ASIA PACIFIC JOURNAL of MEDICAL TOXICOLOGY
APJMT 9;4 http://apjmt.mums.ac.ir December 2020

ORIGINAL ARTICLE

Comparative Study on Lead, Cadmium and Arsenic Concentration of Alfalfa forage in Tehran Province

MAHMOUD TOHIDI MOGHADAM, SEYED SOHEIL GHAEMMAGHAMI*

Ph.D in Feed Hygiene, Departments of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran.
Institute of Agricultural Education and Extension, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

Abstract

Background: This research aims to evaluate heavy metals contamination of Alfalfa forage and soil in industrial and non-industrial regions of Tehran province. The soil and plant samples were collected from Alfalfa fields situated in industrial and non-industrial areas during two harvests of Alfalfa harvest.
Methods: The Electrothermal atomizers spectroscopy technique was employed to measure the heavy metals (lead, arsenic, and cadmium) levels in the samples. A flame atomic absorption spectrometer equipped with acetylene-air flame was employed.
Results: The highest heavy metals concentrations were detected in the soil and Alfalfa samples from the industrial regions. Lead had the highest level in the soil followed by arsenic and cadmium; the same trend held for the Alfalfa as well. Moreover, the heavy metal content in Alfalfa declined from the first to second harvest, while it increased in the soil. The heavy metals content of Alfalfa was below the maximum permitted concentration for feedstuffs and livestock. The highest soil-plant transfer factor of heavy metals was detected in the first harvesting. The highest arsenic transfer factor was recorded in the industrial areas.
Conclusion: The findings of the current study can be helpful in the determination of healthy forage in various areas.

Keywords: Heavy metals, Alfalfa, Soil Pollutants, Animal Feed

How to cite this article: Tohidi Moghadam M, Ghaemmaghami SS. Comparative study on lead, cadmium and arsenic concentration of Alfalfa forage in Tehran province. ***Asia Pac J Med Toxicol*** **2020; 9(4):129-135.**

INTRODUCTION

Heavy metals (HMs) accumulation in soil and agricultural crops has become a major challenge throughout the world. HMs refer to environmental contaminants capable of influencing agricultural plants. They can indirectly or directly enter the human food chain through livestock. HMs (lead, arsenic, and cadmium) contamination in plants used as livestock feed contaminates the human diet (1). As one of the prominent classes of pollutants, HMs can enter the environment through various industrial and agricultural activities (1). The significance of HMs has resulted in the establishment of strict regulations regarding dietary exposure in husbandry practices, dietary ingredients, soil ingestion or spurious soil contamination in foliage (2-4). In this regard, the impact of animal feed contaminants on animal and human health have been comprehensively reviewed (4).This has provided information about the natural amount / background of trace elements in soil, biological indicators of the chemical status of the environment, soil remediation and excessive accumulation of trace metals in soil (5). Soil is the major medium for root activity and irrigation with industrial effluent can lead to significant accumulation of toxic metals in plants(6, 7). Several studies have addressed the content of potentially toxic elements in plants cultivated near or far from the cities(8, 9). Soil contamination of agricultural fields in the vicinity of large cities has become a serious environmental challenge(10). Some elements such as cadmium, lead, and arsenic are more important in terms of toxicity. Lead poisoning in livestock, which is very common, originated from several industrial activities (e.g. lead paint, lead accumulator batteries and mines) (11). Cadmium contamination is primarily due to the direct uptake from cadmium-polluted soils(12). The European food safety authority (EFSA)panel evaluated the risk of arsenic contamination of the food chain on human health(4). As one of the highly toxic metalloids, arsenic could be released into nature through a wide variety of industrial activities(13). Upon HMs exposure, plants adapt several defense mechanisms including elevated proline synthesis, osmotic regulation to prevent metal transfer to the aerial organs, preservation of enzymes involved in maintaining protein synthesis, and enhanced activities of antioxidant enzymes (e.g. catalase, peroxidase) (14, 15). As one of the prominent forage crops, Alfalfa (*Medicago sativa*) can reach high biomass yields with superior nutritional value applicable to the livestock feed. Alfalfa is a perennial herb of the legume family that can be harvested about four times per year in cold climates and up to 12 times per year in tropical regions. It is one of the most important plants for foraging in the world(16). In addition to the accumulation of several heavy elements (zinc, lead, chromium, nickel, copper, , etc.) in various

