JJBS

Jordan Journal of Biological Sciences

Volume 13, Number 3, Supplementary Issue, December 2020
ISSN 1995-6673
Pages 589 - 595

Chronic Effects of Lead Exposure on Oxidative Stress Biomarkers in Feral Pigeon (*Columba livia*) from Smelter Area in Kosovo

Sheval F. Memishi[1,*], Qerim I. Selimi[2], Kasum Rr. Letaj[3] and Isa R. Elezaj[3]

[1]Deprtment of Biology, Faculty of Mathematical and Natural Sciences, University of Tetovo, "Ilinden bb" 1200, Tetovo, North Macedonia.[2] Faculty of Education, University of Mitrovica,"Isa Boletini", 40,000, Mitrovica, [3]Deprtment of Biology, Faculty of Mathematical and Natural Sciences, University of Prishtina "Hasan Prishtina", 10, 000, Prishtina, Kosovo.

Received: January 8, 2020; Revised: February 6, 2020; Accepted: February 21, 2020

Abstract

The effects of lead exposure on oxidative stress biomarkers in feral pigeon (*Columba livia*) from Mitrovicë town (situated in close vicinity of ormer lead and zinc smelter) were studied through the use of blood Pb (PbB) levels, d-aminolevulinic acid dehydratase (δ-ALAD) activity, level of plasma δ-Aminolevulinic acid (ALA), Glutathione (GSH), Malondialdehyde (MDA), Uric acid (UA), Urea (U), and creatinine (CR). Lead levels in pigeons from Mitrovicë were significantly elevated (P<0.001) compared to the control. Elevated PbB in pigeons from Mitrovicë was also accompanied by significantly inhibited (P<0.001) δ-ALAD activity in blood, significantly decreased levels of GSH and MDA (P<0.001; <0.05), and significantly elevated levels of ALA, UA, CR (P< 0.001) compared to the control. Negative correlation was observed between PbB and δ-ALAD activity (r= -0.409; P<0.05) in birds in Mitrovicë. This study provides important evidence about the chronic effects of lead on the analyzed oxidative stress biomarkers. In addition, the study proves that the pigeons in Mitrovicë remain chronically exposed to harmful effects of lead, and that the close vicinity of the former smelter "Trepça" still represents a source of exposure to lead for the health of biota and humans.

Keywords: lead level, oxidative stress biomarkers, δ-ALAD, feral pigeon, Mitrovicë.

1. Introduction

The long-term pollution activities from smelting factories may disturb or destroy ecosystems, thereby making them less suitable for wildlife. In fact, these polluted sites may produce bioavailability and increased levels of toxic compounds such as heavy metals, which affect multiple generations of the population as a result of their extended bioavailability (Dimitrov *et al.,* 2016).

Heavy metals are highly reactive and often toxic at low concentrations. They may remain in the environment for years, posing long-term risk to life long after pollution sources have been removed (Gall *et al.,* 2015). Therefore, the use of monitoring in pollution assessment is of paramount importance.

Lead contamination is a global problem affecting the health of different bird species worldwide (Haig *et al.,* 2014). In this sense, lead (Pb) is a naturally occurring but nonessential element that is highly toxic at elevated concentrations having the potential to affect most body systems and the health of animals. Moreover, this toxic metal is considered one of the most significant threats to several species (Haig *et al.,* 2014; Wiemeyer *et al.,* 2017; Isomurso *et al.,* 2018; Helander *et al.,* 2019; Ecke *et al.,* 2017).

Birds are the most studied and probably the most affected species to lead poisoning (Pain *et al.,* 2019; Plaza and Lambertucci, 2019). In particular, wild birds have been disclosed to be useful bio-indicators because they are sensitive to pollutants and are important structural components of the ecosystem (Kekkonen *et al.,* 2012; Matheo-Thomas *et al.,* 2016).

Negative impacts of pollution from mining and smelting complexes have been observed in wild bird species for decades. Although the technological advances and legislation have resulted in reduced impact from metal industries in terms of metal emissions, polluted soils may still constitute a major source of heavy-metal exposure for wild birds, even if the atmospheric deposition declines (Berglund *et al.,* 2010; Elezaj*et al.,* 2013).

In the last years, the number of studies evaluating the effects of heavy metals on oxidative stress biomarkers in free-living birds exposed to heavy metals under natural conditions has increased (De la Casa *et al.,* 2015; Espin *et al.,* 2014a, 2014b; Koivula *et al.,* 2011; Martinez-Haro*et al.,* 2011; Rainio*et al.,* 2013). However, inter-specific differences due to different tolerance to metals have been found by some researchers (Espin *et al.,* 2014a, 2015; Hernandez-Garcia, 2010; Koivula and Eva, 2010), and the specific resistance of species to metals is still unclear for many birds (Espin *et al.,* 2016).

From this perspective, it is essential to use some biomarkers to detect oxidative stress in birds. This is mainly due to the fact that different antioxidants are involved in the protection against reactive oxygen species

* Corresponding author e-mail: sheval.memishi@unite.edu.mk.

