



**BIOACCUMULATION OF LEAD IN TISSUES OF *PASSER DOMESTICUS* AS A  
BIOINDICATOR SPECIES IN BIRJAND**

**Seyed Hosein Seyed Khorami<sup>1\*</sup>, Mojtaba Zamani<sup>2</sup> and Mohammadreza Usefpur<sup>3</sup>**

<sup>1</sup>\*Department of Environmental Science, Faculty of Natural Resources, College of Agriculture & Natural Resources, University of Tehran, Daneshkadeh Ave., Karaj-Iran.

<sup>2</sup>Tarbiat Modares University, School of Natural Resource and Marine Sciences, Noor, Mazandaran, Iran.

<sup>3</sup>Islamic Azad University, Lahijan Branch, Department of Electronic, Lahijan, Guilan, Iran.

**\*Corresponding Author: Seyed Hosein Seyed Khorami**

Department of Environmental Science, Faculty of Natural Resources, College of Agriculture & Natural Resources, University of Tehran, Daneshkadeh Ave., Karaj-Iran.

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**ABSTRACT**

Recently development of human lifestyle and urbanization have resulted in elevated levels of different contaminants in our environment. Heavy metals specially lead (Pb) is a main issue worldwide, regional and local level impacts the functional of an organism. In current study, House Sparrow (*Passer domesticus*) investigated for Pb concentrations, using liver and feathers from two areas. Samples were caught, prepared and digested and the contents of Pb was analyzed. The mean of Pb concentration in female feathers and liver was significantly different than that found in male feathers and livers from Shokat site. In addition, the average of Pb concentration of birds in Shokat site were significantly higher than those of reference site in both liver and feathers. Based on the results, it could be concluded that House Sparrow have a great capability to apply as a bioindicator species in future monitoring studies.

**KEYWORDS:** Lead, Liver, Feathers, Birjand, *Passer domesticus*, bioindicator.

**INTRODUCTION**

Human populations are becoming increasingly in urban area. Urbanization and industrialization that serve to provide the necessities of the population are well known source of environmental pollution. Large quantities of pollutants such as medical waste, polycyclic aromatic hydrocarbons, organic solvents, pesticides, perfluorinated chemicals and heavy metals have continuously been introduced into ecosystems as a consequence of urbanization and industrial processes.<sup>[1-4]</sup>

Heavy metals pollution resulted from intense industrialization and urbanization has become a serious concern in many developing countries.<sup>[1,5]</sup> Metals are pollutants that can bioaccumulate in the tissues of organisms also they can change the biological equilibrium of existing ecosystems.<sup>[6]</sup> This has led to the development of monitoring schemes aimed at direct measurement of contaminant levels in various organisms, and biomonitoring schemes that use indicator species to estimate the levels in other parts of the ecosystem.

There are three different categories of experiments to evaluate the behavior of xenobiotics in the body of organism: *in vitro* studies, *in vivo* studies, and *in silico* experiments. *In vitro* experiments refer to the studies of performing a given procedure in a controlled environment outside of a living organism<sup>[7,8]</sup>, while *in*

*vivo* investigations apply a whole, living organism as opposed to a partial or dead organism.<sup>[2,9]</sup> Moreover, *in silico* studies investigate the behavior of xenobiotics in body of animals via computer simulation with various software.<sup>[10,11]</sup> Over the past years, several *in vivo* studies have been performed in the monitoring of ecosystem health after environmental contamination by applying different animals such as rodents and birds.<sup>[9,12,13]</sup> In order to estimate this level, there are lots of approaches including the smart approaches in which we are going to analyze the materials of the air based on the experiments that have been done. In other words, we try to find a pattern in the experiments results helping us to determine all the features.<sup>[14,15]</sup>

Birds are much easier to study than other bioindicator and this makes them ideal for monitoring researches.<sup>[16]</sup> Birds have been used successfully for biomonitoring of the different concentrations and effects of heavy metals in the environments, because they occupy a relatively high trophic position in the aquatic/marine and terrestrial food chains abundant, sensitivity and being the first target of atmospheric pollution, important structural components of the ecosystem, having fast metabolic rate, easy to identify, long-lived and are common and widespread.<sup>[17-19]</sup> Previous studies in avian natural population, show a positive correlation between the degree of pollution and accumulation of some heavy

metals in different tissues, of different animals, such as *Hirundo rustica*.<sup>[20]</sup>, *Larus argentatus*<sup>[21]</sup>, *Parus major* and *Parus caeruleus*<sup>[22]</sup>, *Otus lempiji*, *Bubo bubo* and *Nixos scutulata*<sup>[13]</sup>, *Oxyura leucocephala* and *Marmaronetta angustirostris*<sup>[23]</sup>, *Bubulus ibis*<sup>[24]</sup>; *Buteo buteo*<sup>[25]</sup>, *Ficedula hypoleuca* and *Parus major*<sup>[26]</sup> and *Puffinus gravis*.<sup>[27]</sup>