*Correspondence to: Seyed Soheil Ghaemmaghami, Ph.D in Feed Hygiene (D.V.M Ph.D), Institute of Agricultural Education and Extension, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
Email: Soheil_ghaem@yahoo.com, Tel: 02166940731

plant components, it may alter nutrients in soil which can be beneficial for some and dangerous for others (3, 9). The use of HM-contaminated plants as the feed for domestic animals can decrease their milk and meat production and decrease their resistance against diseases (17).Exposure of livestock and humans to bioavailable heavy metals shows evidence that HMs accumulate in the soil and transfer to plants (18, 19). A limited number of works have investigated HMs accumulation in Alfalfa during different harvest times in industrial and non-industrial regions. In this context, the current study is devoted to assessing the influence of HMs (lead, arsenic, and cadmium) in industrial and non-industrial areas and their content in Alfalfa, during 2 different harvest periods.

METHODS

This study measured the HMs levels (lead, cadmium, and arsenic) in agricultural soil and Alfalfa grown in two regions of Tehran province. 36 samples from soils and plants were collected in 2 harvests. First, the selected agricultural farms of industrial and non-industrial regions were divided into 3 farms; then, each farm was separated into three parts (locations beginning, middle, and end) to randomly sample Alfalfa and soil. The sampling of each area was independent during the first and second Alfalfa harvestings. Moreover, water samples were obtained from two areas. Soil samples were collected from an approximate depth of 15 cm. The collected samples were put in polyethylene bags, washed with detergent, and preserved in a container including 5% nitric acid and water, and sent to the laboratory.

2.1 Industrial Area

The industrial area is located near Ray city in the south of Tehran. This area is surrounded by small polluting industries (for example, copper smelters, battery factories, and oil and gas storage tanks) and large Tehran oil refineries. Other industrial sources nearby are the Ray city landfill and the Tehran cement factory.

2.2 Non-Industrial Area

An area near Varamin (south of Tehran province) was chosen as the non-industrial area as it is situated far from the industrial factories.

2.3 Sample Preparation

Alfalfa samples were dried at 105 °C in an oven for 24 h. The dried samples were then milled by a 2-mm grid and kept in a desiccator to determine their HMs contents(20). The soil samples were dried at 70 °C for 24 h. A 2-mm sieve was used to eliminate stones and other solids as described in Sposito (1989) methods(21).

2.4 Measurements of heavy metals

HMs content of the soil and plant samples was assessed using an atomic absorption instrument. For this purpose, a flame atomic absorption spectrometer equipped with acetylene-air flame was employed**.** The detection limit was 0.1 ppm in the digested solution. The sample is simply diluted and then aspirated directly into atomic absorption device that HMs levels were lower than the limit of detection of the sensors device.

2.5 Calculation of transfer factor

The soil-plant transfer factor of HMs(TF) was also computed by the following schemes (22, 23)**:**

TF= C PLANT/C SOIL

C PLANT= Concentration of HMs in plant (mg/kg)

C SOIL = Concentration of HMs in soil (mg/kg)

2.6 Data Analysis

Data analysis was conducted by SPSS 18 software through ANOVA. Duncan's multiple range tests were also employed for the comparison of the mean values if the variances were uniform. A t-test was also applied for comparing the mean of two harvests in the industrial and non-industrial areas in which P <0.05 indicated significance.

RESULTS

3.1 Heavy metals concentration in agricultural soil

According to Table 1**,** the concentration of the investigated HMs in the soil of three farms in the two harvest stages showed no significant difference.

