



**EVALUATION OF HEAVY METALS ENVIRONMENTAL CONTAMINATION
BASED ON THEIR CONCENTRATIONS IN TISSUES OF WILD PHEASANT
(*PHASIANUS COLCHICUS* L.)**

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ABSTRACT

The aim of the study was to determine the level of heavy metals (cadmium and lead) in pheasants tissues (*Phasianus colchicus* L.) and their impact on other essential metals metabolism. The samples of liver, kidneys, testes, breast muscle, heart and feet were collected from pheasants (n=6) shot in the area of Rzeszow and analyzed using atomic absorption spectrometry (AAS) method. The accumulation of both toxic metals was observed mainly in kidney ($P < 0.05$), followed in liver. Specific distribution of lead in birds feet was observed. Traces of heavy metals were found in heart and breast muscle, whereas high level of lead and cadmium in testes were determined. The concentration of microelements (zinc, manganese and calcium) in the examined organs did not exceed physiological levels reported in the literature for other animals. However, certain significant correlations between toxic and essential metals were observed in examined tissues.

Keywords: ring-necked pheasant, bioindicator, heavy metals, accumulation, AAS method

INTRODUCTION

Toxic heavy metals in air, soil, and water are global problems that are a growing threat to humanity. There are hundreds of sources of heavy metal pollution, including the coal, natural gas, paper and chlor-alkali industries, phosphate fertilizers in agriculture, etc. One of the largest problems associated with the persistence of heavy metals is the potential for bioaccumulation and biomagnification in the food chains (**Zhuang et al., 2009**). Thus, a control of accumulation of toxic substances in ecosystems is of great value in the context of global environment pollution.

In recent years there has been growing interest in using bioindicators for monitoring environmental pollution with heavy and toxic metals. The principle behind the bioindicator approach is the analysis of an organism heavy metal contents compared to the background metals level. The advantages of biological monitoring are often easier analysis of entire live organisms than abiotic samples, independence on fluctuations of toxic emissions as well as possibility of monitoring interactions and danger of pollution due to seasonal and weather fluctuations (**Lebedeva, 1997**).

Birds are traditional objects of biological monitoring in polluted ecosystems, especially in territories adjacent to stationary sources of pollution. Extensive studies on heavy metal concentration in birds have been conducted in many polluted regions (**Dmowski, 1999; Mochizuki et al., 2002; Kalisińska et al., 2004**). It is very important to keep several criteria when selecting the bird species for biomonitoring. Among them common occurrence, easy capturing, clearly identified individual territory size, homogeneity of the material, well known biology of species, breeding possibility for laboratory tests as well as bioaccumulation capacity were taken into account (**Dmowski, 1999**).

From this point of view, pheasants play a valuable role as bioindicators because of their vulnerability to human exploitation, sensitivity to habitat degradation, and central position in the food web (**Koréneková et al., 2008; Džugan et al., 2010**). Therefore, the suitability of ring-necked pheasant (*Phasianus colchicus* L.) as an indicator of heavy metals pollution was studied.

MATERIAL AND METHODS

Birds

Male pheasants were shot in the area of Rzeszów by hunters using lead shots in the urban area (group A, n=3), while the other (group B, n=3) were shot in the ecologically clean region (20 km from the city). Tissue samples were collected immediately and transported on ice to the laboratory. They were stored at -20°C until analyzed.

Metals examination

The heavy metals concentration (Cd, Pb) as well as biometals level (Zn, Ca, Mn) were assayed by AAS method using Hitachi Z-2000 spectrophotometer equipped with a graphite furnace after prior microwave mineralization in HNO₃ (Speedwave Four Berghof). All analysis has been done in three independent repetitions for each sample. The metal concentrations were expressed on a wet weight basis (mg/kg).

Statistical analysis

Differences between tissues were assessed using Kruskal-Wallis and Mann-Whitney U-tests (P<0.05). Coefficients of correlation were calculated using Spearman's correlation analysis.

