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## Detection of milk's sheep quality indicators by studying the contamination of milk with some heavy metals in some areas of Hadramout Coast – Yemen

Ali Mohammed Abdel-bari Ba-abbad<sup>1, 2\*</sup>, Alwi Ahmad Alwi Maknoon<sup>3</sup>, and Yaslam Khaled Yaslam Bin-Zaidan<sup>3</sup>

<sup>1</sup> Food Science and Technology Department, Faculty of Environmental science and Marine Biology, Hadhramaut University, Hadhramaut, Yemen.

<sup>2</sup> Clinical Nutrition and Dietetic Program, Faculty of Medicine and Health Sciences, University of Science and Technology- Aden.

<sup>3</sup> Department of Zoology, Faculty of science, Hadhramaut University Hadhramaut, Yemen.

### ABSTRACT

An experimental study was conducted with the aim of using sheep milk as bioindicators of environmental pollution for cadmium, lead and arsenic in some areas of Hadhramout coast in the period from November 2021 to March 2022, 45 samples of milk were collected from five areas of the Hadhramaut coast (Fowah, Al-Ghaleelah Thelah, Shaheer and Al-Khareebah). Cadmium and lead concentrations were measured by (FAAS), while arsenic concentrations were measured by (AAS- MHS 15). The results of study showed that the mean concentrations of cadmium, lead and arsenic in milk samples are 0.0087 ppm, 0.055 ppm and 0.105 ppm; respectively. Concentration of cadmium were higher than the permissible limit according to Europe Union and Codex Alimentarius milk 0.01 ppm in urban areas (Fowah, Al- Ghaleelah, and Shaheer). Lead concentration was found higher than the maximum permissible limit for Europe Union and Codex Alimentarius of lead in milk 0.02 ppm in areas (Fowah, Al- Ghaleelah, and Shaheer). Arsenic concentration was found higher than the maximum permissible limit for Europe Union and of arsenic in milk 0.1 ppm in Thelah area, While Al-Khareebah area was in maximum permissible limit Europe Union. The statistical results showed that there were significant differences between the average concentrations of cadmium, lead and arsenic at a significant level ( $p < 0.05$ ) in milk samples in the different study areas. This study confirmed that sheep milk are good bioindicators for detecting environmental pollution with heavy metals. The study concluded that the main reason for the high concentrations of cadmium and lead in urban areas are human industrial activities, all process of combustion and industrial and agricultural wastes. The study recommends periodic monitoring and examination of heavy metals in the environment using bioindicators and identifying the main sources of their spread in the environment.

**Keywords:** Bioindicator, Cadmium, Lead, Arsenic, Sheep milk, Pollution, Hadhramaut Coast.

\*Corresponding author: Ali Mohammed Abdel-bari Ba-abbad - [E-mail:alabbady72@gmail.com](mailto:alabbady72@gmail.com)



## Introduction

Pollution is the addition (or addition in high concentration) of certain substances to the environment (water, air, and soil), making it less suitable or uninhabitable for life (1). Atomic weights and densities of heavy metals exceed 5 g/cm<sup>3</sup> (2, 3). Thus, they (heavy metals) are characterized by their ability to bioaccumulate in food (4). The type of effect caused by toxic substances, and the effectiveness of that effect, determine the toxicity of the substance. The effect occurs when exposed to this toxic substance through inhalation, ingestion, or direct contact. They cause health effects that appear immediately or appear delayed later in life, as a result of long-term (chronic) and short-term (acute) exposure to these substances (5).

Cadmium (Cd) is non-essential element, is toxic at very low doses, and non-biodegradable, one of the most common and harmful transition metals presents in our environment (6). It has highly soluble in water as compared to other heavy metals (7). Its metal found in the earth's crust, associated with zinc, lead, and copper ores and pure (8, 9).

Cadmium reaches humans through their diet, drinking water or air, or through biomagnification and slowly accumulates in the human body (10). According to (11) the toxicity of cadmium is due to the long biological half-life of this metal, which is (10-30) years in human tissues, which makes it a threat and danger to many organs and systems inside the human body, particularly the kidneys. Lead is the 37th most abundant element of the earth crust. Naturally, it accounts for high crustal abundance of 14 ppm (12). Lead is one of the earliest metals discovered by the human and its distinctive nature, such as pliability, ductility, higher malleability, low melting point and corrosion resistant, make its widespread usages in numerous industrial process (13). Lead enters the human body when eating foods, sweets, or water contaminated with lead, or inhaling lead dust that arises from lead-containing paints, lead-contaminated soil, and from color additives in

paints and cosmetics. In some special cases, children may use toys that contain lead (14). Lead affects various parts of the human body, due to its toxic effect on them, causing physical and mental damage. Its effect on children and women appears when they are exposed to long-term doses of lead, which leads to disorders in the reproductive system in women and damages the endocrine glands in children (15). Arsenic has affected humans more than any other toxic element or compound for thousands of years (16).