The House Sparrow (*Passer domesticus*) is a species of passerine bird of the sparrow family if Passeridae. Sparrow historically is known as one of the birds in the urban environment worldwide that had a close relationship with people and known as the bird with the highest spread on the planet.<sup>[28]</sup> The House Sparrow is one of the most successful birds in the urban environment and has a global distribution. It is introduced nearly in all continents, and distributed to most of Europe and Central Asia and is a non-migratory sedentary bird. Sparrow is primarily a seedeater and has closest radius (300 meters from the nest).<sup>[29]</sup> Sparrow can be applied as a proper bioindicator species for monitoring purposes due to occupying variety of environments along the urban gradient, foraging in small home ranges, high reproductive rate, and high tolerant of urban environmental stress. Moreover, they are useful as bioindicators of urban pollution and based on our review of the literature, several studies applied sparrow as a bioindicator species in different areas.<sup>[24,30-32]</sup>

In general, heavy metal concentrations in the external tissues like feathers are representative of long-term exposure, whereas the internal tissues such as liver concentrations reflect short-term exposure.<sup>[21]</sup> Feathers represent exogenous contamination of metals as well as representing metal concentrations in the bloodstream during growth.<sup>[33]</sup> The advantage of using feathers is the non-destructive and non-invasive sampling technique. Exploring the relationship between feather metal concentrations and the absorption in internal tissues cause to extend the non-destructive method. Non-destructive methods impose minimal stress to populations. Due to practical and ethical reasons inhibit the sacrifice of free-living animals, several studies have

been used feathers as methods for non-destructive biomonitoring of heavy metal accumulation.<sup>[24,34-37]</sup>

Recently, Birjand has transferred from a normal city to the capital South Khorasan, therefore it has faced a rapid increase in industrial, economic, cultural, political, commercial and educational activities. The rapid extension of Birjand cause several problems such as rural-urban migration, population increasing, destroying agricultural land, parks and open space, industrial development and more industrial effluents, high traffic density and high consumption of natural resources as well as windblown dusts which the main source of different kinds of environmental pollution especially heavy metals pollution. However, the environments in Birjand are subject to increasing pollution, in particular air pollution resulting from transportation. Here, we measured the concentration lead (Pb) in feather and liver of the House Sparrow as a bioindicator species. Our objectives are to 1. Quantify the distribution of Pb in sparrow, 2. Assessing the use of feathers as proxy for internal metal accumulation as a basis for future studies using only non-destructive methods 3. Provide baseline information for comparison with future studies.

## MATERIAL AND METHODS

### Study area and sampling

Birjand, the capital of South Khorasan, is a fast-growing city, thus becoming one of the major centers in the East of Iran. The area of Birjand is 42.7 km<sup>2</sup> and located in 32°52'N 59°12'E and an altitude of 1459 m above sea level. Birjand has a dry climate with cold winters and very hot and dry summers. Nowadays, the use of chemicals is increasing in different parts such as industries, home, farms and vehicles with growing populations of Birjand.

We consider two sampling areas, one close to Birjand-Zahedan road (Shokat site) and one sampling area located in rural area near Birjand (Bagheran site) as a reference site. High transportation observes near Shokat site (Fig 1). This region is located near the Birjand-Zahedan road which has high volume of traffic every day.

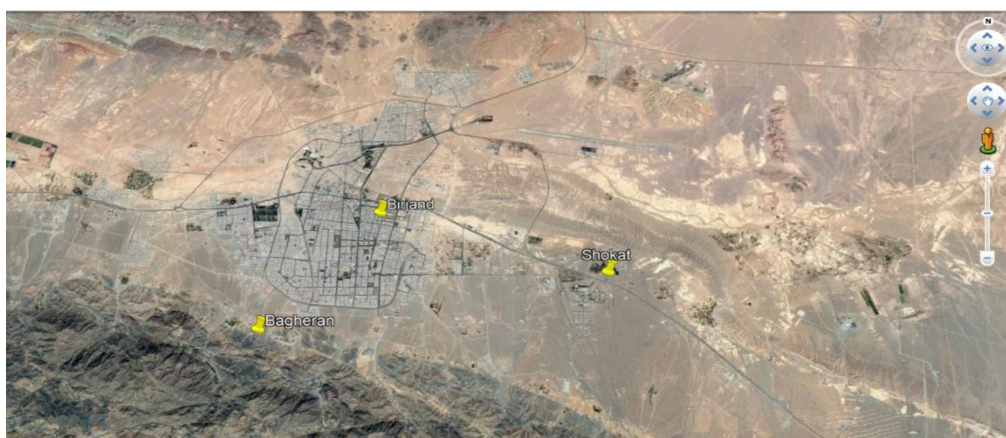


Fig 1: The location of sampling areas.

