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Letter to Editor

Copper, Zinc, Lead and Cadmium Content in Liver and Muscles of Mallards (*Anas Platyrhychnos*) and Other Hunting Fowl Species in Warmia and Mazury in 1999-2000

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Abstract

This paper presents the results of a study on the content of Cu, Zn, Pb and Cd in livers and breast muscles of mallards obtained in Warmia and Mazury in 1999-2000. Samples from 32 mallards hunted in Łężany, Zembrze, Szestno and Nidzica, as well as samples from 7 pheasants and 6 mallards from the area of Śląsk were analyzed. Concentration of all the four metals was higher in the liver than in breast muscles. Mean contents of copper in livers of juvenile and adult mallards from Warmia and Mazury were similar (6.567 and 8.632 mg/kg w.w.). Levels of zinc were approximately the same in both age groups (23.911 mg/kg w.w. in young birds and 25.418 mg/kg w.w. in adults). As regards lead, older mallards from Warmia and Mazury had twice as much of the metal in the liver (0.417 mg/kg w.w.). The content of Pb in the liver was similar in both age groups of the birds from Śląsk. The differences in the concentration of cadmium in the mallards from Warmia and Mazury were smaller. Cd in the amount of 0.128 mg/kg m.m. was found in the young mallards and 0.171 mg/kg m.m. in the adult mallards. The birds from the area of Śląsk contained much higher concentrations of Cd in their tissues, over 1 mg/kg m.m. in the liver and over 0.200 mg/kg m.m. in the breast muscles.

Keywords: copper, zinc, lead, cadmium, *Anas platyrhynchos*, Warmia and Mazury

Introduction

The mallard (*Anas platyrhynhos*) is the most popular species of hunting fowl and the most numerous one in Poland. In very few areas (e.g. Słoński Nature Reserve) do wild geese outnumber mallards. In the early 1980s the population of mallards in Europe was estimated at 2 million birds [31]. Since that time the number of mallards and other species of wild ducks has decreased considerably [26].

This was believed to have been caused by excessive dewatering of land (elimination of small water ponds and marshes), abuse of mineral fertilisers and pesticides [27, 28, 34], predator stress[5], and industrial chemicals and heavy metals[12].

The presence of xenobiotics in tissues of wild animals may be a sign of environmental pollution with these compounds. Negative effects of heavy metals is particularly dangerous to birds, whose metabolism is more rapid compared to other groups of animals. They are, therefore, exposed to a greater threat of accumulating heavy metals in their bodies [12]. Of the four metals (zinc, copper, cad-

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mium and lead), cadmium and lead are characterised by the highest accumulation factor in living organisms; they are easily absorbed from atmospheric air and from the digestive tract[20]. Warmia and Mazury are free from large industrial centres. Any pollutants travel here in air masses from other regions of Poland and other countries or come from deposits of heavy metals in soil and lake sediments, which have been formed due to human activities. Regular analyses of the content of heavy metals and other xenobiotics in tissues of young ducks may help to monitor the rate of pollution or regeneration of a given area of the province or the country. An essential fact in such investigations is that in spring a female mallard returns with her partner to a nesting area which is usually her birthplace. If the nesting is successful and young ducks are bred the female will return to the same place every year.

Material and Methods

Samples (about 50-70 g) of liver and breast muscles were taken from 32 mallards, 7 tufted ducks (*Aythya fuligula*) and 2 garganeys (*Anas querquedula*) obtained in Warmia and Mazury in 1999 and 2000 (Fig. 1). For the sake of comparison, liver and breast muscle samples were collected from 6 mallards and 7 pheasants (*Phasianus colchicus*) from the area of Śląsk, where extractive and metallurgical industries are located. Frozen samples in polyethylene bags were delivered to the laboratory. After defrosting, 10 g of each sample was dry-mineralized. In order to determine cadmium and lead, the mineralysate was transferred to the organic phase. Determinations of Cu, Zn, Pb and Cd were performed using flame atomic absorption spectrometry. An AAS Unicam 939 spectrophotometer fitted with an ADAX data base, background correction and suitable cathode lamps was applied. The determinations were executed in two replications or, in case of large deviations of the results, in three to four replications. The detectability of the determination methods applied for the elements was: Cu – 0.075 μg/g; Zn – 0.025 μg/g; Cd – 0.0125 μg/g; Pb – 0.075 μg/g tissue. Samples with low lead content (under 0.1μg/g) and samples with a low cadmium content (under 0.05 μg/g) were subject to flameless atomic absorption spectrometry of the detectability of 0.0025 μg/g tissue for Pb and 0.001 μg/g tissue for Cd. The results were statistically analyzed. Coefficient of variation and standard deviation were estimated by variance analysis and T-Student's test. STATISTICA 6.0 Pl software was used for statistical calculation.

