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Levels of metals in blood samples from Mallards (*Anas platyrhynchos*) from urban areas in Poland

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ABSTRACT

In this paper we present the studies conducted on blood samples taken from Mallards (*Anas platyrhynchos*). Birds were captured for ringing purposes ($n = 43$) in two small and two big towns (including highly urbanized areas). For comparison samples of blood from birds shot on fish ponds were used ($n = 26$). Based on the body mass all sampled individuals can be assessed as being in good condition. Levels of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) in blood samples were measured with AAS. Concentrations of metals did not differ statistically between sexes and made up a following order: Fe > Zn > Cu > Cr ≈ Ni > Pb > Cd. Mallards from towns revealed lower concentrations of Zn and Cu but higher concentration of Fe. There was no difference in exposition to Pb between birds from towns and fish ponds.

1. Introduction

The Mallard (*Anas platyrhynchos*) is one of the most numerous and widespread waterfowl in the northern Hemisphere with its population estimated on 7.5 million birds in Europe and over 28 million in the world (Wetlands International, 2006). This species shows a great ecological plasticity and is observed in majority types of wetlands including urban habitats. In general, Mallards breeding in temperate zone migrate from northern breeding grounds to wintering areas situated in less harsh winter climate. However, there are also non-migratory populations in Western and Southern Europe, which make only short distance movements (Cramp and Simmons, 1977). Birds living in cities are sedentary (Zárybnický and Klvaňa 2008, authors, unpublished data) even in regions of harsh winters (Avilova, 2008). Mallard is proposed as an effective biomonitor of metal pollution, because samples are easy to collect (by ornithologists during ringing and in the way of hunting), the species is spread all over the world, the literature needed for comparison is available from different parts of the world and this duck meat is edible (Kalisińska et al., 2004). However the vast majority of available data came from research carried out in the natural or semi-natural habitats (Pain, 1991; Szymczyk and Zalewski, 2003; Kalisińska et al., 2004; Taggart et al., 2009). In urbanized areas there are several sources of metal pollution. Apart from the air which is rich in metals (mostly because of industry and transport activities), also water may be a significant carrier of these pollutants. This can be observed through high metal concentrations in deposits (Grodzińska and Szarek-Łukaszewska, 2001). Thus, birds staying in the cities seem to be exposed to higher concentration of metals, than those living in natural and semi-natural habitats.

Our study was undertaken in order to compare concentrations of seven metals (Fe, Zn, Cu, Cr, Ni, Pb, and Cd) in Mallards from urban and non-urban populations and to check if concentrations of metals in blood of birds living in urbanized areas are higher than in birds from fish ponds localized far from the city.

2. Materials and methods

Research was done on Mallards and was carried out in five areas in Poland (Fig. 1). The sample collected outside the cities consists of 26 birds shot by hunters between 16 and 18 August 2009 in Milicz fish ponds. Immediately after the collection, blood samples were taken directly from the heart and frozen (in −18 °C). The second sample of 43 birds came from four Polish cities, where Mallards were caught for ringing between 28 June and 25 August 2011 (Fig. 1, Table 1). Warsaw and Gdansk are large towns with 1.7 and 0.5 million inhabitants respectively, whereas Ilawa and Ostroda have population of about 32–33 thousands. After ringing c.a. 1 mL of blood was taken from metatarsal vein and frozen (in −18 °C) (sampling was conducted according to the permit of The Local Ethics Committee in Gdansk no 23/2010). The sex of birds was determined using plumage characteristics. Ageing was done according to the method described by Baker (1993) based on the size and

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Fig. 1. Sample sites of the research.

shape of the black dot on the 4th–6th grater primary coverts. Only adult Mallards (older than 1 year) were used in this study.

The samples of blood after defrosting in the laboratory (Institute of Environmental Sciences, Jagiellonian University, Cracow, Poland) were weighed (Metler AE240) and put into mineralizer pots. All the samples were mineralized in the hot nitric acid (HNO_3 65% Ultranal®, POCH). Next, the mineralized samples were analysed in the aspect of metals. Flame AA spectrometer (PerkinElmer AAnalyst 200) was used to determine iron and zinc concentrations and spectrometer with the graphite furnace (PerkinElmer AAnalyst 800) to determine cadmium, chromium, copper, nickel and lead. Each sample was analysed twice and the mean value was used in the inquiry. If the precision was insufficient (Relative Standard Deviation larger than 10%), the sample was reanalysed.

