

Analysis of some heavy metals (Cd and Pb) in the Şanlıurfa province using Feral pigeon blood samples

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Abstract: Birds serve as good biomonitors in determining environmental pollution. As for indicators of environmental pollution, we detected blood heavy metal levels in 32 female and 32 male local Feral pigeons in the central districts of Şanlıurfa, located in southeastern Turkey, in one month of each season. The samples were analyzed by coupled plasma-mass spectrometry. Lead (Pb) levels were measured as 233.1 ± 25.7 µg/kg for Haliliye, 101.2 ± 29.7 µg/kg for Karaköprü, 81.3 ± 22.7 µg/kg for Eyyübiye, and 48.1 ± 27.1 µg/kg for Suruç, whereas cadmium (Cd) levels were measured as 30.6 ± 12.6 µg/kg for Haliliye, 35.7 ± 9.2 µg/kg for Karaköprü, 28.1 ± 8.7 µg/kg for Eyyübiye, and 16.8 ± 4.1 µg/kg for Suruç. The results showed that Pb and Cd levels were higher in males. Pb levels were highest in Haliliye, which is a heavy traffic zone, and Cd levels were highest in the Karaköprü where unplanned settlement is observed. Seasonal monitoring demonstrated that Pb levels were highest in spring, and Cd levels were highest in spring and autumn. Conclusively, in this air pollution study considering the central districts of Şanlıurfa, it is suggested to increase the use of public transport, encourage the use of alternative energy resources, to adopt a planned urbanization approach and increase afforestation.

Key words: Feral pigeons, biomonitoring, cadmium, blood, lead

1. Introduction

Heavy metal pollution is a major factor, which affects the structural and functional integrity of the environment at both the regional and local levels [1]. The bioaccumulation of metals in the body causes pathological changes. The toxic effects exerted by heavy metals on living organisms are dose-dependent. Cadmium (Cd) and lead (Pb) show toxic effects on the immune system [2]. Bird populations are sensitive to manmade environmental pollution. Birds are used as biomonitors of environmental pollution because their physiological and biological processes, including nutrition, growth, aging, reproduction, and molting, are affected by the bioaccumulation and distribution of heavy metals in their body [3,4]. In general, birds are exposed to heavy metals through contaminated food and water, polluted soil and air, dermal contact, and feather cleaning [5–8]. It has been reported that exposure to high levels of heavy metals may cause eggshell thinning, reproductive failure, immunosuppression, embryonic development disorders and deformities, and death-related population decline in birds [9,10]. Heavy metals may increase the frequency of mutations in birds by causing oxidative stress-induced DNA damage [11–13].

Several studies have been conducted in wild birds and semi-domesticated Feral pigeons (*Columba livia domestica*) for the assessment of atmospheric pollution [3,7,14–16]. Given their longevity (+ 18 years) and because of the atmosphere and living conditions they share with humans, Feral pigeons are considered as potential biomonitors for the detection of environmental pollution. Besides, they live in a limited location less than 2 km throughout their survival. This limited living area makes them specific location bioindicators to investigate heavy metals and other bioavailable toxicants [17]. To date, pigeons have been used in environmental pollution research conducted in the USA, Bangladesh, China, the Netherlands, Korea, Kosovo, and Chile [7,18,19]. Many studies were carried out related to heavy metal analyses in pigeon blood samples worldwide [2, 20,21]. Many different techniques such as graphite furnace atomic absorption spectrometry (GFAAS), flame atomic absorption spectrometry (F-AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray fluorescence spectrometry (XRF) are used in determining the levels of trace elements. ICP-MS is used effectively and widely because of its high sensitivity, accuracy, wide dynamic range in determining multiple elements at trace levels [22].

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Feral pigeon breeding is very popular in the central and peripheral districts of Şanlıurfa, Turkey, both for economic concerns and fun. This may help to investigate air pollution biomonitoring around Şanlıurfa. On purpose blood samples from pigeons were collected from three central districts, Haliliye, Karaköprü and Eyyübiye, and one peripheral district, Suruç. Considering urbanization characteristics, it can be said that Haliliye is the main settlement with heavy traffic, Karaköprü is the new settlement, and Eyyübiye is the suburb region. Suruç is the preferred peripheral district because most excessive pigeon breedings are made here. This is the first study conducted in Turkey to detect air pollution in the central districts of a metropolis by determination of Cd and Pb concentrations by inductively coupled plasma-mass spectrometry (ICP-MS) in male and female. Feral pigeon blood samples were collected from several districts of the Şanlıurfa province in different seasons.