Fig. 1. Locations of shooting birds obtained in Warmia and Mazury and in Śląsk in 1999-2000.

Results and Discussion

The mallards we observed in the course of our research from Warmia and Mazury in autumn and winter would typically remain beyond the scope of interest as they were birds coming from the northern or eastern Europe[12]. Young birds were mainly sampled because the concentration of heavy metals in their tissues doubtless depends on the rate of contamination of water, air, soil and vegetation in the given area [9].

A component of the enzymes responsible for metabolism of proteins and carbohydrates, zinc is an essential element in the animal organism. High concentrations of zinc occur in the liver, kidneys and bones. Deficiency of zinc causes loss of appetite, loss of body weight, impairment of epidermal products, impairment of reproductive functions and changes in bones, especially in birds due to osteogenesis [7, 16, 24]. The concentration of zinc (tab.1) determined in the liver of the mallards was within the range of 8.329 mg/kg w.w to 43.031 mg/kg w.w. (mean 24.288 mg/kg w.w.). In the other species under study, liver zinc was at a similar level. Dańczak [8], while studying the *Anseriformes* order of birds, obtained similar Zn values in the Long-tailed duck (*Clangula hyemalis L*) and Domestic goose (*Anser anser f domestica*) and higher in mallards (43.6 mg/kg w.w. in males and 53.9 mg/kg w.w. in females). Higher values were also reported for the lesser scaup (*Aytha affinis*) living in the Indiana Harbour Canal (55.205 mg/kg w.w. in adults and 46.322 mg/kg w.w. in juveniles) [6]. The Zn content in duck breast muscles (tab.2) from Warmia and Mazury was level and the means ranged from 8.739 to11.750 mg/kg w.w., which resembles the results obtained from samples of ducks from poultry plants in north-eastern Poland [11] and scaup ducks (*Aythya marila L.*) in Gdansk Bay [36]. The pheasants and mallards from Śląsk had slightly higher Zn concentration, i.e. 15.535 and 19.803 mg/kg w.w. Zn concentrations in the liver and breast muscles of the birds appear to have physiological values, much below toxic levels, e.g. 1,200 mg/kg d.w. (360 mg/kg w.w.) determined for mallards by Gasaway and Buss [14].

Copper, like zinc, is an essential metal for the well--being of an organism. Of all the body organs, the liver, brain, kidneys and heart are the richest in copper. Cop-

per deficiency is as dangerous as its excessive amounts. Deficiency of copper is a rare ailment, with anaemia, retarded growth, disturbances of ossification and feather pigmentation or disturbances in the nervous system being the symptoms. Haemoglobinaemia, haemoglobinuria, jaundice and liver problems, on the other hand, are symptomatic of copper poisoning [8, 16, 24]. The mean copper concentration determined in the liver of the mallards was 7.083 mg/kg w.w. (Tab.1), and in the breast muscles – 4.785 mg/kg.w.w.(Tab.2) The other hunting fowl species under study had similar Cu concentrations. No significant difference was found between the Cu content in juveniles (average 6.567 mg/kg w.w. in liver and 4.128 mg/kg w.w. in breast muscles) and adults (average 8.623 mg/kg w.w. in liver and 5.853 mg/kg w.w. in breast muscles). Copper in the livers of mallard males and females analysed by Dańczak *et al*. [8] was on average 29.7 mg/kg w.w. and 18.6 mg/kg w.w., respectively, and 15.5 mg/kg w.w. in scaup ducks from Gdansk Bay [36]. Younger lesser scaups had 21.45 mg Cu/kg w.w. and adults 15.713 mg Cu/kg w.w.[6]. Our results on the content of copper in the liver seem low compared to those reported by other authors, although they remained on the physiological level.

Cadmium is a strongly toxic element. If absorbed into the organism through the digestive and pulmonary systems, cadmium forms complexes with proteins, in which it is easily transported and stored, mainly in the liver and kidneys and, in smaller quantities, in the pancreas, intestines and bones. Cadmium is also responsible for upsetting the activity of a number of cellular enzymes [1, 12, 19, 22]. It produces tetratogenic, mutagenic and carcinogenic effects. The concentration of cadmium in the liver of the analyzed mallards was within the range of 0.014 to 0.394 mg/kg w.w. (mean 0.139 mg/kg w.w.-Fig.2) and in the breast muscles from 0.025 to 0.215 mg/kg w.w. (mean 0.082 mg/kg w.w.-Fig.3). Both the juveniles and adults had twice as high a content of cadmium as mallards from

Table 1. Concentrations (mg/kg wet wt.) of Cu, Zn and Pb in livers.

