Journal of Human Environment and Health Promotion
Print ISSN: 2476-5481 Online ISSN: 2476-549X

Survey on Lead Contamination of Brown Rats (*Rattus norvegicus*) as a Bioindicator in Urban Areas of Golestan and Mazandaran Provinces

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ARTICLE INFO

Article type:
Original article

Article history:
Received: 1 January 2024
Revised: 21 January 2024
Accepted: 3 February 2024

https://doi.org/10.61186/jhehp.10.1.52

Keywords:
Heavy metal
Rodent
North Iran
Golestan
Mazandaran

ABSTRACT

Background: Heavy metals, such as lead harms animals' health. The objective of this study was to evaluate the level of Pb pollution in the cities of Golestan and Mazandaran Provinces by measuring Pb concentrations in the bloods of brown rats, which serve as omnivorous bioindicators living in the same habitat as humans.
Methods: Eight highly populated cities were selected, with 10 rats trapped in each city. Blood samples were taken from the rats, and Pb measurements were done by atomic absorption spectrometry.
Results: All sampled rats were Pb-contaminated. The mean Pb concentrations in different cities of Golestan Province were statistically similar ($p > 0.05$). In Mazandaran cities, however, significant differences in mean Pb concentrations were observed. The highest contamination was recorded in Neka (10.28 ± 0.97 µg/dL), while the lowest contamination was observed in Babol (7.3 ± 1.36 µg/dL). The mean Pb concentrations in the studied cities of Golestan (9.43 ± 1.81 µg/dL) and Mazandaran (9.02 ± 1.07 µg /dL) Provinces, as well as in male (10.38 µg/dL) and female (9.25 µg/dL) rats were similar.
Conclusion: The Pb contaminations observed in the sampled rats are indicative of Pb contaminations of the sampled cities and the presence of Pb sources in these areas. It seems necessary to take precautionary procedures to prevent the entry of polluted effluents into the sampled cities and the consumption of Pb-contaminated gasoline in these areas, to prevent Pb contamination of human populations.

1. Introduction

Despite the need for some essential metals such as iron and calcium for various metabolic activities in organisms, even low concentrations of heavy metals, such as lead (Pb), in animal bodies can be detrimental and toxic. Interestingly, Pb is widely used worldwide, following iron, aluminum, copper, and zinc [1, 2]. Pb is utilized in various industries such as paints, water pipes, ceramics, cables, medicines, cosmetics, and gasoline. Animals are exposed to this heavy metal through skin absorption, inhalation of polluted air, and consumption of contaminated food [1]. Pb accumulation in the bodies of living organisms can cause severe acute or chronic disorders. Acute exposure may lead to loss of appetite, hypertension, abdominal pain, renal dysfunction, fatigue, sleeplessness, arthritis, hallucinations, and vertigo. Chronic presence of Pb in animals' bodies also may cause dysfunction in the kidneys, liver tissues, and reproductive system. Unfortunately, this metal can be transferred from mother to fetus through the placenta, leading to defects in the fetus and ultimately malformations [1, 2]. The nervous system is also affected by Pb. Headache, irritability, memory loss, and fatigue are among the first signs of Pb exposure in the central nervous system (CNS) [2, 3]. Notably, 5 µg/dl of Pb in children's blood has adverse impacts on intelligence quotient and intensifies aggression and loss of concentration

How to cite: Shahbazadeh Bengar A, Namroodi S, Galdavi S. Survey on Lead Contamination of Brown Rats (Rattus norvegicus) as a Bioindicator in Urban Areas of Golestan and Mazandaran Provinces. *J Hum Environ Health Promot.* 2024; 10(1): 52-6.