(ROS) through close interaction between them, and that the antioxidant defense may respond differently depending on the species (Berglund *et al.,* 2007; Constantini and Verhulst, 2009; Koivila and Eva, 2010).

The concentrations of lead produce harmful effects on bird's health like inhibition of enzymes that are important for metabolic functions (Espín *et al.,* 2015). One of the prime targets of lead toxicity is the heme synthesis pathway. In this pathway, δ-ALAD is the most sensitive and specific biomarker of the low lead (Pb) exposure in humans and wild animals including bird species (Elezaj *et al.,* 2013).

In this sense, ALA-D inhibition results in correlated decrease in hem production of hemoglobine and accumulation of Aminolevulinic acid (ALA), which can be oxidized to generate ROS. Consequently, enhanced lipid peroxidation and DNA damage may occur. The δ-ALAD can serve as a valuable biomarker of oxidative stress in hematological system exposed to lead and act also as a biochemical indicator of lead exposure (Gurer-Orhan *et al.,* 2004).

Recent studies have also explained the role of antioxidants in wild birds, with particular attention to low-weight non-enzymatic molecules such as Glutathione (GSH) and Uric acid (UA) (Constantini *et al.,* 2008; Cohen and McGraw, 2009; Perez-Rodrigez *et al.,* 2009; Koivula and Eva 2010; Sánchez-Virosta *et al.,* 2019; Dolan *et al.,* 2017).

Lipids are the most involved class of bio-molecules of the biological targets of oxidative stress. Lipid peroxidation induces a number of secondary products, among which Malondialdehyde (MDA) is the major and most studied product of saturated fatty acid peroxidation. MDA is highly toxic molecule and should be considered as more than just a marker of lipid peroxidation. Its interaction with DNA and proteins has often been ascertained as potentially mutagenic and atherogenic (Del Rio *et al.,* 2005).

The aim of this study was to assess potential effects of blood Pb on oxidative stress biomarkers in free-living feral pigeons (*Clombalivia*) from Mitrovicë town, situated to a close vicinity of former smelter "Trepça", North East of Kosovo, and Lukinë village-rural area. For this purpose, the study analyzes the blood Pb level (PbB), and some biomarkers of oxidative stress such as δ-ALAD activity, levels of ALA, GSH, MDA, UA, U, and CR.

2. Material and Methods

2.1. Study area

Mitrovicë (Fig.1) had the largest metallurgic and mining complex "Trepça" in Europe, which commenced activities in 1939 with the extraction of lead, cadmium, and zinc. Many industrial plants existed in the complex such as lead smelter, fertilizer production plant, refinery, battery factory, zinc electrolysis facility, and a sulfuric acid plant. The significant amount of heavy metal pollutants was released to the surrounding area, including residential areas, associated with biota and human health risk (Borgna *et al.,* 2009).

The study data collected from the close vicinity of Mitrovicë show that the average content of Pb in soil is 20-fold higher than the European median; Cd 11-fold, Hg 5.5-fold, As 4.6-fold, Zn 4.2, and Cu 3.2-fold higher.

In the narrower vicinity of Mitrovicë and Zveçan, the content of the aforementioned elements is even higher than the intervention values, according to the New Dutch list, and were exceeded in 152 km 2 of the investigated area. (Šajn *et al.,* 2013).

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

Figure1. Localities ithe Map of Republic of Kosovo

2.2. Experiments

Specimens of Feral pigeons (*Columba livia*), a total of 20 birds of both genders from each locality were collected in March 2017from Mitrovicë and Lukinë. In urban areas, this species is sedentary, forming discrete flocks which remain faithful to specific feeding and roosting areas. For this reason, a relatively small sample of birds is expected to reflect any variation in metal exposure at the local level.

In laboratory setting, blood samples were collected by heparynized syringes directly from heart. Blood Pb level (PbB) was determined with atomic absorption spectrometry with an atomic absorber- Varian spectra AA 640Z Zeeman AAS, equipped with a GTA 100 graphite furnace (Varian, USA) and PSD 100 auto sampler (Varian, USA).

Erythrocyte δ-Aminolevulinic acid dehydratase (δ-ALAD) activity was measured according to the CEC standardized method. The level of δ-Aminolevulinic acid (δ-ALA) in plasma was determined by the spectrophotometric method (Berlin and Schaller, 1974). The level of malondialdehyde (MDA) in plasma was determined by the spectrophotometric method (Uchiama and Michara, 1978). Buetler's method was used to determine the total level of reduced glutathione (GSH) in the blood (Buetler *et al.,* 1963). The level of plasma uric acid (UA), urea (U) and creatinine (CR) was determined spectrophotometrically using Bio-La-Tests (Humana-Germany).

2.3. Data analysis

Data 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. Data were expressed as means ± SD.

3. Results

Our results of blood Pb level (PbB), δ-aminolevulinic acid dehydratase activity (δ-ALAD), level of ALA, MDA, GSH, UA, U and CR in individuals of Feral pigeon (*Columba livia*) from Mitrovicë and Lukinë are presented in the table and figures (table 1 and figures 2 and 3).

