



## Evaluating Mercury Pollution in the Blood of Free-Roaming Dogs in Gorgan City, Golestan Province

Mahdiyeh Ghadimi Aliabadi <sup>a</sup> , Somayeh Namroodi <sup>a\*</sup> , Alireza Yousefi <sup>b</sup> , Hajar Abyar <sup>a</sup>

*a. Department of Environmental Sciences, Faculty of Fisheries and Environment Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Golestan Province, Iran.*

*b. Vorkan Veterinary Hospital, Gorgan, Golestan Province, Iran.*

**\*Corresponding author:** Department of Environment, Faculty of Fisheries and Environment Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Golestan Province, Iran. Postal Code: 66177-13446. E-mail: namroodi@gau.ac.ir

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### ABSTRACT

**Background:** Mercury is a dangerous biological pollutant that contaminates food chains and enters the bodies of animals. Since free-roaming dogs are omnivorous and cover a large geographical area, this study measured mercury levels in the blood of free-roaming dogs in Gorgan City.

**Methods:** After recording the sampled dogs' characteristics, blood samples were collected, and mercury concentrations were measured using Coupled Plasma Mass Spectrometry (ICP-MS).

**Results:** The mean mercury concentration in the blood samples was 24.9 µg/L. Statistical analysis revealed no significant differences in mercury concentrations between male and female dogs ( $P = 0.669$ ) or across different age groups ( $P = 0.486$ ).

**Conclusion:** These findings underscore the presence of mercury pollution in Gorgan City, as blood mercury levels can indicate recent environmental contamination. The average mercury concentration in various areas of the city was below the toxic threshold of 105 µg/L. However, even low levels of mercury exposure can be toxic to mammals. Since industrial and hospital waste are recognized as major sources of mercury, it is recommended that these wastes be treated before being released into the environment. The research also indicated that domestic dogs could serve as valuable bioindicators for monitoring environmental contamination by hazardous metals, such as mercury.

## 1. Introduction

Heavy metals are significant environmental pollutants that negatively impact the health of living organisms, even at very low concentrations, by disrupting the biological structures of animals (Yildiz et al., 2023). Some metals, such as calcium, magnesium, phosphorus, copper, zinc, and iron, are essential for the proper functioning of the body. However, metals like mercury pose severe health risks and interfere with normal body functions. Mercury exists in metallic, organic, and inorganic forms (Chung et al., 2023). Its toxicity depends on its chemical structure, with methylmercury being the most hazardous and stable due to

its high affinity for sulfhydryl groups. This property enables it to bind with plant and animal proteins. This enables it to enter the food chain and spread contamination across various trophic levels (Noto, 2021; Klepac et al., 2000). Mercury can enter animals' bodies through multiple pathways, including skin contact, inhalation, and ingestion of contaminated food (Noto, 2021). Symptoms related to mercury toxicity in animals often appear after chronic exposure and can include compromised immune function, skin allergies, deformities, and cancer (Beyersmann & Hartwig, 2008; Lehmann et al., 2010). For example, mercury concentrations in seals exceeding 60 µg/g have been linked to liver damage (Bełdowska & Falkowska, 2016). Generally,



mercury levels in the blood and kidneys of most species are considered normal if below 0.1 mg/kg of wet weight. Concentrations above 6 mg/kg are indicative of mercury poisoning (Blakley, 2021). Various domestic and wild animals have been studied to assess environmental mercury contamination. Free-roaming dogs, in particular, serve as suitable indicators of heavy metal exposure due to their close environmental interactions. They roam an average home range of 0.65 square kilometers (65 hectares), with a maximum distance of 1.05 kilometers (Perez et al., 2018). Given their close interaction with humans, analyzing mercury levels in free-roaming dogs' blood can provide insight into the extent of urban ecosystem contamination with mercury (Zaccaroni et al., 2014; Park et al., 2005). Serpe et al. (2012) studied mercury levels in the liver tissues of domestic and stray dogs in Nepal to monitor environmental contamination. Their findings highlighted the role of these animals as effective bioindicators of mercury pollution in the ecosystem. Gorgan, the capital of Golestan Province in northeastern Iran, is surrounded by numerous villages with moderate weather conditions. Consequently, many owned dogs roam freely across the city, as well as in industrial and rural areas (Sharbati, 2017). Due to their omnivorous diet and extensive movements, analyzing mercury levels in their blood provides a valuable means of assessing mercury contamination in both animal and human populations in Gorgan (Lawler et al., 2008). This study aimed to measure mercury levels in the blood of free-roaming owned dogs in Gorgan and to explore the relationship between the dogs' age and gender with mercury concentrations in their blood.

