

EVALUATION OF LEAD EXPOSURE ON BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS OF FERAL PIGEON (*COLUMBA LIVIA*) LIVING IN URBAN AREAS

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Abstract

This study estimate the effect of environmental pollution insome oxidative stress biomarkers in feral pigeon (*Columba livia*), from polluted area. Specimens of feral pigeons (*Columba livia*: 20 birds male and female), were collected in Peja town (urban area) and in Lukinë village - Prizren (rural area).Blood lead level (BLL) was determined with atomic absorption spectrometry with atomic absorber with a GTA 100 graphite furnace and PSD 100 autosempler. Erythrocyte δ -aminolevulinic acid dehydratase (ALA-D) activity was measured according to the CEC standardized method of Berlin and Schaller. The hematocrit (Hct) was determined in heparinized capillary tubes. Hemoglobin (Hb) was measured with Drapkin's reagent by a standard cyanmethemoglobin method of Van Kampen and Zijlstra. The blood lead level (BLL), and hematocrit (Hct) were significantly higher ($P<0.001$), in feral pigeons of Peja, compared to Lukinë pigeons. The δ -Aminolevulinicacid dehydratase (δ -ALA-D) activity, of feral pigeons from Peja, was significantly inhibited ($P<0.001$), compared to control group. The amount of hemoglobin in both groups was different, but not statistically significant. The results of this study suggest that feral pigeon may be used as model for monitoring lead toxicity, on some biochemical and hematological parameters. The ability of pigeon to accumulate lead draws attention to the potential hazard of children ingesting street dust from such areas.

Keywords: Blood, Lead, ALA-D, Hct, Hb, Feral pigeons

1. Introduction

Urbanization is expanding worldwide, with over 50% of humans now inhabiting urban areas. Urban ecosystems are polluted as a consequence of urbanization and industrial processes (Lee et al, 2016). Heavy metals are released into the environment by both anthropogenic and natural sources. When metals release into the environment, by human activities, they reside long time in soil, water, and exert harmful effects on the ecosystems long after the source of pollution has ceased to operate. They accumulate in different animal and human tissues such as: blood, liver, kidney, bones and feathers (Frantz et al, 2012). Therefore pollution assessment by monitoring is of paramount importance. Species useful for purpose must be representative for the area under investigation, sedentary, accumulators of environmental pollution (Elezajet al, 2013), e.g. by heavy metals, and useful for long term storage also from the view of retrospective analysis within environmental specimen banking.

Negative impacts from urban pollution, have been observed in wildlife species, as well as impaired health on reproduction terrestrial bird species (Koivulaand Eeva, 2010) have occurred at a variety of pollution sources. However, polluted soils may still constitute a major source of lead exposure to wildlife even through the atmospheric deposition declines (Elezajet al, 2013).

In Kosovo, one of the urban lead polluted environments is also the area of Peja town.

The Feral pigeon (*Columba livia*), and House sparrow (*Passer domesticus*) have been studied for the pollution

gradient with lead, what induces oxidative stress and inhibit the activity of enzyme ALA-D, as on biomarker of this stress (Elezajet al, 2012, 2013).

The primary objective of this study, were focused on responses to substantial urban pollution measured with blood lead level (BLL), and some biochemical and hematological parameters (ALA-D, Htc, Hb), in Feral pigeons (*Columba livia*), living in urban lead polluted area. The enzyme δ -Aminolevulinic acid dehydratase (ALA-D; E.C.4.2.24), catalysis the second step on haeme biosynthesis pathway and is required to maintain the hemoglobin and cytochrome content in red blood cells. ALA-D is not only found in bone marrow cells, the major site of haem synthesis, but also in circulating erythrocytes. The level of ALA-D inhibition in erythrocytes has been widely accepted as a standard bioassay to detect acute and chronic lead exposure in humans and birds. The value of this parameter as an indicator of environmental lead pollution has been reported in doves (Espinete al, 2015). Lead inhibits haem biosynthesis and causes anemia, basophilic stippling, a decrease in ALA-D activity and increase in urinary δ -Aminolevulinic acid (ALA), urinary coproporphyrine, erythrocyte zinc protoporphyrine(ZPP). In this sense, ALA-D inhibition results in corresponding decrease in haem production and accumulation of ALA that can be oxidized to generate reactive oxygen species-ROS (Espinete al, 2015). Consequently, enhanced lipid peroxidation and DNA damage may occur. Several authors have studied ALA-D activity in different avian species exposed to Pb experimentally or under natural conditions (Berglund et al, 2010; Gomez-Ramirez et al, 2011). A marked increase in urinary excretion of ALA, the substrate that accumulates as a result of decreased ALA-D- activity has been used in the past as a biomarker for lead toxicity.