2. Materials and methods

This study was aimed at the assessment of the degree of air pollution at the district level in different seasons to determine the impact of air pollution on Feral pigeons in Şanlıurfa province and its vicinity. The study was conducted according to the 2016/24 numbered and 22/07/2016 dated

decision of the Local Board for Animal Experiments of Dollvet Industry and Trade Inc. All Feral pigeons were raised on the hygienic feed by breeders in Suruç, Haliliye, Karaköprü and Eyyübiye districts of Şanlıurfa province (Figure 1) [23] in November 2016 (autumn), February 2017 (winter), May 2017 (spring), and August 2017 (summer) to represent all four seasons. Sixty-four Feral pigeons in total, eight pairs per location were enrolled, and blood samples of two pairs (one male and one female) of pigeons per season and location were pulled from vena cutanea ulnaris located under the wings. The blood was taken to an eppendorf tube without anticoagulants and kept at $-20\text{ }^{\circ}\text{C}$ until analyses.

During preparation and measurements of blood samples, a sample of blood was placed in a Teflon reactor, with nitric acid (HNO_3) at 65%, and then 30% hydrogen peroxide (H_2O_2) added. Thermal digestion was carried out in an oven at $90\text{ }^{\circ}\text{C}$. Depending on the volume of blood contained in the tubes, different amounts of HNO_3 and H_2O_2 were used until the digestion of blood samples were complete, after (24–48 h) the samples were left in the oven. The proportion of 1:5:5 was applied to the mixture of blood, HNO_3 , and H_2O_2 , respectively. After digestion, the sample was allowed to cool, brought up to a volume of 20, 30, or 40 mL with tetra-distilled purified water, and