Table1. Blood Pb level (PbB), δ-aminolevolunic acid dehydratase activity (δ-ALAD), level of (ALA), Glutathione (GSH), Malondialdehyde (MDA), Uric acid (UA), Urea (U), and Creatinine (CR), in Feral pigeon (*Columba livia*), in Lukinë and Mitrovicë.

Parameters	No\Loc	Lukinë (A)	Mitrovicë (B)	Significance *P*<
PbB (µg/dL)	20	4.7 ± 1.1	17.1 ± 10.3	A:B; *P*<0.001
ALA-D (U/LE)	20	45.8 ± 11.5	17.6 ± 12.6	A:B; *P*<0.001
ALA mmol/L	20	0.11 ± 0.09	0.66 ± 0.41	A:B; *P*<0.001
GSH (g/%)	20	35.4 ± 12.7	22.7 ± 3.9	A:B; *P*<0.001
MDA (µmol/L)	20	2.7 ± 1.4	1.04 ± 0.6	A:B; *P*<0.001
UA (µmol/L)	20	215 ± 65	326 ± 85	A:B; *P*<0.001
U (mmol/L)	20	0.5 ± 0.3	0.6 ± 0.2	NS
CR (mmol/L)	20	34.6 ± 4.0	39.1 ± 4.0	A:B; *P*<0.01

Note: The results are expressed as means X and standard deviation SD. NS-non significant.

The blood Pb level (PbB) in the Feral pigeons from vicinity of former lead and zinc smelter "Trepça"in Mitrovicë was significantly higher (*P*<0.001) with values of (17.1+10.3 µg/dL), compared with the rural area of Lukinë (4.6+11.2 µg/dL).

Blood δ-ALAD activity was inhibited (*P*<0.001), level of MDA and GSH significantly decreased (*P*<0.05; <0.001), while ALA and UA values were significantly higher (*P*<0.001) in pigeons from Mitrovicë compared with controls, while CR was significantly (*P*<0.01) higher.

There was negative correlation established between blood lead level and δ-ALAD activity (r= -0.409; *P*<0.05) in birds of Mitrovicë.

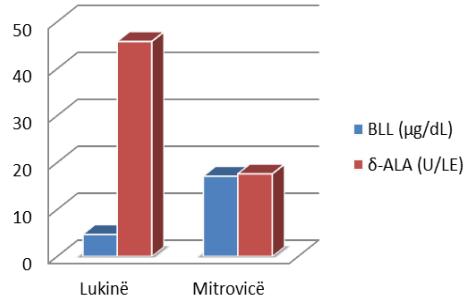

Figure 2. Blood lead level (PbB) and δ-ALAD activity in feral pigeons of Lukinë and Mitrovicë.

The level of plasma ALA in birds from Mitrovicë was 6.0 fold higher compared with that in Lukinë.

The level of MDA in plasma of pigeons from Lukinëis was significantly higher compared with pigeons in Mitrovicë (2.7 ±1.4; 1.0 ± 0.6; respectively; *P*< 0.01). The level of plasma MDA in birds from Mitrovicë is 1.7 times lower compared with the plasma MDA of pigeons in Lukinë.

A level of plasma GSH in pigeons from Mitrovicë (22.7 ± 3.9) was significantly lower (*P*<0.001) compared with birds from Lukinë (35.4 ± 12.7).

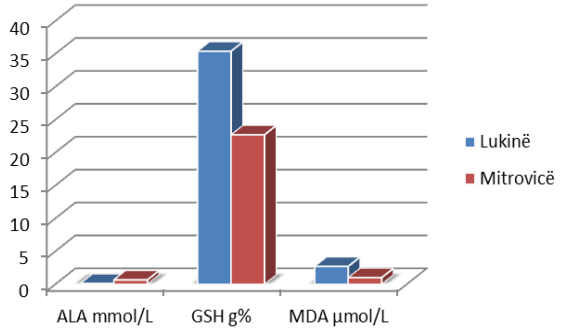

Figure 3. The level of ALA, GSH and MDA in feral pigeons of Lukinë and Mitrovicë.

Plasma uric acid level (UA) in Mitrovicë (326 ± 85) is significantly higher compared with the plasma UA of birds in Lukinë (251 ± 65; *P*<0.001), while the urea (U) in pigeons from Mitrovicë (0.6 ± 0.2), although not significantly, is higher than in plasma (0.5± 0.3) of pigeons from Lukinë.

The plasma level of creatinine (CR) in pigeons from Mitrovicë (39.1 ± 4.0) is significantly higher (*P*< 0.01) compared with pigeons in Lukinë (34.6 ± 4.0).

4. Discussion

The results of Pb level in the blood of Feral pigeons (Columba livia) from Mitrovicë (17.1+10.3 µg/dL) are significantly higher (P<0.001) compared with the control group of Lukinë pigeons (4.6+11.2 µg/dL). This confirms the presence of the risk of lead contamination in this area not only for the pigeons but also for the humans and other animal species.

