1cm) were taken on the each individuals (table 1).
The muscles samples for analyses were taken from the large side muscle of fish body above the lateral line. There were chosen for analyses individuals with similar biometric measurements. Due to a relatively low amounts of meat obtained from white bream and common bleak, the material from individuals of similar body length was combined (about 2-3 pieces).

Figure 1. Map of Poland. The place of fish collection.

Table 1. Biometric measurements for common bream (*Abramis brama* L.), white bream (*Blicca bjoerkna* L.) and common bleak (*Alburnus alburnus* L.) caught from the Vistula River

Fish species	n	Body length (cm) range (mean value)	Body weight (g) range (mean value)	Age
Common bream (*Abramis brama* L.)	10	13.3-15.6 (14.5)	36.90-63.45 (52.30)	2+-3+
White bream (*Blicca bjoerkna* L.)	10	11.1-13.2 (12.2)	28.40-50.30 (39.10)	2+
Common bleak (*Alburnus alburnus* L.)	10	10.0-13.5 (12.3)	12.35-19.50 (17.35)	3+

n – number of samples

The samples of fish meat were immidietely frozen after preparation and kept in the deep freezer before analyzing. All frozen samples were freeze dried in a Finn-Aqua Lyovac GT2 freeze drier (parameters: temperature -40°C, pressure 6 10^{-2} mbar, duration at least 48h). The freeze dried samples were mineralized in microwave mineralizator Ethos Plus, Milestone. For the mineralization 0,1g of the tissue was weighted and then HNO_3 and H_2O_2 were added in ratio 4:1. During the first 10 minutes, the temperature was increased to 190 °C. During the next 7 minutes the temperature was kept at a level of 190 ± 5 °C. The mineralized samples have been carried quantitatively to the measuring flask with a capacity of 50 ml.
Pb concentrations were determined by atomic absorption spectrophotometer Solaar 939 QZ, ATI Unicam. Tissue concentrations of minerals have been reported as $\mu g \cdot g^{-1}$ wet weight ($\mu g \cdot g^{-1}$ ww). The accuracy of the analyses was controlled by adding standard solutions (Recipe Chemicals + Instrument GmbH, Germany). Analyzes were carried out in a certified laboratory. The results showed that the recovery percentage was in the range from 95 to 101% and this values were included in the

final results. Lead was determined using the analytical wavelength 283,3 nm. All analytical samples and blanks were prepared in triplicate.

Statistical analysis

Data analyses were performed by using the Statistica 8.0 software (StatSoft, USA). Significance of differences in the average content of lead in the meat of fish were calculated by one-way analysis of variance (ANOVA). Tukey's test was applied, and statistically significant differences were evaluated as significant at $p \leq 0.05$. The normality of the data was tested using the Shapiro-Wilk's test ($p=0,00012$) and the homogeneity of variance by means of Levene's test ($p=0.58126$).

Results and Discussion

Analyzed fish were collected near the wastewater treatment plant. One-way analysis of variance indicated that the mean value of Pb was highest in the meat of common bream (0.086 $\mu g \cdot g^{-1}$ ww) and the lowest amounts were determined in the meat of white bream (0.075 $\mu g \cdot g^{-1}$ ww) (table 2).

Table 2. The mean content of lead ($\mu g \cdot g^{-1}$ wet weight) in the meat of common bream (*Abramis brama* L.), white bream (*Blicca bjoerkna* L.) and common bleak (*Alburnus alburnus* L.) caught from the Vistula River

Fish species	n	Content of lead ($\mu g \cdot g^{-1}$) mean ± SD
Common bream (*Abramis brama* L.)	10	0.086 ± 0.016^{a}
White bream (*Blicca bjoerkna* L.)	10	0.075 ± 0.009^{a}
Common bleak (*Alburnus alburnus* L.)	10	0.079 ± 0.011^{a}

The values marked with the same letters in the column are not significantly different ($p \leq 0,05$, Tukey's test),

