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Metal Bioaccumulation, Enzymatic Activity, Total Protein and Hematology of Feral Pigeon (Columba Livia), Living in the Courtyard of Ferronickel Smelter in Drenas

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Abstract: This study was undertaken to evaluate the effects of Ferronickel smelter in concentration of lead, cadmium, zinc, copper and nickel in tissue of femur, tibia, liver kidney and testes, levels of plasma alanin aminotransferase (ALT), aspartat aminotransferase (AST), alkaline phosphatase (AP) blood δ-aminolevulinic acid dehydratase (ALA-D), serum total proteins, hematocrit and hemoglobin in Feral pigeons. Pb was detectable only in tissues of femur and tibia; Cd and Cu in liver and kidney, Zn in all tissues while Ni only in tissues of pigeons of courtyard smelter. Zn concentration was significantly higher ($P<0.05$) in kidney of courtyard smelter in comparison with reference. Plasma AP was significantly higher ($P<0.001$) in pigeons of reference in comparison with pigeons of courtyard smelter, while ALT and AST were relatively unchanged. The ALA-D activity of pigeons from courtyard of smelter was significantly inhibited ($P<0.001$) and an inverse correlation between Pb concentration in femur and tibia and ALA-D activity ($r = -877$; $P<0.001$; $r = -0.787$; $P<0.01$) was established. The total serum proteins of pigeons of courtyard of smelter was significantly lower ($P<0.001$). Hematocrit and haemoglobin were unchanged. Suggestion: Feral pigeons as worth biomonitoring organisms for evaluation of environmental pollution based on Ferronickel industry.

Keywords: Metals; pigeon; enzymes; hematology

INTRODUCTION

Nickel has toxic effect on living organisms and is often considered as contaminant and weak carcinogen (Stawarz et al., 2003) .Upon ingestion, absorption of nickel is influenced by many factors including dose, age and diet. Studies in rats, dogs and mice indicate that only 1-10% of orally administrated nickel (Ni, $NiCl_2$ and $NiSO_4$) is absorbed by the gastrointestinal tract from diet or drinking water (Ho and Furst 1973; Schroder et al., 1974). After being absorbed, nickel is distributed through the body and accumulates primarily in the kidneys. Numerous studies on rats (Wanger 1973), ducks (Cain and Pafford 1981), livestock (O'Dell et al., 1971; Spears et al., 1986), and wild mammals and birds (Rose and Parker 1983; Outridge and Scheuhammer 1983) , have indicated that nickel accumulates extensively in kidneys of animals. However, there was no accumulation of nickel observed in the tissues of rats exposed to 5 $mg.L^{-1}$ in drinking water (Schroeder et al., 1974). Animals have a high capability to eliminate assimilated nickel. The majority of nickel that is absorbed by animals is unlikely to be retained in the body. Tedeschi and Sunderman (1957) noted that dogs excreted 90% of ingested nickel in faces and 10% in urine. The lowest effect dose was 25 mg Ni Kg $^{-1}$ per day in reducing growth and enzymatic activity of rats (Whanger, 1973). A number of studies have been carried out on the effect of environmental pollution of urban and different smelter vicinities in birds (Ohi et al., 1973, Hutton and Goodman, 1980, Drasch et al., 1987, Elezaj et al., 2008, Selimi et al., 2008). There is, however, little information with the regard to the biological availability of above metals from the vicinities of ferro-nickel smelter.

We therefore determined the bio accumulation of lead, cadmium, zinc, copper and nickel in tissues , the values of three plasma enzymes (often used to assess hepatic damage) : alkaline phosphatase (AP; EC 3.1.3.1), alanin aminotransferase (ALT; EC 2.6.1.2) and aspartat aminotransferase (AST: EC 2.6.1.1), the activity of blood δ-aminolevulinic acid dehydratase (ALA-D; EC4.2.1.24),serum total proteins, hematocrit value and hemoglobin amount in Feral pigeons (Columba livia), living in courtyard of Ferronikeli smelter in Drenas, and in the group of pigeons from Lubizhdë village reference site.

