

## Study of Cadmium Absorption and Accumulation in Different Parts of Four Forages

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**Abstract:** Wastewater reuse such as industrial wastewater for different applications like irrigation, particularly in dry and semi-dry regions is considered as a useful method for minimizing problem of water shortage. This method, apart from helping compensation for water shortage, may bring back into the earth some useful elements such as Phosphorus and Nitrogen which are useful for growing plants. However, wastewater may contain different types of pollutions like heavy metals. Of these metals, Cadmium (Cd) can be absorbed more easily and quickly. Once Cadmium accumulates in plants in quantities greater than the standard values, it can cause a variety of symptoms in living animals. In this experimental work, the rate of Cadmium accumulation in plant organs of four forages commonly used in Iran was studied. For each plant type, three different treatments were carried out: one with no Cadmium added to soil (control treatment) and two containing 50 and 100 ppm Cadmium concentration in soil. The results showed that Cadmium absorption by the plants are greater in the samples containing 50 and 100 ppm Cadmium, compared with the control treatment and are various in different plant parts. Moreover, there is a higher inclination in Clover for absorbing Cadmium, compared to other species. Cadmium accumulation in Alfalfa is mostly seen in the aerial parts (i.e. leaf). For Sorghum, there is low difference in Cadmium concentrations in different organs. Overall, Sorghum seems to have a lower potential for absorbing Cadmium ions, compared to other three types. Comparisons made between different parts of Sainfoin revealed that its root and stem have greater potential for absorbing this metal, compared to leaf. By making a comparison between the amount of Cadmium accumulated in different organs belonging to the four plants and the existing standards, it was revealed that there may be no danger of intoxication for farm animals.

**Key words:** Cadmium • Wastewater • Forages • Contaminated soil

### INTRODUCTION

Increasing growth of world population along with the expansion of agricultural and industrial activities for improving supply of nutritional materials and the occurrence of several consecutive years of drought, are the reasons why available water resources in most countries located in the dry-region zone have been used with the highest degree, such a condition may naturally exert a great deal of pressure on water resources.

Water supply is considered as one of the most fundamental concerns of many governments at present and in the future and large investments have been done in this area. It should be accepted that one of the main options is using of water with different quality for different consumptions exists [1]. Undoubtedly, wastewaters reuse in agriculture sector is

one of the most significant parts of the usage-chain. Moreover, having access to wastewater and industrial sewage as an assuring and permanent source of water and nutritional materials can provide the necessary water and fertilizer for agriculture products. In addition, the availability of wastewater with an approximately fixed volume throughout the whole year makes it possible for farmers to have more freedom for choosing the type of product and the cultivation method [2, 3].

As mentioned earlier, Cadmium is an environment pollutant which may be found in all ecosystems, water, air and plants. Consequently, different living species, including humans, animals and plants are always in danger of being polluted by this metallic element. Cadmium concentration inside the body of living animals can reach an amount higher than its standard value because of its accumulative characteristic. As result, different symptoms may appear [4-6].

Cadmium may be easily absorbed by plants and it may also be accumulated in agricultural products, in concentrations that can even be considered toxic for some plant species. Excessive accumulation of this element in soil can reduce growth in plants [7]. Use of polluted plants by farm animals and domestic animals and even directly by humans is considered significant in two respects. Firstly, using polluted forages may cause some disturbances in farm animals as well as domestic animals, including reduction in milk and in rate of animal growth, reduction in resistance to some diseases and infections and disruption in animal reproduction process. Secondly, if polluted materials produced from animals are used by humans, a variety of disturbances may be seen [8].

Intoxication by Cadmium can cause variety of symptoms in animals, including blood iron deficiency, liver diseases and brain and nerve damages. Moreover, studies show that Cadmium can cause liver cancer as well as cancer of testicles in animals [9].

Of adverse effects caused by this heavy metal in humans are accumulation in kidneys and in liver, Urea Protein clotting, obstruction of urethra, accelerating nephrolith formation, heart diseases and hypertension, osteoid pains, diarrhea and prostate cancer [7].