The limits of the analytical method were calculated for each metal according to the procedure described by Fleming et al. (1997). The precision and accuracy of the whole procedure were checked against the certified reference material (Table 2). Next to that, spikes were run regularly after each ten samples.

In Mallards, body mass is a good proxy for condition index (Whyte et al., 1986; Boos et al., 2000). Hence, to check if birds used in this study were in good condition all birds were weighed with accuracy of 1 g and their body mass was compared with the data on mallards caught in the same summer period (Folk et al., 1966). Analyses were performed separately for males and females. In males three groups of birds were distinguished consisting birds from big towns, small towns and fish ponds. The low number of females caught in cities caused that they were classified only into two groups: birds from towns and birds from fish ponds. The distributions of the data of metals concentrations significantly differed from the normal one (Shapiro Wilk test, $p < 0.05$ in all cases) so nonparametric tests were used in all analyses. All calculations were done with Excel 2010 (Microsoft) and Statistica 10 (StatSoft Inc.).

3. Results

Body masses of birds used in this study were in the range of those given in literature sources for summer period (Fig. 2). Thus, it may be assumed that samples were obtained from Mallards being in good condition.

Table 1
Sample size collected at different sites.

Category	Place	Males	Females	Total
Big towns	Warsaw	10	2	12
	Gdansk	10	5	15
Small towns	Ostroda	8	2	10
	Ilawa	5	1	6
Fish ponds	Milicz	15	11	26
Total		48	21	69

Table 2
Limits of detection (LoD) and quantification (LoQ), recoveries for reference materials (RM) and relative standard deviation (RSD) between duplicates.

Metal	LoD	LoQ	RM	Recovery [%]	RSD [%]
Cd	0.024 μg/L	0.057 μg/L	ERMCE195	90.9	4.4
Cr	0.252 μg/L	0.387 μg/L	BCR185R	97.3	2.3
Cu	0.027 μg/L	0.130 μg/L	SRM1577b	99.5	1.9
Fe	0.058 mg/L	0.415 mg/L	SRM1577b	100.7	7.0
Ni[a]	1.858 μg/L	4.573 μg/L	–	–	–
Pb	0.530 μg/L	1.478 μg/L	ERMCE195	92.4	9.0
Zn	0.011 mg/L	0.024 mg/L	SRM1577b	107.3	2.7

[a] Appropriate nickel CRM were not found on the market.

Concentrations of all the metals did not differ statistically between sexes among the birds in towns (Mann–Whitney test, $p > 0.05$ in all cases) as well as the ones in the pond area (Mann–Whitney test, $p > 0.05$ in all cases). Concentrations of studied metals in blood of Mallards in all groups made up the following order:

Fe > Zn > Cu > Cr ≈ Ni > Pb > Cd

The highest concentrations were determined in the case of iron which maximally reached 535.667 μg/g w.w. (Fig. 3). Significantly higher values were found in birds from towns than in those from fish ponds (Kruskal–Wallis test, $H_{2,48} = 21.62$, $p < 0.0001$ for males and Mann–Whitney test, $U = 19.0$, $p = 0.0124$ for females).

Much lower concentrations were recorded in the case of zinc. Its highest concentration was found in female bird from a town group (24.470 μg/g w.w.). Concentrations were higher in birds collected on fish ponds (Kruskal–Wallis test, $H_{2,48} = 22.89$, $p < 0.0001$ for males and Mann–Whitney test, $U = 11.0$, $p = 0.0022$ for females) (Fig. 4).

Median accumulation of copper varied between 0.597 μg/g w.w. in drakes form towns and 2.730 μg/g w.w. in females from the fish