[1]. Due to the highly toxic effects of Pb on living organisms, the use of Pb additive in automotive gasoline, and paint pipes has been banned in many advanced countries such as the United States [1]. Unfortunately, in Iran, limitation laws about Pb usage are not well implemented and Pb contamination continues to threaten the health of humans and other animals [4]. Golestan and Mazandaran Provinces, known for their temperate and humid climate conditions, are among the most populous Provinces in Iran. Agricultural and industrial activities are concentrated in these areas [5]. The results of surveys on soil and water resources in non-urban areas of the aforementioned Provinces have shown Pb contaminations of the mentioned resources. Studies by Nazari (2017) and Mirzaei *et al.* (2015) have documented Pb contamination along the west and east coasts of Golestan Province and in surface soils of Golestan Province, respectively [6, 7]. However, there is a lack of data about Pb contamination in urban ecosystems of Golestan and Mazandaran Provinces. Therefore, an evaluation of Pb pollution in the cities of these two Provinces is required to ensure the well-being of the native population. The accumulation of elements or chemicals in an organism is called bioaccumulation [1]. Omnivorous animals such as rodents, which have a low home range, can be suitable bioindicators to assess Pb contamination in differentecosystems [8]. Brown Rats (*Rattus norvegicus*), considered suitable bioindicators, belong to the rodent family and are abundant in the northern cities of Iran. These rats inhabit human-dependent habitats and close to water ecosystems [8, 9]. As there is a lack of information about Pb contamination in urban areas of northern Iran, we aimed to analyze Pb contamination in highly populated cities of Golestan and Mazandaran Provinces by measuring Pb concentration in blood samples of brown rats.

2. Materials and Methods

2.1 Sampling

During the spring and winter of 2018, the eight most populated cities in Golestan (Bandar-e-Gaz, Aq Qala, and Azadshahr-Minoodasht) and Mazandaran (Babol, Amol, Neka, and Babolsar) Provinces were selected. These cities have different temperature ranges, rainfall patterns, altitudes, and humidity levels (Figures 1 and 2). A total of 80 brown rats (*Rattus norvegicus*) were trapped by handmade live mouse traps. After taking blood samples from the tail, the sampled rat was released into the place where trapped. The whole blood sample was transferred to the laboratory and kept at -20 ˚C in the refrigerator.

2.2 Pb measurement

Blood samples were diluted with tretinoin solution (Merck co. Germany) with a ratio of 1:11 and then Pb concentration was measured by atomic absorption spectroscopy (model AA-7003) [10]. The heating program of the diluted samples was as follows: drying for 10 s at a temperature of 85 ˚C, drying for 25 s at a temperature of 95 ˚C, drying for 5 s at a temperature of 120 ˚C, ashing for 5 s at a temperature of 500 ˚C, ashing for 10 s at a temperature of 500 ˚C, ashing for 2 s at a temperature of 500 ˚C, atomization for 0.8 s at a temperature of 2100 ˚C, atomization for 1s at a temperature of 2100 ˚C, and cleaning out for 2s at a temperature of 2700 ˚C. The Pb concentration of the standard solution was 5 ppb and the detection limit of Pb was 0.005 ppb.