Considering above-mentioned points, it's necessary to study the manner and rate of accumulation of heavy metals in plants. A variety of researches have been carried out in this area in different countries including Iran. Daneshmand [10] showed that Cadmium absorbs in aerial parts of plants and amount of accumulation is lower than permissible limits in these food plants.

The results of investigations conducted by Forushany [11] on the effects of using wastewater ooze on performance and absorption of heavy metals in barley showed barley plant has high potential for absorbing heavy metals.

Molla-Hosseiny [12] in his studies about accumulation of heavy metals in Sorghum showed concentration of Cadmium increases in Sorghum plant when cultivated in a soil polluted by heavy metals and root has higher concentration as compared to other plant parts.

Atash-Nama [13] worked on accumulation of heavy metals in three forages, Rangzan *et al.* [14] studied the effect of industrial water remains quality on heavy metals accumulation in Sorghum and Clover, Gzygloti *et al.* [15], Sim and Kline [16] and Bahman-yar *et al.* [17] studied the effect of irrigation with water-remains on the amount of heavy metals accumulation in soil. Results of these

researches showed that the level of concentration of heavy metals in the crops depends on the type of metal, soil characteristics and type of plant, however generally, concentration in aerial parts of a plant or crop, such as leaves and stem is significantly higher than in other parts and significantly lower in seeds, as compared to leaves and stems.

Many standards and a great deal of recommendations exist all over the world regarding the amount of heavy metals in different plant types, which can be consumed by humans. Also, there are many standards corresponding to the amount of heavy metals in sewers which may be released into the environment. There are some standards showing permitted levels of the heavy metal, Cadmium:

- The World Health Organization (WHO) has presented the maximum permitted level of Cadmium accumulation in human diet 0.1 mg/l [18].
- United Nation standard for nutritional and agricultural materials has determined a max level of 0.01 ppm for Cadmium accumulation in irrigational waters and a max level of 7  $\mu\text{gkg}^{-1}$  body weight for humans' daily diets [19].
- The United States Environment Protection Agency (USEPA) has presented a standard which permits a maximum amount of 85 ppm for Cadmium in ooze which may be deposited in agricultural lands [20, 21].
- The American Food Industries has introduced a standard which permits a maximum amount of 92  $\mu\text{gday}^{-1}$  for Cadmium existing in human's daily diet [20].
- Miller has recommended a maximum amount of 0.5 ppm for Cadmium accumulated in plants that are eaten by human beings [7].
- Channey [5] and Okoronkwo *et al.* [6] have recommended permissible level of 70  $\mu\text{gday}^{-1}$  for Cadmium consumption for human beings.
- Kebatta-Pendias [22] has determined a max permissible amount of 10-20 ppm for Cadmium accumulated in plants used by farm animals.

With regard to the fact that Alfalfa, Clover, Sorghum and Saifoin are among the most important nutritional materials used by farm animals and also the information of amount of Cadmium accumulation in different plant parts in the same studies is limited, the first objective of this study is to research experimentally and compare the potential for absorbing Cadmium and the rate of Cadmium accumulation in different organs of the four

aforementioned plants. It can be discovered what effects of cultivating these plants in polluted soil may be on the rate of absorption of Cadmium by the same plants, also in which plant parts the absorbed metal has the maximum accumulation and in which organs, the minimum.

## MATERIALS AND METHODS

**Cultivating Different Experimental Plants:** At the first stage, a suitable place was needed for cultivating and investigating different species of studied plants (Clover, Alfalfa, Sorghum and Sainfoin). In order to prevent pollution of cultivation soil by Cadmium heavy metal, it was used several cylindrical pots having a diameter of 40 cm and a height of 60 cm. It should be mentioned that the soil was chosen from among various samples and collected from a 400ha farmland of the Seed and Plant Improvement Institute (S.P.I.I.) in Karaj. The soil had some specifications which have been presented in Table 1.