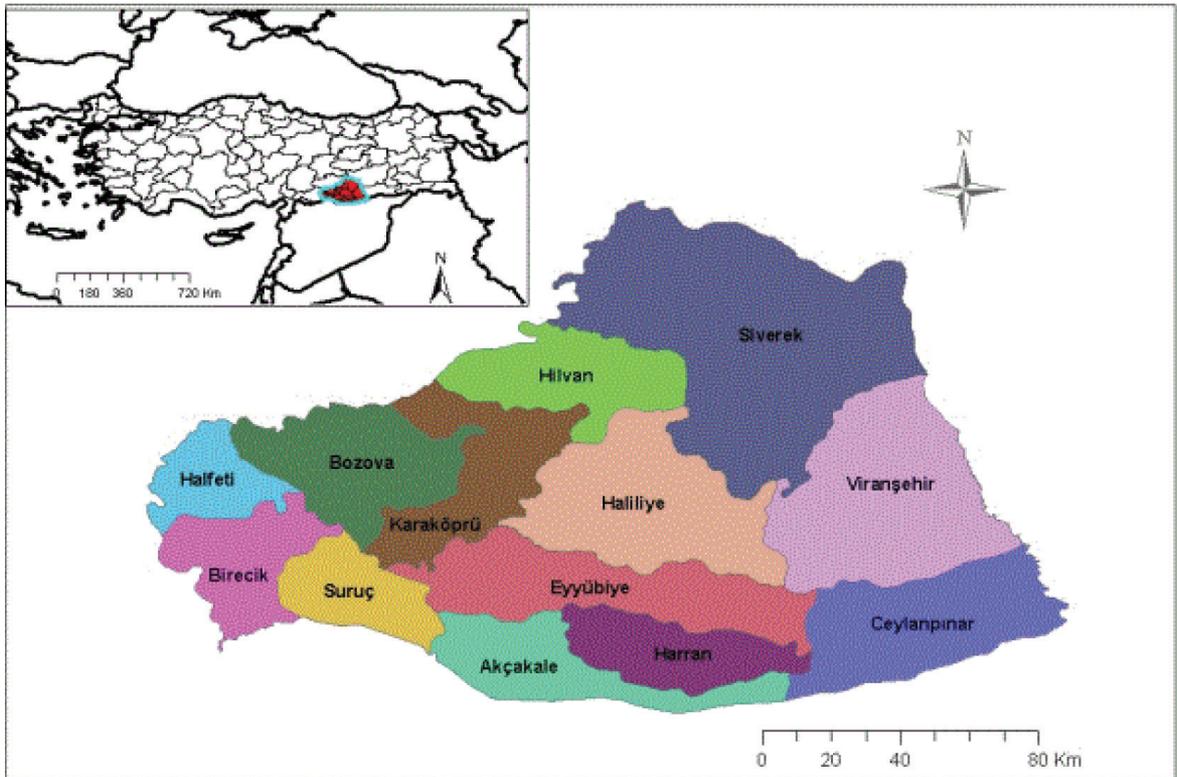


Figure 1. Sixty-four blood samples obtained from pigeons located in three central districts, namely Haliliye, Karaköprü and Eyyübiye, and one peripheral district, Suruç

transferred to the measuring vessel. The teflon reactors used for the wet digestion were previously washed with 3 mL of HNO₃, left in the oven for 2 h, and then rinsed twice with tetra distilled water and finally dried in an oven at 100 °C. Blood concentrations of Cd and Pb were measured by inductively coupled plasma mass spectrometry [24]. The device was Perkin-Elmer SCIEX ELAN 6000 ICP-MS (PerkinElmer, Inc., Waltham, MA, USA). Table 1 presents the device conditions under which blood samples were measured. The limits of detection (LOD) value, which is measured as three times the standard deviation of the reagent blanks, were 0.042 (Cd) and 0.025 (Pb) µg/L. The limits of quantification (LOQ) were 0.312 (Cd) and 0.269 (Pb) µg/dL. The recovery rates were calculated as 94.5% for Cd and 101.8% for Pb.

2.1. Statistical analyses

Descriptive and inferential statistical analyses were performed using IBM SPSS 21.0 packet program (SPSS Inc., Armonk, NY, USA). Data were log-transformed to obtain normal distributions that satisfied the homogeneity of variance required by one-way analysis of variance (ANOVA). Metal concentrations in all sex groups, locations, and seasons were evaluated for normal distributions and, if needed, the least significant difference (LSD) posthoc test was used in the comparison of averages of features, which were found significant. A P value less than 0.05 was considered statistically significant. The concentrations of metals in tissues were expressed as µg/kg (parts per billion) dry weight.

3. Results

The analyses of the blood samples taken from 64 Feral pigeons raised by private breeders in the Haliliye, Eyyübiye, Karaköprü and Suruç districts of the Şanlıurfa province revealed Pb concentrations of 233.1 ± 25.7 µg/kg for Haliliye, 101.2 ± 29.7 µg/kg for Karaköprü, 81.3 ± 22.7 µg/kg for Eyyübiye, and 48.1 ± 27.1 µg/kg for Suruç, and Cd concentrations of 30.6 ± 12.6 µg/kg for Haliliye, 35.7 ± 9.2 µg/kg for Karaköprü, 28.1 ± 8.7 µg/kg for Eyyübiye, and 16.8 ± 4.1 µg/kg for Suruç. The highest Pb levels were measured in the blood samples taken from the pigeons raised in the Haliliye district (Table 2). The mean values and standard deviations (± SD) of the Cd and Pb levels measured in the blood samples of male and female Feral pigeons raised in the districts of the Şanlıurfa province are presented in Table 3. The seasonal distribution of the Cd and Pb levels measured in blood samples from the central districts of the Şanlıurfa province is shown in Figure 2.

Higher Cd and Pb concentrations were detected in the blood samples of male Feral pigeons, compared to those of female pigeons. Similar Pb levels were detected in the blood samples taken in November, February, and August from pigeons raised in the central districts of

Table 1. ICP-MS (Inductively coupled plasma – mass spectrometer) device conditions under which blood samples were measured (RF: radio frequency. STD/KED Mode: Standard/ Kinetic Energy Discrimination Mode).