Compared with our earlier results (Elezaj et al., 2008, 2013) about PbB in pigeons in Mitrovicë from 2000 to 2003 (the year when the smelter was closed down), these results are 4.9 times lower, 5.5 (2001); 2.6 (2002); 4.0 (2003) and 8.7 times lower than in 2012. The intermittent analysis of blood Pb levels (from 2000-2017) in the free-living pigeons from vicinity of lead and zinc smelter in Mitrovicë revealed the progressive decrease of blood lead levels in pigeons of Mitrovicë over the seventeen years (Elezaj et al., 2008, 2013; our data, 2017).

Our results of blood Pb level in birds from Mitrovicë are consistent with the results of Vanparys (Vanparys et al., 2008) who in the blood of great Tit (Parus major) from polluted areas collected 500-1000mcaused by a non-ferrous metallurgic smelter in the south Antwerp (Belgium). Vanparys found significantly higher blood Pb levels (5.8 times higher) compared with reference.

Coeurdassier (Coeurdassier et al., 2012), established higher levels of Cd and Pb in the blood and erythrocytes of European Black birds (Turdus merula) caught up from a contaminated site-smelter of Metaleurop Nord, North France.

Our results of blood Pb level in pigeons from Mitrovicëare are consistent with the results of Cai and Calisi (Cai and Calisi, 2016) who in pigeons (Columba livia) from the Soho/Greenwich Village neighborhood revealed highest levels of lead in their blood (X=23.121µg/dL), followed by pigeons from Lower Manhattan/Lower East Side (X=22.708 µg/dL) and Upper West Side (X= 19.957 µg/dL). Correlation data revealed that levels of blood of pigeons from the Manhattan neighborhoods positively correlate with the rates of children in the same neighborhoods with the elevated levels of blood lead (r = 0,647, N=8, P=<0.042).Whatever sources may be causing lead toxicity in their subjects, there exists a parallel between high blood levels in the pigeons sampled and high rate of children with blood Pb levels of 10µg/dL or more in NYC neighborhoods. They (Cai and Calisi, 2016) provide a powerful example of how monitoring pigeon biology may help us to better understand the location of prevalence of lead with the aim of providing greater awareness and devising prevention measures.

The results indicate a decrease of Pb exposure in the last 17 years in Feral pigeons (Columba livia) and House sparrows (Passer doemsticus) free-living in Mitrovicë town (Elezaj et al., 2013; Millaku et al., 2015), which could be explained by the closure of lead and zinc smelter "Trepaça" in Mitrovicë in the year 2000.

Our results of δ-ALAD activity in the blood of feral pigeons from Mitrovicë (inhibited 62.4 % v-s reference) explain the sensitivity of this biomarker of lead exposure. The results also confirm that this inhibition of δ-ALAD activity occurs due to the prevailing high level of lead pollution in this area.

The results are consistent with our former results (Elezaj et al., 2012) found in feral pigeons from the same area in 2012 when the δ-ALAD activity was 94.9% inhibited v-s reference (1.2 ± 0.9; 23.4 ± 3.0 U/LE respectively), while blood lead level was 149 ± 60 µg/dL v-s 5.4 + 0.6) µg/dL in the reference. In 2013, Elezaj (Elezaj et al., 2013) in the farm pigeons from Mitrovicë, established 77.8 % inhibition of δ-ALAD activity of pigeons from Mitrovicë v-s reference (5.2 ± 2.8; 23.4 ± 3.0 U/LE), while blood lead level was 50 ± 25; 5.4 ± 0.6 µg/dL respectively. The inhibition of blood δ-ALAD activity (61%) was marked also in the feral pigeons from courtyard of ferronickel smelter in Drenas (Elezaj et al., 2011).

Due to lead exposure across the observed range of Pb concentrations (0.01–0.34 µg/g), in nestling of Golden Eagle (Aquila chrysaetos), in western United States (California, Idaho, Oregon, Wyoming), Herring (Herring et al., 2020), found 68% reduction in delta-aminolevulinic acid dehydratase (δ-ALAD) activity.

Our results of inverse correlation established between blood Pb level (PbB) and δ-ALAD activity (r=-0.409, P<0.05) in feral pigeons from Mitrovicë explain the effect of the continuous increase of lead in blood in the continuous increase inhibition of activity of this oxidative stress biomarker.

These are consistent with the results of Elezaj's studies (Elezaj et al., 2011) that established inverse relationship between Pb concentrations in femur bone (51 µg/g d.w) and tibia (35.9 µg/g d.w.), v-s blood δ-ALAD activity (r=-877, P<0.001; r=-787, P<0.01 respectively) in free-living feral pigeons (Columba livia) in the courtyard of Ferronickel smelter in Drenas, Kosovo.

Correlation between delta-aminolevulinic acid dehydratase (δ-ALAD) activity and whole-blood lead (Pb) concentration in nestling Golden Eagles (Aquila chrysaetos) is also established in the western United States (California, Idaho, Oregon, Wyoming). Delta-aminolevulinic acid dehydratase activity declined 68% across the observed range of (0.01–0.34 lg/g) Pb concentrations (Herring et al., 2020).