The order to the mean accumulation levels of HMs in the soil of the two areas was as follows: Pb> As > Cd.The variations in the HMs of the soils sampled in 2 harvests are

Table 1. Concentration of heavy metals on the soil in the Industrial and Non-industrial area (mg.kg^{-1})

Stages	Sites	Non-industrial area			Industrial area		
		Pb	Cd	As	Pb	Cd	As
Cut1	Farm 1	265.3	2.7	3.8	308.3	4.2	5.3
	Farm 2	278.3	3.7	4.8	318	4.3	6.3
	Farm 3	273.5	2.7	4.2	312	5	6
	Mean ±SD	272.4 ±3.5	3.0 ±0.6	4.3 ±0.3	312.8 ±4.4	4.5 ±0.4	5.9 ±0.5
	Significance	0.3	0.1	0.5	0.4	0.3	0.7
Cut2	Farm 1	278.3	4.2	5.9	368.7	5.3	6.5
	Farm 2	296.7	4.8	6.2	378	5.4	7.7
	Farm 3	291.7	4.5	6.2	374.7	6	6.6
	Mean ±SD	288.9 ±6.7	4.5 ±0.3	6.1 ±0.2	373.8 ±8.1	5.6 ±0.4	6.9 ±0.2
	Significance	0.2	0.6	0.5	0.9	0.6	0.2

±SD: Standard deviation; BDL: below detection limits; sig: significance level among differences areas for each parameter shown in (figure 1). Despite the incremental trend of the mean HMs contents of the soils from first to the second harvests, this trend was not significant.

A significant difference was observed in the mean As, Pb, and Cd accumulations in the Alfalfa agricultural soil in the industrial and non-industrial regions for each harvesting time (Table 2).

3.2 Heavy metals concentration in Alfalfa

The HMs contents of Alfalfa grown in farms located in the two areas in both harvests are presented in Table 3.No significant difference was detected in the 3 farms with respect to lead, cadmium, and arsenic.

The mean HM accumulation in Alfalfa grown in the two areas varied in the following order: Pb> As > Cd. The variation of HM in alfalfa is shown in figure 2. As can be seen, HMs concentration showed a declining trend from the first to the second harvest in each area. No significant difference was detected in the average Pb and Cd contents in the two harvests; the mean arsenic content, however, exhibited a reduction from first to the second harvest in the non-industrial area (P≤0.05).

Moreover, a significant difference was observed in the mean As, Pb, and Cd accumulations in the Alfalfa cultivated in the industrial and non-industrial regions in each harvesting time (Table 4**)**. The average Pb accumulation was higher in Alfalfa grown in the industrial area for both harvests. Besides, the two regions were significantly different in terms of Cd and As (P≤0.05).

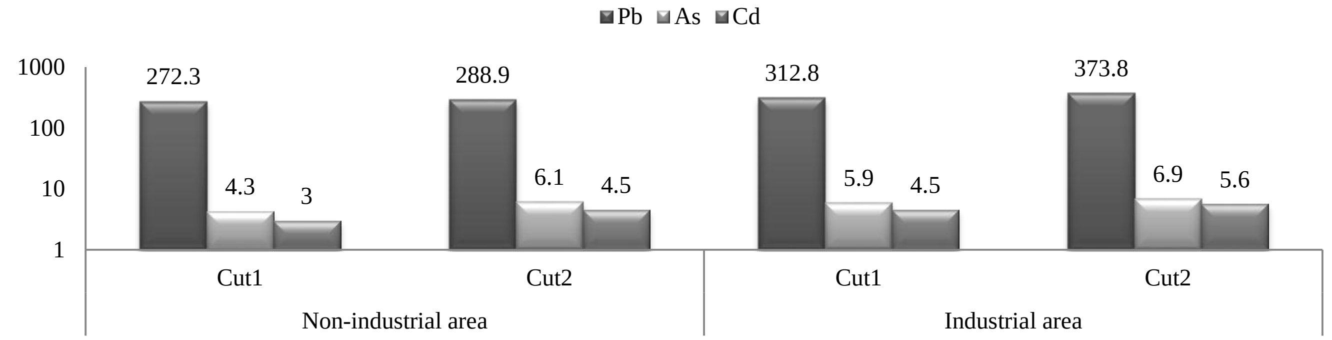


Figure 1. Means of heavy metals in soils in two harvesting (mg.kg^{-1})