STADY AREA, MATERIAL AND METHODS

The smelter Ferronikeli is located in Drenas. Drenas is located 20 kilometres far from Prishtina (capital of Republic of Kosovo) in the West. The smelter has operated since 1982. Ferronikeli has three open pit mines: the Dushkaja mine with

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estimated reserves of 6.2 million tonnes; the Suka mine-0.8 million tonnes and the Gllavica with 6.8 million tones. The plant is located on an industrial area of 81 ha. An internal slag disposal is located 500 m far from the East boundary of the plant. Its area is around 32 ha. This slag disposal contains around 3 milion tons of granulated slag from the past. Ferronikeli smelter is well-known for final production of Ferro- nickel. In metallurgical processing the mineral base is treated by an oxide mineral of nickel (two sources) with the following average chemical structure: Ni+Co=1.2%; Fe=26.0%; SiO2=47.0%; CaO=2.5%; Cr_2O_3=1.2%; MgO=11.0%. The produced slag has the chemical structure as follow: Ni-0-08%; SiO_2 55-57 %; MgO 10.0%; Fe_{total} 20%; Ca) 4.0%. The capacity of the smelter in the technological lines (rotating furnace and electrical furnace) is about 12.000 t Ni/year (Rizaj et al., 2008). In recent time, the smelter plant is active and it is known as "New CO Ferrocickel". Vast quantities of sulphur dioxide and heavy metals emitted during the last 30 years have caused widespread destruction and toxic effects of soils, vegetation and animals around the smelter. Specimens of feral pigeon (Columba livia; 20 birds, males and females), were collected in ferronickel smelter courtyard (on April, 2009) and in Lubizhdë village (control area). The blood samples were collected in heparinised tubes by puncturing the alvar vein in the region of the carpal vein joint, after first removing feathers in the area and thoroughly cleaning the skin. Birds were then killed by cervical dislocation and the tibia, femur, liver and testes excised, precautions tissues were dried at thermostat at 100 ^{0}C for 48 h and dry ashed as described by (Blanusa and Breski, 1981). Tissue concentration of Pb, Cd, Zn, Cu and Ni was measured by flame atomic absorption spectrophotometry (UNICAM 929). Erythrocyte ALA-D activity was measured according to the CEC standardized method (Berlin and Schaller 1974).). Serum Alanin aminotransferase (ALT; EC 2.6.1.2) and Aspartate aminotransferase (AST; EC 2.6.1.1) were measured by the IFCC methods for measurement of catalytic concentrations of enzymes (Bergmeyer and Horder, 1980).
Alkaline phosphatase (EC 3.1.3.1) was estimated by the method of (Tietez et al., 1983).Total proteins were measured by biuret colorimetric method (Reinhold , 1953) . The hematocrit was determined in heparynized capillary tubes, centrifuged for 8 min at 11.000 rpm (Haemofuge, Heraues). Hemoglobin was measured with Drabkin's reagent by a standard cyanmethemoglobin method (Van Kampen and Zijlstra 1961). Student's t test was applied to determined the statistical significance.

RESULTS AND DISCUSSION

Table 1 summarizes the tissue concentration of Pb, Cd, Zn, Cu and Ni obtained on pigeons from courtyard of Ferronickel smelter of Drenas town and Lubizhdë- reference site. The Pb was detectable only i femur (54.1+43 µg/g d.w. / min/max 7.2-82.2/; and tibia (35.9+34.4 µg /g d.w; /6.2 - 47.5./) of pigeons from smelter courtyard, while in all other examined tissues of feral pigeons of both groups, Pb concentration was under the limit of detection. The moderate Pb concentrations in femur and tibia of pigeon's from courtyard smelter are in accordance with results of (Elezaj et al., 2008), who recorded moderate Pb concentration in femur and tibia of urban population of feral pigeon's in Prishtina - the capital of the Republic of Kosovo. Since the feral pigeon's habit of feeding at ground level, on pavements and in the gutter of roads, offers an explanation for the moderate lead contents in bones of pigeon's from courtyard smelter.