2. Materials and methods

Specimens of Feral pigeons (*Columba livia*: 20 birds male and female), were collected in March 2017, in Peja town (urban area) and in Lukinë village- Prizren (rural area) (Fig . 1) Pejë town is located in northern of Kosovë, in the geographical coordinate: 42 ° 39 V and 20° 18 L., with about 96.500 inhabitants. In the past, the city was on the important trade route between Dubrovnik and Istanbul. However, Pejë remains its status as the northern traffic junction of the landscape Rrafshi i Dukagjinit. Following the 1999 conflict, the vehicles number in Kosovë has drastically increased. The total amount of registered vehicles in Kosovë is 300 000, about 100 000 are registered in the town of Pejë. The petrol in Kosovë, still contain alkyl lead compounds as anti-knocked agent.

Lukinë village is located in southern west part of Kosovë, 120 km away from Mitrovicë town and 70 km away from Pejë (Fig . 1).

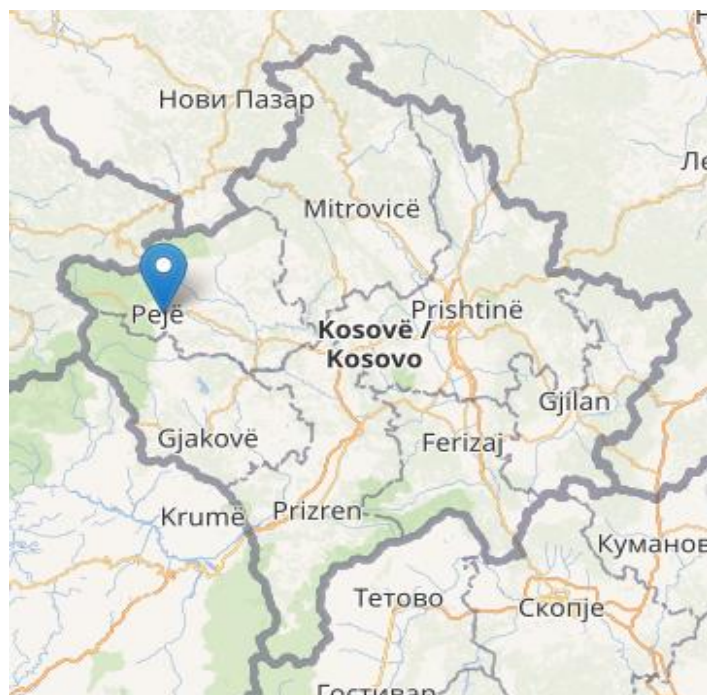


Fig 1. Location of Peja in the map of Kosovo

In laboratory blood samples were collected in heparinized syringes direct from heart. Blood lead level (BLL) was determined with atomic absorption spectrometry with atomic absorber from type Varian spectra AA 640Z Zeeman AAS, abular with a GTA 100 graphite furnace (Varian, USA) and PSD 100 autosempler (Varian, USA). Erythrocyte δ -aminolevulinic acid dehydratase (ALA-D) activity was measured according to the CEC standardized method (Berlin and Schaller, 1964).

The hematocrit (Hct) was determined in heparinized capillary tubes, centrifuged for 5 minutes at 11.000 rpm (HaemofugeHeraues). Hemoglobin (Hb) was measured with Drapkin's reagent by a standard cyanmethemoglobin method (Van Kampen and Zijlstra, 1961).

3. Data analysis

Statistical analysis of the results was carried out with Sigma stat 32 programs (2004 STAT) Software. For each continuous variable, a distribution form was determined, and significant differences between means were checked by Student's t test. Parson's correlation test was performed to examine the relationship between δ -ALAD activity and Pb concentrations; and between δ -ALAD activity and other oxidative stress biomarkers. A value of $p < 0.05$ was considered statistically significant.

4. Results and discussions

Blood lead level, ALA-D activity, Hct and Hb amount have been presented in tabular and graphic form.

