

Threat of Copper, Zinc, Lead, and Cadmium in Alfalfa (*Medicago scutellata*) as Livestock Forage and Medicinal Plant

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Background: Concentrations of 4 toxic metals, viz. Cd, Cu, Pb, and Zn in the soil and alfalfa samples collected from Borujerd, Iran, was determined. The capability of alfalfa to accumulate heavy metals from soils was assessed in terms of Biological Concentration Factor.

Materials and Methods: The alfalfa and soil samples were collected from 20 different farms, including 13 wastewater-irrigated and seven underground-irrigated farms. After acid digestion, the samples were analyzed using atomic absorption spectrophotometer.

Results: The levels of Cd, Pb, and Zn in the soils of wastewater-irrigated farms were higher than those from the groundwater-irrigated farms. With the exception of Cu, concentrations of heavy metals in the alfalfa crop were higher in wastewater-irrigated farms compared to well water. Also, in the case of BCF, both Cd and Cu values decreased with increasing metal concentration in soil. The order of BCF of heavy metals in alfalfa was in order of Cu>Cd>Zn>Pb in well water-irrigated and Zn>Cd>Cu>Pb in wastewater-irrigated samples.

Discussion and Conclusions: The findings remarked that the levels of Cu, Cd, and Pb in alfalfa were exceeding the permissible levels suggested by the Joint FAO/WHO Expert Committee on Food Additives. These outcomes propose that the consumption of alfalfa plants is potentially threatening both animal and human health.

Keywords: Alfalfa, BCF, Borujerd, Heavy metal (Cu Zn, Pb and Cd), Medicinal plants

1. Background

Medicinal plants are widely used throughout the world as raw materials for self-administered pharmaceutical treatments and other supplementary products (proteins, vitamins, minerals) in the public population. The medicinal materials are derived from various parts of plants (1). The medicinal plants are

increasingly used for their reasonable price and assumption of safety of these natural products (2, 3). A considerable percentage of the people in the world use medicinal plant. The majority of people believe that medicinal plants have phytotherapy property without any side effects (4).

Heavy metals can easily be absorbed by medicinal plants and accumulated in their tissues from the soil, water and air. Soil is exposed to contamination via wet and dry atmospheric deposition of heavy metals from point sources and non point sources, such as metalliferous mining, smelting, industrial activities, fertilizers pesticides, sewage sludge, organic manure, and compost (5). The contamination of heavy metals is a potential risk for crop plants, animals, and human because of their toxicity, persistent nature, and bioaccumulation and biomagnification in the food chain (6). Considering both biological and environmental properties, heavy metals can be classified into two categories: essential (biometals) and non-essential (or toxic metals). Essential metals (Co, Cu, Fe, Mn, and Zn) can also produce toxic effects on organism at high levels, while toxic metals (Cd, Pb, Al, Sn, Hg,) are toxic to human health and environment even at low concentrations (7). Therefore, there is a need to monitor the heavy metal concentrations of plants to ensure that the levels of these metals are not increased before they reach to the consumer's level. Many studies have been conducted on metal contamination in medicinal or dietary plants around the world (1, 3, 4, 6, 8, 9, 10), which indicate the importance of this field.

Since the issue of metal pollution in alfalfa, as leaf forage and medicinal plant, from various regions of Iran is limited, the present study was undertaken to obtain data from contamination of alfalfa with heavy metals, together with information on soil contamination.

2. Objective

The present study was designed to investigate the content of some heavy metals and their bioaccumulation in soil, and alfalfa irrigated with sewage and underground water. Measuring of heavy metals in plants from polluted and non-polluted areas can provide

scientific data that will help in evaluating the health risks posed especially to the local community that use these plants in various illnesses.