Table 1.Tissues accumulation of metals (µg/g d.w.) of feral pigeon (Culumba livia) from Ferronickel area (Drenas) and Lubizhdë (reference)

Tissue	Element	Lubizhdë	Drenas
Femur	Pb	ND	51.4±43 (10)
	Zn	101±9 (10)	95.9±18 (10)
	Ni	ND	9.3±10 (10)
Tibia	Pb	ND	35.9±34 (10)
	Zn	90±5 (10)	86.5±12 (10)
	Ni	ND	0.41±2 (10)

Table 2.Tissues accumulation of metals (µg/g d.w.) of feral pigeon (Culumba livia) from Ferronickel area (Drenas) and Lubizhdë (reference)

Tissue	Element	Lubizhdë	Drenas
Liver	Cd	ND	0.85±0.2 (2)
	Zn	71±27 (10)	72.6±22 (10)
	Cu	8±4 (10)	10.5±5.3 (10)
	Ni	ND	7.4±15.5 (7)
Kidney	Cd	10±11 (3)	19.1±3.6 (2)
	Zn	62±12* (10)	87.1±33 (10)
	Cu	18±23 (2)	9.8±8.9 (10)
	Ni	ND	21.4±38 (10)
Testes	Cd	ND	ND
	Zn	36±7 (10)	34.3±13 (10)

Note: Values are expressed as means ± SD. In parenthesis, number of animals * $P < 0.05$.

Cadmium concentration was detected in the liver and in the kidney of two (2) pigeons from both groups (among the ten pigeons on each group), and the values are not statistically different in comparison with reference. The association of cadmium and zinc in kidney and liver of courtyard smelter and control pigeons is thought to be a reflection of the interaction which is known to occur between these two metals (Frieberg et al.,1974).The co-accumulation of tissue cadmium and zinc has also been reported in horses (Frieberg et al., 1974), seabirds (Hutton, 1979) and cadmium-dosed rats (Stonard and Webb, 1976).

Copper was detected on the liver and kidney of pigeons of both groups and there were statistically no difference between groups. A lower concentration of Cd and Cu in the kidney and liver of pigeons from smelter courtyard (contaminated area) my explained by the fact that Cd and Cu do not participate on the composition of the nickel ore.

Zinc was detected in all examined tissues and it was significantly higher in the kidney of pigeons from smelter courtyard in comparison with reference. Highest Zn concentrations were found in femur, followed by tibia, kidney, liver and, lastly testes. The statistically similar bone zinc concentrations of pigeons from courtyard smelter and control birds are in accordance with results of (Hutton and Goodman, 1980), who found the similar zinc concentration of pigeons of London and control. Evidence arising primarily form courtyard smelter pigeons indicates that zinc co-accumulates with lead in the bone tissue.

At present it is not known whether this co-accumulation reflects an interaction of toxicological significance. Zinc has been shown to antagonize lead toxicity by reducing tissue lead accumulation and by reactivating lead-inhibited ALA-D, an enzyme involved in haem synthesis (El-gazar et al., 1978; Hutton, 1979).

Nickel was detectable in all examined tissues (except testes) only in feral pigeons (Columba livia) from smelter courtyard. The highest concentration of Ni was recorded in kidney (21.4 µg /g d.w; / 5.2-67.7/), femur (9.3 µg /g d.w; /3.9-29.0), and in liver (7.4 µg /g d.w; /0.56 - 48.5). Our results of Ni concentration in tissues of feral pigeons from smelter courtyard are in accordance with results of Korenekova (Korenekova et al., 2008) recorded in pheasants (Phasianus colchicus) from defined locality burden with pollution of metallurgical industry in Eastern Slovakia. These data support other studies showing elevated levels of Ni in the areas closest to the Russian smelters (Aamlid, 1992, Siversten et al., 1992).