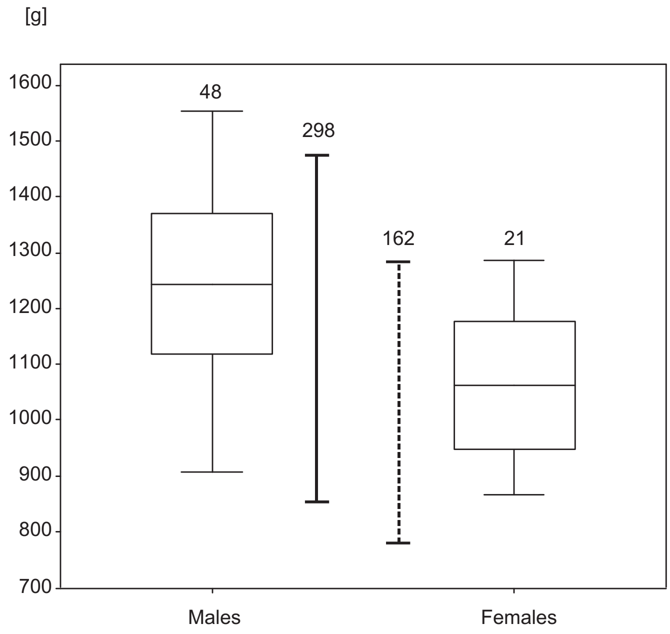

Fig. 2. Body mass of Mallards taken for this study. Horizontal line – mean, rectangle – standard deviation, vertical line – range. Thick vertical lines show range of body mass of adult males (solid line) and adult females (dotted line) of Mallards from wild populations weighed between June and August in former Czechoslovakia (Folk et al., 1966). Numbers above indicate sample sizes.

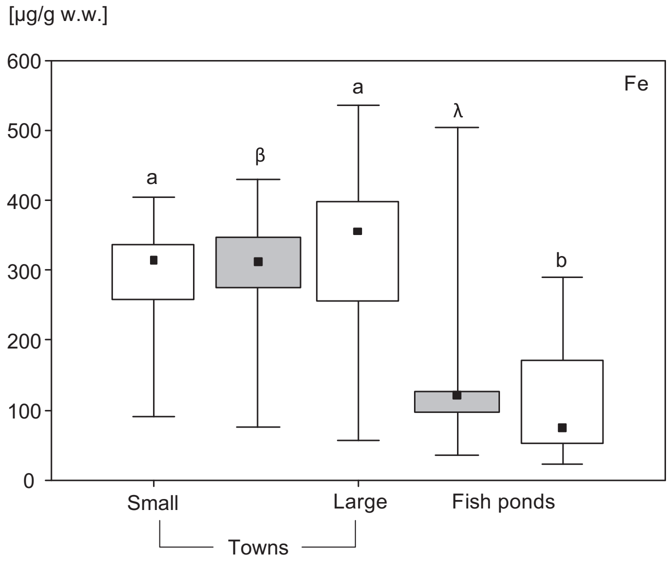

Fig. 3. Iron concentrations in blood of males (white) and females (grey) of Mallard sampled in towns and fish ponds. Dot – median, box – interquartile range, whiskers – range, statistically significant differences according Kruskal–Wallis and Dunn post-hoc tests for males and Mann–Whitney test for females are marked with different letters (separately for males and females).

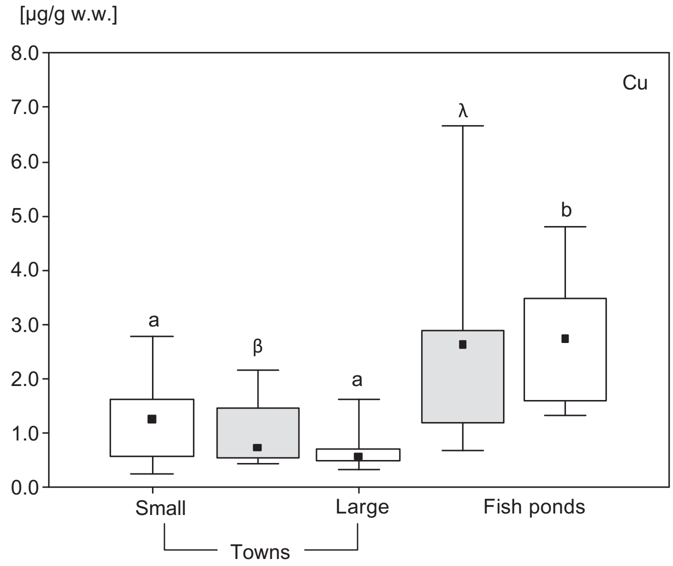

Fig. 5. Copper concentrations in blood of males (white) and females (grey) of Mallard sampled in towns and fish ponds. Legend as on the Fig. 3.

pond group. The highest concentrations occurred in two females from fish ponds group (6.768 and 6.361 µg/g w.w.). Similarly to zinc, statistically higher concentrations were found in birds shot in the area of fish ponds (Kruskal–Wallis test, $H_{2,48} = 23.42$, $p < 0.0001$ for males and Mann–Whitney test, $U = 15.0$, $p = 0.0054$ for females) (Fig. 5).