Natural individuals of the population of House Sparrow were collected by mist nets during May to July of the year 2016. We caught 19 sparrows from Shokat site and 22 from the reference site. The gender of each birds was determined. The 41 sparrows comprised 24 males (14 reference site and 10 Shokat site) and 17 females (8 reference site and 9 Shokat site). Each bird was euthanized using ether after capture and then weighed and sexed under clean condition. Each bird was euthanized using ether after capture and then weighed under clean condition. Polyethylene bags were applied for storing birds and then samples were transferred directly to the lab and kept at deep freeze (-18 °C), until they were analyzed to prevent tissue lysis and possible redistribution of Pb between tissues.

### Sample preparation

After biometric measurements, birds were carefully dissected to avoid external contamination, and liver was removed and weighed to an accuracy of  $\pm 0.001$ g. Breast feathers are considered to be more representative of exposure to metals than are other feathers, and do not reflect molt sequence as do flight feathers. Therefore, a pinch of feathers from each side of the sparrow's breast was plucked with a stainless scissors. In the lab, feathers were washed three times with acetone, alternating with deionized water to remove loosely adherent external contamination (Burger, 1993). Feathers and liver were placed in an oven (60°C) for 24 h and the dry weight was determined. Dried sample was digested with 5 mL of nitric acid (65% HNO<sub>3</sub>) and 1 mL hydrogen peroxide (30% H<sub>2</sub>O<sub>2</sub>) in the digestion system for 30 min. All

samples were diluted to 50 ml with deionized water and were preserved at -18°C until analysis. The Pb concentrations were measured with ICP-OES (VARIAN, 725-ES) instrument. Blank solution was prepared and carried through each 5 samples to check for contamination during the digestion procedure and sample manipulation. The blank concentrations were lower than the respective detection limits, and detection limits was 0.05 ppm for Pb.

### Statistical analysis

Significant differences were assumed at  $p < 0.05$ . All statistical analyses were conducted using the Minitab (v.17) program. All data were tested for normality by using Kolmogorov--Smirnov one-sample tests. Due to normal distribution and homogeneity of variance of Pb levels in the tissues, parametric statistics were employed.

### RESULT AND DISCUSSION

The concentrations of Pb in the liver and feather of both male and female between areas are presented in Tables 1 and 2 expressed as  $\mu\text{g} / \text{g}$  dry weight (dw). Adrian and Stevens (1979) indicated that analyses based on dry tissues increase the reliability and consistency of an experiment. The average levels of Pb in the liver of House Sparrows were 24.32 and 10.26  $\mu\text{g} / \text{g}$  dw in Shokat and reference sites which represents significant difference between the areas ( $P < 0.01$ ). Moreover, there was a significant difference between the average concentration of Pb in feathers of birds in the Shokat site (39.96  $\mu\text{g} / \text{g}$  dw) and reference site (18.26  $\mu\text{g} / \text{g}$  dw) (Fig. 1).