3. Materials and Methods

Twenty alfalfa farms were selected from an agricultural plain of Borujerd, north-east of Lorestan province, Iran (Figure 1), of which thirteen farms were irrigated with wastewater and the other seven were irrigated with wells water. A total of 20 samples from the aerial part of alfalfa along with soil from 0–20 cm depth were collected through composite sampling technique. Samples were stored in polythene bags and transported to the laboratory for further analysis. All plant samples were rinsed with deionized water prior to drying in room-temperature. Samples (soil and plant) were air-dried and sieved prior to digestion and analysis and stored in polyethylene bags, then ground, sieved, and digested with acid mixture of HCl, HNO₃, HClO₄ at ratio of 3:1:1 (V/V) for one gram soil (7) and mixture of HNO₃ and HClO₄ at ratio of 3:1 (V/V) for one gram plant (9). The solution was heated until clear solution appeared. The solution was diluted with deionized water to make a final volume of 25ml. All samples were analyzed in triplicate using Flame or Graphite-Furnace atomic absorption spectrophotometer. The concentrations of Pb and Cu in soil samples and Zn in soil and plant samples were determined by flame atomic absorption spectrometry (FAAS, Model: Younglin AAS 8020), while Pb and Cu in plant and Cd in soil and plant samples were determined by graphite-furnace atomic absorption spectrometry (GFAAS). Metal levels were expressed as mg kg⁻¹ on a dry-weight (dw) basis of the soil and plant samples. Precision was expressed in terms of relative standard deviation (RSD) that was less than 10%. The limits of detection (LOD) of the FAAS for Pb, Cu and Zn were 0.8, 1.15 and 1.3

mg kg⁻¹ respectively, while detection limits of the GFAAS for Pb, Cu and Cd were 0.04, 0.06 ad 0.09 mg kg⁻¹, respectively. Also limits of quantification (LOQ) of the FAAS for Pb, Cu and Zn were 2.1, 2.65 ad 2.9 mg kg⁻¹, respectively, while limits of quantification (LOQ) of the GFAAS for Pb, Cu and Cd were 0.13, 0.17 and 0.25 mg kg⁻¹, respectively.

Bioconcentration factor

Bioconcentration factor (BCF), defined as the concentration ratio of a particular metal in the edible parts of plants to that in the soil, was applied in risk evaluation models for estimating the element concentrations in plants, then used

for the quantification of the exposure route associated with human consumption of vegetables contaminated with heavy metals (11, 12).

All data sets were analyzed using SPSS 20.0 at the P-value of p<0.05. To evaluate the degree of correlation between the variables (Pb, Cd, Cu and Zn), the Pearson correlation was performed. A Shapiro-Wilk test was conducted to check the data for goodness of fit to a normal distribution. One sample t-test was applied for the comparison of the heavy metals in the alfalfa with World Health Organization standard for edible plants.