RESULTS AND DISCUSSION

Among the analyzed elements calcium showed the highest content in all examined organs of pheasants, while the lowest value was marked by lead (table 1). The concentration of elements in examined pheasant tissues declined in the order:

liver: Ca> Zn> Mn> Cd> Pb,
kidneys: Ca> Zn> Cd> Mn> Pb.
testes: Ca> Zn> Mn> Cd> Pb
breast muscle: Ca> Zn> Mn> Pb≥Cd
heart: Ca> Zn> Mn> Cd> Pb
feet: Ca> Zn> Mn> Pb>> Cd

Heavy metals accumulation

Table 1 Toxic and essential metals concentrations (mg.kg⁻¹ wet weight) in tissues of shot pheasants (*Phasianus colchicus* L.)

Tissue Metal	Metal content [mg.kg ⁻¹ wet weight]					
	Liver	Kidneys	Testis	Breast muscle	Heart	Foot
Cadmium						
mean (n=6)	0.721 ^a	4.308 ^b	0.069 ^c	0.006 ^d	0.007 ^d	0.007 ^d
±SD	±0.837	±2.764	±0.044	±0.004	±0.003	±0.009
min.	0.026	1.069	0.014	0.002	0.004	0
max.	2.008	7.479	0.128	0.011	0.012	0.020
variability [%]	116.1	64.2	63.8	63.8	47.4	135.0
Lead						
mean (n=6)	0.059 ^a	0.096 ^a	0.053 ^a	0.007 ^b	0	0.840 ^c
±SD	±0.028	±0.037	±0.058	±0.005	0	±0.258
min.	0.040	0.055	0.009	0	0	0.552
max.	0.113	0.134	0.164	0.012	0	1.116
variability [%]	47.7	38.5	109.9	61.6	0	30.7
Zinc						
mean (n=6)	20.008 ^a	16.019 ^a	19.409 ^a	4.571 ^b	24.218 ^a	57.339 ^c
±SD	±3.257	±1.624	±3.262	±0.680	±1.129	±11.600
min.	15.641	14.245	15.625	3.619	23.606	45.685
max.	23.850	18.835	24.324	5.495	26.231	69.615
variability [%]	16.3	10.1	16.8	14.9	4.7	20.2
Manganese						
mean (n=6)	2.839 ^a	2.051 ^b	0.675 ^c	0.162 ^d	0.650 ^c	1.533 ^{a,b}
±SD	±0.908	±0.181	±0.280	±0.025	±0.179	±0.242
min.	1.596	1.743	0.337	0.136	0.436	1.269
max.	4.193	2.235	1.053	0.192	0.907	1.800
variability [%]	32.0	8.8	41.6	15.1	27.5	15.8
Calcium						
mean (n=6)	32.711 ^a	59.538 ^b	39.708 ^a	12.654 ^c	12.556 ^c	52854 ^d
±SD	±7.436	±20.268	±13.612	±4.942	±2.589	±7492.3
min.	19.47	39.586	22.66	8.53	9.30	47059
max.	39.88	91.536	56.76	20.88	16.30	63778
variability [%]	22.7	34.0	34.3	39.1	20.6	14.2

^{a,b} means in row with different letters are significantly different

Enhanced levels of both heavy metals were observed mainly in kidneys, liver and testis, in opposite to heart and breast muscle tissues. Kidneys were the major site of heavy metals

accumulation, the levels of Cd found in liver and gonads were 6-fold and 60-fold lower ($P < 0.05$), respectively. Similarly, Pb concentrations observed in the liver and testes were by 40% and 50% lower than in kidney, respectively. Specific high accumulation of lead in pheasant's feet was observed ($P < 0.05$).

The elevated levels of cadmium was most of all observed in tissues of birds collected from urbanized area (group A), which caused the great variability in Cd level for total group of birds, from 60 to 135% depending on the tissue (figure 1a). Meanwhile, the minor individual differences in Pb level (figure 1b) were observed. Due to small amount of analyzed samples these results needs confirmation.

It is commonly accepted that kidney and liver are the target organ for heavy metal toxicity (Szkoda *et al.*, 2011). However, the level of cadmium observed in tested birds was by 2-fold higher than described for pheasants from Eastern Slovakia (Koréneková *et al.*, 2008) and comparable with obtained for hens from Lower Silesia, the most polluted smelting region in Poland (Kolacz *et al.*, 2003). The high lead concentration observed for foot confirmed the Ca-replacement by this metal in skeletal system (Wiemann *et al.*, 1999). A major storage site for Ca^{2+} is the calcified bone matrix which incorporates also cations precipitating as insoluble phosphates. Consequently, a long-term exposure to Pb^{2+} results in a deposition of lead in bone where relatively high concentrations of Pb^{2+} (>20 mg/g bone) were detected (Goyer *et al.*, 1994).