The undetectable nickel in femur, tibia, liver, kidney and testes of feral pigeons from reference site are in accordance with the results of Rose and Parker (1983), who in field study found reduced accumulation of nickel in terrestrial wild birds and noted concentrations in body tissue of ruffed grouse (Bonasa umbellus) 10 times lower than those in dietary items of grouse collected in the contaminated area. A review by Outridge and Scheuhammer (1993), reported organ concentrations of Ni in most avian species from uncontaminated sites range from 0.1-5 mg/kg dry weight, and there is some evidence that birds may tend to accumulate higher Ni burdens in polluted habitats than do mammals. However, Ni seems to be only sparsely absorbed from gastrointestinal tract, and in mammal's excretion in urine is nearly complete in a few days after an intake (Amadur et al., 1986). Hence, no strong Ni-accumulation can be expected by dietary intake of Ni.

Table 3 summarizes the plasma values of AP, ALT, AST, total serum proteins, blood ALA-D activity, hamtocrit value and haemoglobin amount obtained from pigeons from Drenas and Lubizhdë. The level of plasma AP was significantly higher ($P<0.001$), in the group of feral pigeons from reference site (Lubizhdë village) in comparison with activity of plasma AP of pigeons from courtyard smelter. Our results of inhibition of plasma AP feral pigeons from contaminated area are in discordance with the results of (Whanger, 1973), who in Weanling rats fed diets containing 500 and 1000 ppm nickel acetate found tissue inhibition of alkaline phosphatase activity. The higher level of plasma AP of the feral pigeons from reference site can be caused from the different toxicants which the birds consumed from the nearby agro- field of village.

Table 3. Some enzymes (AP, ALT, AST, ALAD), total proteins, Hct and Hb of feral pigeon (Culumba livia) from Ferronickel area (Drenas) and Lubizhdë (reference)

Parameters	N	Lubizhdë	Drenas
AP *** (IU/L)	10	327±88	135±57
ALT (IU/L)	10	17±9.8	17±3.3
AST (IU/L)	10	175±110	172±49
ALAD (U/LE)***	10	31±14	19±7.4
Total protein ** (g/L)	10	63.8±15.8	45.2±12.7
Hct %	10	46.8±5.2	49.2±4.1
Hb (g/L)	10	154.4±37	143.7±24

Note: Values are expressed as means ± SD. N; number of animals.** $P<0.01$; *** $P<0.001$.

The values of plasma ALT and AST were relatively unchanged. Our results concerning the unchanged plasma activity of ALT and AST in both groups of pigeons are in accordance with results of Eastin and O'Shea (1981), who found no significant differences of plasma activities of ornithine carbamoyltransferase and alaninamino-transferase in mallards treated with Ni sulphate through breed mash (0,12.5, 50.0, 200,0, or 800.0 ppm Ni). On the other hand, Kalahasthi and co-workers (2006), found significantly positive correlation between urine nickel level and levels of serum alaninetransferase and aspartate transaminase in nickel planting exposed workers. The serum levels of ALT and AST were significantly increased in high and moderate exposed workers as compared to control group.

The blood ALA-D activity of pigeons from smelter courtyard was significantly inhibited ($P< 0.001$), in comparison with reference. There was significantly inverse correlation between Pb concentration in femur and tibia and blood ALA-D activity ($r = -877$; $P<0.001$ and $r = - 0.787$; $P<0.01$ respectively). The inverse correlation between Pb concentration in femur and tibia and blood ALA-D activity can be explain by the fact that bone is the target organ of Pb accumulation in the chronic intoxication by the Pb lower doses.

In humans, lead blood of 50-60 µg/100 ml results in a decrease of ALAD by about 75 % (Roels et al., 1974), while in pigeons the same lead blood caused decrease of about 90%. According to Finley and co-workers (Finley et al., 1976), blood ALAD in avian species is more sensitive to lead than in mammals. Thus, the usefulness of blood ALAD activity as an index of lead exposure is limited to the most lightly contaminated individuals.

