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Survey on the lead concentration of brown rats (*Rattus norvegicus*) and the effect of environmental factors on Pb distribution pattern in rural- agricultural areas using GIS (Golestan and Mazandaran Provinces)

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Abstract

Heavy metals have contaminated various ecosystems. Omnivorous animals such as rodents with high fertility, abundance and low motility can be good indicators for heavy metal contamination of their habitat. Rural-agricultural areas around seven cities in Golestan and Mazandaran Provinces were selected to trap five brown rats (*Rattus norvegicus*) from each region. After the preparation of serum samples, Pb concentration was measured using atomic absorption spectrometry. Also, GIS software was used to survey the effect of environmental factors on the Pb distribution pattern. The average Pb concentration was similar in males and females and in rural areas of Golestan and Mazandaran Provinces was 6.7 and 6.3 µg/dL, respectively ($p \geq 0.05$). The highest average Pb concentration (7.55 µg/dL) belonged to rats sampled in areas around Ghargomishan road close to Neka, and the lowest average Pb concentration (5.20 µg/dL) belonged to rats sampled from Kaleh agricultural areas (between Bahnamir and Babolsar Cities). Using the sensitivity method of the linear multiple regression model, it was found that the temperature and humidity have the highest effect and altitude and precipitation have the lowest impacts. The results of this study showed rural-agricultural areas of Golestan and Mazandaran Provinces are contaminated by Pb and the transmission risk of this hazardous metal to humans and other animals living in these areas is high. Therefore, the communication of information to the authorities and the use of adequate measures to prevent Pb entrance to the studied areas is necessary.

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Introduction

Developments in the industrial and agricultural sectors and improved human livelihood in recent decades have made the use of heavy metals inevitable in various fields (Esmaili Sari, 2002). Heavy metals are released into the environment through various methods such as mining, use of chemical fertilizers in agriculture, rainfall polluted with environmental pollutants, and the discharge of industrial waste (Esmaili Sari, 2002). Among various heavy metals, lead (Pb) has no application in animal biological activities and is toxic to animals at very low concentrations (Esmaili Sari, 2002). The presence of Pb in animals' bodies can affect all parts of the body and have many harmful effects such as learning and behavior troubles, anemia, increase in blood pressure and fertility decrease (Ab Latif Wani et al., 2015; Rubin et al., 2002).

Mazandaran and Golestan Provinces, with many villages, are two important agricultural hubs in Iran due to the moderate and humid climatic conditions. The consumption of agricultural pesticides, which are common ways of environmental pollution with heavy metals, is high in these two provinces (Sharbati, 2012). Studies on the surface soils of the northern Provinces of the country also showed the heavy metals contamination of soils in different parts of Golestan Province (Mirzaee et al., 2014, Mirzaee et al., 2015). Measurement of the concentration of the pollutants in animals' bodies can be beneficial in assessing the quality of the environment in which they are living (Ceruti et al., 2002). Rodents are omnivorous animals with high reproductive capacity and live in all geographical areas with limited home ranges (Ziaie, 1996). Therefore, it seems that analyzing the level of pollutants such as heavy metals in these animals can help measure heavy metal environmental contamination and predict the heavy metal contamination in other animals' species in an ecosystem.

Since previous studies in the northern regions of the country were limited to soil samples, we attempted to measure Pb contamination in the blood of brown rats (*Rattus norvegicus*) as sentinel species in rural areas of Golestan and Mazandaran Provinces. Also, the results were surveyed using GIS software to prepare the map of the Pb distribution and analyze the effect of environmental factors on the Pb distribution pattern (Zhang, 2006).

Materials and methods

During 2018-2019, seven rural-agricultural areas around seven cities from Golestan (36°30' to 38°8' N and 53°57' to 56°22' E) and Mazandaran (36° 33′ to 56″ N and 53° 03′ to 32″ E) Provinces were chosen (Table 1) and using hand-made traps, five brown rats (*Rattus norvegicus*) were trapped from each area (Sharbati, 2012). After recording basic characteristics such as sex, they were transferred to the laboratory, and blood sample was taken from them using the tail vein. The captured rats were released after sampling at the site. Blood samples were centrifugated, and the obtained serums were stored in a freezer at -20 °C. Digestion was carried out according to the Abdul-Sada's recommendations (Abdul-Sada, 2019). Serum Pb concentrations were measured using the graphite furnace atomic absorption spectrometry, Model AA-7003. Chi-square test was used to compare Pb concentration between males and females. The Garmin Etrex 30x GPS device was used to record the the sampled areas (Mahiny and Turner, 2003). Using ArcGIS10.6, the map of Pb concentration of the sampled points was zoned, and while preparing the map of pollution density of the affected areas, the effect of different environmental factors, altitude, rainfall, temperature and humidity, on Pb distribution pattern was examined throw sensitivity analysis by multiple linear regression (Zhang, 2012).