In the experiment carried out, two different concentrations of Cadmium in soil were used, 50 and 100 ppm. Also a control treatment with no addition of Cadmium to the soil was chosen. Cadmium nitrate ( $\text{Cd}(\text{NO}_3)_2$ ) with a molecular mass 234.42 was considered as a source of Cadmium in soil for making homogeneous mixtures. After preparing the soil and pots, three pots for each plant type were used in every treatment. There were 12 pots all together on which cultivation was carried out. This study were done under laboratory conditions, the test plan using the factorial testing plan in random blocks model, in three treatments and three repetitions.

**Irrigation and Harvesting:** Irrigating each type of plants was done by considering its water need as approved by Iranian Department of Agriculture and by using scaled containers. So the amount of water required by each plant type was determined from the corresponding table. Then, at a particular time (once in two or three days and with attention to climate parameters and various stages of plant growth), water was added to the soil with a uniform distribution [23]. After giving an appropriate amount of time to each plant for growing and yielding product, it was time to take samples.

As mentioned earlier, in order to have high-precision results, each 50 and 100 ppm treatment as well as the control treatment involved three repetitions. At the time of taking samples, four samples of each plant organs (roots, stems and leaves) were collected and examined from each pot.

Table 1: Physical and chemical properties of the soil sample

Soil characteristics	Values
CEC (mg/100g)	0.11
Organic Carbon (%)	1.25
$\text{HCO}_3^-$ (meq/l)	4.20
Cl (meq/l)	3.32
$\text{SO}_4$ (meq/l)	3.80
Ca (meq/l)	4.12
Mg (meq/l)	3.31
Na (meq/l)	2.72
Clay (%)	23.00
Silt (%)	42.00
Sand (%)	35.00
Cd (ppm)	0.03
pH	7.40

**Washing and preparing the samples:** At the end of growing season, samples were taken from different plant organs in order to determine the amount of accumulated Cadmium. Then, different parts of the collected samples were washed and separated using a plastic knife, again washed in distilled water and kept in a ventilated oven at a temperature 70°C for 72 hours to be dried. Then, the dried parts were ground and changed into powder. An appropriate amount of the resulting powder (5g.) was burn to ashes in a muffle furnace by gradually increasing temperature from 25°C to 450°C over a one and half hour period, followed by 2 hours at 450°C, in the end was suspended in 20 ml of aquaregia ( $\text{HCl}:\text{HNO}_3$ , 3:1 v/v) and diluted (1:20) with de-ionized water in order to be prepared for Cadmium measurement.

**Experiments:** Plant samples were prepared in accordance with the standard 3050 presented by the United States Environment Protection Organization [20, 21]. However for measuring the amount of accumulated Cadmium the method of Atomic Absorption has been used. This method of measuring metals concentration involves a high degree of sensitivity, precision and accuracy and therefore, it is considered as the most common method at the present [24]. In this study, for measurement of Cadmium resulting of extraction the graphite furnace atomic absorption spectroscopy (GFAA, Perkins-Elmer model 4100ZL, Cupertino, CA) was selected [25].

In order to analyze the results, statistical ANOVA was carried out on the data obtained from the measurement. This task was done using the statistical software SPSS. Moreover, the results obtained from data analyzes were then statistically compared using the Duncan's Multiple Range test at 1% and 5% probably levels. For graphical description, EXCEL software was used. Summarize of the results are presented in Tables 2 to 4.

## RESULTS AND DISCUSSION

**Results Analysis of Variance:** Table 2 shows results analysis of variance in plants under study.

The results of ANOVA table show that in each of organs (root, leaf and stem), the effect of species, treatment and also interaction effect between species and treatment on amount of accumulated Cadmium has been significant at the level of 1%.

The effect of investigated plant species on amount of accumulated Cadmium: Investigating the effect of plant type on average amounts of accumulated Cadmium in different plant parts as stated in Table 3 that for each organ, all the investigated species reside in different groups, on the basis of Cadmium concentration.