Table 2. Compare heavy metals in industrial and non-industrial areas in soil (mg.kg^{-1})

Harvesting	Cut1			Cut2		
Heavy metals	Pb	Cd	As	Pb	Cd	As
Non-industrial	272.4 ±3.5	3.0 ±0.6	4.3 ±0.3	288.9 ±6.7	4.5 ±0.3	6.1 ±0.2
Industrial	312.8 ±4.4	4.5 ±0.4	5.9 ±0.5	373.8 ±8.1	5.6 ±0.4	6.9 ±0.2
Significance	0.001	0.09	0.07	0.007	0.1	0.1

±: Standard deviation; BDL: below detection limits; sig: significance level among differences areas for each parameter

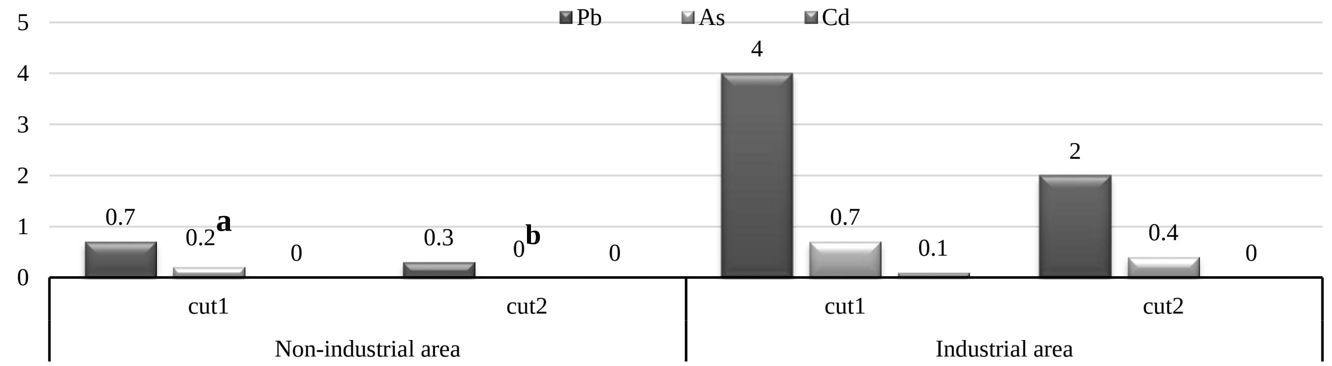


Figure 2. Mean of heavy metals in Alfalfa in two harvesting (mg.kg-1)

Table 3. Concentration of heavy metals on Alfalfa in Industrial and Non-industrial area (mg.kg^{-1})

Harvesting	Sites	Non-industrial area			Industrial area		
		Pb	Cd	As	Pb	Cd	As
Cut$_1$	Farm 1	0.6	BDL	0.1	3.5	0.11	0.55
	Farm 2	0.8	BDL	0.2	4.5	0.1	0.8
	Farm 3	0.7	BDL	0.2	4	0.13	0.75
	Mean ±SD	0.7 ±0.08	-	0.2 ±0.04	4 ±0.5	0.1 ±0.02	0.7 ±0.1
	Significance	0.1	-	0.3	0.3	0.6	0.5
Cut$_2$	Farm 1	0.25	BDL	BDL	1.9	BDL	0.37
	Farm 2	0.4	BDL	BDL	2.1	BDL	0.45
	Farm 3	0.3	BDL	BDL	2	BDL	0.38
	Mean ±SD	0.3 ±0.01	-	-	0.2 ±0.1	-	0.4 ±0.04
	Significance	0.1	-	-	0.7	-	0.2

SD: Standard deviation; BDL: below detection limits; sig: significance level among differences areas for each parameter