Table 1: Average Pb concentrations in sampled brown rats base on of sampled areas

Province	City	Sampled area	Number of sampled brown rats	Average Pb concentration (µg/dl)
Mazandaran	Babol	Agricultural area around Dunesar village	5	5.93
	Amol	Agricultural areas of Rudbar Dasht/ Mohammadi Rice factory	5	6.53
	Babolsar – Behnamir	Agricultural areas around Kaleh factory	5	5.20
	Neka	Agricultural area around Gomishan road	5	7.55
Golestan	Bandargaz	Agricultural areas around the highway along the river	5	6.5
	Agh-ghala	Agricultural areas around Mahmudabad-Ghorbanabad villages	5	6.38
	Azadshahr/ Minudasht	Agricultural areas around Chehel Chay river	5	7.20

Table 2. Results of Chi-Square test on Pb concentration of male and female rats

sig	df	Average	number	Gender
0.157	1	10.31	13	Male
		9.5	22	Female

Table 3. Results of linear multiple regression model

Model parametres, $Y = (a + b_1x_1 + b_2x_2 + \ldots + b_nx_n)$					
R^2	b_4x_4	b_3x_3	b_2x_2	b_1x_1	a
0.31	0.006	0.1	0.09	0.08	9.97

Results

In the present study, the highest concentration of Pb (7.55 µg/dl) was observed in the rural-agricultural areas around Ghargomishan road (around Neka City) in Mazandaran Province with an average humidity of 66.75 %, average annual rainfall of 497.8 mm, average annual temperature of 16.82°C, and average altitude of 550 meters (Figure 1, Table 1). The lowest average Pb concentration (5.20 µg/dl) was seen in rats sampled from agricultural areas around the Kaleh factory in Mazandaran Province with an average humidity of 80%, average annual rainfall of 899 mm, average annual temperature of 17.1°C and an average altitude of 1981 meters (Figure 2).

The results of t-test on Pb concentration in sampled rats of Golestan (6.7 µg/dl) and Mazandaran (6.3 µg/dl) Provinces indicated no significant differences between the level of Pb concentrations in the two Provinces ($p \geq 0.05$). In this study, 13 male and 22 female brown rats were sampled, and their average Pb concentrations were 10.3 and 9.5 µg/dl, respectively. The results of the Chi-square test showed that the difference between the average Pb concentration of males and females was not significant, and the sex of the sampled rats was not an effective factor on the amount of Pb contamination in brown rats (Table 2).

The results of interpolation method through Inverse Distance Weighted (IDW) on Pb concentration in each area are provided as maps. In these maps, it can be

seen that the highest Pb concentration in Golestan Province belonged to Minudasht, a city with an average humidity of 71.95 %, average annual rainfall of 680.62 mm, average annual temperature of 13.6°C, and an average altitude of 851.11 meters (Figure 1) and the highest concentration of Pb concentration in Golestan Province belonged to rural agricultural areas around Ghargomishan road.