## 2. Materials and Methods

### 2.1 Study area and sampling

Gorgan City with an area of 3,567 hectares in northern Iran (located between 54°24' to 54°28' east longitude and 36°49' north latitude) is the capital of Golestan Province. (Emadodin & Namjoo, 2014; Aghili et al., 2022). During a four-month period in 2022, blood samples were collected from 80 free-roaming dogs brought to a veterinary clinic (Figure 1). Data including the age, gender, and living location of each dog were recorded. A 5 mL blood sample was taken from the cephalic vein as described by the National Institutes of Health (NIH) Guidelines for Research Involving Animals and was stored at -20°C until analysis (Park et al., 2005; Sousa et al., 2013).

### 2.2 Mercury Measurement and Statistical Analysis

To digest the samples, 0.1 mL of blood was diluted with 14.5 mL of 0.5% ultrapure HNO<sub>3</sub>, 0.05% Tritonx, and 2% methanol in a 15 mL Falcon tube. An ICP-MS model 4500HP (made in England), equipped with automatic 520Asx sampling, was used to analyze mercury concentration. The detection limit for mercury was 0.01 µg/L, with accuracy verified by spiked recovery tests yielding > 95% recovery. Accuracy was validated using certified reference material

(CRM, NIST 1643e, trace elements in water) containing mercury. The CRM was analyzed in triplicate, yielding a recovery of 99.8 ± 0.5%. Additionally, spike-and-recovery tests were conducted by adding known amounts of mercury (2.5 and 5.0 µg/L) to the samples. The recovery percentages ranged from 97.2% to 101.5%, confirming the accuracy of the method. Precision was assessed by analyzing six replicates of a single sample with a mercury concentration of 5.0 µg/L. The relative standard deviation (%RSD) was 1.8%, indicating high repeatability. Inter-day precision was evaluated by analyzing the same sample across three days, resulting in a %RSD of 2.1%. To perform descriptive analyses in SPSS 26 software, with the normality of the data tested using the Kolmogorov-Smirnov test; the Shapiro-Wilk test was also employed for reinforcement. Q-Q plots were examined for further validation. An ANOVA test was used to compare mercury concentration in different age groups. Outliers can have a significant impact on the results of statistical tests. Box plots were used to identify outliers. Additionally, an independent samples t-test was performed to examine the effect of dogs' gender on blood mercury concentration.

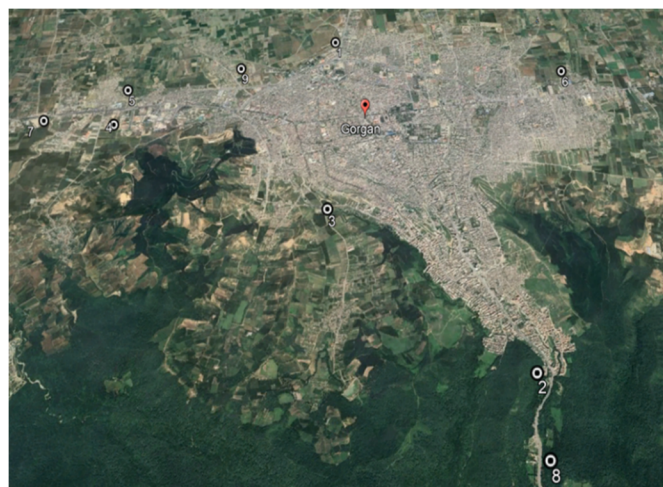


Figure 1. Geographical location of sampled points in Gorgan City (Source: Google Earth 2022, edited by the author using ArcGIS 10.8)

## 3. Results and Discussion

According to the results of the Kolmogorov-Smirnov test, the data had a normal distribution of *p-value* = 0.149 (Table 1). T-test and ANOVA parametric tests were used to analyze the data.