n – number of samples

There were no statistical significant differences in the lead content between the analyzed fish species (at $p \leq 0.05$). The maximum lead level permitted determined by the WHO (1996) is 0.4 $mg \cdot kg^{-1}$ and 0.1 $mg \cdot kg^{-1}$ ww by the FAO (1986). The mean concentration of lead in the muscles of common bream caught from lakes in the Olsztyn Lake District ranged from 0.027 to 0.182 $mg \cdot kg^{-1}$ ww (Łuczyńska and Brucka-Jastrzębska, 2005) and below 0.1 $mg \cdot kg^{-1}$ ww. in the same species collected from the Lake Łańskie, Pluszne, Dłużek and Maróz (the Olsztyn Lake District) (Łuczyńska and Brucka-Jastrzębska, 2006). Those values were very similar to this presented in the work. The same concentrations of lead were determined in the fish from the Lakes Kisajno, Dargin and Niegocin (Łuczyńska, et al., 2001). The mean content of this metal ranged from 0.020 to 0.075 $mg \cdot kg^{-1}$ww (in the meat of common bream these values ranges from about 0,04 to 0,06 $mg \cdot kg^{-1}$ww). Concentration of lead in the meat of common bream collected from the two basin of Lake Balaton (the western and eastern) was 1.53 and 0.85 $mg \cdot kg^{-1}$ dw (about 0.306-0,17 $mg \cdot kg^{-1}$ww, assuming a water content of 80%), respectively (in October) and 0.098 and 0.086 $mg \cdot kg^{-1}$ ww (in

May) (Farkas, et al., 2002). The average Pb concentration in the muscles of common bleak and common bream collected from the Šalek lakes (Slovenia) ranged from 0.01 to 0.06 mg·kg^{-1} ww and from < 0.01 to 0.10 mg·kg^{-1} ww, respectively (Petkovšek, et al., 2012). As these study showed, Pb concentration were similar to those of unpolluted areas. Previous research conducted by Stanek (2005) indicated that the highest concentration of Pb was determined in the gills and those values ranged from 0.0764 to 0.0854 mg·kg^{-1} ww (common bream), from 0.0586 to 0.1096 mg·kg^{-1} ww (perch) and from 0.0512 to 0.0896 mg·kg^{-1} ww (white bream). Lead content in common bleak from the Zhrebchevo dam Lake (southeastern Bulgaria) was 0.06 mg·kg^{-1} ww (Zhelyazkov, et al., 2014) and was similar to the values obtained in this work. Pb content in the fish meat analyzed by Lidwin-Kaźmierkiewicz, et al. (2009) ranged from 0.01 to 0.02 mg·kg^{-1} ww. These values were low-similar or lower than those reported for fish by other authors. In the meat of perch (*Perca fluviatilis* L.) from the Pomeranian Bay and Szczecin Lagoon Pb concentration were ranged within 0.007-0.033 mg·kg^{-1} ww and 0.013 mg·kg^{-1} ww, respectively (Szefer, et al., 2003). Much higher concentration of Pb was reported by Jurkiewicz-Karnakowska (2001) in fish from Zegrzyński Reservoir (1.78-2.54 mg·kg^{-1} ww) and Özparlak et al. (2012) in the meat of *Alburnus akili* (1.05 mg·kg^{-1} ww).

As Protasowicki (1982) indicated the location of the reservoir impact on the content of lead in the fish body. Furthermore, the concentration of metal in fish tissues is generally related to the age of a fish and consequently on its size and length (Petkovšek, et al., 2012) and type of food intake (Łuczyńska, et al., 2000).

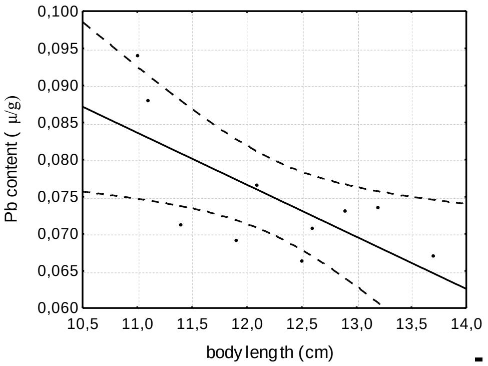
Analysis of correlation indicated a negative and statistical significant correlation between the fish body length and Pb concentration (table 3, figure 2).

As a earlier investigations show, a negative correlation between metal concentration and body length may be due to dilution of these elements with the increase of fish body, reducing the rate of nutrition and food ratios and mineral excretion by the gills, skin, and feces. Investigation have show that the biocumulation of heavy metals (Cr, Mn, Ni) decreased as fish body length increased (negative correlation) (Canli and Atli, 2003).

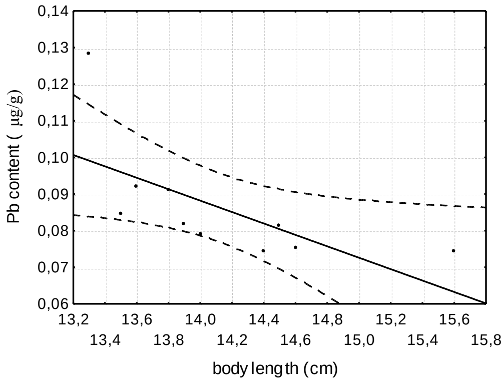
The main explanation of this correlation is a higher ability to metabolize compounds for the younger fish than for the older individuals. The next explanation is the fact that the mechanism of the neutralization of harmful metals are not developed sufficiently in the young organisms. Therefore, larger amounts of minerals can be accumulate in their body (Kljaković Gašpić, et al., 2002).