Millions of people are being chronically exposed to elevated doses of arsenic from air, food, water, and soil. It has the ability to readily change its oxidation state and bonding configuration, thus showing diverse chemical behavior in the environment and forming large numbers of organic and inorganic compounds (17). As a result of these malpractices, arsenic concentrations in groundwater exceeded the safe limits for arsenic (10 µg/L), as recommended by the World Health Organization (18). Arsenic is colorless, odorless and tasteless, so its presence in drinking water is not obvious, but greater concern is ongoing (chronic) exposure to smaller amounts of arsenic over time (19). Arsenic reaches the human body through drinking water contaminated with it, or through eating foods produced from contaminated lands, crops irrigated with contaminated water, or marine organisms in environments contaminated with arsenic (20). The concentration of arsenic in foods contaminated with it exceeds the doses to which humans are exposed through drinking water, soil or air (21).

## Bioindicators

Living organism's plants, animals or microorganisms that are exploited to detect pollutants in the ecosystem (22). There are criteria for selecting and using the appropriate living organisms as bioindicators are:

- An ability to accumulation (or selection of specific compounds).
- Occurrence in large populations.



- Their presence cannot be limited to only a single habitat.
- Ease of identification and representative sampling.
- Samples should be easy for chemical analysis (23)

The importance of monitoring and following up the behavior of these elements in the environment, measuring the negative effects of industrial activity, and searching for the possibility of using some types of living organisms as bioindicators to assess the level of pollution (24).

### ***Milk Sheep***

Sheep milk is a good indicator of pollution with heavy metals, due to the behavior of these animals in grazing and searching for water and food, as these animals occupy a larger area than cows, so they move in search of water and food for very long distances, which makes them vulnerable to various types of pollutants through eating food and water heavy metal contamination (25). The chemical composition of milk (nutrients or toxic contaminants) varies according to some internal factors (lactation status, animal type, and health condition such as mastitis), and other external factors such as: production season, animal nutrition and environment) (26). the ability of animals to adjust to a range of environmental conditions such as grazing in hot, arid conditions under direct solar radiation in poor pasture (27). It has the ability to deal with diversified grazing, which is, eating different types of food (28). Thus, heavy metal concentration in milk can be an indicator of environmental pollution (29).

### ***Problem statement:***

Milk is an essential food product for humans, the research was interested in studying the extent of milk contamination with heavy metals, to reveal milk quality in terms of heavy metal contamination.

## **Methods**

### ***Study Area & Sample Size***

Non-probability simple random sampling

technique was used to select the samples in this study. A total of 45 milk samples were collected from five areas in Hadhramaut Coast included Fowah, Al-Ghaleelah, Thelah, Shaheer and Al-Khareebah for heavy metals analysis included in this study.

### ***Samples collection***

Samples of sheep's milk were randomly selected, to study milk contamination, and affected by various human activities, samples were collected from different areas as follows:

- Geographic location: Coastal areas are near cities and other far from sea coast.
- Human activities: Some study areas are close to large population centers, and other rural areas and mostly sparsely populated.

### ***Samples preparation***

The samples were dried by drying and sterilization oven J.P Selecta. Each 30 mL of milk samples was placed in heat-resistant glass dishes and then placed in a dry oven at 70° C overnight, then it was put in the desiccator for an hour, and then it was crushed well (31). Samples were digested by microwave digestion system Model START D (32). The digestion method of milk samples was used (HPR-FO-24) according to the " Milestone Application Book" which attached to the device. The digested samples were placed in plastic bottles and the size was completed to 50mL with Bi-distilled water from Ultra-Pure Water System.

### ***Sample Analysis***

Cadmium and lead concentrations were measured by Flame Atomic Absorption Spectrophotometer (Appendix E) Model UP 9100 (33). While Arsenic by Atomic Absorption Spectrophotometer (Appendix E) Model Pin AAcle 900F (34). Witch Mercury Hydride System 15 (MHS15) (35).

Method of preparing solutions and running the devices has been adopted for analysis elements according to (36). The limit detection of cadmium, lead and arsenic was identified by method produce signal/noise ratio of 3 Through the equation:



The detection limits of cadmium, lead and arsenic were 0.008, 0.012 and 0.0013 ppm.

$$\text{Detection Limit} = \frac{(\text{Standard Conc.}) \times 3 (\text{Std. Dev.})}{\text{Mean}} \quad (36)$$

### Statistical analysis

The data were statistically analyzed by Statistical Package for Social Sciences (SPSS) version 24, using ANOVA Table one way, and the significance of differences between the means were tested by Least Significant Difference Test (L.S.D) with statistically significant with a probability level (< 0.05 P).