Component / Parameter	Type / Value / Mode
Nebulizer	Mainhard (concentric)
Spray chamber	Glass cyclonic
Triple cone interface material	Nickel
Plasma gas flow	18.0 L/min
Auxiliary gas flow	1.2 L/min
Nebulizer gas flow	0.94 L/min
Sample uptake rate	1 mL/min
RF power	1500 W
Replicates per sample	3
Mode of operation	STD/KED Mode Collision (using He gas)

Table 2. The mean values and standard deviations (± SD) of the Cd and Pb concentrations detected in the blood samples of male and female pigeons raised in three central districts, namely Haliliye, Karaköprü and Eyyübiye, and one peripheral district, Suruç.

Sex	Female (n = 32)	Male (n = 32)
Lead (µg/kg)	100.75 ± 22.17	128.81 ± 29.72
Cadmium (µg/kg)	26.64 ± 1.94	29.35 ± 2.10

Table 3. The mean values and standard deviations (± SD) of the Cd and Pb concentrations (µg/kg) detected in the blood samples of pigeons raised in three central districts, namely Haliliye, Karaköprü and Eyyübiye, and one peripheral district, Suruç.

Districts of Şanlıurfa	Metals	
	Cd	Pb
Eyyübiye (n = 16)	28.1 ± 8.7	81.3 ± 22.7
Haliliye (n = 16)	30.6 ± 12.6	233.1 ± 257.5
Karaköprü (n = 16)	35.7 ± 9.2	101.2 ± 29.7
Suruç (n = 16)	16.8 ± 4.1	48.1 ± 27.1

Şanlıurfa province. However, the Pb levels measured in the blood samples taken in May selected to represent the spring season, statistically differed from the levels detected in the samples taken in the other three seasons. The Pb concentrations detected in the blood samples taken in

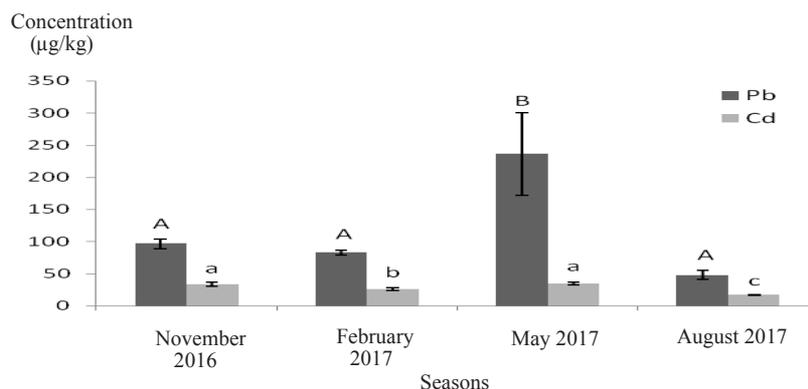


Figure 2. The seasonal distribution of the Cd and Pb concentrations ($\mu\text{g}/\text{kg}$) detected in pigeons blood samples obtained from the districts of the Şanlıurfa province ($n = 16$ in total for each season).

May were significantly higher. The Cd levels detected in the samples taken in November and May were similar, but the levels detected in the samples taken in February and August showed statistically significant differences ($p < 0.05$). Higher Cd levels were detected in the samples collected in November and May, compared to those collected in February and August.