Data in Table 3 showed that the maximum amount of Cadmium accumulated in the root is 2.308 mg kg<sup>-1</sup> which corresponds to clover and the minimum amount was 0.507 mg kg<sup>-1</sup> which corresponds to Sorghum. Alfalfa and Sainfoin had concentrations 1.198 mg kg<sup>-1</sup> and 1.714 mg kg<sup>-1</sup>, respectively and were between those two types.

For stem, the results showed Clover has the highest Cadmium concentration with 1.747 mg kg<sup>-1</sup> and Sorghum with 0.287 mg kg<sup>-1</sup> of Cadmium concentration has the least. Alfalfa and Sainfoin were between those two plant types with the same letters.

For leaves, Alfalfa ranked first with 1.886 mg kg<sup>-1</sup> of Cadmium concentration and Sorghum with 0.356 mg kg<sup>-1</sup> ranked last among the studied plants. A comparison of the results can be easily glanced from Fig. 1.

The effect of investigated treatments on amount of accumulated Cadmium: Table 4 shows the effect of different levels of treatments on amount of accumulated Cadmium in different parts of the four plant types.

Investigating the effect of different levels of Cadmium concentrations in experimental treatments as stated in Table 4 that the amount of Cadmium accumulation in plant organs (root, stem and leaf) significantly increased at the level of 1% in third treatment (100 ppm), compared with other two treatments (one with a concentration 50 ppm and the other with no Cadmium), the second treatment (50 ppm) and the control treatment took the second and third places in the Duncan's Statistical grouping, respectively. This finding is comparable with results carried out by Molla-hoseini *et al.* [12], Gardiner *et al.* [26] and Ramos *et al.* [27].

The results in Table 4 and Fig. 2 for all plant types also showed that the maximum amount of Cadmium accumulation is in root and the minimum is in stem.

Table 2: Variance analysis of discussed characteristic in studied species

S.O.V	df	MS		
		Root	Leaf	Stem
Species	3	5.277*	4.322*	3.237*
Treatments	2	21.393*	11.325*	8.842*
Treatments×Species	6	1.745*	1.545*	0.934*
Error	24	0.0045	0.0048	0.0057
C.V.	-	4.42	7.21	7.89

\* =significant at 1% probably level

Table 3: Comparison of average Cadmium concentration in different parts of discussed species

Species	Cadmium(mg kg <sup>-1</sup> )		
	Root	Leaf	Stem
Alfalfa	1.198 <sup>c</sup>	1.886 <sup>a</sup>	0.890 <sup>b</sup>
Sorghum	0.507 <sup>d</sup>	0.356 <sup>d</sup>	0.287 <sup>c</sup>
Clover	2.308 <sup>a</sup>	1.153 <sup>b</sup>	1.747 <sup>a</sup>
Sainfoin	1.714 <sup>b</sup>	0.532 <sup>c</sup>	0.918 <sup>b</sup>

\* Figures bearing similar letters show that in the probability level of 1% there has been no significant difference

Table 4: Comparison of mean Cadmium concentration in different parts of plant species in different treatments

Treatment	Cadmium(mg kg <sup>-1</sup> )		
	Root	Leaf	Stem
Control	0.055 <sup>c</sup>	0.043 <sup>c</sup>	0.037 <sup>c</sup>
50 ppm	1.518 <sup>b</sup>	1.108 <sup>b</sup>	0.920 <sup>b</sup>
100 ppm	2.722 <sup>a</sup>	1.983 <sup>a</sup>	1.735 <sup>a</sup>

\* Figures bearing similar letters show that in the probability level of 1% there has been no significant difference

Table 5: Comparing of interaction effect between species and treatments on Cadmium accumulation amount