## Result

### *Cadmium, lead and Arsenic concentrations in milk samples in study areas*

The results of cadmium, lead and arsenic concentrations were varying in milk samples between study areas Table 1. The highest concentrations of cadmium were in Al-Ghaleelah 0.0136 ppm and Fowah 0.0113 ppm, followed by Shaheer 0.01 ppm then Thelah 0.0086 ppm. The concentration of cadmium in Al-Khareebah was below detection limit <0.008. The general average of cadmium concentrations in sheep milk samples in all study areas was 0.0087 ppm.

The highest concentration of lead was in Fowah 0.098 ppm, followed by Al-Ghaleelah 0.085 ppm then Shaheer 0.074 ppm, while the lowest concentration was in Thelah 0.019 ppm. The concentration of lead in Al-Khareebah was below detection limit <0.012. The general average of lead concentrations in milk samples in all study areas was 0.55 ppm. The highest concentration of arsenic was in Thelah 0.163 ppm, Al-Khareebah was 0.130 ppm then Shaheer that was 0.098 ppm and Al-Ghaleelah 0.097 ppm, while the lowest concentration was in Fowah 0.040 ppm. The general average of arsenic concentrations in milk

samples in all study areas was 0.10 ppm.

**Table 1:** Cadmium, lead and Arsenic concentrations (ppm) in milk samples in study areas

No.	Area	Cadmium	Lead	Arsenic
1	Fowah	0.0113	0.098	0.040
2	Al-Ghaleelah	0.0136	0.085	0.097
3	Thelah	0.0086	0.019	0.163
4	Shaheer	0.01	0.074	0.098
5	Al-Khareebah	BDL*	BDL*	0.130
<b>Mean</b>		<b>0.0087</b>	<b>0.055</b>	<b>0.10</b>
* Below detection limit				

### Results of Statistical Analysis

The results of statistical analysis showed no significant differences in cadmium concentrations between Al-Ghaleelah and Fowah, between Fowah and Shaheer and between Shaheer and Thelah. There are significant differences between Al-Ghaleelah and

Thelah and between Al-Ghaleelah and Shaheer, while Al-Khareebah were below detection limit.

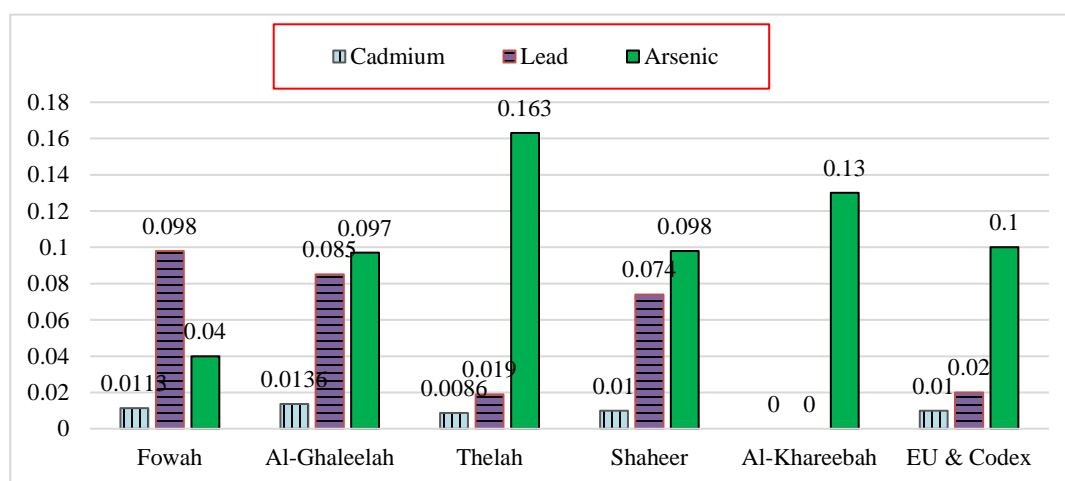
The results of statistical analysis showed significant differences in lead concentrations between Fowah and all study areas, but there are no significant differences between Shaheer and Al-Ghaleelah. Al-Khareebah was below detection limit. The results of statistical analysis showed significant differences in arsenic concentrations between all study areas except between Al-Ghaleelah and Shaheer and between Shaheer and Fowah. These results are shown in Table 2.