Species	Cu			Zn			Pb		
	X (x_{min}-x_{max})	s	cv	X (x_{min}-x_{max})	s	cv	X (x_{min}-x_{max})	s	cv
Mallard W&M									
J, n = 24	6.567 (2.006-18.802)	4.442	67.6	23.911 (8.329-43.031)	11.454	47.9	0.155[a] (0.050-0.441)	0.104	67.1
A, n = 8	8.632 (2.355-17.449)	6.170	715	25.418 (11.749-34.112)	7.806	30.7	0.417 (0.055-0.892)	0.313	75.0
All, n =32	7.083	4.905	69.2	24.288	10.561	43.5	0.225	0.213	94.7
Tufted duck W&M									
J, n= 4	5.552 (4.27-7.593)	1.436	25.9	23.557 (13.109-30.4310)	7.628	32.4	0.165 (0.043-0.3)	0.116	70.2
A, n = 3	6.663 (6.343-6.972)	0.315	4.7	25.01 (22.011-29.051)	3.634	14.5	0.139 (0.041-0.291)	0.133	96.0
All, n = 7	6.028	1.191	19.7	24.180	5.839	24.1	0.154	0.113	16.5
Garganey W&M J, n =2	4.751 (4.491 and 5.011)	0.368	7.7	15.393 (9.975 and 20.811)	7.662	49.8	0.476 (0.431 and 0.521)	0.064	13.4
Mallard Śl.									
J, n = 4	8.383 (6.663-9.591)	1.237	14.7	25.455 (23.660-26.642)	1.304	5.1	0.202 (0.173-0.240)	0.032	15.9
A, n = 2	9.899 (9.355 and 10.443)	0.769	7.8	27.426 (27.299 and 27.554)	0.180	0.6	0.255 (0.249 and 0.262)	0.009	3.6
All, n = 6	8.888	1.284	14.4	26.112	1.437	5.5	0.220	0.037	17.0
Pheasant Śl.									
J n = 5	5.937 (4.987-7.058)	0.901	15.2	24.013 (20.998-26.070)	2.111	8.8	0.182 (0.134-0.222)	0.034	18.8
A, n =2	7.489 (7.151 and 7.827)	0.478	6.4	26.820 (26.390 and 27.251)	0.609	2.3	0.165 (0.163 and 0.167)	0.003	1.7
All, n =7	6.380	1.074	16.8	24.815	2.216	8.9	0.177	0.029	16.5

n– number of samples; x-arithmetic mean; s- standard deviation; x_{min}- minimum; x_{max}-maximum; a- valence not included in mean: 6.766 and 6.514; cv- coefficient of variation (%); W&M- Warmia and Mazury; Śl .-Śląsk

the environs of Żnin and Bydgoszcz, where adults had 0.092 and 0.052 mg/kg w.w., and juveniles 0.037 and 0.055 mg/kg w.w., respectively [12]. Long-tailed ducks from Gdansk Bay had 0.675 mg Cd/kg w.w. in the liver and about 0.007 mg Cd/kg w.w. in breast muscles [35]. Similar values were reported for lesser scaups in the Indiana Harbour Canal (0.694 and 0.915 mg Cd/kg w.w. in juvenile and adult birds respectively) [6]. A higher level of Cd in liver (average 1.274 and 1.121 mg/kg w.w –Fig.2) and in the breast muscles (average 0.432 and 0.469 mg/kg w.w. –Fig.3) was found in the mallards and pheasants from Śląsk, which is due to the environmental pollution of that region. It is estimated that over 60% of environmental pollution by cadmium originates from zinc and other non-ferrous metallurgy [19], and Śląsk is an area with developed extractive and metallurgical industries. Higher levels of cadmium in the liver were also determined in the ducks from Słoński Nature Reserve (mean 1.68 mg/kg w.w.) [8]. Some authors [15, 33] consider cadmium at the level of <3 mg/kg d.w., that is 0.9 mg/kg w.w., to be the background level for freshwater waterfowl. Therefore, cadmium in the liver and breast muscles of the mallards analysed in the course of our investigation does not exceed this level.