Plant type	Cd ppm	Cadmium(mg kg <sup>-1</sup> )		
		Root	Leaf	Stem
Alfalfa	0	0.050 <sup>i</sup>	0.050 <sup>g</sup>	0.030 <sup>f</sup>
	50	1.413 <sup>f</sup>	1.807 <sup>c</sup>	0.997 <sup>d</sup>
	100	2.130 <sup>d</sup>	3.800 <sup>a</sup>	1.643 <sup>c</sup>
Sorghum	0	0.060 <sup>i</sup>	0.040 <sup>g</sup>	0.040 <sup>f</sup>
	50	0.527 <sup>h</sup>	0.427 <sup>f</sup>	0.337 <sup>e</sup>
	100	0.933 <sup>g</sup>	0.600 <sup>e</sup>	0.483 <sup>c</sup>
Clover	0	0.062 <sup>i</sup>	0.030 <sup>g</sup>	0.030 <sup>f</sup>
	50	2.463 <sup>c</sup>	1.030 <sup>d</sup>	2.057 <sup>b</sup>
	100	4.400 <sup>a</sup>	2.400 <sup>b</sup>	3.153 <sup>a</sup>
Sainfoin	0	0.050 <sup>i</sup>	0.050 <sup>g</sup>	0.050 <sup>f</sup>
	50	1.670 <sup>c</sup>	0.417 <sup>f</sup>	1.043 <sup>d</sup>
	100	3.423 <sup>b</sup>	1.130 <sup>d</sup>	1.660 <sup>c</sup>

\* Figures bearing similar letters show that in the probability level of 1% there has been no significant difference

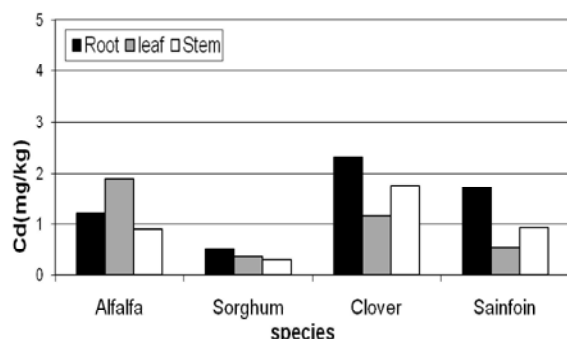


Fig. 1: Comparison of average Cadmium concentration in different parts of discussed species

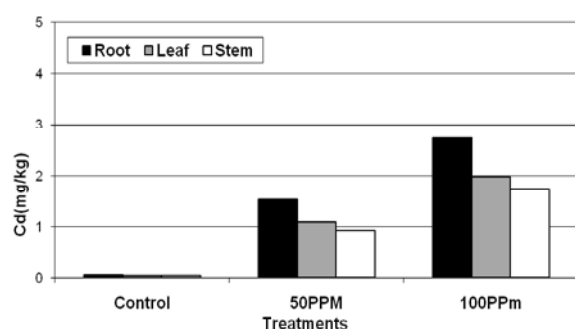


Fig. 2: Comparison of mean Cadmium concentration in different parts of plant species in different treatments

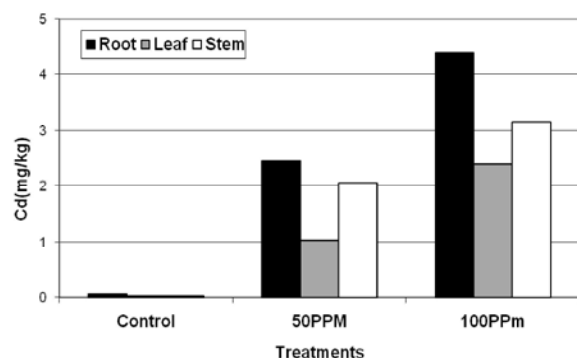


Fig. 3: Cadmium concentration in different organs in clover for different treatments

Investigation the interaction effect between treatments and species on amount of Cadmium accumulation: Table 5 shows the effect of experimental treatments and plant species on amount of Cadmium accumulation in different organs of the plants under study.

Data presented in Table 5 showed that the maximum amount of Cadmium accumulation in plant parts is in treatment 100 ppm. Cadmium accumulation in organs of four plant species as stated in below:

Control treatment < treatment 50 ppm < treatment 100 ppm

Data also showed that concentration of Cadmium in organs is various in different plant types.