Table 1. Data related to Kolmogorov-Smirnov test

	Kolmogorov-Smirnov		
	Statistic	df	<i>p-value</i>
Mercury	0.138	30	0.149

In this study, all the blood samples from the studied dogs were found to be contaminated with mercury. In total, blood samples were collected from 80 free-roaming dogs (36 females, 44 males) aged between one and nine years, and the mean results are presented in Table 2. The average

concentration of mercury in the blood of the sampled dogs in Gorgan City was 24.9 µg/L. The highest concentration of mercury was 49.1 µg/L while the lowest was 7.07 µg/L. The identified mercury concentration in the dogs studied in this research, although below the critical threshold of 105 µg/L defined for acute toxicity, can still have significant negative effects. Also, the mercury level identified in this study (mean = 24.9 µg/L) is sufficient to cause chronic and long-term negative effects on the health of the dogs (Blakley, 2021). Even low concentrations of mercury can damage nerve cells and disrupt the functioning of the nervous system, immune system, and endocrine glands leading to behavioral problems, weakness, and being more vulnerable to infections (bacterial, or viral diseases). These results underscore the importance of monitoring environmental pollution sources and taking action to reduce mercury emissions in local ecosystems. In humans, a mercury blood concentration of 5 µg/L is considered safe, but concentrations between 10-15 µg/L may have mild effects on the nervous system, particularly in children and pregnant women. A blood concentration of 50 µg/L or higher is considered highly dangerous and is associated with serious neurological and kidney effects (Zulaikhah et al., 2020). Therefore, the mercury levels detected in the blood of the sample dogs in the current study could be toxic to humans in Gorgan City.

**Table 2.** The average concentration of mercury metal in sampling points

Region	Number of samples per region	Average age (years)	Average mercury contamination (µg/L)
1	8	5	18.20
2	8	6	9.13
3	10	1	32.70
4	12	3	15.00
5	8	9	49.10
6	8	4	39.30
7	10	4	21.80
8	8	3	7.07
9	8	2	31.80

Similar to the results of the current study, previous studies on the soil, water, and fish of Golestan Province have confirmed mercury contamination in this Province. Agah et al. (2011) reported mercury concentrations in surface sediments of Gorgan Bay ranging between 10 and 44 ng/g. Rajabi and Ghorbani (2015) collected samples from drinking water wells in Garambadasht, Golestan province, to assess mercury levels in groundwater sources. The results showed that mercury concentrations in all samples exceeded the World Health Organization's standard limit. Heidary et al. (2012) conducted a study to assess mercury levels along the southern shores of the Caspian Sea in Golestan province. They collected kidney and fish samples from the region's fish population and found that mercury accumulation in fish was influenced by the concentration of the metal in the coastal sediments. In another study, Namroodi et al. (2017) measured mercury concentrations in the tissues of wild and domestic geese from Golestan province. They captured 15 wild gray geese from the Gomishan wetland and Gorgan Bay, along with 25 domestic gray geese, and collected samples of tail feathers and internal organs for mercury analysis. The mercury contamination in the tail feather samples was found