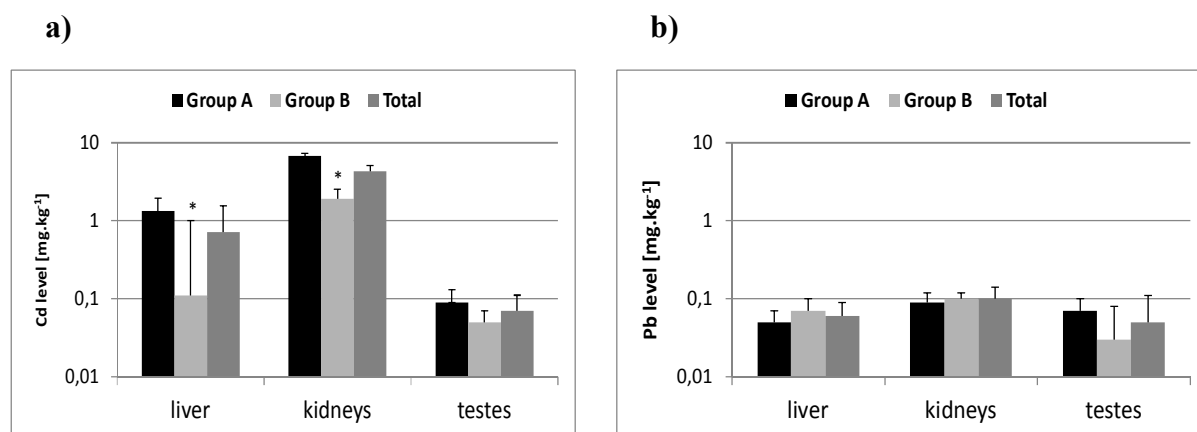


Figure 1 The effect of environmental pollution on the level of cadmium (a) and lead (b) in tissues of pheasants derived from urbanized area (Group A) and ecological region (Group B). *significant differences ($p < 0.05$)

Moreover, Cd level found in the kidneys was several times above the maximum level specified in Polish legislative framework for poultry, hence intake of pheasants offal poses a threat to consumer health.

The concentration of microelements (Zn, Mn, Ca) in the examined organs (table 1) did not exceed physiological levels reported in the literature for other animals (**Chudzicka-Popek and Majdecka, 2010; Długaszek and Kopczyński, 2011; Skibniewska et al., 2011**). Furthermore, slight individual differences in essential metals level were observed, the coefficient of variability was lower than 30%.

Metal-metal specific interactions

Antagonistic correlations between the Cd-Zn and Cd-Ca concentration in the kidney were demonstrated (table 2). Such interactions are well known and were frequently reported (**Króliczewska, 2002; Marchewka, 2009**) The synergistic relation between Zn-Cd levels observed in testis may be result of zinc capture from the whole body, which could protect reproductive organs against Cd-induced damage. By contrast, there was no relationship between lead and cadmium accumulation in liver and kidney ($P > 0.05$).

Table 2 Spearman correlation analysis of metal – metal interactions in pheasants tissues.

Metal-metal pair	Spearman's rank correlation coefficient (r_s)		
	Liver	Kidneys	Testes
<i>Cd – Pb</i>	-0.09	-0.14	0.37
<i>Cd – Zn</i>	0.09	-0.6	0.89
<i>Cd – Mn</i>	0.2	0.37	-0.49
<i>Cd – Ca</i>	0.43	-0.54	0.37
<i>Pb – Zn</i>	0.14	0.23	0.31
<i>Pb – Mn</i>	0.26	0.23	0.14
<i>Pb – Ca</i>	-0.09	0.12	-0.54
<i>Zn – Mn</i>	0.77	0.09	-0.2
<i>Zn – Ca</i>	0.37	-0.26	0.49
<i>Mn – Ca</i>	0.71	-0.49	-0.37

CONCLUSION

It was shown that pheasant can be used as biomonitor of heavy metals in contaminated environment. The evaluation can be conducted by direct examination of heavy metals content in target organs (kidney and liver) as well as by studying an interactions between toxic and essential metals. Due to small birds population used in experiment it requires further study.

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