Based on the collective data of the Federal National Resource Damage Assessment regulations (NRDA; regulation 43 CFR 11.62) have set the Pb-poisoning injury level in wildlife at >50%, decrease in blood δ-ALAD activity compared with unexposed reference animals, which again include birds (Van der Merwe et al., 2011). Taken together, these findings suggest that the recommendations of the acceptable lower threshold-limit for blood lead in feral pigeons and different free-living bird species require reexamination.

Our results of higher inhibition index of δ-ALAD activity (38.4%) can be explained by the fact that negative inversion between blood lead level and blood δ-ALAD activity above > 20 µg/dL disconnects the progressive linearity.

The results of higher Aminolevulinic acid (ALA) level (6.0-fold higher), as a precursor to δ-ALAD in the plasma of Feral pigeons from Mitrovicë town are consistent with the results of Costa (Costa et al.,1997), who in lead exposed workers established the positive linear relationships between plasma ALA level and blood lead level. Our data are in line with free radical hypothesis for lead poisoning, where ALA distribution to and accumulation in several organs may trigger oxidative stress response, not only in the case of lead exposed workers (Costa et al.,1997), but also in wild animals (birds), in conditions of environmental pollution with lead.

The correlation data between levels of Aminolevulinic acid (ALA-P), PbB, and δ-ALAD activity to our knowledge so far have not been investigated not only in wild pigeons but also in other birds. Thus, this draws attention to the fact that the amount of plasma ALA may have contributed to causing oxidative stress in individuals of these populations through the formation of free radicals, similar to causing oxidative stress in workers exposed to lead poisoning.

According to our data of the measure of the values of ALA in the feral pigeons, it turns out that the obtained results of this biomarker of oxidative stress in the feral pigeons are among the first to be investigated in these species.

It is important to explain that many metals are capable of generating Reactive oxygen species (ROS) and include oxidative damage and may therefore lead changes in oxidative regulation (Rianio et al., 2013). Oxidative state (in terms of GSH-reduced and GSH/GSSG-oxidized ratio), did not vary among species, suggesting that different species may employ different antioxidant pathways to achieve the same oxidative state. According to them, the effects on oxidative status observed in one species cannot be generalized to the other ones (Rainio et al., 2013). Of all the biomarkers that we examined, only glutathione peroxydase (GP) and glutathion-S-transferase (GST) activities in blue tits and GSH level in pied flycatchers

showed any association with metal contamination (Rainio et al., 2013).

Our results of lower concentration (34 %) of GSH in plasma of feral pigeons from smelter vicinity compared with the reference pigeons are consistent with the results of Sugawara (Sugawara et al., 1991), who in the erythrocytes of workers exposed to lead established lower concentration of GSH. In addition, previous studies have also observed that Pb concentrations above 10-15 ug/dL (or even lower) may produce inhibition of antioxidant enzymes, depletion of GSH, and induction of Thiobarbituric acid reactive substances (TBARS) in erythrocytes of Griffon vultures and Eagle owls (Espin et al., 2014).

Our results of lower level in the plasma of MDA in feral pigeons from smelter vicinity are consistent with the results of Dobrakovsky (Dobrakovsky et al., 2017), who in occupationally workers exposed to lead for 36 to 44 days (with blood Pb level 49.1 + 14.1 ug/dL at the end of study) established no change of malondialdehyde (MDA) level and activities of catalase (CAT) and superoxide dismutase (SOD). According to them, short-term exposure to lead induces oxidative stress associated with elevated level of lipid peroxides (LPH), but not MDA.

In addition, our results of higher level of plasma uric acid, urea, and creatinine in the birds from smelter vicinity are consistent with results of Hamidipour (Hamidipour et al., 2016), who in blood of Japanese quails exposed to 0.4 mg/kg diet of “Lead Acetate” for 21 days found significant increase of enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid dehydrogenase (LDH), glucose, creatinine, and uric acid, compared with the control group.

Additionally, creatinine concentration in blood plasma is nearly constant in natural conditions. Excessive amounts are also excreted through glomerular filtration. Its sharp increase in plasma is a sign of impaired glomerular filtration and renal dysfunction (Newman and Price, 1999).

The increase of plasma creatinine may be due to the adverse impact of lead or other metals on kidney function and glomerular filtration in birds from smelter vicinity. Uric acid rise may be due to the adverse effect of lead on kidney function. Because birds are uricotelic, measurements of uric acid are more valuable than creatinine and urea to asses kidney function (Khaki et al., 2011).

The level of uric acid, in addition to being a waste product, is also considered a biomarker for many physiological characteristics in vertebrates, including birds, because it cannot be broken down in vivo. It is the most dominant antioxidant defense system for birds and can be linked to their ability to cope with the heightened metabolic ROS production throughout their lives (Carro et al., 2012).

Urea serves an important role in the metabolism of no nitrogen containing compounds in animals. Urea is present in very small amounts in avian plasma, and plasma urea concentration has traditionally been considered an inappropriate parameter to evaluate renal function in birds (Lumeij and Remple, 2007).