Like cadmium, lead is an element that plays no role in metabolic processes of animal organisms. It is an extremely toxic element with a wide range of harmful effects. Exposure to lead may cause kidney and nervous system problems. It can also inhibit heme synthesis [3]. The lead concentration determined for the mallards was from 0.050 mg/kg w.w. to 0.892 mg/kg w.w. (mean 0.225 mg/kg w.w.) in the liver (Tab.1) and 0.038 mg/kg w.w. to 0.283 mg/kg w.w. in breast muscles (Tab.2). Similar results (average 0.2 mg/kg w.w.) were obtained by Holt *et al*.[18], who investigated waterfowl in Norway, and by Fabczak *et al*.[10], who studied cormorants (*Phalacrocorax carbo*) in northeastern Poland. Two mallards shot near Nidzica were found to have a much higher content

Table 2. Concentrations (mg/kg wet wt.) of Cu, Zn and Pb in breast muscles.

Species	Cu			Zn			Pb		
	X (x_{min}-x_{max})	s	cv	X (x_{min}-x_{max})	s	cv	X (x_{min}-x_{max})	s	cv
Mallard W&M									
J, n = 13	4.128 (2.095-18.802)	2.016	48.8	11.085 (9.217-16.320)	1.811	16.3	0.122a (0.038-0.283)	0.078	64.4
A, n = 8	5.853 (1.482-10.021)	2.876	49.1	9.359 (3.223-16.470)	4.409	47.1	0.154 (0.103-0.209)	0.046	28.0
All, n =32	4.785	2.464	51.5	10.428	3.084	29.6	0.136	0.067	49.6
Tufted duck W&M									
J, n= 4	5.835 (3.394-6.812)	1.631	28.0	10.654 (8.178-12.758)	1.884	17.7	0.036 (<0.01-0.069)	0.028	77.4
A, n = 3	6.103 (5.234-6.534)	0.752	12.3	13.212 (11.184-14.734)	1.828	13.8	0.042 (0.035-0.051)	0.008	19.1
All, n = 7	5.950	1.241	20.8	11.750	2.181	18.6	0.039	0.021	53.2
Garganey W&M J, n =2	6.020 (5.840 and 6.600)	0.254	4.2	8.739 (6.440 and 11.039)	3.252	37.2	0.246 (0.208 and 0.285)	0.054	22.1
Mallard Śl.									
J, n = 4	6.383 (5.254-7.057)	0.830	13.0	19.659 (18.175-20.630)	1.049	5.3	0.095 (0.073-0.120)	0.022	23.2
A, n = 2	7.327 (7.154 and 7.500)	0.245	3.3	20.093 (19.088 and 21.098)	1.421	7.1	0.074 (0.057 and 0.092)	0.025	33.2
All, n = 6	6.698	0.814	12.1	19.803	1.056	5.3	0.088	0.023	25.9
Pheasant Śl.									
J n = 5	4.211 (3.765-4.783)	0.434	10.3	15.088 (14.054-16.309)	0.807	5.3	0.083 (0.057-0.125)	0.025	30.4
A, n =2	6.168 (5.958 and 6.379)	0.297	4.8	16.651 (16.364 and 16.939)	0.406	2.4	0.055 (0.053 and 0.058)	0.003	6.4
All, n =7	4.770	1.026	21.5	15.535	1.022	6.6	0.075	0.025	32.9

n– number of samples; x-arithmetic mean; s- standard deviation; x_{min}- minimum; x_{max}-maximum; a - valence not included in mean: 6.799 and 8.597; cv- coefficient of variation (%); W&M- Warmia and Mazury; Śl .-Śląsk

of lead, both in the liver (5.514 and 6.766 mg/kg w.w.) and in breast muscles (6.799 and 8.597 mg/kg w.w.). Concentrations above 5 mg/kg d.w. (1.5 mg/kg w.w.) [17] are considered abnormal. Lead content in the liver exceeding 20 mg/kg d.w. (6 mg/kg w.w.) is thought to be a sign of acute lead poisoning, usually associated with severe physiological effects [13]. This means that the two ducks from Nidzica environs must have been poisoned with lead. Mateo and co-authors [25] reported that 26% of the mallards shot by hunters in Spain contained above 1.5 mg Pb/kg w.w. in the livers. The content of lead in the liver of juvenile and adult mallards analysed in our study (0.155 and 0.417 mg/kg w.w.) is much lower than in livers of mallards from Idaho, USA (0.534 and 1.767 mg/kg w.w. respectively) [4]. Krieger [21] found that ducks shot by hunters in the Lower Coeur d'Alene River, Idaho, USA contained about 8 mg Pb/kg w.w. in the liver, and 26% of those ducks had lead shot in their gizzard. Scanlon *et al*.[32] noticed that the mallards with lead shot in gizzards had about 9 mg Pb/kg w.w. in the liver, whereas the livers of other mallards contained 2 mg Pb/kg w.w. According to the report of Bagley and Locke [2] the mean Pb content

