

ORIGINAL ARTICLE

Lead Effect on Aminolevulinic Acid Dehydratase Activity of Feral Pigeon (*Columba livia*) in Drenas

Albana Plakiqi Milaimi^{*1}, Qerim I. Selimi¹, Kasum Rr.Letaj¹, Artan Trebicka²

¹Department of Biology, Faculty of Mathematical and Natural Sciences, University of Pristina, 10.000 Pristina, Republic of Kosovo

²Department of Biology, Faculty of Natural Sciences, University of Tirana, Republic of Albania

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ABSTRACT: This study was aimed to investigate the effects of environmental pollution with heavy metals from ferro-nickel smelter on Aminolevulinic Acid Dehydratase (ALAD) activity, and to analyze the blood lead level of feral pigeon (*Columba livia*) in ferro-nickel smelter courtyard in Drenas City, Republic of Kosovo. For this purpose, twenty specimens of feral pigeon (20 birds, males and females), were collected in Drenas city which were living in ferro-nickel smelter courtyard, and 20 specimens in Lubizhdë village as control group (non-contaminated area). ALAD activity in Drenas group was significantly inhibited ($P < 0.001$), compared with ALAD activity of controls. The blood lead level was significantly increased ($P = 0.015$) compared to control group. Correlation between ALAD and blood lead level in Drenas group was negative ($r = -0.117$; $P > 0.050$) and positive in Lubizhdë group ($r = 0.452$; $P > 0.050$), but not in significant difference between the input groups. Feral pigeons can play an important role as bioindicators, which can be used to monitor the environmental pollution with heavy metals that may originate from Nickel metallurgy.

INTRODUCTION

Environmental pollution with heavy metals means the presence of these contaminants in the natural environment that can cause harmful effects to living organisms. These pollutants can originate from natural and anthropogenic sources. Except of these sources of

pollution, Industrial Revolution has brought to accumulation of untreated chemicals and their wastes into the environment. Due to the rapid development of industry, lifestyle, today our environment is becoming problematic and unsafe for living organisms.

* Corresponding author: aplakiqi@ymail.com (A. Plakiqi Milaimi).

Approximately 30 metals, such as organometallic compounds or as salts, are used in the industry. More troubling is the fact of the uses and realizing of heavy metals in the environment from metallurgies that brought to high level of pollution with these metals. Many scientific papers reported for risk of heavy metals for living organisms. Heavy metals after their thermal and volatile processes released into the atmosphere such as oxides. Heavy metal metallurgies, such as Nickel metallurgy, can release heavy metal compounds, which can bring to serious effects on human health [1].

These heavy metal compounds such as; arsenium (As), chromium (Cr); cadmium (Cd), lead (Pb), mercury (Hg), nickel (Ni) and zink (Zn), cause the higher risk, because during their combustion, waste products can have a detrimental effect on people who are exposed to them [3]. Some of these metals are dangerous to the environment (such as Hg, Cd, As, Pb, Cr), some can cause corrosion (Zn, Pb), while others are carcinogenic, mutagenic, theratogenic or toxic. Also, Aluminium, Arsenium, Lead, Lithium (Li), Manganese (Mn), Mercury and Thalium (Tl) have negative effects on nervous system [4]. Cd, Hg and Pb may cause toxic effects on kidney [5]. Hg, Pb, Cd, Cu have toxic effects on liver, while Ni, Cd, Cu, Cr on skin, bones and teeth. Pb, Cu, Mn, Zn, Cd and Ni compounds can be released by burning oil and gasoline from traffic vehicles [6]. In addition, Zn and Cd can be released from damaged batteries; Ni, Cr, W and Mo, from their car waste.