Creatinine is reportedly of questionable value for evaluating renal function in birds because birds excrete creatine before it has been converted to creatinine. Some data suggest that creatine and creatinine may act as precursors of food mutagens and uremic toxins. Recent identification and purification of many of the enzymes involved in creatinine metabolism have just opened the door to wide variety of biological, physiological, as well as clinical investigations and applications (Lumeij and Remple, 2007).

5. Conclusion

The results of this study support the effects of lower levels of PbB on analyzed oxidative stress biomarkers. Additionally, the results confirm that feral pigeons in Mitrovicë remain chronically exposed to harmful effects of lead levels, and the close vicinity of smelter "Trepça" still poses a threat for the health of humans and biota.

Conflict of Interest

The authors have no conflicts of interest to declare.

References

Berglund AMM, Sturve J, Förlin L and Nyholm NEI. 2007. Oxidative stress in pied flycatcher (Ficedula hypoleuca) nestlings from metal contaminated environments in northern Sweden. *Environmental Research*, **105**: 330-339.

Berglund AMM, Ingvarsson PK, Danielsson H and Nyholm NEY. 2010. *Lead exposure* and *biological effects* in *pied flycatchers (Ficedula hypoleuca) before* and *after* the *closure* of a *lead mine* in *northern Sweden. Environmental Pollution, **158**:1368-1375.*

Berlin A and Schaller KH. 1974. European standardized method for the determination of ALA-D activity in blood. *Zetschrift fur Klinische Chemie und klinische Biochemie,* **12**: 389-390.

Borgna L, Di Lella LA, Nannoni F, Pisani A, Pizzetti E, Protano G, Riccobono and Rossi S. 2009. The high contents of lead in soils of northern Kosovo. *Journal of Geochemical Exploration,* **101**: 137-146.

Buetler E, Duron O and Kelly BM. 1963. Improved method for the determination of blood gluthatione. *The Journal of laboratory and clinical medicine,* **61(5)**: 882-888.

Cai F and Calisi RM. 2016. Seasons and neighborhoods of high lead toxicity in New York City: The feral pigeon as bioindicator. *Chemosphere*, **161**: 274-279.

Carro MD, Settle T and Klandorf H. 2012. The role of uric acid in the avian species. *Nova Science publishers, Division of Animal and Nutritional Sciences,* **70**: 127-134.

Coeurdassier M, Fritsch C, Faivre B, Crini N and Scheifer R. 2012. Partitoning of Cd and Pb in the blood of European blackbirds (*Turdus merula*) from smelter contaminated site and use for biomonitoring. *Chemosphere,* **87**: 1368-1373.

Cohen AA and McGrave KJ. 2009. No simple measures for antioxidant status in birds: complexity in inter- and intraspecific correlations among circulating antioxidant types. *Functional Ecology,* **23**: 310-320.

Constantini D and Verhulst S. 2009. Does high antioxidant capacity indicate low oxidative stress? *Functional Ecology*, **23**: 506-509.

Costa AC, Trivelato GC, Pinto AMP and Bechara ECH. 1997. Correlation between plasma 5-aminolevulinic acid concentrations and indicators of oxidative stress in lead-exposed workers. *Clinical chemistry,* **7**: 43-47.

De la Casa-Resino I, Castellanos A, Soler F and Pérez-López M. 2015. Biomarkers of oxidative status associated with metal pollution in the blood of the white stork (*Ciconia ciconia*) in Spain. *Journal of Toxicological and Environmental Chemistry,* **97(5)**: 588-598.

Del Rio D, Stewart AJ and Pellegrini N. 2005. A rewiev of recent studies on malondialdehyde as toxic molecule and biological biomarker of oxidative stress. *Nutrition, Metabolism and Cardiovascular Diseases*, **15(4)**: 316-328.

Dimitrov HA, Mitovska VI, Tzekov VD and Chasovnikova TG. 2016. Bioaccumulation of Cadmium and Lead in Rodent Species from Region of Lead and Zinc Smelting Factory-Plovdiv (South Bulgaria). *Ecologia Balakanica*, **8(1)**: 9-18.

Dobrakovsky M, Boron M, Birkner E, Kasperczyk A, Chwalinska E, Lisovska G and Kasperczyk S. 2017. The Effect of a Short-Term Exposure to Lead on the Levels of Metal Ions, Selected Proteins Related to Them, and Oxidative Stress Parameters in Humans. *Hidawi. Oxidative Medicine and Cellular Longevity*, **2017**: 8763793.

Dolan KJ, Ciesielski TM, Lierhagen S, Eulaers I, Nygård T, Johnsen TV, Gómez-Ramírez P, García-Fernández AJ, Bustnes JO, Ortiz-Santaliestra ME and Jaspers VLB. 2017. Trace element concentrations in feathers and blood of Northern goshawk (*Accipiter gentilis*) nestlings from Norway and Spain. *Ecotoxicology and Environmental Safety*, **144**: 564-571.