4. Discussion

Of the central districts of Şanlıurfa province included in the present study, Haliliye is characterized by heavy vehicular traffic, presented with high Pb levels in the tested Feral pigeon blood samples ($233.1 \pm 25.7 \mu\text{g}/\text{kg}$). Schilderman et al. [14] reported pigeon blood Pb concentrations as $253 \pm 182 \mu\text{g}/\text{kg}$ for heavy vehicular traffic zones, $73.3 \pm 18.6 \mu\text{g}/\text{kg}$ for moderate vehicular traffic zones, $33 \pm 30.4 \mu\text{g}/\text{kg}$ for Maastricht, and $26.6 \pm 17.9 \mu\text{g}/\text{kg}$ for Assen. In their studies on Pb concentrations in pigeon blood samples from Morocco, Elabi et al. [25] reported levels as $16.4 \pm 1.60 \mu\text{g}/\text{kg}$ for Kamra, $22.12 \pm 2.56 \mu\text{g}/\text{kg}$ for the town centre, $31.33 \pm 2.85 \mu\text{g}/\text{kg}$ for Oulja, and $11.17 \pm 2.14 \mu\text{g}/\text{kg}$ for Allal Behraoui, whilst Kouddane et al. [21] reported $206.36 \pm 34.97 \mu\text{g}/\text{kg}$ for industrial zones, $167.54 \pm 31.65 \mu\text{g}/\text{kg}$ for the town centre, $68.98 \pm 22.58 \mu\text{g}/\text{kg}$ for moderate vehicular traffic zones, and $25.14 \pm 6.02 \mu\text{g}/\text{kg}$ for less polluted zones. In their research on the use of blood for the detection of Pb exposure, Scheuhammer et al. [26] reported that blood Pb concentrations of $48 \pm 33 \mu\text{g}/\text{kg}$ should be interpreted as normal and indicated that while a level of $150 \mu\text{g}/\text{kg}$ would indicate the absence of abnormal Pb exposure, a blood concentration of $200 \mu\text{g}/\text{kg}$ would indicate mild exposure to Pb. The Pb concentrations detected in the present study in Feral pigeon blood samples from the Haliliye district are similar to the levels reported by Schilderman et al. [14] and Kouddane et al. [21], who attributed very high Pb levels to heavy vehicular traffic. In the present study, the highest Pb levels were measured

in the pigeon blood samples from Haliliye, which is the largest central district of the Şanlıurfa province and is characterized by heavy vehicular traffic.

Pain [27], Franson [28], and Friend and Franson [29] have indicated that blood Pb concentrations reaching a level of 0.5–1 ppm (approximately 2.6–5.2 ppm) are associated with biological function disorders and the onset of clinical signs. In their research on songbirds, Beyer et al. [30] reported that while increased blood Pb concentrations were associated with the inhibition of delta-aminolevulinic acid dehydratase at a level of more than 50%, Cd exposure had no such effect. Furthermore, it has been reported that, in birds, blood Pb levels above $400 \mu\text{g}/\text{kg}$ disrupt physiological functions [31], and blood Pb levels above $200 \mu\text{g}/\text{kg}$ are associated with subclinical signs related to the inhibition of delta-aminolevulinic acid dehydratase [32].

Several studies have been conducted to investigate blood Cd concentrations. In their study, in which they tested pigeon blood samples from four different regions of Amsterdam, Schilderman et al. [14] reported Cd concentrations of $5.24 \pm 2.48 \mu\text{g}/\text{kg}$ for heavy vehicular traffic zones, $6.20 \pm 2.96 \mu\text{g}/\text{kg}$ for moderate vehicular traffic zones, $7.58 \pm 2.60 \mu\text{g}/\text{kg}$ for Maastricht, and $6.67 \pm 2.0 \mu\text{g}/\text{kg}$ for Assen. In a study on pigeon blood samples from Morocco, Elabidi et al. [25] reported Cd concentrations of $1.58 \pm 0.35 \mu\text{g}/\text{kg}$ for Kamra, $2.24 \pm 0.3 \mu\text{g}/\text{kg}$ for the town centre, $1.13 \pm 0.3 \mu\text{g}/\text{kg}$ for Oulja and $0.34 \pm 0.04 \mu\text{g}/\text{kg}$ for Allal Behraoui. In another study on pigeon blood samples from Morocco, Kouddane et al. [21] reported Cd concentrations of $0.89 \pm 0.34 \mu\text{g}/\text{kg}$ for less polluted zones, $2.48 \pm 1 \mu\text{g}/\text{kg}$ for moderately polluted zones, 6.2 ± 2.71 for the town centre, and 5.97 ± 2.27 for industrial zones. In the present study, the highest Cd concentration was detected in samples from the Karaköprü district ($35.7 \pm 9.2 \mu\text{g}/\text{kg}$). When compared to the results of previous research, the Cd concentration detected in the present study in Feral pigeon

blood samples from the Karaköprü district were observed to be very high.