Many studies provide evidence for a progression of adverse health effects of lead in animal and human populations. Toxic negative effects of lead may cause many disorders on many organisms, including nervous system, urinary, reproductive and blood system. Like other heavy metals, due to high density, lead can accumulate in many organs, such as liver, kidney, especially on solid tissues (femur and tibia), affecting aminolevulinic acid activities of these target organs [7]. Lead has long been known to alter the hematological

system by inhibiting the activities of several enzymes involved in heme biosynthesis. Particularly, sensitive to lead action is delta-aminolevulinic acid dehydratase (ALAD). ALAD is zinc-dependent enzyme, which catalyse porphobilinogen synthesis, including heme synthesis [7]. Lead can inhibit ALAD synthesis by changing their activity in quaternary structure of this enzyme and its activity, because lead have antagonist role of zinc [8]. As a result, lead may cause anemia. The anemia induced by lead is primarily the result of both inhibition of heme synthesis and shortening of erythrocyte lifespan, but lead also can induce inappropriate production of the hormone erythropoietin leading to inadequate maturation of red cell progenitors, which can contribute to the anemia.

Lead can affect ALAD activity on feral pigeon living in urban and industrial areas when lead concentration was three times higher than the control group [7]. In addition, ALAD activity is inhibited by the air pollution, which results from lead and zink metallurgy [7]. Even low level of blood lead concentration can inhibit ALAD activities on pigeon populations living in urban area [9]. However, there is little information about effects of Nickel metallurgy on ALAD activity in living organisms. We therefore determined the ALAD activity and blood lead level in feral pigeons (*C. livia*).

MATERIALS AND METHODS

The smelter Ferronikeli is located in Drenas, 20 km far away from Prishtina (capital of Republic of Kosovo) in the West, including a space of 81 hectares [10]. The final productions of this smelter are ferro-nickel. Drenas is a city with traffic, urban and metallurgical pollution. Chemical composition of the air consists of Ni, SiO₂, Fe, FeO, CaO, MgO, Cr₂O₃, Al₂O₃. Also, noted the presence of Fe, Zn, Cu, Cd, Pb, Ni, Cr and Mn into the water of Drenica river [10], which is near of ferro-nickel smelter, where discharge its metallurgical waste. Area near the

smelter is populated by humans near 70 meters and this results even a higher risk for human beings.

Twenty specimens of feral pigeon males and females were collected on May 2014 in ferro-nickel smelter courtyard and twenty specimens in Lubizhdë village (control area). The blood samples were punctured from alvar vein, and then they were removed of feathers. Blood lead level was determined with flame atomic absorption spectrometry (UNICAM 929) [11]. Blood was mineralized with concentrated nitric acid and then softened hydrochloric acid was added, where the lead has passed into lead chloride. Five milliliter of blood were punctured from heart and put into Erlenmeyer flask of 100 ml. Samples were mineralized in high temperature with the addition of concentrated nitric acid (three times out of 5.0 ml until foam appeared). Then, being in this Erlenmeyer flask 5.0 ml HCl 6 N was added and the solution was well mixed. After that, into Erlenmeyer flask, 10 ml ethyl ether was added to digest emulsion and this content was transferred to the separated funnel. The bottom layer of acid-water was transferred back to the previous cup and was dried almost entirely. Residue of the glass was softened with redistilled water up to 2.5 ml.

The solution was absorbed directly into flame atomic absorber spectrometer (UNICAM 929) and absorbance was read at the wavelength of 283.3 nm. Blood lead level was counted in µg/dl.

The activity of ALAD (EC.4.2.1.24) was estimated according to the European standardized method of Berlin and Schaller (1974) [12].

Blood was taken directly from heart with heparinised syringe and then was carried into test tubes for centrifugation, which was previously obscured by aluminum folio and contained 0.02 ml heparin/ml blood. In each one of three test tubes by 2.6 ml of deionized water, 0.4 ml of blood was added. After well blended, test tubes were incubated for 10 minutes at a temperature of 37°C. One of three test tubes served as a

blind test, two others were parallel samples for determining the activity of the enzyme ALAD. In the blind, 2.0 ml of trichloroacetic acid 10% (TCA) test was added that was mixtured with 0.1 M HgCl₂ and was well blended. In each test tubes 2 ml 0.01 M of aminolevulinic acid substrate were added prepared by phosphate buffer (pH = 6.40 ± 0.01) and incubated in the temperature 37°C ± 0,2 °C for 60 minutes. The incubation time was calculated from the moment of adding the aminolevulinic acid substrate.