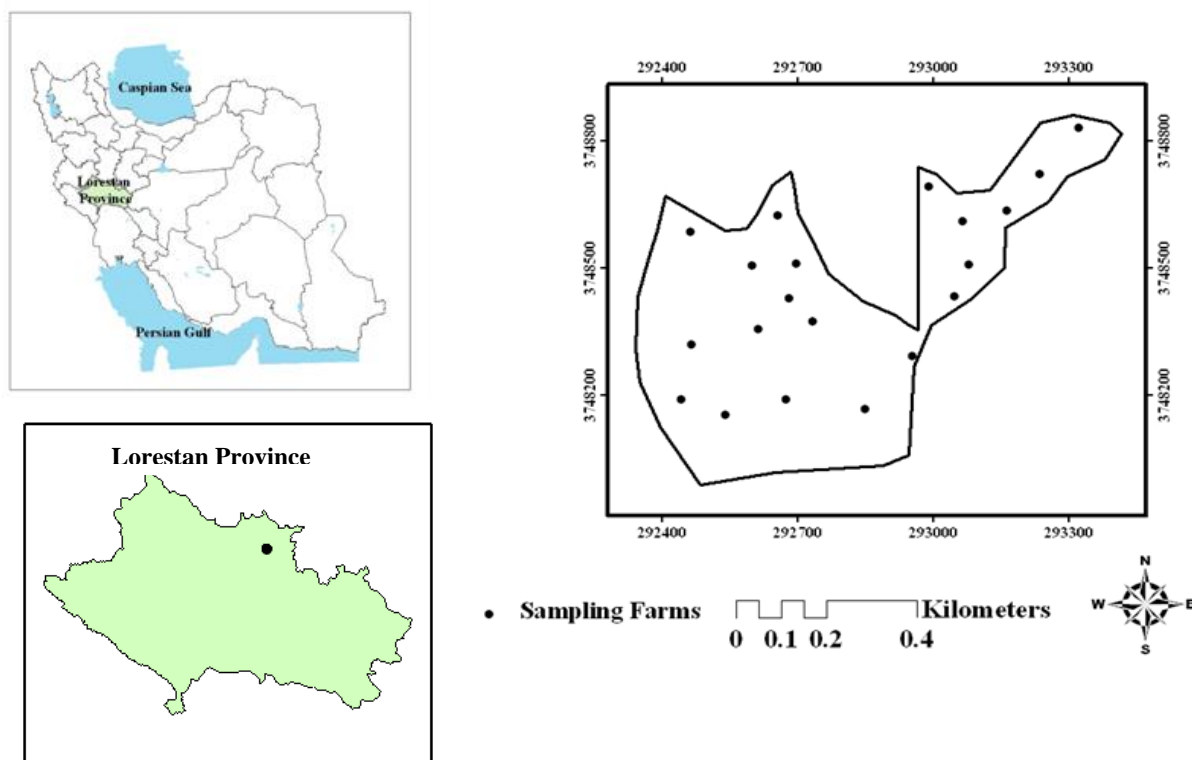


Figure 1 Location of the farms for plant and soil sampling in Borujerd

4. Results and discussion

In the present research, alfalfa and soil samples were analyzed for Cu, Cd, Pb, and Zn in order to determine their metals concentration and monitor accumulation risks of these heavy metals in alfalfa leaf, as forage and medicinal plant, and in soil. Mean levels and standard deviation of Cu, Cd, Pb, and Zn in the soil samples of twenty farms (irrigated with well water and wastewater) are listed in Table 1. Concentrations of Cd, Cu, Pb and Zn in the farms irrigated with well water were 6.3 ± 0.96 , 21.82 ± 8.07 , 53.9 ± 4.006 , and 35.28 ± 5.69 mg kg^{-1} , respectively, while in the wastewater-irrigated farms were 10.18 ± 3.33 , 7.9 ± 4.84 , 64.32 ± 11.9 , and 92.04 ± 25.48 mg kg^{-1} , respectively. The Cd, Pb, and Zn concentrations in the soil samples from wastewater-irrigated farms were higher, but Cu concentration had a higher level in the farms irrigated with well water. Earlier studies have also reported that irrigation with wastewater significantly contributed to the heavy metal concentration of soils (13, 14). According to the results presented in the Table 1, the mean contents of heavy metals concentrations in the topsoil were in the order of $\text{Pb} > \text{Zn} > \text{Cu} > \text{Cd}$ and $\text{Zn} > \text{Pb} > \text{Cu} > \text{Cd}$ for well water and wastewater irrigation, respectfully. In Table 2, the results of this study were compared with the normal amount of these metals in soils. The concentrations of Cd, Pb, and Zn in surface soils of the study area were higher than their concentrations in typical agricultural soils ($\text{Cd}=0.35$, $\text{Cu}=25$, $\text{Pb}=19$ and $\text{Zn}=60$ mg kg^{-1}), while the concentration of Cu was not above the typical range. This indicates that the plants such as medicinal plants that grow on these polluted soil scan easily absorb and accumulate heavy metals (11).

The average concentrations (mg kg^{-1} dry weight) of selected heavy metals in alfalfa from different farms are given in Table 3. Higher metal levels were observed in samples from wastewater-irrigated fields, except for Cu.