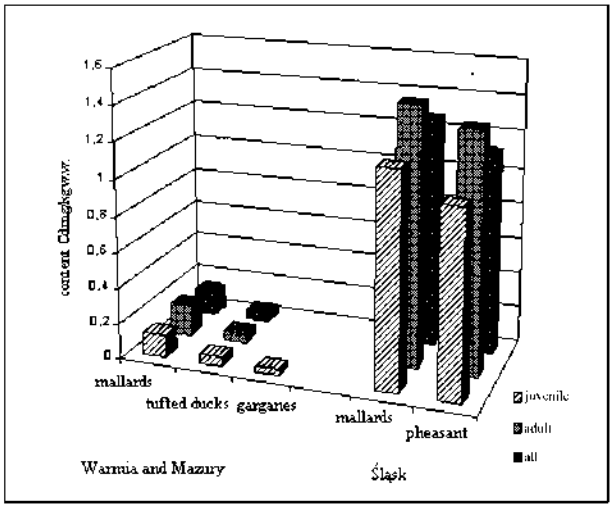

Fig. 2. Concentrations (mg/kg wet wt.) of Cd in livers.

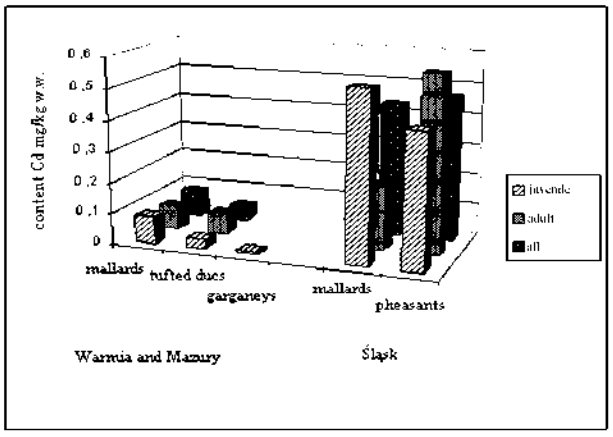

Fig. 3. Concentrations (mg/kg wet wt.) of Cd in breast muscles.

in the liver of 11 species of waterfowl ranged between 0.5 to 1.5 mg/kg w.w., which is similar to the results obtained in our study.

Among the birds included in the authors' study, teals and adult mallards from Warmia and Mazury had the highest concentration of Pb in the liver (over 0.4 mg/kg w.w.) and breast muscles (0.246 and 0.154 mg/kg w.w.). It is worth mentioning that the birds from Śląsk were obtained from an area where hunting occurs very rarely, once or twice a year, and the ducks from Warmia and Mazury were obtained in hunting grounds where hunting occurs regularly. Therefore, there may be large amounts of lead shot, which is the kind of ammunition commonly used for fowl hunting in Poland. According to Sanderson and Bellrose [30] mallards are highly susceptible to lead poisoning due to their tendency to ingest lead shot as grit and because of their eating habits (mainly cereal grains and weed seeds). This diet, rich in carbohydrates but poor in proteins, calcium and phosphorus, accelerates absorption of lead into blood. Blood, liver, heart and kidneys constitute the so-called 'quickly exchangeable pool'. The level of lead in the liver of mallards is indicative of the actual supply of lead in the environment [10, 23]. The regulation of the Ministry of Health and Social Security of 31 March 1993 set the permissible level of lead in livers of poultry at 0.5 mg/kg. Although the lead concentration determined during our research was twice as low as the highest permissible level, it must be remembered that even as little as 0.16 mg Pg/kg body weight of the bird has a toxic effect on its organism [38].

In summary, the examined mallards from Warmia and Mazury contained in their tissues copper and zinc at the physiological level. The concentration of lead and cadmium (elements which do not perform any functions in metabolic transformations) was at the characteristic level for this group of birds. Moreover, young mallards which hatched in the area under study had a lower content of lead in the liver than adult mallards. The results obtained may be evidence of a low degree of pollution of the area of Warmia and Mazury with the xenobiotics under study. This is also proved by a soil examination conducted by the Institute of Soil Science and Plants, Puławy, Poland, according to which 89% of the area of the land under discussion is covered by soils in which the content of heavy metals remains at the natural level [37]. The results of national monitoring, performed in 1999 by the State Environmental Protection Inspection Office, also indicate that the deposition of heavy metals from the atmosphere in the area of Warmia and Mazury, as compared with other provinces, is lower than the average load of the whole area of Poland [29].

Acknowledgements

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