Chromium and nickel reached similar, low level of accumulations in Mallard blood. Their medians were placed just above limits of quantification. The highest concentration of chromium was found in blood of drake from the small town group (4.776 µg/g w.w.). In males differences among groups were not statistically significant (Kruskal–Wallis test, $H_{2,48} = 5.74$, $p = 0.0567$). In the case of females, statistically lower concentrations were determined among birds from fish ponds (Mann–Whitney test, $U = 25.5$, $p = 0.0405$) (Fig. 6).

Maximum concentration of nickel occurred in drake from fish ponds (3.7 µg/g w.w.). High value was found also in drake from small town group (3.710 µg/g w.w.). Statistically significant differences were found in nickel concentrations between drakes from big towns and fish ponds (Kruskal–Wallis test, $H_{2,48} = 14.98$, $p = 0.0006$) and between females from towns and fish ponds (Mann–Whitney test, $U = 26.0$, $p = 0.04109$). In birds from the fish ponds nickel accumulation was higher than in those from the urbanized areas (Fig. 7).

The highest lead concentrations were noted in birds from towns with the maximum value found in drake from large town

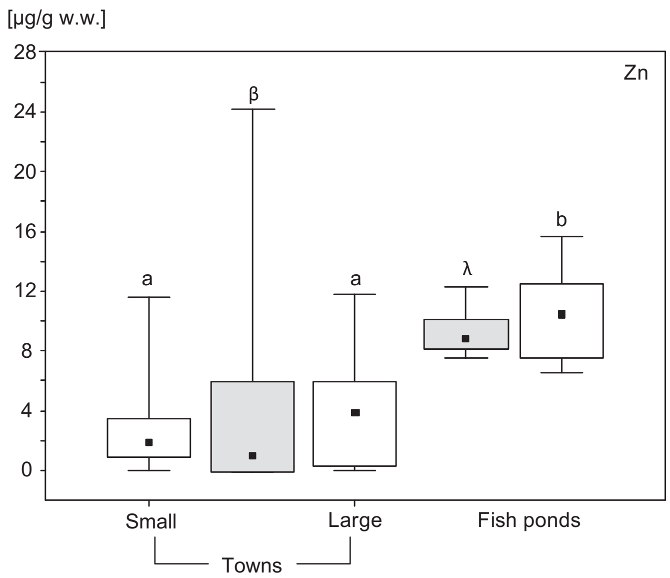

Fig. 4. Zinc concentrations in blood of males (white) and females (grey) of Mallard sampled in towns and fish ponds. Legend as on the Fig. 3.

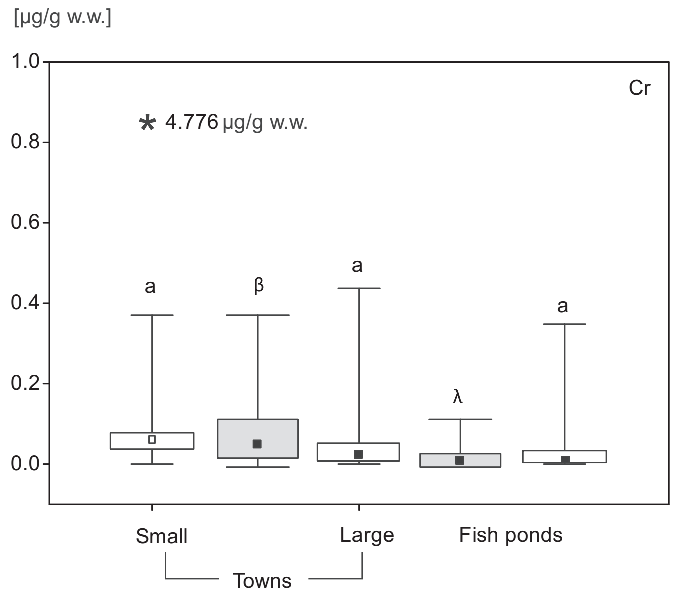

Fig. 6. Chromium concentrations in blood of males (white) and females (grey) of Mallard sampled in towns and fish ponds. Legend as on the Fig. 3. One extreme value found in the sample from small towns was excluded from the analysis and shown as an asterisk.