Mean values of Cd, Cu, Pb, and Zn were 3.8, 5.4, 1.91, 27.66 and 4.4, 5.39, 2.04, 28.16 in alfalfa irrigated with well water and wastewater, respectively. The comparison of the mean levels of these metals with the permissible levels of FAO/WHO (15) indicated that mean concentrations of Cu, Cd, and Pb were significantly above the recommended limit in edible plant (Table 4) and the concentration of Zn was within the permissible limit (Table 4). Also the permissible limit for Cd in medicinal plant is 0.3 ppm and that for Pb is 10 mg kg^{-1} (15), which means that Cd concentration in alfalfa is several folds higher than this value. The alfalfa plant has a high potential to uptake and accumulate toxic heavy metals as Cd and Pb above the tolerance limits of other plants, which may be because of the presence of chemical functional groups that can bind to metal and responsible for metal tolerance and accumulation (16). Comparing the total concentrations of heavy metals in alfalfa were with the normal range in plants, Cu, Pb and Zn were higher both in well and wastewater irrigated alfalfa than the normal range normal range in plants. According to Hajar *et al.* (17), the concentrations of Cd exceeded the normal range in plants. This high level of Cd is toxic and acts as stress to plants for a number of reasons including inhibition of germination process, reduction of plant biomass and chlorosis of leaf (18). Cd accumulates in human tissues such as the lungs, liver, kidneys, bones, and reproductive organs and has a long biological half-life of two to three decades, which affects the lungs, liver, kidneys, bone, cardiovascular system, and immune system (19).

High potentiality of alfalfa for absorption and accumulation of heavy metals in its roots and shoots have already been mentioned by several authors (20, 21, 22), suggesting it as an appropriate option for phytoremediation of heavy metals-contaminated soil. Heavy metals

accumulation can be dangerous for forage and medicinal purpose because of their transfer into the human and animal food chain and cause various disorders. In this research, the

concentrations of Cd, Cu, and Pb in alfalfa plants were higher than those reported by Carrillo and Cajuste in Mexico (23) and by Ebrahim *et al.* (24) in Bahrain.

Table 1 Heavy metals concentrations in soil samples

	Irrigated with well water				Irrigated with wastewater			
	Pb	Cd	Zn	Cu	Pb	Cd	Zn	Cu
Mean	53.9	6.3	35.28	21.82	64.32	10.18	92.04	7.92
Std. Deviation	4.006	0.96	5.69	8.07	11.9	3.33	25.48	4.84

Table 2 Concentrations (mg kg⁻¹) of heavy metals in plants, soils, and toxic soils for plants (17)

Metals	Normal range in plants	Normal range in soils	Toxic soils for plants
Cd	2	0.35	3 – 8
Cu	0.4 – 45.8	30	60 – 125
Pb	3	35	100 – 400
Zn	1 – 160	90	70 – 400

Table 3 Findings of determination of studied metals in analyzed plant (alfalfa) samples by AAS method

	Irrigated with well water				Irrigated with wastewater			
	Pb	Cd	Zn	Cu	Pb	Cd	Zn	Cu
Mean	1.91	3.8	27.66	5.40	2.04	4.4	28.16	5.39
Std. Deviation	0.43	0.69	4.82	3.12	0.61	0.78	11.73	2.82

Table 4 Results of one sample t-test analysis for the comparison of the heavy metals concentration (mg kg⁻¹dw) in alfalfa with FAO/WHO permissible levels

Heavy metals	FAO/WHO edible plant*			FAO/WHO medicinal plant*		
	t	Sig. (2-tailed)	Permissible limit	t	Sig. (2-tailed)	Permissible limit
Pb	12.84	0.00	0.43	-65.76	0.00	10
Cd	22.58	0.00	0.21	22.07	0.00	0.3
Zn	0.27	0.78	27.4	-	-	-
Cu	3.76	0.001	3	-	-	-