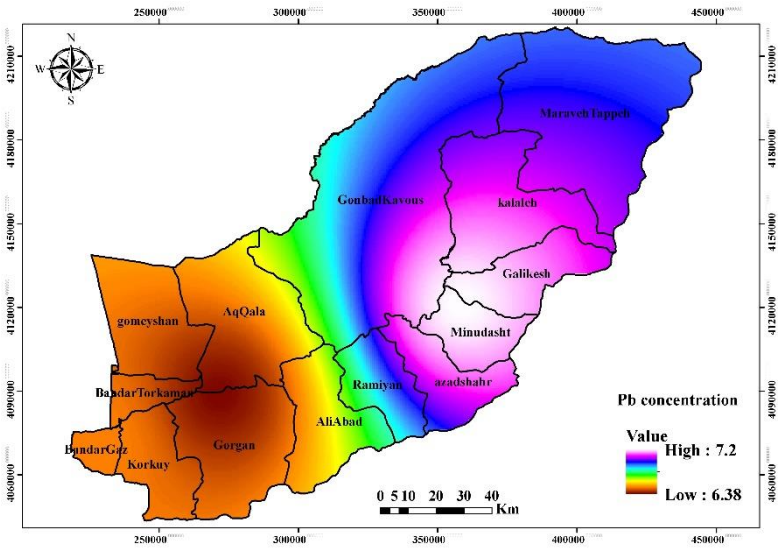

Figure 1. Map of Pb (µg/dl) distribution pattern in Golestan Province using IDW method.

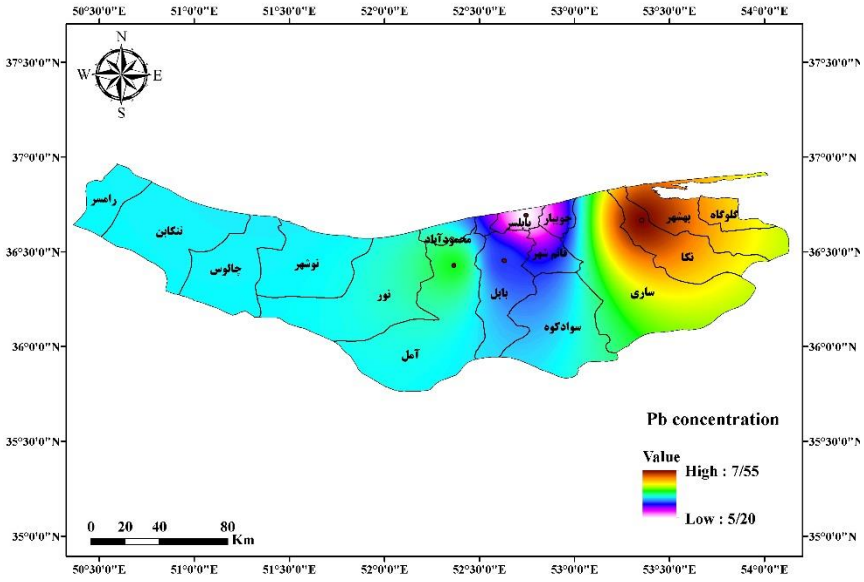

Figure 2. Map of Pb (µg/dl) distribution pattern in Mazandaran Province using IDW method.

In the sensitivity analysis of multiple linear regression, where the acceptable F with a 99% confidence interval is 3.32, the F value was significant comping up at 3.95 in the regression equation. The T value (for significance level of 99% and df=147) was equal to 2.57. Results showed that temperature and humidity were significant at 99%, precipitation was significant at 95% and altitude was not significant. The results of the sensitivity analysis of multiple linear regression are represented in Table 3. The independent variables in the regression were as follows: annual average temperature (X1), altitude (X2), annual average humidity (X3) and annual average

precipitation (X4). The R^2 indicated that the relationship of Pb concentration with environmental factors of annual average temperature, annual average humidity and annual average precipitation is 31.0% in the studied areas. The effect of each environmental factor on the Pb concentration in the studied areas is shown as a coefficient in the output of the multiple linear regression model in Table 3. The higher the coefficient, the greater the effects of environmental factors on the Pb concentration in the studied areas.

Discussion

Rodents are the most populous mammal species in the world, and because of their small size, they have a higher rate of metabolism than other mammals (Gdula-Argasińska et al., 2004). Therefore, rodents are more exposed to environmental pollutants by feeding compared to other species of mammals. In recent years, heavy metal contamination of urban and wild rodents has been monitored worldwide. Here we provide a short summary and comparison of the relevant literature with our study.

Mousavi et al. (2006) surveyed Pb concentration of the bone tissue in brown rats (*Rattus norvegicus*) of Noor, a city in Mazandaran Province. They reported the average Pb concentration of 15.52 µg/g and stated that the Pb concentration in bone tissue was higher than the accepted standards for the presence of Pb (0.001 µg/g) in animals' tissues.

In another study, Zarrintab et al. (2015) investigated the Pb concentration in different organs of brown rats (*Rattus norvegicus*) as a biological indicator of species in Aran and Bidgol, a city in Isfahan Province. They reported the average Pb concentration of 135.06, 87.97 and 61.80 µg/g (dry weight) in liver, kidney and muscle tissues, respectively.