Cadmium concentration in different parts of the tested plant species as demonstrated in Table 5 shows that in Alfalfa, the highest amount of Cadmium accumulation is in Leaves followed by Root and Stem in order., in Sorghum, Sainfoin and Clover, the highest amount of Cadmium accumulation is in roots. The results for other plant types can be investigated in Table 5.

Molla-hoseini *et al.* [12] on Sorghum, Atashnama *et al.* [13] on Alfalfa and Sainfoin and Rangzan *et al.* [14] on Sorghum and Clover showed similar results in this area.

A brief look at Cadmium concentrations accumulated in the organs of four different plants with respect to treatments with 50 and 100 ppm concentrations and comparing them with control treatment shows a potential for an increase in concentration of Cadmium in these plants when cultivated in a soil polluted by heavy metals. Accumulation of Cadmium in different plant parts suggests that with a high degree of movement which this metal has in the soil, it can be easily absorbed by the root and then transferred to different organs of the plant. In other words, accumulation of this element in soil and consequently the undesired increase in the amount of this metal in plants may in the long term, endanger the health of affected plants and consequently, the health of farm animals and humans.

By observing the concentrations of accumulated Cadmium in plant organs corresponding to treatments 50 and 100 ppm, it is found that an increase in Cadmium concentration in soil can result in an increase in Cadmium accumulation in plants cultivated in such a polluted soil. But, this is not the only necessary condition. In other words, the overall concentration of this metal in soil can not by itself be considered as appropriate criterion for determining the amount of Cadmium absorbed by the plant [16]. Moreover, the potential for absorbing heavy metals ions by plants, apart from plant type and ion concentration in root environment, depends on other factors such as its degree of dissolvability in root environment. This factor, itself, dependent on some physical characteristics of soil, like particle size and water-keeping capacity, as well as on some chemical characteristics such as acidity, existence of nutritional materials and existence of organic chemicals excreted from plant root [4, 28]. For this reason, it was observed that Cadmium accumulation in each of Sorghum organs

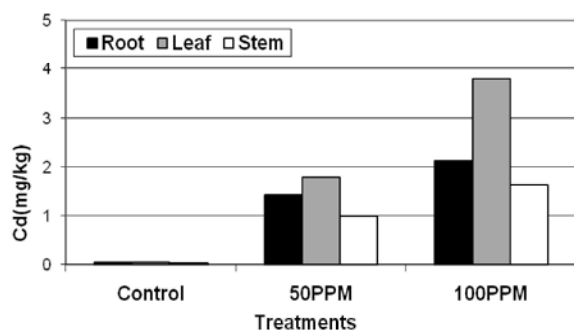


Fig. 4: Cadmium concentration in different organs in Alfalfa for different treatments

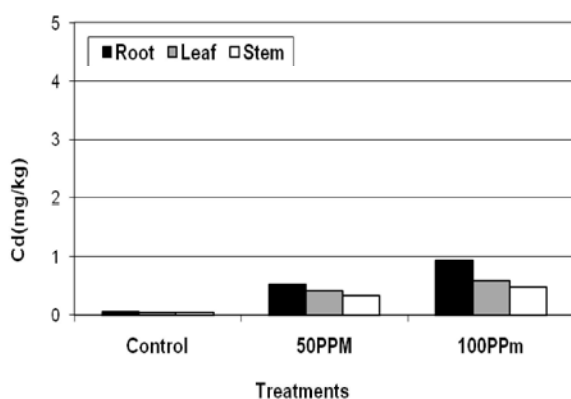


Fig. 5: Cadmium concentration in different organs in Sorghum for different treatments

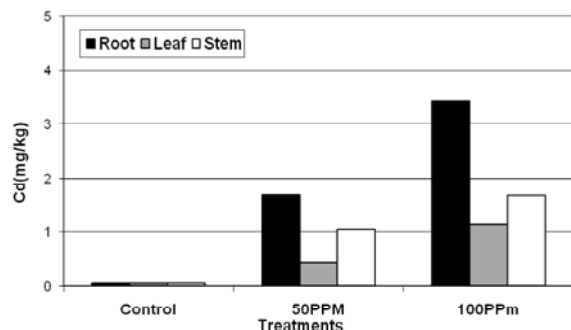


Fig. 6: Cadmium concentration in different organs in Sainfoin for different treatments

does not yield a significant difference with increasing concentration of Cadmium in the soil corresponding to root region.