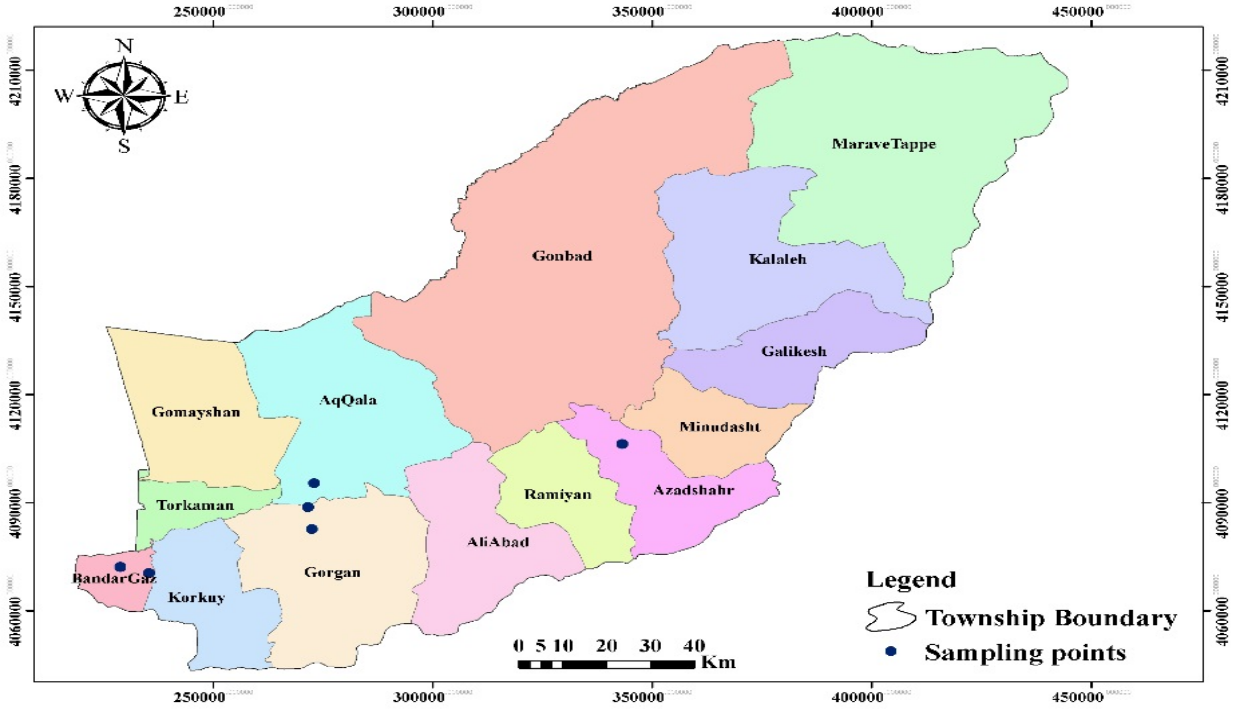

Figure 1. Geographical location of sampled cities from Golestan Province

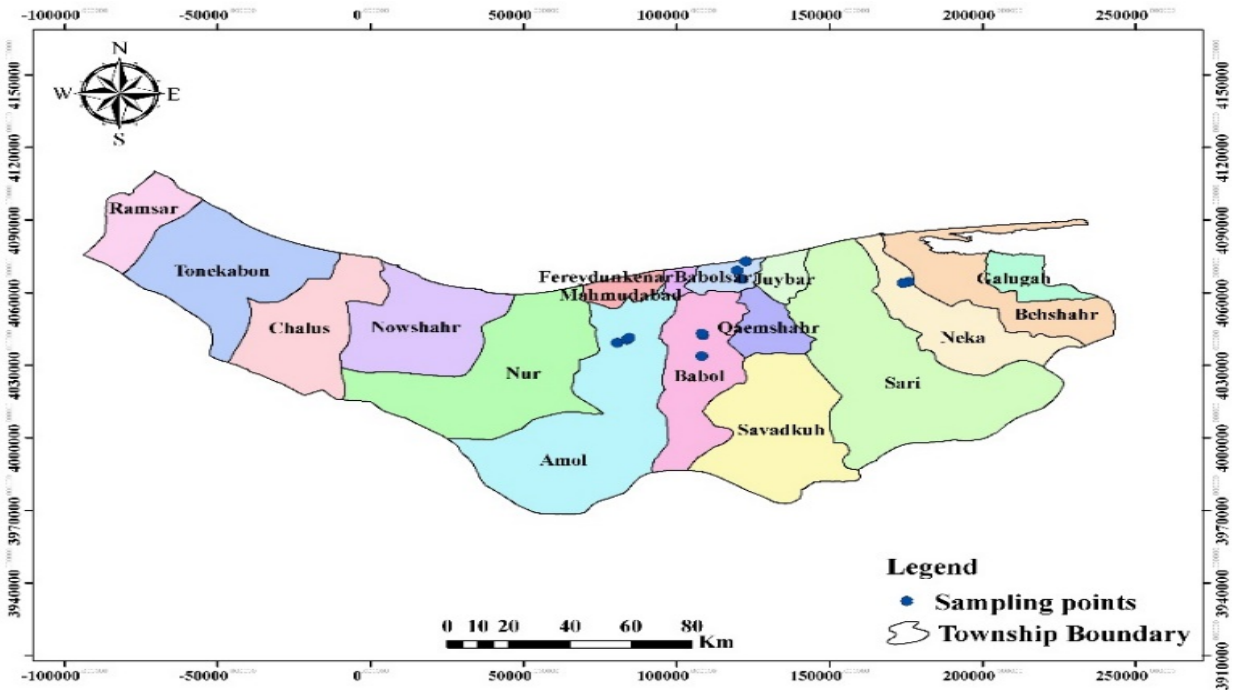

Figure 2. Geographical location of sampled cities from Mazandaran Province

2.3 Analysis

Kolmogorov-Smirnov test was used to determine whether the results were normal or abnormal. Because of normal distributions of data, the Chi-square test and t-test were used to compare Pb concentration between males and females among the sampled cities.

3. Results and Discussion

The results of the Kolmogorov-Smirnov test showed that the data were normal for both Golestan (Sig = 0.306) and Mazandaran (Sig = 0.95) Provinces (Table 1). Therefore, parametric statistical tests (Chi-square and t-test) were employed to analyze the relationship between blood Pb concentrations, sampled location, and sex. Furthermore, the results were compared with a 95 % confidence percentage.