to be  $0.99 \pm 0.07$  ppm. The exact sources of mercury were not surveyed in this study. However, in addition to industrial activities, local sources such as small industrial workshops, car repair centers, and the use of fossil fuels in industries or even in households can be potential sources of mercury emissions. Hospital waste also contains significant amounts of mercury. Furthermore, since Gorgan is surrounded by agricultural areas, the use of fertilizers and pesticides that may contain mercury should also be considered (Blakley, 2021). Most studies in Iran have focused on birds and fish to monitor mercury pollution in ecosystems (Agah et al., 2006; Mashroofeh et al., 2015). The only research addressing mercury contamination in canids was conducted by Malvandi et al. (2010) which analyzed golden jackals in Mazandaran Province. This research identified mercury contamination in the jackals and demonstrated that wild canids can serve as useful indicators for monitoring heavy metal pollution in ecosystems. In Golestan province, limited studies have examined mercury contamination. Heydari et al. (2012), investigated heavy metal pollution, including mercury, along the southern coast of the Caspian Sea. In this study, 40 fish (*Acipenser stellatus*) were collected from the southeastern shores of the Caspian Sea in Golestan Province, revealing an average mercury concentration of  $7.85 \pm 0.48$  mg/L. The findings indicated that the accumulation of heavy metals in fish (*Acipenser stellatus*) is influenced by the concentration of these metals in sediments and the physiological characteristics of the fish. In another study by Aghili and Aghaei Moghadam (2018), the researchers examined heavy metals in the water and sediment of Gorgan Bay before and after fish farming activities. Using atomic absorption spectrophotometry, they measured mercury concentration, which was consistently below 1 µg/L at all three sampling stations. Moreover, Sadeghi et al. (2020) investigated mercury levels in the soil of Gonbad Kavos (Golestan Province) by analyzing mercury concentrations in wheat grains. The average mercury concentration in the wheat samples was  $0.021 \pm 0.019$  mg/kg, which was below the acceptable standard limits. Malvandi et al. (2022) assessed heavy metal contamination, including mercury, by collecting fish samples from Turkmen Port. They reported mercury levels in the fish muscle tissue to be below the limits established by the World Health Organization. The results of this study also showed mercury pollution in the urban areas of Golestan Province. Given the toxic effects of mercury on mammals, domestic dogs have been used in other studies to monitor the level of mercury pollution in the environment. Sousa et al. (2013) investigated mercury concentrations in the blood of 26 domestic dogs in Portugal. The average concentration of mercury in the examined dogs was  $13.38 \pm 0.16$  µg/L, which was lower than the average concentration of mercury in the dogs studied in current research. In another study, Altinok-Yipel et al. (2022) investigated the concentration of blood mercury in 140 shelter and owner dogs from different cities in Turkey. The average mercury concentration was 0.74 µg/L, which was higher than the average mercury concentration in this study. Zaccaroni et al. (2014) assessed mercury concentrations in domestic dogs



from three regions in Campania, Italy, with an average of 460 µg/L, significantly higher than the levels found in the current study. Furthermore, Park et al. (2005) examined mercury levels in the serum of 204 urban dogs from various parts of Korea, reporting an average concentration of  $255.3 \pm 40$  µg/L. The mercury levels in the dogs of the present study were also lower than those of the Korean dogs. Various factors, such as the mercury concentration in soil and diet, age, sex, habitat conditions, and the sample size of sampled dogs in each study, may contribute to the differences observed in similar research conducted on domestic dogs in different parts of the world. Other similar studies have been done on the tissues of domestic dogs. Lopez-Alonso et al. (2007) evaluated mercury concentrations in kidney and liver tissues of rural and urban dogs in northwestern Spain. The average concentration of mercury in all dogs was calculated to be 53.4 µg/kg in the

kidney and in the liver, it was 32.7 µg/kg. Serpe et al. (2012) investigated mercury concentration in the kidneys and liver of domestic and stray dogs in Nepal. The average mercury concentration in the kidney was 54 µg/kg and in the liver was 40 µg/kg. In addition, Kral et al. (2015) investigated mercury contamination in hair samples from urban dogs in the Czech Republic, with an average concentration of 33.5 µg/kg, which exceeded the mercury levels reported in the current study. As can be seen, the results of all research on mercury levels in the tissues consistently showed higher concentrations than those focused on blood samples due to the potential for mercury bioaccumulation in tissues over time (Serpe et al., 2012). According to the results in Table 3, the average mercury concentration in females (23.14 µg/L) was higher than in males (21.15 µg/L), but this difference was not statistically significant ( $p$ -value = 0.669).