The analysis of the dependence of the content of mineral elements in particular organs and the body length of the fish from the Żnin Duże Lake indicated a positive correlation for Cu, Fe and Ni, and negative correlation for Zn and Mn (Stanek, et al., 2005). Analyses carried out by Petkovšek, et al. (2012) indicated that the concentration of metals in analyzed tissues did not correlate strictly to fish length. Negative correlations were found for Zn concentration in the muscles of roach and muscles and gills. Those observation was in good agreement with Farkas, et al. (2003) who found a negative size-related correlation for cadmium, lead and zinc in the muscles and gills. The similar results were observed by Łuczyńska and Brucka-Jastrzębska (2005) and Dobicki and Polechoński (2003). From many researches it appears that this interdependence was varied and dependent on the characteristics of individual species. In most of cases there were observed a negative correlation between the age and the metal concentration in their tissues.

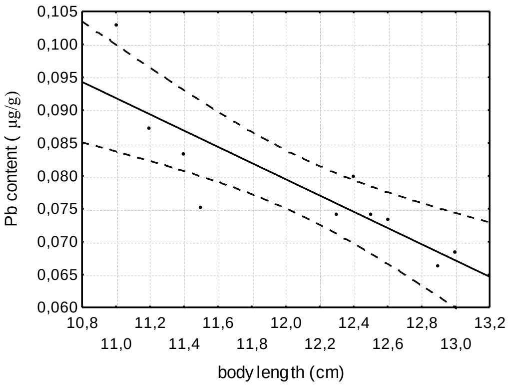
As indicated analyses the muscle tissue are not considered to the specific physiological sites for lead (Al-Yousuf, et al., 2000), but as a literature review shows, numerous factors (biological and environmental) affect the degree of contamination of fish muscle tissue with lead, mercury and cadmium. Pb accumulate in the largest amounts in bones.



white bream (*Blicca bjoerkna* L.)



common bream (*Abramis brama* L.)



common bleak (*Alburnus alburnus* L.)

	r	p
common bream	-0.655549	0.039604
white bream	-0.696262	0.025308
common bleak	-0.847267	0.001972

Figure 2. Correlation between the mean content of lead (µg·g^{-1}) and body length (cm) for common bream (*Abramis brama* L.), white bream (*Blicca bjoerkna* L.) and common bleak (*Alburnus alburnus* L.) caught from the Vistula River.

As Perkowska and Protasowicki (2000) indicated cadmium and lead accumulate in different ratio in the liver and kidney. In all analyzed species the content of these metals were higher in the gills than in the muscles. It proves that the respiratory system is the main way of acquisition of this metals by fish. Higher cadmium concentrations were found in the liver of red gurnard (*Trigla cuculus*), whole lowest cadmium levels were always found in muscles tissues of the analyzed fish. Lead concentration were much higher than cadmium and the liver and gills accumulated the great amounts of this metal (Canli and Atli, 2003). Yilmaz, et al. (2010) reported that the highest lead concentration were determined in the liver of *Solea lascaris* (2.98 µg·g^{-1} wet weight) and the lowest lead concentration (0.14-0.39 µg·g^{-1} wet weight) were always found in muscle tissues of fish. The skin accumulated more lead than did muscle tissue. Jarić, et al. (2011) indicated that heavy metals accumulations in analyzed tissues (muscle, gill, liver and intestine) differed statistically significant in the case of all assessed metals. The highest concentration of most of the analyzed metals and trace elements were recorded in the liver, while the lowest one were determined in the muscle. Analyses carried out by Petkovšek, et al. (2012) indicated that the accumulation of Pb was most pronounced in gills than in muscle and liver. Authors suggest that elevated content in gills reflect high content of those element in plankton and are correlated with feeding habits.

Conclusions

There were not statistically significant differences in lead content between analyzed fish species, especially between benthic fish (common bream and white bream) and pelagic species (common bleak). Despite the common bleak feeds in the upper layers of the reservoir, the results of Pb concentrations did not differ from those determined for common bream and white bream. This explains the homogeneous conditions of nutrients in the river.

Data obtained in this study are comparable to the values determined by other authors for the same fish species collected from different types of reservoirs and environmental conditions.

The concentration of Pb in the meat of analyzed fish were lower than the maximum levels set by law. Therefore, the meat of those fish is healthy and save for human consumption.

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