Table 4. Comparison heavy metals in industrial and non-industrial areas in alfalfa (mg.kg^{-1})

Areas	Cut1			Cut2		
	Pb	Cd	As	Pb	Cd	As
Non-industrial	0.7 ±0.08	BDL	0.2 ±0.04	0.3 ±0.01	BDL	BDL
Industrial	4 ±0.5	0.1 ±0.02	0.7 ±0.1	2 ±0.1	BDL	0.4 ±0.04
Significance	0.009	0.006	0.007	0.005	BDL	0.004

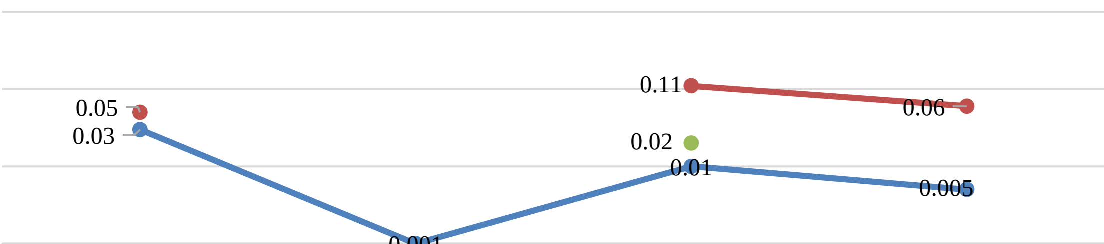
±: Standard deviation; BDL: below detection limits; sig: significance level among differences areas for each parameter

3.3 Transfer factor

Similar HMs transfer factors were calculated for the non-industrial and industrial regions. Comparing the harvests, HMs of the first harvest were higher in both areas. The arsenic transfer factor was the highest in the industrial area compare with other heavy metals (Figure 3).

DISCUSSION

Table 5 presents the standard residual levels of heavy metals in different global organizations and is compared with the obtained levels of these elements in this study.



	Non-industrial cut1	Non-industrial cut2	Industrial cut1	Industrial cut2
Pb	0.03	0.001	0.01	0.005
As	0.05	0	0.11	0.06
Cd	0	0	0.02	0

Figure 3. Transfer factor in the industrial / non-industrial area in two cutting of alfalfa.

Table 5. Compare residual concentration heavy metal this study to others standards (mg.kg^{-1}).

Heavy metal		Pb	As	Cd
This study	Soil	265.3-374.7	0.5-7.7	2.7-5.4
WHO-EU, FAO		20-300	1-5	15-20
This study	Plants	0.25-4.5	BDL-0.8	BDL-0.13
WHO-EU, FAO		0.1-30	0.2-0.8	0.1-5
NRC		5-30	10-100	0.5

BDL: below detection limits; WHO: World health organization; FAO: Food and Agriculture Organization; NRC: National Research Council

4.1 Heavy metals in agricultural soil

The lead, cadmium, and arsenic contents of the farms were below the maximum permitted content during the first harvest for the non-industrial area (Table. 1&5). Esmaeili et al. (2014) assessed the lead and cadmium content of agricultural soils in industrial and non-industrial areas in Iran, and they were 319.3 mg/kg and 8.68 mg/kg and 7.2mg/kg and 0.11 mg/kg respectively(24). According to Kelepertzis in Greece(2014), Pb, Cd, and As contents of agricultural soils ranged from 3.17-48.49mg/kg, 0.07-6.1mg/kg, and 2.7-12.8 mg/kg, respectively. The highest Cd concentration exceeded the maximum permissible concentration (5 mg/kg)(25). Furthermore, the mean heavy metal contents of non-industrial regions were lower than the permissible limit. Pb concentration of the three investigated farms of the industrial area exceeded the maximum permitted concentration (Table.5). As concentration was however below the maximum permitted concentration. Cd concentration exceeded the maximum permitted concentration (1-5 mg/kg) in the second harvest for the industrial area. Moreover, the mean Pb concentration of the industrial area was above the maximum concentration. In addition, HMs contents of the agricultural soils increased from the first to second harvest **(**Figure 1**)**. Some studies have shown that HMs distribution in the soil was not uniform and depended on several factors including fertilization, irrigation, precipitation and physical features of soil(26, 27). Chemical factors play key roles in HMs accumulation in alfalfa, including pH, soil organic matter, interfering ions, and soil texture. Other factors include solubility of HMs, their bonding with organic matter, and capacity and mobility combined with the availability of these elements for the roots and their translocation from soil accumulation in the alfalfa.