Data also showed that the amount of Cadmium absorption in plant organs is varied, that in all plants, except Alfalfa accumulated Cadmium in their roots was higher than the aerial parts (Leaf and Stem).

For Clover in Fig. 3, it was observed that the maximum accumulation of Cadmium took place in its root and its aerial parts absorbed less Cadmium.

This shows that with movement of Cadmium towards upper plant parts, less accumulation take place in organs. As in the case of Alfalfa, a high potential for absorbing Cadmium ions was seen in root environment in this plant. This plant had the highest Cadmium concentration in Root and Stem among the other species.

For Alfalfa, it was observed that, Cadmium accumulation did mostly occur in aerial parts (i.e. leaf) of the plant, so that the maximum accumulation took place in leaves of the plant (with Cadmium concentration of  $1.886 \text{ mg kg}^{-1}$ ) with respect to other plant types (Fig. 4). This situation shows a high potential of Alfalfa for absorbing and keeping Cadmium in its aerial parts. It is worth to mention that Alfalfa, because of such a potential, is used as an accumulator in soils polluted by heavy metals, a concept that is covered in Phytoremediation [29].

For Sorghum in Fig. 5, in the two treatments involving concentrations of Cadmium in soil, there was no high difference between Cadmium concentrations in different parts of the plant, namely leaf, stem and root. In other words, Cadmium accumulation in each organ does not differ with an increase in concentration of Cadmium existing in the soil in root region, the same as the other plant species. Moreover, the maximum concentration can be observed in Sorghum root and the minimum concentration in Sorghum stem. In comparison with other species of plants under investigation, Sorghum seems to have less potential for absorbing Cadmium ions.

For Sainfoin, comparing Cadmium concentrations in different plant parts revealed that its root and stem have more potential for absorbing this heavy metal its leaf has (Fig. 6). This plant type rarely has a high potential for absorbing Cadmium ions.

## CONCLUSION

This study showed the maximum potential for absorbing Cadmium is in Clover, followed by Sainfoin, Clover and Sorghum. It can be a guide for selecting plant species, irrigation methods and suitable plant organs for consumption in farms and fields irrigated by contaminated water. Cadmium accumulation in plants for each organ is in the following order of ranking:

Root : Sorghum < Alfalfa < Sainfoin < Clover  
 Leaf : Sorghum < Sainfoin < Clover < Alfalfa  
 Stem : Sorghum < Alfalfa < Sainfoin < Clover

Cadmium concentration in different parts of the tested plant species is the following order of ranking:

Alfalfa : Leaf > Root > Stem  
Sorghum : Root > Leaf > Stem  
Clover : Root > Stem > Leaf  
Sainfoin : Root > Stem > Leaf

By comparing Cadmium concentrations in different plant parts with those corresponding to the existing standards, it became clear that the amount of absorbed Cadmium in any plant organ is not excessive to endanger the health of farm animals. However as far as the aerial parts of Alfalfa (i.e. leaf) is concerned, which may contain a higher amount of Cadmium and may be considered as a source of nutrition for farm animals, it is essential to make necessary considerations to ensure their health and more studies be carried out in this field, specially with more treatments and higher Cadmium concentrations and for other contaminations.

Since, it is possible that an amount of Sorghum may be directly consumed by human beings; therefore, it is necessary to investigate the issue Cadmium accumulation in nutritional parts of Sorghum and compare it with the existing standards. In this study, the maximum Cadmium accumulation in Sorghum was less than the UN standard set for agricultural and nutritional materials which permits at most a daily use of 35 micro-grams of Cadmium by human beings, The standard set by the American Food Industries which permits a daily use of at most 92 micro-grams of Cadmium by human and the standard set by Miller in his book which has specified a maximum amount of 0.5 ppm to be used by humans.

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