After that, in two parallel test tubes intended for determining the activity of ALAD, 2 ml of 10% TCA solution containing 0.1 M HgCl₂ were added and were well blended. Then, the three test tubes were centrifuged 10 minutes in 3000 rpm. After the centrifugation, from each tube, 2.0 ml of pure supernatant were transferred to three other test tubes; in each of them 2.0 ml of fresh Ehrlich reagent were added incubated for 10 minutes. Absorbance was read compared to H₂O-Re for each tube separately with Spectrophotometer at 555 nm wavelength.

The activity of the enzyme ALAD was expressed per liter of erythrocytes according to equation:

$$\text{ALAD (U/L)} = \text{A555 corrected} / \% \text{ Hct} \times 1881.7$$

Statistical software Sigma stat 3.5 was used for processing results of investigation. Results are presented as average values, standard deviations and correlation. Student test was applied to determine the statistical significance.

RESULTS AND DISCUSSION

Table 1 summarizes the activity of ALAD and blood lead level obtained from pigeons of Drenas and Lubizhdë. ALAD in Drenas group was inhibited ($P < 0.001$), compared to pigeons of control group.

Moreover, blood lead level was significantly increased ($P = 0.015$) compared to control group. Correlation between ALAD and Pb in Drenas group was negative ($r = -0.117$; $P > 0.050$) and positive in Lubizhdë group

($r=0.452$; $P> 0.050$), but not in significant difference between the input groups.

Our results are in accordance with results of many authors [7-9, 13, 16, 17], who found out that high blood lead level inhibited activity of ALAD. On May 2010, the blood ALAD activity of pigeons from ferro-nickel smelter courtyard was significantly inhibited ($P<0.001$) in comparison to reference [13].

We have repeated this experiment to monitor the level of pollution in this area. As results of this, ALAD activity is approximately in the same level.

Same cases of inhibiting ALAD activity on pigeons population were found out in Mitrovica as results of metallurgic activity [7] when blood lead level was 3 times higher compared to control group ($P<0.001$). We found out inverse correlation between blood lead concentration and ALAD activity.

Table 1. Blood Aminolevulinic Acid Dehydratase (ALAD); Blood lead level and correlation between blood ALAD and Pb of feral pigeon (*Columba livia*) from study and control area

PARAMETERS			
LOCALITY	ALAD U/L	Pb µg/dl	Correlation r=
LUBIZHDË	40.78 ± 8.67 (20)	3.33±1.61 (20)	0.45
DRENAS	19.16 ± 11.06 (20)	25.43±20.69 (20)	-0.117
SIGNIFICANCE			$P>0.050$
P	<0.001	= 0.015	$P>0.050$

Note: Values are expressed as means ± Standard Deviation; 20 - number of animals; P-significance

Referring to results of many authors which applied acute intoxication of birds with lead in ducks, Canada geese [14,15] and in natural animal populations who lived nearby the smelters, red blood cell ALAD activity was inhibited when the blood lead concentration has been increased [7, 13]. These results are in accordance with previous studies [16, 17], which found out that ALAD activity was inhibited by increasing of blood lead level of animal and human populations living near Trepça smelter in Mitrovica. Due to realizing of many compounds of heavy metals from smelters, in our case ferro-nickel smelter, perhaps inhibition of ALAD activity maybe came from presence of lead oxides and other lead compounds in air, soil and from traffic pollution. These studies have shown negative correlation between lead concentration and ALAD activity.

Finally, results of many authors indicate that pigeons can be used as biological indicators of air pollution and exposure to heavy metals [13, 18, and 19].

CONCLUSION

Because of small weight, high metabolism, standing on high level of food pyramid, feral pigeon can accumulate lead, which can enter into the body through digestive or respiratory system. For this reason, feral pigeon can be good indicator of environment pollution with lead.

Feral pigeon has been exposed to lead due to realizing of heavy metals from nickel metallurgy and petrol derivatives. Knowing effect of lead on blood, we have found out that even in low level of lead on blood aminolevulinic acid dehydratase (ALAD) activity is inhibited. Therefore, ALAD activity is a reliable biomarker of lead toxicity in birds, because it is very

sensitive even in low doses of lead and can be more convenient than other laboratory tests.

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