Ecke F, Singh N, Arnemo JM, Bignert A, Helander B, Berglund ÅMM, Borg H, Bröjer C, Holm K, Lanzone M, Miller T, Nordström Å, Räikkönen J, Rodushkin I, Ågren E and Hörnfeldt B. 2017. Sublethal lead exposure alters movement behavior in free-ranging golden eagles. *Environmental Science and Technology*, **51**: 5729-5736.

Elezaj IR, Selimi QI and Letaj KRR. 2008. The accumulatin of Pb, δ- aminolevulinic acid dehydratase, hematocrit and hemoglobine amount of Feral pigeons (*Columba livia*) living near lead and zinc smelter "Trepça" in Mitrovica- Kosovo-Four year study. Procedings of the 14th International Conference of the Heavy Metals in the Environment. Taipei-Taiwan.

Elezaj I, Selimi Q, Letaj K, Plakiqi A, Mehmeti IS and Milaimi A. 2011. Metal Bioaccumulation, Enzymatic Activity, Total Protein and Hematology of Feral Pigeon (*Columba livia*), Living in the Courtyard of Ferronickel Smelter in Drenas. *Journal of Chemical Health and Risks*, **1(1)**: 01-06.

Elezaj IR, Selimi QI, Letaj KRR, Millaku LB and Sefaja L. 2012. Metal Accumulation, blood δ-Aminolevulinic Acid Dehydratase Activity and Micronucleated Erythrocytes of Feral pigeons (*Columba livia*), Living Near Former Lead-Zinc Smelter "Trepça"- Kosovo. Proceedings of 16th International Conference on Heavy Metals in the Environment. Rome-Italy.

Elezaj IR, Selimi QI, Letaj KRR, Millaku LB and Sefaja L. 2013. Metal Accumulation, Blood δ-Aminolevulinic Acid Dehydratase Activity and Micronucleated Erithrocytes of Feral pigeons (*Columba livia*) Living Near Former Lead-Zinc Smelter "Trepça"-Kosovo. E3S web Conference 1.34001. DOI: 10. 1051/e3sconf/2013014001.

Espín S, Martínez-López E, Iménez PJ, María-Mojica P, Antonio J and García-Fernández AJ. 2014a. Effects of heavy metals on biomarkers for oxidative stress in Griffon vulture (*Gyps fulvus*). *Environmental Research*, **129**: 59-68.

Espín S, Martínez-López E, León-Ortega M, Martínez JE and García-Fernández AJ. 2014b. Oxidative stress biomarkers in Eurasian Eagle owls (*Bubo bubo*) in three different scenarios of heavy metal exposure. *Environmental Research*, **131**: 134-144.

Espin S, Martínez-López E, Jimenez P, Marija-Mojica P and Garcia-Fernandez AJ. 2015. Delataminolevulinic acid dehydratase (δ ALAD) activity in four free-living species exposed to different levels of lead under natural contitions. *Environmental Research*, **137**: 185-198.

Espín S, García-Fernández AJ, Herzke D, Shore RF, Van Hattum B. Martinez-Lopez E, Coeurdassier M, Eulaers I, Fritsch C, Gomez-Ramirez P, Jaspers VLB, Krone O, Duke G, Helander B, Mateo R, Movalli P, Sonne C and van den Brink NW. 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? *Ecotoxicology*, **4**: 777-780.

Gall JE, Boyd RS and Rajakaruna N. 2015. Transfer of heavy metals through terrestrial food webs: **a review**. *Environmental Monitoring and Assessment*, **187(201)**: 1-21.

Gurer-Orhan H, Sabir HU and Ozgunes H. 2004. Correlation between clinical indicators of lead poisoning and oxidative stress in controls and lead exposed workers. *Toxicology*, **195**:147-154.

Haig SM, D'Elia J, Eagles-Smith C, Fair JM, Gervais J, Herring G, Rivers JW and Schulz JH. 2014. The persistent problem of lead poisoning in birds fromammunition and fishing tackle. *The Condor*, **116**: 408-428.

Hamidipour F, Pourkhabbaz HR, Banaee M and Javanmardi S. 2016. Bioaccumulation of Lead in the Tissues of Japanese Quails and Its Effects on Blood Biochemical Factors. *Iranian Journal of Toxicology*, **10(2)**:13-21.

Helander B, Sundbom M, Runkel AA and Bignert A. 2019. Temporal Changes in Concentrations of Lead and Other Trace Metals in Free‐Ranging Eurasian Eagle Owls (*Bubo bubo*) in Sweden. *Archives of Environmental Contamination and Toxicology*, **77**: 377-389.

Hernández-García D, Wood CD, Castro-Obregón S and Covarrubias L. 2010. Reactive oxygen species: A radical role in development? *Free radical biology & medicine*, **49(2)**: 130-43.

Herring G, Eagles-Smith CA, Buck JA, Sheil AE, Venum CR, Emery C, Johnson B, Leal D, Heath JA, Dudek BM, Preston CR and Woodbridge B. 2020. The lead (Pb) lining of agriculture-related subsidies: enhanced Golden Eagle growth rates tempered by Pb exposure. *Ecosphere*, **11(1)**: e03006. Open access, 17 January 2020.