* FAO/WHO (1984)

Bio concentration factor (BCF) represents plants' ability to absorb metals from soil (25). BCF is ratio of metal concentration in plants to metal concentration in soils (26). Figures 2 and 3 demonstrate the BCF values of the metals in alfalfa from various farms. The mean BCF values for Cu, Cd, Pb, and Zn were, respectively 1.25, 0.53, 0.03, 0.35, and 0.39, 0.50, 0.037, 0.56, for the ground water irrigated fields and wastewater irrigated fields, respectively. The BCF of heavy metals in the alfalfa grown in well water irrigated field indicated a trend in the order of $Cu > Cd > Zn > Pb$ and in waste water irrigated field as $Zn > Cd > Cu > Pb$. The BCF was lower than 1 for all the heavy metals in both wastewater and well water irrigated farms.

These results were in line with the findings of Elouear *et al.* (27) that reported BCF values lower than 1 for Cd, Pb and Zn, showing a more limited soil-to-alfalfa plant transfers of these metals (27). Also, our results showed that in the case of BCF, both Cd and Cu values decreased with increasing metal concentration in soil, which was in agreement with Elouear *et*

al. (27), Rahmanian *et al.* (28), Wang *et al.* (18), and Zhao *et al.* (29). Low values are regarded normal when plants are vegetated on contaminated soils. The decreased BCF values with increasing soil pollution has been attributed to the self-adjusting of plants that plays a significant role on sequestering the heavy metals in their roots (28, 30). Only small amounts of heavy metals are translocated to the above-ground portions of the plant. Another possible reason is that plants grow unfavorably and are not sustained in heavily polluted soils (30). The weak growth of plants has been observed in heavily contaminated soil that in this condition, the uptake of metals by plant species was diminished and subsequently the metal concentrations in plant tissues were decreased. Accordingly, the BCF values for metals for plants in these soils were significantly decreased. High BCF in edible parts of vegetable crops growing in wastewater irrigated farms show high absorption and accumulation of heavy metals, which could easily enter into food chain and create risk for human and other animals (31).

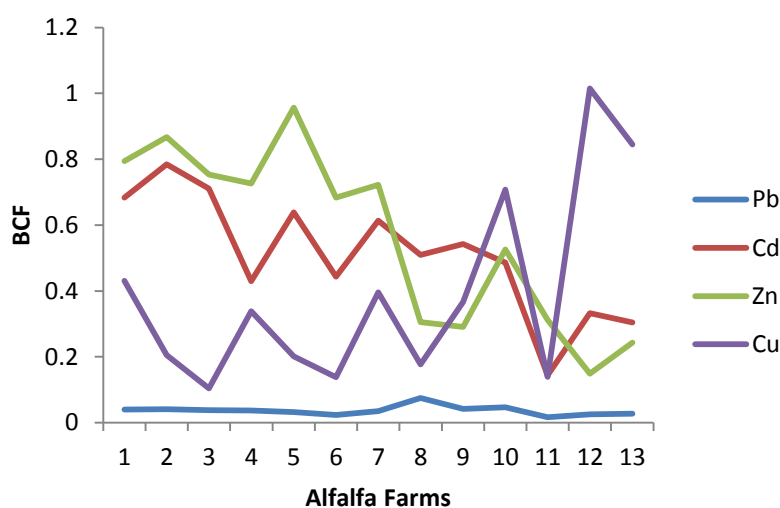


Figure 2 Bio concentration actor (BCF) of soil to alfalfa in the wastewater irrigated field