Numerous studies have been conducted on the investigation of Cd and Pb concentrations in blood samples of various bird species. Brumbaugh et al. [33] reported Cd levels of 140 µg/kg and 4 µg/kg (mean level of 9 ± 6 µg/kg for both species), and Pb levels of 520 µg/kg and 60 µg/kg (mean level of 290 ± 320 µg/kg for both species), respectively, for an American tree sparrow (n= 1) and redpoles (n = 3) live-captured in Aufesi-Alaska; Cd levels of 4 µg/kg and 7 µg/kg and Pb levels of 3 µg/kg and 30 µg/kg, respectively, for American tree sparrows (n = 2) and redpoles (n = 6) captured near a mining haul road of the Delong Mountain Regional Transportation System, and Cd levels of 14 µg/kg and 20 ± 20 µg/kg and Pb levels of 490 µg/kg and 380 ± 140 µg/kg (mean level of 18 ± 5 µg/kg for both species), respectively, for American tree sparrows (n = 2) and Savannah sparrows (n = 6) captured in Newheart-Alaska. In their study, in which they collected blood samples from migratory shorebirds at an early and late period during their migration stopover at Delaware Bay-USA, Tshipoura et al. [34] reported early Cd and Pb levels as 3.94 ± 1.37 µg/kg and 75.38 ± 15.52 µg/kg, respectively, and late Cd and Pb levels as 1.98 ± 0.41 µg/kg and 105.40 ± 18.78 µg/kg, respectively, for red knots (n = 15); early Cd and Pb levels as 2.54 ± 1.42 µg/kg and 145.00 ± 12.56 µg/kg, respectively, and late Cd and Pb levels as 1.49 ± 0.41 µg/kg and 33.60 ± 4.05 µg/kg, respectively, for sanderlings (n = 15); and early Cd and Pb levels as 2.01 ± 0.80 µg/kg and 42.39 ± 8.42 µg/kg, respectively, and late Cd and Pb levels as 1.59 ± 0.55 µg/kg and 77.27 ± 18.57 µg/kg, respectively for semipalmated sandpipers (n = 15). Furthermore, in their study on Cd and Pb analyses in blood samples from young and adult seagulls in Northeast Minnesota, Burger, and Gochfeld [35] reported Cd and Pb levels as 99 ± 16 µg/kg and 12 ± 3 µg/kg, respectively, for young seagulls (n = 14), and Cd and Pb levels of 6 ± 1 µg/kg and 29 ± 10 µg/kg, respectively, for adult seagulls (n = 14). The Cd and Pb levels detected in young seagulls captured in 1993 in New York Bay were reported as 9 ± 1 µg/kg and 94 ± 5 µg/kg, respectively, and according to the analyses performed in

1994, Cd and Pb levels were detected as 47 ± 12 µg/kg and 176 ± 54 µg/kg, respectively, in young seagulls, and as 16 ± 7 µg/kg and 233 ± 61 µg/kg, respectively, in adult seagulls [35].

Different levels of heavy metal bioaccumulation having been detected in different bird species have been attributed to heavy metal absorption rates and sand vs. grit preferences varying with species [36]. Seasonal monitoring has demonstrated that both Cd and Pb bioaccumulation levels are higher during spring. In their research, in which they collected blood samples during different seasons from pigeons raised in various neighborhoods of New York City, Cai and Calisi [20] determined that blood Pb levels were highest in summer. The researchers attributed high Pb bioaccumulation in summer to the increased seasonal activity of pigeons in this season, which led to their increased exposure to atmospheric Pb. Likewise, in the present study, the Cd and Pb levels detected in Feral pigeon blood samples being highest in spring could be attributed to the increased seasonal activity of these birds.

5. Conclusion

Pb levels were highest in Feral pigeon blood samples from the Haliliye district, which is a heavy vehicular traffic zone, and Cd levels were highest in the Karaköprü district, where unplanned settlement is observed. Seasonal monitoring demonstrated that Pb levels were higher in spring (May), and Cd levels were higher in spring and autumn. Based on the results of this study, aimed at determining the level of air pollution in the central districts of Karaköprü, Haliliye, Suruç, and Eyyübiye of the Şanlıurfa province, it is suggested to increase the use of public transport, encourage the use of environmentally kind alternative energy resources, adopt a planned urbanization approach, and increase afforestation. For heating purposes, the use of natural by-products such as olive pomace should be encouraged and increased as an alternative to conventional fuels, such as coal and wood.

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