Isomurso M, Koivusaari J, Stjernberg T, Hirvelä-Koski V and Venäläinen ER. 2018. Lead poisoning and other human-related factors cause significant mortality in white-tailed eagles. *Ambio*, **47**: 858-868.

Kekkonen J, Hamski IK, Vaisanen RA and Brommer JE. 2012. Levels of heavy metals in House Sparrows (*Passer domesticus*), from urban and rural habitats of southern Finland. *Ornis Fennica*, 89:91-98.

Khaki ZZ, Amoli SJ, Lesan V and Esfahani AT. 2011. Changes of serum biochemistry in short term toxicity with lindane pesticide in broiler chicken. *Journal of Veterinary Research*, **66(1)**: 1-7.

Koivula MJ and Eeva T. 2010. Metal-related oxidative stress in birds. *Environmental pollution*, **158**: 2359-2370.

Koivula MJ, Mirella K, Juha-Pekka S, Miko N and Eeva T. 2011. Metal pollution indirectly increases oxidative stress in great tit (*Parus major*) nestlings. *Environmental Research*, **111**: 362-370.

Lumeij JT and Remple D. 2007. Plasma urea, creatinine and uric acid concentrations in relation to feeding in peregrine falcons (*Falco peregrines*). *Avian pathology*, **20**: 79-83.

Martinez-Haro M, Green AJ and Mateo R. 2011. Effect of lead exposure on oxidative stress biomarkers and plasma biochemistry in waterbirds in the field. *Environmental research.* **111**: 530-538.

Mateo-Tomás P, Olea PP, Jiménez-Moreno M, Camarero PR, Sánchez-Barbudo IS, Rodríguez Martín-Doimeadios RC and Mateo R. 2016. Mapping the spatio-temporal risk of lead exposure in apex species for more effective mitigation. *Procedings of the Royal Society B*, **38**: 20160662. https://doi.org/10.1098/rspb20160662.

Millaku L, Imeri R and Trebicka A. 2015. Bioaccumulation of heavy metals in tissues of House sparrow (*Passer domesticus*). *Research Journal of Environmental.* **9(2)**: 107-112.

Newman D and, Price CP. 1999. **Renal function and nitrogen metabolism**, In: Burtis C.A., Ashwood E.R., editors. Tietz. Text book of Clinical Chemistry third ed. W B Saunders, Philadelphia.

Pain DJ, Mateo R and Green RE. 2019. Effects of lead from ammunition on birds and other wildlife: A review and update. *Ambio*, **48**: 935-953.

Plaza PI and Lambertucci SA. 2019. What do we know about lead contamination in wild vultures and condors? A review of decades of research. *Science of the Total Environment*, **654**: 409-417.

Perez-Rodrigez L. 2009. Carotenoids in evolutionary ecology: Re-evaluating the antioxidant role. *Bio Essays*, **31(10)**:1116-1126.

Rainio MJ, Kanerva M, Salminen JP, Nikinmaa M and Eeva T. 2013. Oxidative status in nestlings of three small passerine species exposed to metal pollution. *Science of the Total Environment*, **454**: 466-473.

Šajn R, Aliu M, Stafilov T and Alijagic J. 2013. Heavy metal contamination of topsoil around a lead and zinc smelter in Mitrovicë, Kosovë. *Journal of Geochemical Exploration*, **134**: 1-16.

Sánchez-Virosta P, Espín S, Ruiz S, Stauffer J and Eeva T. 2019. Effects of calcium supplementation on oxidative status and oxidative damage in great tit nestlings inhabiting a metal-polluted area. *Environmental Research*, **171**: 484-492.

Sugawara E, Nakamura K, Miyake T, Fukumura A and Seki Y. 1991. Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. *British Journal of Industrial Medicin*, **48**: 239-242.

Uchiyama M and Mihara M. 1978. Determination of Malonaldehide Precursor in Tissues by Thiobarbituric Acid Test. *Analitycal Biochemistry*, **86**: 271-278.

Van der Merrwe D, Carpenter JW, Nietfeld JC and Miesner JF. 2011. Adverse health effects in Canada geese (*Branta canadensis*) associated with waste from zinc and lead mines in the Tri-State Mining District (Kansas, Oklahoma, and Missouri, USA). *Journal of wildlife diseases*, **47(3)**: 650-60.

Vanparys C, Dauwe T, Van Campenhout K, Bervoets L, De Coen W, Blust R and Eens M. 2008. Metallothioneins (MTs) and δ-aminolevulinic acid dehydratase (ALAd) as biomarkers of metal pollution in great tits (*Parus major*) along a pollution gradient. *Science of the Total Environment*, **401(1-3)**: 184-93.

Wiemeyer GM, Pérez MA, Bianchini LT, Sampietro L, Bravo GF, Jácome NL, Astore V and Lambertucci SA. 2017. Repeated conservation threats across the Americas: high levels of blood and bone lead in the Andean condor widen the problem to a continental scale. *Environmental Pollution*, **220**: 672-679.