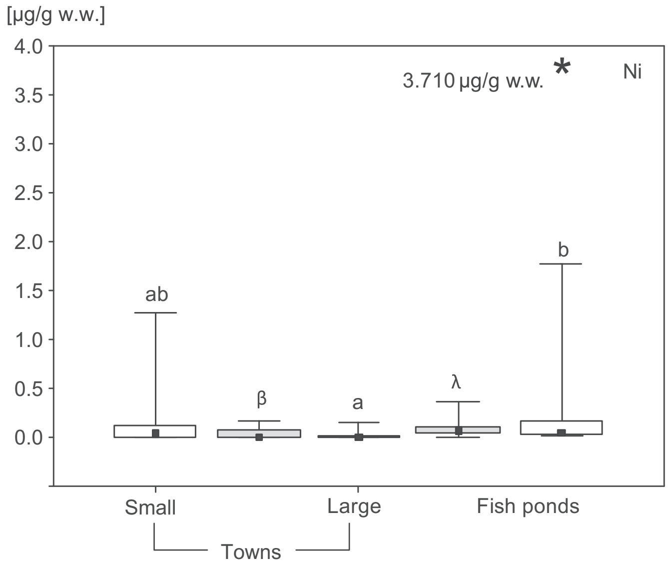

Fig. 7. Nickel concentrations in blood of males (white) and females (grey) of Mallard sampled in towns and fish ponds. Legend as on the Fig. 3. One extreme value found in one male from fish ponds was excluded from the analysis and shown as an asterisk.

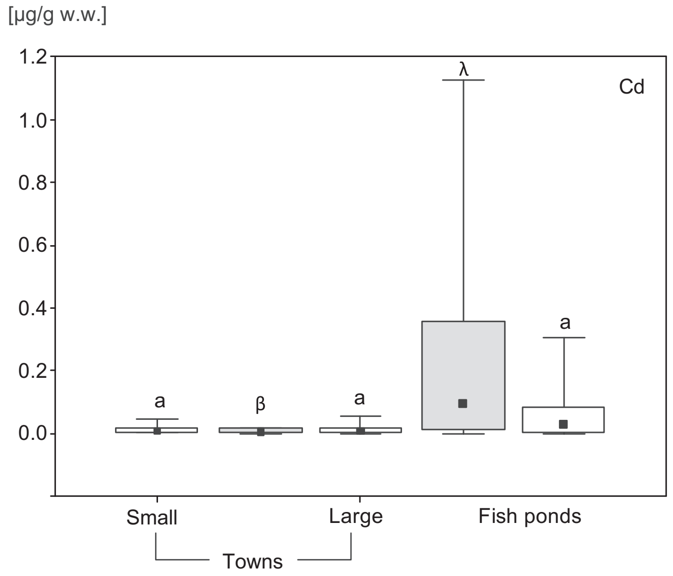

Fig. 9. Cadmium concentrations in blood of males (white) and females (grey) of Mallard sampled in towns and fish ponds. Legend as on the Fig. 3.

(0.690 μg/g w.w.) and in female from small town areas (0.507 μg/g w.w.). There were no statistically significant differences between females from towns and ponds (Mann–Whitney test, $U = 41.0$, $p = 0.3416$). However, significantly lower concentrations of lead were noted among males from the small town group than from the large town one (Kruskal–Wallis test, $H_{2,48} = 6.35$, $p = 0.0419$) (Fig. 8).

Cadmium concentrations did not differ statistically among drakes from different areas (Kruskal–Wallis test, $H_{2,48} = 5.23$, $p = 0.0731$). Significantly higher concentrations of this element were noted in the case of females from fish ponds than those from the urbanized areas (Mann–Whitney test, $U = 0.008$). In that group, also the maximum measured concentration occurred which was equal to 1.128 μg/g w.w. (Fig. 9).