4.2 Heavy metals in Alfalfa

According to Tables 3and 5, the concentration of heavy metals in alfalfa in industrial and non-industrial areas was below the permitted concentration for animal feed and other standard limits. Comparing the total HMs concentrations, HMs content of in Alfalfa grown in industrial areas was higher than those cultivated in non-industrial areas for both harvesting stages.

According to Al-Rashidi and Sulayman in Oman (2013), the element concentrations in Alfalfa plants are closer to Blatter and their distributions were consistent in two distances from the same industrial areas(2). GhaziFard and Sharifi (2003) studied Pb accumulation (1.25 mg/kg dry matter) in different plants near Iran around IRANKUH Lead and Zinc mine. They also compared the Alfalfa (in different harvesting stage) with the rest of the plants and Alfalfa plants grown in the Cd-free areas. These results were in line with the present study(28). Pb and Cd concentrations in leguminous plants vary in different countries and range from1-18.8 mg/kg and 0.22-0.2 mg/kg dry matter, respectively(6). In general, As residual concentration in the plants and crops cultivated on non-contaminated soils is less than 0.5 mg/kg dry matter(29). Arsenic content in non-contaminated straw and Alfalfa feedings were recorded <0.1 mg/kg(30). In the industrial area, the content of the plant was measured at 6.2 mg/kg(6). Based on a study conducted by Rosas et al. (1999), the As content of the Alfalfa differed in various harvesting stages in different Mexican cities and ranged from 0.5-2 mg/kg(31). Khan et al. in Pakistan (2011) revealed the different impacts of irrigation waters (tube well water and effluent) on the HMs content of Alfalfa. Accordingly, wastewater irrigation increased the Cd and Pb concentrations. Cd concentration in the first and second cutting ranged from 0.3-1.41 mg/kg and 0.21-0.99 mg/kg. Concerning Pb, its range in first and second cutting was 1.30-13.53 mg/kg and 0.56-12.44 mg/kg, respectively. Figure 2 shows the mean of heavy metals in Alfalfa during two harvests: the concentrations of these elements decreased nearly by half from the first to second cutting. Thus, under normal irrigation, the Pb and Cd contents declined from 1.30 and 0.3 mg/kg in the first cutting to 0.56 and 0.2 mg/kg in the second cutting. These variation trends of HMs accumulation are consistent with the present study(32). Li. D et al. in United state (2005) measured Pb, Cd, and As contents of Alfalfa as 1.2-0, 0.47-0.017, and 0.3-0 mg/kg, respectively(33). Concerning the HMs concentration in Alfalfa, Alizadeh et al in Iran (2009) stated that some plants, like Alfalfa, tend to distribute Cd in their roots, whereas others (e.g. lettuce) transfer it to their leaves, probably due to low levels in the plant(34). Cd absorption of the plants largely depends on the total Cd level of the soil as well as the plant species(35).

4.3 Evaluation of the transfer of heavy metals from soil to plant

The mean HMs transfer factors in the first and second harvests were similar in the non-industrial and industrial regions. Al-Rashidi and Suleiman (2013) declared similar findings in line with this study(2). Some studies expressed that the transfer factors of HMs depend on various factors including dissolving ability, element capacity, soil texture, and plant capture ability in the harvesting stage(6). In general the concentration of metals is not directly or automatically reflected from the soil to the plants. It depends on the fraction of the metals that are bioavailable to plants by root uptake (Figure 3). In the industrial area, concentration ranged from 0.05 to 0.1 (the maximum standard limit) (36).