Table 1. Data related to Kolmogorov-Smirnov test

Sampling location	Number	Mean (µg/dL)	Standard deviation (Std)	Sig (0.05)
Golestan Province	40	9.43	± 4.94	0.306
Mazandaran Province	40	9.02	± 2.29	0.95

The mean Pb concentrations in cities of Golestan Province were similar ($P > 0.05$), however, the average Pb concentrations in sampled cities of Mazandaran Province were different, with the highest and lowest Pb concentrations observed in Neka (10.28 ± 0.97 µg/dL) and Babol (7.35 ± 1.36 µg/dL), respectively (Table 2). This difference shows that the location has an effect on serum Pb contamination levels, which is consistent with the findings by Sánchez-Chardi *et al.* (2007) [11]. Results of the t-test revealed that mean Pb concentrations in winter and spring did not show a significant difference and it can be explained by the presence of the same Pb contamination sources in spring and winter (Sig = 0.91).

Table 2. Mean Pb concentrations in studied rats by city

Sampled Province	Studied city	Number of samples	Mean (µg/dL) ± Standard deviation (Sd)
Golestan	BandarGaz	10	9.25 ± 0.57
	AqQala	10	9.78 ± 2.49
	Azadshahr	10	9.31 ± 2.53
	Gorgan	10	9.40 ± 0.51
	Mean		9.43 ± 1.81
Mazandaran	Babol	10	7.35 ± 1.36
	Amol	10	8.98 ± 1.59
	Babolsar	10	9.50 ± 0.97
	Neka	10	10.28 ±0.97
	Mean		9.02 ± 1.07

Mean Pb concentrations of sampled cities from Golestan (9.43 ± 1.81 µg/dL) and Mazandaran Provinces (9.02 ± 1.07 µg/dL) were not significantly different (Sig = 0.577) (Table 3). Considering that the number of sampled rats in this study was small, it is impossible to explain observed similarity with certainty. However, the presence of similar sources of Pb pollution in surveyed cities of Golestan and Mazandaran Provinces can explain the obtained result. Regarding the obtained results, Pb contamination was observed in all the sampled rats, indicating brown rats are suitable bioindicators. A similar conclusion has been reported by Beernaert *et al.* (2007), Damek-Poprawa *et al.* (2003), and Tête *et al.* (2015) who studied Pb contaminations in rodents [12-14].

Table 3. Results of the t-test on mean Pb concentration of sampled rats in Mazandaran and Golestan Provinces

Equality of variance test	F	Sig	t	df	Sig. (2-tailed)	Mean Difference	Std. Error	95% Confidence Interval	
								Lower	Upper
Assuming equality of variances	0.037	0.85	- 0.567	19	0.577	-0.41	0.73	- 195	1.12
Assuming inequality of variances			- 0.554	15.7	0.587	-0.41	0.75	- 2.01	1.18