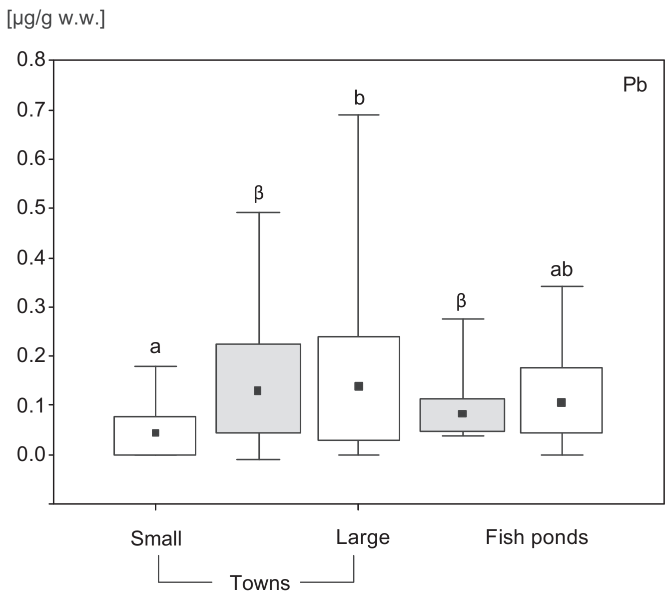

Fig. 8. Lead concentrations in blood of males (white) and females (grey) of Mallard sampled in towns and fish ponds. Legend as on the Fig. 3.

There was a very high positive correlation between the level of nickel and chromium in blood of Mallards from towns ($r = 0.96$) and fish ponds (Pearson correlation coefficient $r = 0.90$). As regards other metals birds from fish ponds revealed moderate positive correlation only between the level of chromium and zinc ($r = 0.40$), whereas in blood of Mallards caught in towns levels of nickel and copper, chromium and copper were moderately and weakly positively correlated ($r = 0.74$ and $r = 0.32$ respectively).

4. Discussion

Concentrations of metals among different waterbirds species are being documented all over the world (Di Giulio and Scanlon, 1984; Kalisińska et al., 2007; Braune and Scheuhammer, 2008; Binkowski et al., 2013a,b). However most of these research studies concern metal concentrations in tissues sampled during the necropsy. There is a significantly lower number of blood studies (Pain, 1989; Garcia-Fernandez et al., 1996; Van Eeden and Schoonbee, 1996) and in the case of a few metals (e.g. chromium) we cannot find any published data. Blood analyses always demand sampling of live specimens (e.g. during ringing) or from shot animals but just after the shot which is hard to carry out (presence of different specialist is demanded in the field). It is the probable reason of scarce amount of this kind of data. We assumed that there is no difference between the blood taken in different ways (from the ventricle of the heart and from the metatarsal vein). Such comparison is not available in the literature. However, our current research showed no difference in that aspect in enzyme activities, haematological parameters and metals concentrations (Binkowski, unpublished data).

Critical issue in the biomonitoring use of animals is their connection with the habitat (Walker et al., 2006). Migratory species do not inhabit a given area during the whole year so it is essential to choose the appropriate time of sampling where specimens spent a few months or weeks in the research area. In the period when samples were taken for this study majority of Mallards finished their breeding period (Cramp and Simmons, 1977). Those from towns were sampled earlier than birds from fish ponds. Majority of males were moulting whereas females were before moulting. In Polish wetlands the increase of the Mallard number prior autumn migration is observed in July or August (Cieślak et al., 1991; Kunysz and Hordowski, 1992; Furmanek, 2000; Meissner et al., 2011). Thus, birds shot in August in fish ponds were after moult and probably in the premigratory stage prior the departure or some of them might be migrants stopping there during the early stage of migration towards wintering grounds. All birds used in our study were in good condition, so metal concentrations noted among the studied Mallards of both sexes seemed to be within the physiological tolerance.

Iron was the only metal the concentrations of which in birds from towns were statistically higher than in birds from fish ponds (Fig. 3). Urban Mallards are mostly sedentary (Zárybnický and Klvaňa 2008, authors, unpublished data) and probably the reason of this difference is the much higher iron concentration in towns where steel constructions (also near river banks), industry production and sewages (which contain pharmaceuticals metabolites) are abundant (Nordberg et al., 2007). Elevated concentrations of iron in Mallards collected in polluted areas have been already noted in northern Poland (Kalisińska et al., 2004) and in other parts of the Europe (Kozulin and Pavluschick, 1993). Iron is an essential element, which is especially important as the component of haemoglobin. In strongly increased concentrations its side effects can be also visible in any species (Nordberg et al., 2007). Toxic effects may occur by ingestion or parenteral administration (Gupta, 2012). The data presenting toxic levels of these metals is scarce. Among people (only available data), concentrations over 350 mg/dL (~3800 μg/g w.w.) are considered as toxic (Pagana and Pagana, 2010). The above mentioned values are substantially higher than noted among the studied birds.