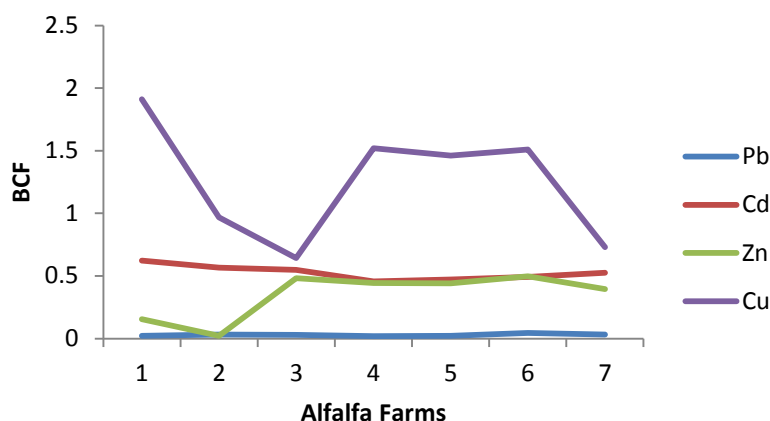


Figure 3 Bio Concentration Factor (BCF) of soil to Alfalfa in the groundwater irrigated field

5. Conclusion

The investigation of heavy metal pollution in soil and alfalfa as medicinal plants in ground water and wastewater irrigated farms demonstrated a higher level of heavy metals in farms irrigated with wastewater. The mean total concentrations of Cd, Cu, and Pb in alfalfa were higher than the acceptable limits defined by the FAO/WHO for edible plant and the concentration of Zn was within the permissible limit. Higher heavy metals concentrations in alfalfa from both farms (irrigated with well water and wastewater) was alarming condition for their safety. Therefore before applying this plant for animal consumption or for preparation of medicinal aims, it is essential to check heavy metal concentrations, particularly from polluted area.

Conflict of Interest

The Authors declare that have no conflict of interest.

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Authors' Contributions

Each of the authors contributed to the development of the paper.

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تهدید مس، روی، سرب و کادمیوم در یونجه (*Medicago scutellata*) به عنوان علوفه دام و گیاه دارویی

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مقدمه: غلظت ۴ فلز سمی Cu, Cd, Pb و Zn در نمونه‌های خاک و یونجه از مزارع یونجه بروجرد (ایران) ارزیابی شد. همچنین توانایی یونجه برای تجمع فلزات سنگین از خاک از نظر فاکتور تغلیظ زیستی مورد بررسی قرار گرفت.

مواد و روش‌ها: نمونه‌های یونجه و خاک از بیست مزرعه (سیزده مزرعه آبیاری شده با فاضلاب و هفت مزرعه آبیاری شده با آب چاه) جمع‌آوری شد. پس از هضم اسیدی، نمونه‌ها با استفاده از دستگاه اسپکترو فتومتر جذب اتمی مورد آنالیز قرار گرفتند.

نتایج: سطوح Cu, Pb و Zn در خاک مزارع آبیاری شده با فاضلاب بیش‌تر از مزارع آبیاری شده با آب زیرزمینی بود. به جز Cu، غلظت فلزات سنگین در یونجه مزارع آبیاری شده با فاضلاب نسبت به آب چاه بیش‌تر بود. همچنین نتایج ما نشان داد که در مورد BCF، مقدار Cu و Cd با افزایش غلظت فلز در خاک کاهش می‌یابد. ترتیب BCF فلزات سنگین در یونجه آبیاری شده با آب چاه به شکل $Zn > Pb > Cd > Cu$ و $Pb > Cu > Zn > Cd$ برای مزارع آبیاری شده با فاضلاب بود.

بحث و نتیجه‌گیری: نتایج نشان داد که سطوح Cu، Cd و Pb در یونجه بیش‌تر از سطوح مجاز توصیه شده کمیته کارشناسی افزودنی‌های غذایی FAO/WHO و غلظت Zn در محدوده مجاز بود. این نتایج نشان می‌دهد که مصرف گیاه یونجه به طور بالقوه سلامت حیوان و انسان را تهدید می‌کند.

کلمات کلیدی: BCF، بروجرد، فلزات سنگین (Cu، Zn، Pb و Cd)، گیاهان دارویی، یونجه