CONCLUSION

HMs as agricultural and environmental pollutants can directly or indirectly affect the human food chain through animal husbandry. Some studies have been published on the accumulation of HMs in soil and their transfer to plants. HMs-contaminated livestock feedstuffs can threaten public health by influencing the disease development in the animals. This research revealed the significant HMs accumulation in the soil of industrial areas. Thus, Alfalfa crops cultivated in such regions are hazardous to livestock, in particular for ruminants and other species of animals. Apparently though, enhancement of the HMs content of the soil in the industrial regions did not elevate their accumulation in Alfalfa. It depends on the fraction of the metals that is bioavailable to plants by root uptake. Regarding a decline in the pollution of heavy metals in alfalfa for the second harvest (both industrial and non-industrial regions), this research supports employing the second harvest for livestock feeding.

Funding/Support

The current study was partially supported by the agricultural research, education and extension organization (AREEO) (project code: 2-01-01-005- 960566).

Conflict of interest: None to be declared.

REFERENCES

1. Rezaeian M, Tohidi Moghadam M. Determination of heavy metal in agricultural soils near and far from the cement factory in Tehran, Iran. Iranian Journal of Toxicology. 2016;10(5):23-6.
2. Al-Rashdi TT, Sulaiman H. Bioconcentration of heavy metals in Alfalfa (Medicago sativa) from farm soils around Sohar industrial area in Oman. APCBEE procedia. 2013;5(1):271-8.
3. Chaurasia S. Effect of cement industry pollution on chlorophyll content of some crops at Kodinar, Gujarat, India. Proceedings of the International Academy of Ecology and Environmental Sciences. 2013;3(4):288.
4. Authority E. Dietary exposure to inorganic arsenic in the European population. EFSA J. 2014;8(28):36-7.
5. Fink-Gremmels J. Animal feed contamination: effects on livestock and food safety: Elsevier; 2012.
6. Kabata-Pendias A. Trace elements in soils and plants: CRC press; 2000.
7. Khan S, Cao Q, Zheng Y, Huang Y, Zhu Y. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environmental pollution. 2008;152(3):686-92.
8. Amin N, Ibrar D, Alam S. Heavy metals accumulation in soil irrigated with industrial effluents of Gadoon Industrial Estate, Pakistan and its comparison with fresh water irrigated soil. Journal of Agricultural Chemistry and Environment. 2014;3(02):80.
10. Rezaeian M, Moghadam MT, Kiaei MM, Zadeh HM. The effect of heavy metals on the nutritional value of Alfalfa: comparison of nutrients and heavy metals of Alfalfa (Medicago sativa) in industrial and non-industrial areas. Toxicological Research. 2019:1-11.
11. Lavado RS. Concentration of potentially toxic elements in field crops grown near and far from cities of the Pampas (Argentina). Journal of environmental management. 2006;80(2):116-9.
12. Taghipour H, Mosaferi M, Armanfar F, Gaemmagami S. Heavy metals pollution in the soils of suburban areas in big cities: a case study. International Journal of Environmental Science and Technology. 2013;10(2):243-50.
13. Ramtahal G, Yen IC, Bekele I, Bekele F, Wilson L, Maharaj K, et al. Relationships between cadmium in tissues of cacao trees and soils in plantations of Trinidad and Tobago. Food and Nutrition Sciences. 2016;7(01):37.
14. Montoya R, Airam E, Montañez Hernández LE, Luévanos Escareño MP, Balagurusamy N. Impacto del arsénico en el ambiente y su transformación por microorganismos. Terra Latinoamericana. 2015;33(2):103-18.
15. Sharma P, Dubey RS. Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. Journal of plant physiology. 2005;162(8):854-64.
16. Anjum SA, Tanveer M, Hussain S, Ashraf U, Khan I, Wang L. Alteration in growth, leaf gas exchange, and photosynthetic pigments of maize plants under combined cadmium and arsenic stress. Water, Air, & Soil Pollution. 2017;228(1):13.
17. Pond WG, Church DB, Pond KR, Schoknecht PA. Basic animal nutrition and feeding: John Wiley & Sons; 2004.
18. Reis LSLdS, Pardo PE, Camargos AS, Oba E. Mineral element and heavy metal poisoning in animals. Journal of medicine and medical Sciences. 2010:560-79.
19. Rajaganapathy V, Xavier F, Sreekumar D, Mandal P. Heavy metal contamination in soil, water and fodder and their presence in livestock and products: a review. Journal of Environmental Science and Technology. 2011;4(3):234-49.
20. Li N, Kang Y, Pan W, Zeng L, Zhang Q, Luo J. Concentration and transportation of heavy metals in vegetables and risk assessment of human exposure to bioaccessible heavy metals in soil near a waste-incinerator site, South China. Science of the total environment. 2015;521:144-51.
21. AOAC. AOAC Official Method 999.10 Lead, Cadmium, Zinc, Copper, and Iron in Foods Atomic Absorption Spectrophotometry after Microwave Digestion'. Gaithersburg, MD, Edition 18. 2005. 2002.
22. Sposito G. The surface chemistry of natural particles: Oxford University Press on Demand; 2004.
23. Grytsyuk N, Arapis G, Perepelyatnikova L, Ivanova T, Vynograds' Ka V. Heavy metals effects on forage crops yields and estimation of elements accumulation in plants as affected by soil. Science of the Total Environment. 2006;354(2-3):224-31.
24. Lotfinasabasl S, Gunale V. Studies on heavy metals bioaccumulation potential of mangrove species, Avicennia marina. International Journal of Engineering Science and Technology. 2012;4(10):4411-21.
25. Esmaeili A, Moore F, Keshavarzi B, Jaafarzadeh N, Kermani M. A geochemical survey of heavy metals in agricultural and background soils of the Isfahan industrial zone, Iran. Catena. 2014;121:88-98.
26. Kelepertzis E. Accumulation of heavy metals in agricultural soils of Mediterranean: insights from Argolida basin, Peloponnese, Greece. Geoderma. 2014;221:82-90.
27. Peralta-Videa JR, Lopez ML, Narayan M, Saupe G, Gardea-Torresdey J. The biochemistry of environmental heavy metal