Table 1. Blood lead level (BBL), δ -aminolevulinic acid dehydratase activity (δ -ALAD), hematocrite (Hct) and hemoglobin (Hb), in feral pigeon (*Columba livia*), in Lukinë and Pejë

Parameters	Number	Lukinë	Pejë	Significance P<
BLL ($\mu\text{g/dL}$)	20	4.68 \pm 1.12	7.2 \pm 1.2	P<0.001
ALAD (U/LE)	20	45.83 \pm 11	29.12 \pm 5.05	P<0.001
Hct (%)	20	40 \pm 6.2	51.8 \pm 4.9	P<0.001
Hb (g/L)	20	157.2 \pm 7.4	159.9 \pm 15.6	NS

Note: values are expressed as means \pm standard deviation SD; 20-number of pigeons. NS=no significant.

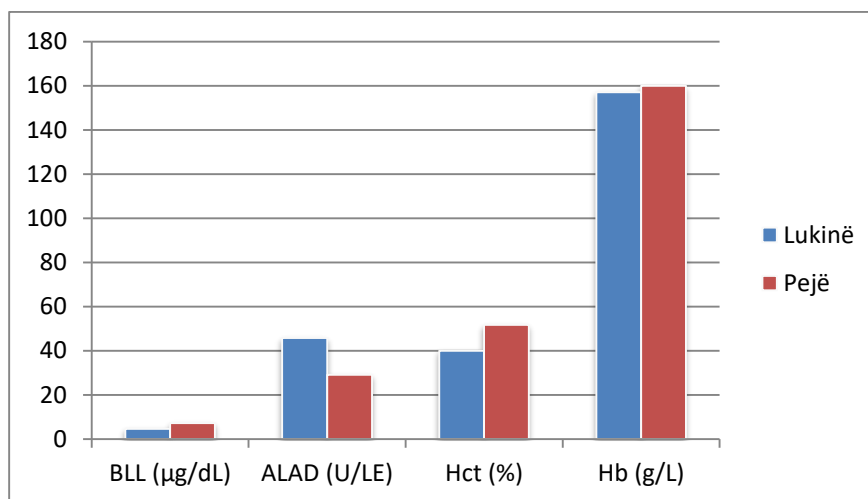


Fig 2. Blood lead level (BLL $\mu\text{g/dL}$), (δ -ALA-D U/LE), (Hct %)and (Hb g/L), in Feral pigeon (*Columba livia*), in Lukinë and Pejë

More than 20 years in Kosovo is observed environmental pollution by elevated Pb blood levels and altered values of specific Pb biomarkers such as ALAD, in different animals, especially in feral pigeon and house sparrow. In addition, antioxidant mechanisms and plasma biochemistry also appear to be affected by the level of Pb exposure.

The blood lead level (BLL) in pigeons of Pejë town was significantly higher ($P<0.001$), in comparison with reference group, and the blood δ -ALAD activity of feral pigeons from Pejë town was significantly inhibited ($P<0.001$), in comparison with control group.

Our results of inhibited activity of δ -ALAD (29.1 U/LE), in pigeons from Pejë, are in accordance with results of (Plakiqi-Milaimiet *al*, 2015], who in feral pigeons (*Columba livia*) of Drenas established inhibited activity of δ -ALAD (19.2 U/LE).

Our results of inhibited activity of δ -ALAD (29.1 U/LE), in pigeons from Pejë are in accordance with results of (Elezajet *al*, 2012), who in the House sparrow (*Passer domesticus*) in the city of Prishtinë, established (27.5 U/LE), inhibited values of activity of this enzyme.

The pronounced inhibition of blood δ -ALAD activity in feral pigeons from Pejë town, confirms once more the sensitivity of this enzyme to lead. Our results of inhibited activity of δ -ALAD are in accordance with results of (Martinez-Haro *et al*, 2011), in waterbirds (mallard, common coot, common pochard and moorhen). Negative relationships between ALAD ratio and PbB were found in both species (mallard: $r = -0.885$, $P<0.001$) and (coot: $r = -0.533$, $P<0.001$).

Inhibition of D-AAL enzyme activity in feral pigeon's blood in Pejë at a relatively low BLL of (7 $\mu\text{g/dL}$), is in accordance with the results of (Espinat *al*, 2015), which in four species of different birds exposed to natural environments with different levels of Pb, also found inhibition of D-AAL enzyme activity in their blood. The author finds inhibition of D-AAL enzyme activity in Eagle-owl blood, when Pb values in blood are (5 $\mu\text{g/dL}$);

in Griffon vulture, are ($8\mu\text{g/dL}$), and in birds Slender-billed gull and Audouin gull, ($2>\mu\text{g/dL}$). Her results show that Eagle owl and Griffon vulture reflect inhibition of enzyme activity of 79% and 94%, respectively, when BLL concentration exceeds 19 and $30\mu\text{g/dL}$, respectively.