The amount of total serum proteins of pigeons from Drenas was significantly lower ($P<0.001$) in comparison with reference, while the hematocrit value and haemoglobin amount were relatively unchanged. Our results of lower level of serum total proteins are in the accordance with the results of ((Kalahasthi et al., 2006), who determined decreased serum total proteins and albumins in high and moderate nickel exposed workers as compared to the control group , but this decrease was not significant. Finally, the unchanged values of hematocrit and hemoglobin of feral pigeons can be comment based on the fact that lead anemia is a later clinical manifestation of lead plumbism. Finally, examination of the biological impact of nickel exposure upon urban pigeons (Columba livia) is expected to be of more relevance in assessing the implications of low level chronic nickel contamination than certain laboratory studies in which high doses are administered over short time periods.

REFERENCES

Aamildl, D.1992. The concentration of nickel and copper in some important plants in South Varanger, Norway.-In Kismul, V., Jerre.J. & Lobersli, E., eds. Effects of air pollution on terrestrial ecosystems in border between Russia and Norway. SFT Document 92, 04:202-209.

Amadur, M.O., J. Doull, and C.D. Klassen, 1986. Toxicology. The basic science of poisons. Fourth Ed. – Pergamon Press, New York.

Bergmeyer, H.V., Horder K, 1980. IFCC methods for measurement of catalytic concentrations of enzyms. Clin Chim Acta., 105: 147-172 F.

Berlin, M. and K.H. Schaller.1974.European standardized method for the determination of δ-aminolevulinic acid dehydratasae activity in blood. Z.Klin.Chem.Biochem., 12, 389-390.

Cain, B. W., Pafford E A, 1981. Effects of dietary nickel on survival and grouth of mallard ducks. Environ. Contam. Toxicol. 10:737-745.

Drasch, G.A., 1987. The urban Pigeon (Columba livia-Forma urbana)- a biomonitor for the lead burden of the Environment.Environmental Monitoring and Assessment. 9, 223-232.

Eastin W.C. Jr., and T.J. O'Shea.1981. Effects of dietary nickel on mallards. J Toxicol Environ Health., 7, (6), 883-92.

Elezaj, I.R., Q.I.Selimi, K.Rr.Letaj, 2008. The accumulation of Pb, δ-amino levulinic acid dehydratse, hematocrit and hemoglobin amount in blood of Feral Pigeons (Columba livia) living near lead and zinc smelter "Trepça" in Mitrovica-Kosova-Four year study. Proceedings of 14 th International Conference on Heavy Metals in the Environment. November 16-23, 2008, Taipei, Taiwan, 269-272.

El-Gazzar, R.M., V. N. Finelli, J. Boiano, and H.G. Petering.1978. Influence of dietary zinc on lead toxicity in rats. Toxicol. Lett., 1, 261-269.

Finley, M., M.P. Dieter and L.N. Locke. 1976. δ-Aminolevulinic acid dehy dratase: Inhibition in ducks dosed with lead shot. Environ . Res. 12, 243-249.

Frieberg, L., M. Piscator, G. Nordberg, and T. Kjellstrom.1974. Cadmium in the environment, 2nd edn. Clevevlend, Ohio, CRC Press.

Ho W., and Furst A. 1973. Nickel excretion by rats following a single treatment. Proc. West. Pharmacol. Soc. 16: 245-248.

Hutton, M. 1979. An experimental survey of the impact of heavy metals on selected species of birds from contaminated environment. PhD thesis, University of London.

Hutton, M., G.T. Goodman, 1980: Metal contamination of feral pigeons (Columba livia) from the London area: Part 1-Tissue accumulation of Lead, Cadmium and Zinc. Environmental Polllution (Series A)., 40,207-217.

Kalahasthi R.B., H.R. Rajmohan and K. B. Rajan. 2006. Assesment of functional integrity of liver among workers exposed to soluble nickel compounds during nickel planting. Indian

Journal of Occupational and Environmental Medicine. 10, (2), 78-81.