Hazratian et al. (2017) surveyed liver Pb concentration of brown rats (*Rattus norvegicus*) as a biological indicator in Tehran. The average Pb concentrations in liver tissues of the sampled rats from the central and northern parts of Tehran were 73.06 µg/g and 1.85 µg/g, respectively.

As it can be seen, the average tissue Pb concentrations of all previously mentioned studies were higher than the average Pb serum concentration of this study. This could be due to a higher chance of Pb bioaccumulation over time in different tissues than the Pb serum concentration, which is mostly an indicator of recent Pb contamination of the sampled rodents (Ab Latif Wani et al., 2015).

However, the average Pb concentrations of tissues in some similar studies were lower than the average serum Pb concentration in the current study. For example, Hamidian et al. (2015) evaluated the Pb concentration in different tissues (bone, liver, heart tissues and hair) of Persian jirds and reported Pb concentrations below the detection limit.

Nakata et al. (2017) studied the kidney Pb concentrations of the brown rats (*Rattus norvegicus*) in some Japan cities. They reported an average Pb concentration of 4.58 mg/kg, 3.42 mg/kg, and 3.40 mg/kg in the sampled rats of Kyoto, Osaka and Tokyo, respectively.

In another study, Ceruti et al. (2002) measured Pb concentration in the kidney of 21 brown rats (*Rattus norvegicus*) in rural areas of Milan to analyze the degree of Pb contamination of rural areas. The average Pb concentration was reported to be 0.6 µg/g. The lower tissue concentration of Pb in mentioned studies compared to the serum Pb concentration of this study could be due to lower Pb contamination of other studied areas than Golestan and Mazandaran Provinces.

However, various other factors such as the studied species, number of the studied rodents, diet, age and sex of the studied rodents may also explain the observed difference in the results of similar studies on various rodent species in the world (Komarnicki, 2000). Nevertheless, the sex of rodents showed different effects on the rodents' heavy metals concentration in different studies.

The results of Zarrintab et al. (2015) study of the Pb concentration in various organs (muscle, kidney and liver tissues) of brown rats (*Rattus norvegicus*) in Aran and Bidgol showed higher Pb concentration in

males than females. Female animals are said to be able to excrete Pb from the body in each childbirth and lactation. (Burger et al., 1999; Komarnicki 2000). This can explain the higher tissue concentration of Pb in male brown rats than in female brown rats in the study of Zarrintab et al. (2015).

However, the results of the present study showed similar serum Pb concentrations in male and female sampled brown rats (sig = 0.157). The young age of the sampled female rats and their immaturity can explain the similarity of Pb contaminations in the sampled males and females in the present study.

The effect of environmental factors on Pb concentration in different sampled areas was surveyed using GIS software and a linear multiple regression model. We found that temperature and humidity had the greatest effect on the Pb concentration while altitude and precipitation had less effect in this study. Rural-agricultural areas of Minudasht, with moderate climatic conditions, are most prone to presence of Pb in terms of environmental factors.

Appropriate temperature and humidity have a direct effect on the type of land use, and areas with moderate temperatures and humidity, are suitable for agricultural activities and also the presence of humans as important Pb importers in the environment (Sharbati 2012). Hence, the observed positive relationship between Pb concentration and temperature and humidity observed in this study can be justified. The results of the current study provided suitable data for GIS software to predict the amount of Pb contamination in other agricultural-rural areas of Golestan and Mazandaran Provinces through analyzing their environmental factors (Maas et al., 2010; Mirzaei et al., 2015). Ieradi et al. (2003) reported that heavy metals contaminated soil in rodent habitats is the main reason for their contamination with heavy metals. So, we can postulate that the soil of areas with good temperature and humidity (Minudasht and Babolsar) may have higher Pb contamination than other sampled agricultural-rural areas of Golestan and Mazandaran.

Conclusion

The results of this study showed that brown rats could act as a suitable biomarker for the study of ecosystem Pb contamination and the danger of Pb transmission to humans and other animals living in sampled areas. So, conveying information obtained by the findings of this study to the authorities and taking adequate measures to prevent Pb release into the agricultural-rural areas in Golestan and Mazandaran Provinces is necessary.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

No conflict of Interests has been declared by the authors.

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