**Table 1: The concentration of Pb (averages  $\pm$  SE,  $\mu\text{g}/\text{dry weight g}$ ) in liver.**

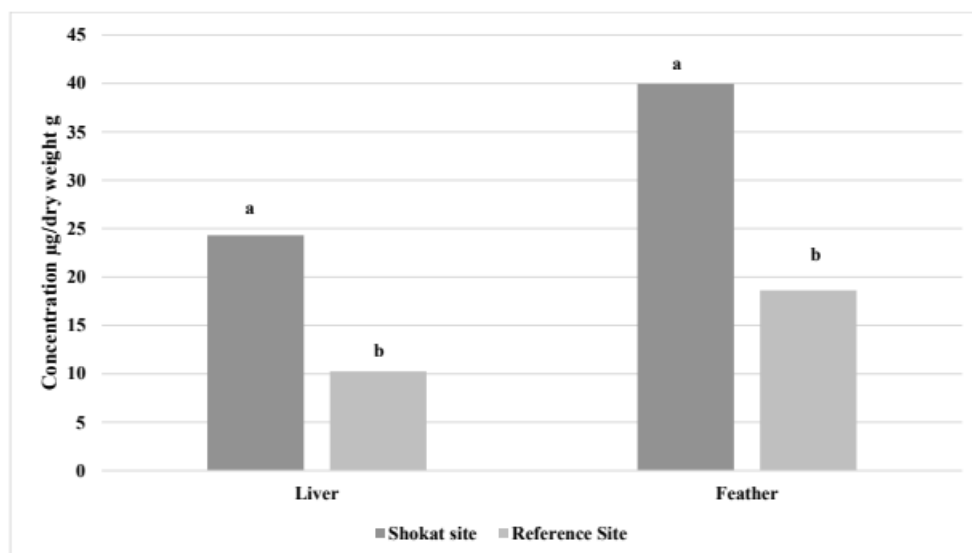
Area	Shokat site		Reference Site	
	Male	Female	Male	Female
Average	21.09 $\pm$ 6.67 <sup>a*</sup>	27.55 $\pm$ 7.14 <sup>b</sup>	7.42 $\pm$ 3.22 <sup>a</sup>	13.1 $\pm$ 3.22 <sup>b</sup>
Minimum	14.6	12.59	8.96	8.47
Maximum	24.87	31.02	29.45	36.08
Total Average	24.32 <sup>a</sup>		10.26 <sup>b</sup>	

\*Letters denote statistically significant differences ( $P < 0.05$ ) between genders in Pb concentrations (a>b)

**Table 2: The concentration of Pb (averages  $\pm$  SE,  $\mu\text{g}/\text{dry weight g}$ ) in feather.**

Area	Shokat site		Reference Site	
	Male	Female	Male	Female
Average	34.29 $\pm$ 12.53 <sup>a*</sup>	45.63 $\pm$ 10.28 <sup>b</sup>	17.12 $\pm$ 8.5 <sup>a</sup>	20.12 $\pm$ 6.96 <sup>b</sup>
Minimum	19.33	25.67	8.96	8.47
Maximum	43.28	53.24	29.45	36.08
Total Average	39.96 <sup>a</sup>		18.62 <sup>b</sup>	

\*Letters denote statistically significant differences ( $P < 0.05$ ) between genders in Pb concentrations (a>b).



**Fig 1: The average concentration of Pb in liver and feather of House Sparrows in Shokat and reference site.**

Our results are in accordance with previous studies that show elevated levels of heavy metals in the body of birds living in the urban areas. Kekkonen *et al.* 2012 found the levels of heavy metals in the livers of House Sparrows sampled in the urban areas higher than in rural areas. In addition, previous studies also reported the high concentration of heavy metals in the tissues of House Sparrows living in urban regions.<sup>[32,38,39]</sup> Moreover, Millaku *et al.* (2015) reported the higher concentration of some heavy metals to be significantly higher in sparrows from the polluted area when compared to tissues from the reference site.

In order to justify high concentrations of Pb in Shokat site in both liver and feathers of birds in compare with reference site, the results of the study should compare with the level of CO and NO<sub>x</sub> to understand the source of pollution. Probably the high volume of transportation in Shokat site cause increasing the level of Pb. The concentration of Pb in feather was higher than livers in our study. Franson *et al.* (1996) reported that 2 mg/kg dw of Pb causes subclinical physiological impacts on different bird species. The concentration of Pb in the liver of birds in our study in both areas was higher than amount of Pb in liver of House Sparrows from polluted and reference sites in Millaku, Imeri and Trebicka (2015) research. Due to heavy metal contamination by secretory products and exposure to the environment, it is hard to interpret the concentration of Pb in the feathers.<sup>[42]</sup> Generally, species, age, sex, tissues and environmental factors are important features affecting the bioaccumulation of heavy metals in the body of organisms.<sup>[43]</sup>

The results of study indicated that there is a significant difference between the concentrations of Pb of male and female in Shokat and reference sites ( $P < 0.05$ ). Female birds show higher concentration of Pb in the liver and feather in both areas in our study (Table 1 and 2). Eeva, Hakkarainen and Belskii (2009) reported that male

House Sparrows represent high concentration of heavy metals in the liver than female. Some reasons might justify the high concentration of heavy metals in the body of House Sparrow females such as higher reproductive efforts and differences in dispersal. In addition, females tend to dispersal more form their habitats than males.<sup>[32,44,45]</sup> In the other hand, Swaileh and Sansur 2006b did not report any significant difference between the sexes.

Bioindicator species could help to monitor the contaminants and determine the quality of environment. Bioindicator species applying in monitoring studies should have a large population, high metabolic rate, clear gender differences, sampled easily, response to different level of pollution, large geographical distribution and are typically found in both contaminated and noncontaminated area.<sup>[9]</sup> In consequence, House Sparrows could consider as a proper bioindicator species in future studies.

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