**Table 2 :** Cadmium, lead and arsenic concentrations (ppm) in milk samples, the values are (mean  $\pm$  SD n=3)

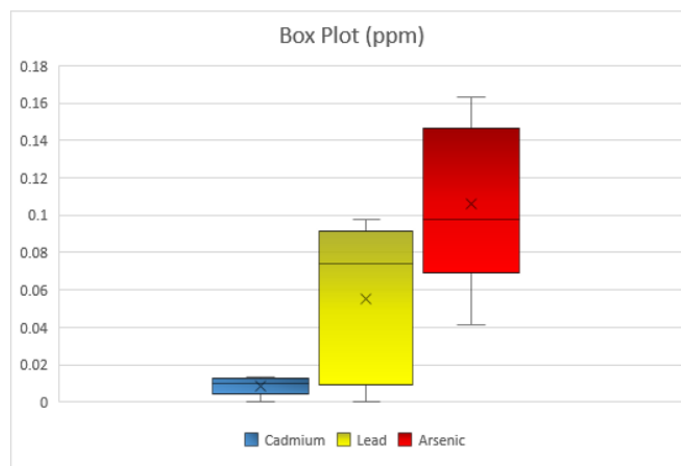
Area	Cadmium	Lead	Arsenic
Fowah	0.0113 $\pm$ 0.0011 <sup>ab</sup>	0.098 $\pm$ 0.132 <sup>a</sup>	0.040 $\pm$ 0.0015 <sup>e</sup>
Al-Ghaleelah	0.0136 $\pm$ 0.0020 <sup>a</sup>	0.085 $\pm$ 0.0061 <sup>a</sup>	0.097 $\pm$ 0.0040 <sup>dc</sup>
Thelah	0.0086 $\pm$ 0.0011 <sup>c</sup>	0.019 $\pm$ 0.0068 <sup>c</sup>	0.163 $\pm$ 0.0035 <sup>a</sup>
Shaheer	0.010 $\pm$ 0.0020 <sup>bc</sup>	0.074 $\pm$ 0.010 <sup>b</sup>	0.098 $\pm$ 0.0070 <sup>c</sup>
Al-Khareebah	BDL	BDL	0.130 $\pm$ 0.0034 <sup>b</sup>
Total	0.0087 $\pm$ 0.0049	0.055 $\pm$ 0.040	0.105 $\pm$ 0.042
<i>P-value</i>	<0.001	<0.001	<0.001
Detection limit	0.008	0.012	0.003

Statistical significance difference at  $P < 0.05$ . The values are (mean  $\pm$  SD n=3)



**Figure 1:** Comparison of cadmium, lead and arsenic concentrations in milk between (EU & Codex) and study areas

The similar letters in same column means no significant differences



**Figure 2 :** Box Plot of cadmium, lead and arsenic concentrations (ppm) in sheep milk samples



## 4. Discussion

### *Cadmium in milk samples*

In this study, the cadmium concentrations were <0.008 ppm, 0.0086 ppm, 0.1 ppm, 0.0113 ppm and 0.0136 ppm (Table 1) in milk samples collected from Al-Khareebah, Thelah, Shaheer, Fowah and Al-Ghaleelah; respectively. These results partially agree with results in north-east Hungary 0.006-0.020 ppm (37) and Egypt 0.004-0.029 ppm (38). Less than these results were reported by (39) 0.00059-0.00077 ppm, (26) 0.00079-0.00611 ppm, (2016) (40) 0.00071-0.009 ppm and (41) 0.0099-0.00044 ppm. Higher than these results were reported by (42) 0.05-0.10 ppm. The highest cadmium concentrations in this study was 0.0136 ppm in Al-Ghaleelah (Table 1) This is due to the type of water and food in addition to the style of grazing for sheeps. Stilled water is widely spread in the region and through the free grazing style. Sheep depend on this water as a source of drinking and the remnants of waste that are collected in large quantities before burning as it is within the scope of grazing for sheep. (43 and 44) Confirmed the presence of cadmium in solid waste, while (45 and 46) indicated the presence of cadmium and other elements in the soil surrounding the solid waste landfill.

Results in exceeded levels of cadmium standards of WHO to reach levels of eco-toxicological risk and the high accumulation of heavy metals in the edible plant parts of the widely consumed vegetable (47). Despite this, the concentrations of cadmium in the milk samples that analyzed in all areas in this study (Table 1) were found below the Codex Alimentarius maximum permissible limit of cadmium in milk 0.01 ppm (48). Except Fowah, Al-Ghaleelah and Shaheer, which has reached the cadmium concentration to maximum permissible limit of cadmium in milk for Codex Alimentarius Commission Standards.