Also of interest are the results of (Rodriguez *et al*, 2009), which in three bird species (Eagle-owl, Griffon vulture and Audouin's Seagull), found the negative correlation between blood lead level and activity of the D-AAL enzyme ($r = -0.439$, $P < 0.001$; $r = -0.367$, $P < 0.002$; $r = -0.552$, $P < 0.003$, respectively), also found high values of hematocrit compared to control.

Earlier studies in the same species (Eagle-owl, Griffon vulture) found that levels of PbB at values between $2\mu\text{g/dL}$ and $15\mu\text{g/dL}$ were sufficient to generate oxidative stress in relation to Pb, (Espineta *al*, 2014a, 2014b). Whereas in this study, PbB levels between $5\mu\text{g/dL}$ in Eagle owl and $8\mu\text{g/dL}$ in Griffon vulture are the lowest values that have significant effect on D-AAL enzyme activity, (Espineta *al*, 2015).

Martinez-Haro (Martinez-Haro *et al*, 2011), in wild geese exposed to lead, also found a negative correlation ($r = -0.53$) between the BLL and D-AAL enzyme activity.

In the case of exposure to inorganic lead workers, (Stoleski *et al*, 2008), also found a negative correlation ($r = -0.54$), between the level of Pb and the activity of the D-AAL enzyme.

Inhibition of D-AAL enzyme activity at a rate of 38.4%, in feral pigeons in Peja, where blood lead level was $7.0\mu\text{g/dL}$, and negative correlation BLL and ALA-D activity ($r = -0.409$; $P < 0.05$), confirms once again the sensitivity of this enzyme to relatively low levels of lead in the blood.

On the other hand, moderate levels of lead in urban pigeon blood ($7.0\mu\text{g/dL}$), which are two times higher than the levels of lead in blood of control group, are several times lower (about 5 times), compared to the lead levels in feral pigeon blood of the city of Prishtinë, analyzed in 2000, a period when leaded gasoline was still used in Kosovë.

Reduction of lead level in pigeon blood in Pejë, clearly reflects the effect that the implementation of the law on the use of unleaded fuel (in Kosovë) has had in reducing the exposure of leaded urban environments emitted by car gases in urban environments.

Our results of the higher percentage of hematocrit in pigeons in Pejë (51.8%), compared to the control group (40.0%), are in line with Elezaj's results, (Elezaj *et al*, 2013), found in feral pigeon (47.5%) in the city of Mitrovica, compared to the control group (44.0%). These results also correspond to the results of (Elezaj *et al*, 2011), which show higher percentage of hematocrit (49.2%) in feral pigeons (*Columba livia*), which live near the Feronikel Foundry in Drenas, compared with the control group (46.8%).

The higher percentage of hematocrit in the natural populations of feral pigeons, in Pejë, compared to its percentage in the Lukinë group, may indicate the relevance of this hematological parameter to the intoxication of lead and other metals, which are also related to the process of hematopoiesis, respectively the increase in the number of erythrocytes, as a protective reaction of the organism to the action of these metals. Prpic-Majic (1984), found that hematocrit value analysis and hemoglobin quantities, in cases of low lead poisoning, cannot serve as reliable indicators of intoxication with lead.

Our results of the amount of hemoglobin in the blood of pigeons in Pejë (159.7g/L), which show similar values, are in accordance with the results of (Herrera-Duenas *et al*, 2014), which show values of (151.1g/L) in the house sparrow (*Passer domesticus*), in an urban polluted environment. On the other hand, our results are in disagreement with Elezaj's results, (Elezaj *et al*, 2011), which found lower values of hemoglobin (143.7g/L) in urban pigeons (*Columba livia*), which live near the Feronikel foundry in Drenas.

5. Conclusions

Our findings suggest that Feral pigeons (*Columba livia*) free living around urban polluted area, are an useful biomonitor species and representative for lead exposure, and that ALA-D activity, Hct and Hb, may function as a useful biochemical and hematological parameters in free living Feral pigeons in urban areas contaminated with heavy metals.

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