Due to the presence of rodents in large numbers and their feeding behaviors, which used various sources of food, researchers have surveyed heavy metal pollution in different ecosystems by Pb measurement in different species of rodents [15-20]. Mean serum Pb concentrations have been reported at 6.7 µg/dL and 6.3 µg/dL in brown rats from agricultural areas of Golestan and Mazandaran Provinces, respectively. Lower serum Pb concentrations in rural brown rats of these two Provinces highlighted the lower Pb contamination of rural areas than urban and the role of gasoline in Pb distribution in urban areas [21]. Mean blood Pb concentration in wood mice (*Apodemus sylvaticus L.*) has been measured by Rogival *et al.* (2006) in some areas of Belgium and they ranged from 25.96 to 175.81 µg/dL are much higher than the results of our study [22]. Janiga *et al.* (2019) reported a mean Pb concentration of 33.6 µg/dL in the blood of sampled snow voles (*Chionomys nivalis*) and Tatra marmots (*Marmota marmota latirostris*) from the alpine environment which is also higher than results of our study [23]. Nakata *et.al*, (2022) reported very low mean Pb concentration (0/25 µg/dL) in the plasma of wild rodents from Japan [24]. These are the only similar studies on rodents and other studies have investigated Pb tissue contamination in rodents. For example, Zarrin Tab *et al.* (2015) measured Pb concentration in the liver, kidney, and muscle of brown rats (*Rattus norvegicus*) as an indicator species in Aran and Bidgol counties (Isfahan Province) and reported the average Pb concentration of 135.06 µg/g, 87.97 µg/g and 61.80 µg/g dry weight in the liver, kidney, and muscle tissues, respectively [25]. In another study, Hazratian *et al.* (2017) studied Pb contamination of brown rats (*Rattus norvegicus*) in Tehran and reported mean Pb concentrations of 70.06 $\mu g.g^{-1}$ and 85.1 $\mu g.g^{-1}$ in liver tissues in central and northern regions of Tehran, respectively [18]. Despite the presence of Pb in non-urban areas of Golestan and Mazandaran Provinces, only one study has been conducted on Pb contamination in northern cities of Iran (Noor City) [20]. Mousavi *et al.* (2006) reported that the average Pb concentration of 15.52 µg/g in bone tissues of brown rats (*Rattus norvegicus*) from Noor City exceeded the standard level. As Pb has been measured in tissue samples in other studies in Iran, their results are not comparable with our results. There is a higher opportunity for Pb tissue bioaccumulation over time than blood and Pb blood concentration indicates mostly the rate of recent Pb contamination [17]. It should be mentioned that other factors such as the number of samples, amount of pollution in the studied ecosystems, age of the sampled rats, and season of sampling, in addition to the type of biological sample can also affect Pb concentrations in surveyed rats [15]. Results of the Chi-square test that was used to determine the effect of sex on the Pb concentrations in the studied rats indicated that the Pb concentrations in males and females were similar (Sig = 0.123) (Table 4).

Table 4. Results of the Chi-square test

Gender	Number	Mean	df	Fig
Male	39	10.31	1	0.123
Female	41	9.25		

According to the possibility of removing heavy metals from females' bodies through childbirth and lactation, sex is also one of the factors that can reduce Pb concentration in female rats [25]. Results of a study by Zarrin Tab *et al.* (2015) on Pb contamination of brown rats showed that Pb concentrations in females were lower than that of males [25]. However, in this study, Pb concentrations were similar in males and females which was in agreement with the results of Ieradi *et al.* (2006) survey, which reported similar Pb concentrations in males and females house mice (*Mus domesticus*) [15]. Sánchez-Chardi *et al.* (2007) that surveyed bioaccumulation of Pb in small mammals also reported similar results. Sampling immature females or females without lactation and childbirth experiences could explain observed results [11]. The specific sources of Pb contamination in the studied rats are unknown in this study. Drinking water was reported to be contaminated with Pb in the concentration of 4.38 µg/L in Gorgan (capital of Golestan Province) by Dadban Shahamat *et al.* (2022) [26]. However, Saeedi *et al.* (2010) introduced traffic-related Pb as the main reason for Pb contamination in soils around highways [4]. Therefore, it seems that Pb-contaminated water or gasoline could be some sources of Pb contamination in the studied cities. However, further investigations are needed to confirm this hypothesis and also find other Pb contamination sources.

4. Conclusion

The standard concentration of blood Pb in mammals, including rodents, has not been documented. The safe tissue Pb concentration has been reported as 0.001 µg/g. Comparing this standard, which shows Pb concentration over time in tissues, all the sampled rats in this study suffer from high levels of Pb contamination. Such high Pb contaminations of urban sampled rats highlight the potential danger of human Pb contamination in the sampled cities as sampled rats breathe the same air and drink similar sources

of water as urban humans. Therefore, implementing appropriate management programs to find Pb resources and eliminate them in the cities of Golestan and Mazandaran Provinces seems necessary. Additionally, it is recommended to measure Pb in other biological samples from the Northern Provinces of Iran, and the consumption of Pb-free gasoline is suggested.

Authors' Contributions

Amir Shahbazadeh Bengar: investigation; analyzation; resources. Somayeh Namroodi, Somayeh Galdavi: Conceptualization; methodology; validation; formal analysis; supervision; review and edition of the manuscript.

Funding

The study was financially supported by the Gorgan University of natural resources and agricultural sciences, Golestan, Iran under grant number of 60300.

Conflicts of Interest

No conflict of interest has been declared by the authors.

Acknowledgements

We gratefully thank all the collogues who helped us to obtain samples and do laboratory tests.

Ethical considerations

Ethical approval wasn't required for this study because the surveyed rats were live and the research committee of Gorgan University of agricultural and natural resources approved the proposal under number of 9521133130.

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