**Table 3.** The effect of gender on the mercury concentration in the blood sample of the studied dogs using an independent t-test

Briar test of variances	F	<i>p</i> -value	t	df	<i>p</i> -value (2-tailed)	Mean Difference	Std.Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
assuming equality of variances	0.40	0.52	-0.43	28	0.66	-1.99	4.60	-11.43	7.44
assuming inequality of variances			-0.41	21.00	0.68	-1.99	4.81	-12.00	8.01

Park et al. (2005) showed that there is no significant difference between the concentration of mercury in the serum samples of both males and females and the average concentration of mercury in females was higher as in the recent study. Also, Sousa et al. (2013) reported no significant difference in blood mercury levels between male and female dogs, with males having a higher average mercury

concentration than females. Living in the same environment and using common food resources by males and females can justify this similarity (Namroodi et al., 2017). The results of the current study indicated that mercury concentration increased with age (Table 4). but there was no significant difference between average mercury concentration and age groups (1-3 years, 3-6 years, 6-9 years) ( $p$ -value = 0.486).

**Table 4.** The effect of age groups on the concentration of mercury in the blood samples of dogs using one-way analysis of variance

	Sum Squares	df	Mean Squares	F	<i>p</i> -value
Between Groups	229.35	2	114.67	0.74	0.48
Within Groups	4183.10	27	154.93		
Total	4412.45	29			

Few studies have explored the relationship between age and mercury concentration in blood samples. Park et al. (2005) found no significant difference between age and mercury concentration in the serum of urban dogs from various regions in Korea. Similarly, Sousa et al. (2013) did not observe a significant correlation between age and mercury levels in the blood of domestic dogs. Lopez-Alonso et al. (2007) also reported no significant difference based on age. Since mercury concentration in the blood reflects recent exposure levels, the lack of significant differences across age groups in this study and others can be explained. The relatively higher mercury concentrations in older dogs might be attributed to their greater roaming behavior compared to younger dogs, which exposes them to different food resources.

#### 4. Conclusion

The findings of the present study showed that dogs in different parts of Gorgan City were contaminated with

mercury with an average concentration lower than the standards (105 µg/L) provided for this pollutant. However, even low levels of mercury have been shown to be toxic to mammals. The concentration of mercury in the blood indicates the level of recent environmental contamination, suggesting that many areas within Gorgan are affected by mercury pollution, likely from multiple sources. Furthermore, the presence of mercury in the blood samples of free-roaming dogs shows the potential for mercury to enter the local food chain. This underscores the inadequate management of mercury-containing waste disposal in this city. To address these issues, it is essential to implement stricter waste disposal regulations, enforce laws to limit mercury entrance in Gorgan City and raise public awareness regarding the potential mercury exposure. In addition, future research should include longitudinal studies to monitor changes in mercury levels over time, as well as investigations into the health effects of mercury exposure on free-roaming dogs.

## Authors' Contributions

**Mahdiyeh Ghadimi Aliabadi:** Formal analysis; data curation; writing-original draft preparation; visualization; investigation. **Somayeh Namroodi:** Conceptualization; project administration; funding acquisition and methodology; writing-review and editing. **Alireza Yousefi:** Visualization; investigation. **Hajar Abyar:** Writing-review and editing; supervision; software; validation.

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## Conflicts of Interest

No conflict of interest has been declared by the authors.

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## Ethical considerations

All experimental procedures involving animals (blood collection) were approved by the Animal Welfare and Ethics Committee of Gorgan University Agricultural Science and Natural Resources, Gorgan, Iran (approval number: N.T. 8/116709).

## Using artificial intelligence

Artificial intelligence techniques are not used in this research.

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