Contrary to iron, the accumulation of zinc and copper were statistically higher among birds shot in the wetland area (Figs. 4 and 5). There are some suggestions that zinc concentrations in tissues may by significantly influenced by the month of collection which is connected with different stage of molting process and changes in food quality (Parslow, 1982). Concentrations of copper can be highly variable among species and as well as in the case of zinc visible differences, may occur during the year (Parslow, 1982). Contents of both elements in tissues can be also connected with the pollution of the environment (Parslow, 1982; Di Giulio and Scanlon, 1984; Hui et al., 1998; Kalisińska et al., 2004). Its source cannot be clearly pointed but it is highly possible that it is a secondary impact of farming activity where herbicides and fungicides with zinc and copper are used (on the banks of ponds and inflows). A part of these elements can also target the ponds with fish food (artificial feedstuff and natural grain). Higher copper and zinc concentrations as well as their deficiency can be harmful to organism (Mertz, 1981; Nordberg et al., 2007), especially among waterfowl (Beck, 1961). However, no data are available on the toxicity of copper to avian wildlife. Research on the adverse effects of higher concentration concerns only domestic waterfowl which are fed with extremely high doses of copper (diet containing even 500 mg of metal per 1 kg of feedstuff). More common effects on birds of high copper concentrations in the environment can be seen through the trophic chain when the prey of some avian species is eliminated by the pollution (which limits the nutrition sources) (Eisler, 1998). On the contrary, zinc poisoning may be observed more often in nature, but in most cases it is connected with the zinc compounds ingestion. In poisoned birds, the concentration of this metal only in plasma reached over 15 mg/L (~15 μg/mL) (Eisler, 1993). However, the plasma concentration is the least sensitive factor of zinc poisoning which may not be increased even with high concentrations in the environment (Nordberg et al., 2007). The concentrations of both elements among the studied specimens in Poland are comparable to the literature data. Median concentration of zinc in blood of Mallards collected in the period of 2006–2011 in the area of Zator fish ponds in southern Poland (6.22 μg/g w.w., Binkowski, unpublished data) is within the limits found in this study. Other data on mean copper concentration in waterbirds blood were ranging from 0.59 μg/g w.w. in Mallards from Zator fish ponds in southern Poland to 3.1 μg/g w.w. in Red-knobbed Coot (*Fulica cristata*) from South Africa (originally 26.1 μg/g d.w. – recalculated with the assumption of 12% d.w. content in the w.w.) (Van Eeden and Schoonbee, 1996). Quite high copper concentrations in blood of adult Mallards collected within this study on Milicz fish ponds were comparable to the results obtained there between 2006 and 2011 in the same place (median = 2.09 μg/g w.w.). During the same period the concentrations of copper measured in blood of Mallards from Zator area (southern Poland; 0.59 μg/g w.w.) were significantly lower (Binkowski, author, unpublished data). This comparison suggests that copper availability in this place is higher than in other fish ponds and in studied urbanized areas.

Concentrations of chromium and nickel in samples of blood showed small variation among the studied groups of birds (Figs. 6 and 7). Both of these metals play physiological roles in animals, but also their side effects of higher concentrations are known mostly from human studies (Nordberg et al., 2007; Tsipoura et al., 2011). Chromium has high affinity to clay particles so probably its availability for animals (especially from lower levels of food chain) is low. No detectable amounts of this metal were observed even in fish bred in highly polluted ponds (Balasubramanian and Pappathi, 1995). Similarly, nickel concentrations in tissues of various bird species observed all over the world are usually low (Kozulin and Pavluschick, 1993; C Hui, 1998; A Hui et al., 1998). Concentrations of both elements in blood of studied birds were small. Nickel concentrations found by Van Eeden and Schoonbee (1996) were visibly higher than those of birds from Poland (the lowest value noted by those authors was even higher than medians observed in Poland).