### *Lead in milk samples*

In this study, the lead concentrations in milk samples were <0.012 ppm, 0.019 ppm, 0.074 ppm, 0.085 and 0.098 (Table 1) were prepared from Al-

Khareebah, Thelah, Shaheer, Al-Ghaleelah and fowah; respectively. The results in this study partially agree with similar results in Calabria-Italy <0.040-0.09 ppm (49), Iran 0.0046-0.030 ppm (26), and north-east Hungary 0.007-0.040 ppm (37). Lower than lead concentrations in this study were reported by (41) 0.0025-0.033 ppm and higher than lead concentrations in this study also were reported by (38) 0.147-0.737 ppm, (42) 0.11-0.28 ppm and (50) 2.64-5.66 ppm.

Average lead concentrations in Fowah, Al-Ghaleelah and Shaheer are 0.098, 0.085, and 0.074 ppm; respectively (Table 1) were higher than Codex Alimentarius maximum permissible limit of lead in milk 0.02 ppm (51). This is due to the increase in industrial activity in Fowah, a region with high population activity in Mukalla city. It is also a transmission of lead from pollution sources (car exhausts and residues of factories, laboratory, batteries and paint pigments) to milk sheep through the food chain and excessive use of fertilizers for growth plant in invalid places of agriculture which is in the end is used as a feed of sheep. This applies to the Shaheer as well. While the spread of brick and cement coefficient and solid waste burring in Al-Ghaleelah led to high average lead concentration.

According to (52) Free-grazing mammals, have higher levels of lead exposure in urban areas also indicated that the concentration of lead in urban sheep in Egypt that graze on street waste is higher compared to the sheep that were housed indoors in the University Veterinary Teaching Hospital.

### *Arsenic in milk samples*

The results of this study indicated that levels of arsenic in milk samples ranged from 0.040 ppm to 0.163 ppm average (0.10 ppm) (Table 1). The average results in this study partially agree with similar results in Turkey 0.14 ppm (53). Average of arsenic concentrations values above 0.10 ppm were reported by (54) 2.07 ppm and (55) 32.3 ppm. Average of arsenic concentrations values under



0.10 ppm were reported by (41) 0.0051 ppm, (56) <0.030 ppm and (57) <0.03. Average results demonstrate that milk samples from rural areas (Thelah and Al-Khareebah) shows higher values, whereas milk samples from urban areas (Fowah, Al-Ghaleelah and Shaheer) shows lower value (Table 1) The reason for this rise is the contamination of agricultural soil with fertilizers and pesticides may seep into the water which is used for drinking sheep and irrigating fodder. According to (58) concentrations of heavy metals during lactation depend mostly on feed or water intake, and possible consumption of soil. (55) concluded that arsenic concentration in milk samples were directly

proportion to its levels in the drinking water. Average arsenic concentration in milk samples from Thelah area was higher maximum permissible limit for the Europe Union the presence of arsenic in milk 0.1 ppm (59) Al-Khareebah was in maximum permissible limit and this is could be because of Industrial activities near this area and waste from these factories, while urban areas (Fowah, Al-Ghaleelah and Shaheer) were found below maximum permissible limit.

**Table 3 :** A comparison of cadmium, lead and arsenic content (ppm) in milk samples from different countries and present study

Country	Cd	Pb	As	Reference
Present Study	0.0087	0.055	0.10	
Egypt	N.R**	3.96	N.R**	(50)
	<0.022	<0.022	N.R**	(60)
Iran	0.002	0.010	N.R**	(61)
	0.0016	0.014	N.R**	(62)
	0.0033	0.0121	N.R**	(26)
Pakistan	0.102	N.D*	N.R**	(63)
Turkey	0.13	3.17	2.07	(54)
Nigeria	0.30	N.R**	N.R**	(64)
Slovakia	<0.0040	<0.10	<0.03	(57)
Codex Maximum limit	0.01	0.02	N.R**	(48, 51)
EU Maximum limit	-	-	0.1	(59)

\* Not detectable, \*\* Not reported

It is recommended to eliminate of pesticide waste and agricultural waste by coordinating with official authorities such as Ministry of Agriculture and Fish Wealth in order to minimize the risk of heavy metals contaminations.

## Conclusion

This study confirmed that sheep milk is good bioindicator for detecting environmental pollution with heavy metals. The main reason for the high concentrations of cadmium and

lead in urban areas are human industrial activities, all process of combustion and industrial and agricultural wastes. The irregular use of fertilizers and pesticides led to the concentrations of arsenic more in rural agricultural areas than in urban areas.

## Conflict of interest

The authors declare that no conflict of interest.



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