uptake by plants: implications for the food chain. The international journal of biochemistry & cell biology. 2009;41(8-9):1665-77.
28. Darwish MAG, Pöllmann H. Trace elements assessment in agricultural and desert soils of Aswan area, south Egypt: geochemical characteristics and environmental impacts. Journal of African Earth Sciences. 2015;112:358-73.
29. Ghazifard A, Sharafi M. Evaluating the uptake concentration of heavy matals by agricultural products and assessing its environmental pollution around Irankuh lead and zinc mine (LENJAN VALLEY). 2003.
30. Underwood EJ. The mineral nutrition of livestock: Cabi; 1999.
31. Nicholson FA, Chambers BJ, Williams J, Unwin R. Heavy metal contents of livestock feeds and animal manures in England and Wales. Bioresource Technology. 1999;70(1):23-31.
32. Rosas I, Belmont R, Armienta A, Baez A. Arsenic concentrations in water, soil, milk and forage in Comarca Lagunera, Mexico. Water, Air, and Soil Pollution. 1999;112(1-2):133-49.
33. Khan MJ, Jan MT, Mohammad D. Heavy metal content of alfalfa irrigated with waste and tubewell water. Soil & Environment. 2011;30(2).
34. Li Y, McCrory D, Powell J, Saam H, Jackson-Smith D. A survey of selected heavy metal concentrations in Wisconsin dairy feeds. Journal of Dairy Science. 2005;88(8):2911-22.
35. Alizadeh M, FATHI SF, Torabian A. A SURVEY OF HEAVY METALS ACCUMULATION IN FORAGING PLANTS IRRIGATED BY SEWAGE IN SOUTH OF TEHRAN (CASE STUDY: MAIZE & ALFALFA). 2009.
36. Alloway B, Ayres DC. Chemical principles of environmental pollution: CRC press; 1997.