Kampen, E.J., and G.W. Zijlstra.1961. Standardization of hemoglobinometry II. The hemoglobinecyanide method. Clin.Chim.Acta., 6,538-544.

Korenekova ,B., M.Skalicka, I. Kozarova, J.Nagy, D.Mate and P.Nad. 2008. Comparison of cadmium, lead and nickel accumulation in liver, breast and leg muscles of pheasants. Slovak J. Anim.Sci., 41, (4), 184-186.

O'Dell, G. D., Miller W J, Moore S L, King W A, Ellers J C, Jurecek H. 1971. Effect of dietary nickel level on excretion and nickel content of tissues in male claves. J.Anim.Sci. 32:769-773.

Ohi, G., H.Seki, K.Akyama, H. Yagyu,1974. The Pigeon, a Sensor of Lead Pollution. Bulletin of Environmental Contamination & Toxicology., 40 (1), 92-98.

Outridge,P. M., Schuhammer A M. 1983. Bioaccumulation and toxicity of nickel: Implications for wild mammals and birds. Environ. Rev. 1:172-197.

Reinhold, J.G. 1953. Standard methods of clinical chemistry. 1 p 88.

Rizaj, M., Beqiri E, McBow, O'Brein E.Z, Kongoli F, 2008. The mineral base and productive capacities of metals and non-metals of Kosovo. JOM., vol.60 No.8:18-22.

Roels, H.A, J.P. Buchet and R.R. Lauwerys, 1974. Inhibition of human δ-amino levulinic acid dehydratase by lead. Int.Arch. Arbeitsmed . 33, 277-284.

Rose, G. A., Parker G H. 1983. Metal contents of body tissues, diet items, and dung of ruffed grouese near Sudbury, Ontario, Canada. Can J.Zool. 61: 505-511.

Schroeder, H. A., Mitchener M, Nason A P. 1974. Life term effects on nickel in rats: Survivel, tumors, interactions with trakes elements and tissue levels. J.Nutr. 104:239-243.

Selimi, Q.I., I.R. Elezaj, K.Rr.Letaj, Tissue accumulation of Pb, δ-aminolevulinic acid dehydratse, hematocrit and hemoglobin amount in blood of Feral Pigeons (Columba livia) living in Prishtina the capital of Kosova (Four year study). Proceedings of 14 th International Conference on Heavy Metals in the Environment. November 16-23, 2008, Taipei, Taiwan, pp 257-260.

Sivertsen,B., T. Makarova, L. Hagon, and A.A. Baklanov.1992. Air pollution in the border areas of Norway and Russia.- NILU OR: 8/92:1-14.

Spears, J. W., Harvey R W, Samsell L J, 1986. Effects of dietary nickel and protein on growth, nitrogen metabolism and tissue concentrations of nickel , iron, manganese and copper in claves. J. Nutr. 116: 1876-1882.

Stawarz, J., M. Zakezevski., A. Marecnik and S. Hraska. Heavy metal concentration in the toad Buffo buffo from a region of Mochovce, Slovakia. Ecologia (Bratislava), 22, 292-297.

Stonard,M.D., and M.Webb. 1976. Influence of dietary cadmium on the distribution of the essential metals, copper, zinc and iron in tissues of rats. Chem Biol, Interactions. 15, 349-363.

Tedechi, R.E., Sunderam F W. 1957. Ni poisoning (V), metabolism of Ni under normal conditions and after exposure to nickel carbonyl. AMA Arch. Ind. Health 16: 486.

Tietz N.W., C.A. Burtis and P.Duncan, 1983. A reference method for measurement of alkaline phosphatase. Clin.Cem. 29, 5, 302-304.

Whanger, P. D., 1973. Effects of dietary nickel on enzyme activities and mineral contents in rats. Toxicol. Appl. Pharmacol. 25:323-331.