Lead and cadmium are known as xenobiotics (Scheuhammer, 1987; Nordberg et al., 2007). Lead is a serious neurotoxin, which can influence the behaviour and the whole physiology of the organism (Dieter and Finley, 1979; Kalisińska et al., 2004; Nordberg et al., 2007) and may be a very serious reason of mortality (Perrins et al., 2003). Because of that it is probably the most studied element in the environment, mostly on wetlands because of the possibility of lead poisoning from ammunition and fishing sources (Di Giulio and Scanlon, 1984; Sanderson and Bellrose, 1986; Pain, 1990; Scheuhammer and Norris, 1995; Mateo et al., 2000; Kalisińska et al., 2004; Binkowski and Zakrzewska, 2009; Binkowski et al., 2013a). The diagnosis of the problem in a single specimen can be done on the basis of lead concentrations in chosen tissues (Pain, 1990). The content in the whole blood was established at the level of 0.5 μg/mL (c.a. 0.46 μg/g w.w.; Sanderson and Bellrose, 1986). According to this value, two Mallards can be marked as poisoned (female from Ilawa and male from Gdansk). It is worth emphasizing that more restrictive threshold given by Mudge (1983) points out concentrations higher than 0.25 μg/mL (c.a. 0.23 μg/g w.w.) as those present in polluted birds. According to that level 17% of studied birds from both kinds of towns and fish ponds were poisoned. Mallards from bigger towns accumulated bigger quantity of lead than those from small ones (Fig. 8). This difference may be connected with the environmental pollution, which seems to be higher in highly urbanizes areas (Sawicka-Kapusta et al., 2005). Our results clearly show that there is no sharp difference in exposure to lead between birds from towns (places of no hunting activity) and fish ponds (area of hunting) (Fig. 8). Mean lead concentrations in blood of Red-knobbed Coot from South Africa (Van Eeden and Schoonbee, 1996) and Mute Swans (*Cygnus olor*) from England were significantly higher (23.8 μg/g d.w. ≈ 2.9 μg/g w.w. and 3.36 μmol/L ≈ 0.6 μg/g w.w.

respectively) than those noted among the studied Mallards from Poland.

Cadmium is a strong nephrotoxin and its 75% of body burden is accumulated in liver and kidneys. Concentrations in these organs are used as evaluators of the exposition of the animal to this metal (Eisler, 1985). Cadmium in blood is also bound by morphotic elements (Nordberg et al., 1985), but monitoring data usually refer to concentrations in the whole blood (Nordberg et al., 2007). Concentrations of cadmium in studied birds from towns were low and fitted in the narrow range (Fig. 9). Higher diversity of cadmium concentration noted among birds shot on fish ponds (especially in females) may be connected with living in different habitat (Kalisińska et al., 2004). In comparison to concentrations found by Van Eeden and Schoonbee (1996) in various waterfowl species (Mallard 1.8, Reed Cormorant 4.7 μg/g w.w.), the levels of cadmium in birds from Poland were not high.

Significant correlations found in these research studies concern two elements which are so far rarely studied – chromium and nickel (Kalisińska et al., 2004). The relationship between chromium and copper, and chromium and zinc were also noted in liver of waterbirds from Spain (Mateo and Guitart, 2003), but both results are hard to explain. All of these metals play important roles in enzymes activation (Nordberg et al., 2007), thus, the noted correlation may have a physiological base and need further inquiry. The data concerning correlations of nickel concentration were not found in the literature.

Urban areas are usually connected with wide spectrum of pollution (McMichael, 2000). Our studies showed that only concentrations of iron and chromium in females were higher in birds from towns, whereas concentrations of copper, zinc, nickel, and cadmium in females were higher in Mallards from fish ponds. It allows us to infer that even if the main sources of pollution are centralized in urbanized areas, some metals reached higher concentration in Mallards staying outside towns. We cannot either exclude a possibility of early migration among some birds so the image of the local pollution on fish ponds areas can be disturbed by the exposure of bird in previous staging areas. It is known that Mallards can change their sites between urbanized and non-urbanized areas within one season (Figley and VanDruff, 1982; Waterbird Research Group KULING, unpublished data). On the other hand it is also possible that some metal pollution may be higher in fish ponds than in cities due to management towards achieving better conditions for fish production (e.g. controlling of vegetation on pond embankments with pesticides, using grain with metal addition, using synthetic fertilizer, forming embankments from bottom deposits).

It seems that blood samples provide proper material for nondestructive analyses and in the case of sedentary bird species may be used in metal pollution monitoring. The cost of collecting samples is negligible but the results may give a constant image of xenobiotics distribution in the environment and be a fast detecting alarm of pollution.

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