American-Eurasian J. Agric. & Environ. Sci., 3 (3): 357-364, 2008
ISSN 1818-6769

Investigation of Cadmium Absorption and Accumulation in Different Parts of Some Vegetables

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Abstract: Environment pollution through heavy metals is a growing global concern. Most heavy metals accumulate in the top soil and in the long term, increase their concentration in the soil results in increase their absorption and accumulation in plants. The quantity or level of heavy metals absorption in plants, depends not only on the concentration levels of the metals in the physical and chemical composition of the soil, but also varies in different parts of the plant type. Several studies have been conducted on the use of waste water and sewage, carrying heavy metals, for agriculture; however information regarding amount of absorption and accumulation of heavy metals in various parts of different crops and plant types is limited. This study has been conducted with the objective of analyzing the impact of various levels of Cadmium (Cd) concentration in the root region on the quantity of its absorption and accumulation in various parts of seven different types of vegetables, commonly used in Iran. The experiments were carried out under laboratory conditions, the test plan using the factorial testing plan in random blocks model, in four treatments and three repetitions. The treatments includes Cadmium concentration in 4 levels (control treatment, without adding Cadmium, soil with 50 mg kg^{-1} Cadmium, soil with 100 mg kg^{-1} Cadmium, soil with 50 mg kg^{-1} Cadmium and irrigation water with 0.5 molar EDTA). The results show except control treatment, in every other treatment Cadmium accumulation is in excess of permissible limits for human consumption and its accumulation in different parts as stated below: *Root:* leek < parsley < onion < coriander < mint < cress < lettuce *Stem:* leek < parsley < mint < onion < coriander < cress < lettuce *Leaf:* leek < parsley < coriander < mint < onion < cress < lettuce

Key words: Cadmium · contaminated soil · EDTA · wastewater · vegetables

INTRODUCTION

Environment pollution by heavy metals is a problem that has global dimensions and is on the increase. The discharge of heavy metals as a byproduct of various human activities has been accompanied by large scale soil pollution. So much so that contamination levels in these soils were either more than normal levels, or expected to soon reach those levels [1-3].

Research findings show that at least 20 million hectares of land in North and South Africa, South America, Middle East, Southern Europe, South West America, Mexico and a significant part of Central and East Asia is irrigated by raw sewage, mainly for cultivation of vegetables. Consequently, this usage ends to soil contamination and heavy metals accumulation both in soil and crops [4, 5].

The main source of heavy metals in soil is the use of urban and industrial waste water, chemical phosphate fertilizers, sludge from waste water treatment plants in cities and metal extraction mines. The primary heavy metals present in sewage, include Zinc (Zn), Copper (Cu), Lead (Pb), Cadmium (Cd), Nickel (Ni), Chromium (Cr), Tin (Sn) and Arsenic (As). Most heavy metals infiltrate and accumulate in the top soil. Accumulation of heavy metals in soil is incremental and in the long term results in increased levels of contamination; so much so that contamination levels may reach limits that can constitute a real threat to food safety for human civilization [6-8].

Iran and other countries located in the arid belt of the world, face severe water scarcity and to partially meet the demand for water by their large urban populations, are compelled to re-use a significant volume of urban and industrial waste water (contaminated by heavy metals),

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for agriculture. Investigations conducted earlier indicate that on an average, more than 6 m^3/s waste water and surface water discharged by the urban conglomerate Tehran, through drains and canals which collect its various urban and industrial wastes, is used for the purpose of irrigating fields and farmland located in the south of Tehran [9]. Long-term use of this waste water, which is mainly used for cultivation of leafy and other vegetables, has resulted in the accumulation of heavy metals in the soil and their transfer to the various crops under cultivation, with levels of contamination that exceed permissible limits [9].

Prolonged use of waste water for irrigation of fields and farmland in Ghazvin has caused concentration of Lead, Copper, Cadmium and Zinc to exceed permissible limits several times over [10].

This situation is not an exception but the rule for a significant part of the country's agricultural land, especially land surrounding large cities and the trend is growing. Investigation of soil pollution levels in fields and farmlands in the country has revealed that the quantity of Cadmium and Lead in samples collected from contaminated areas in the provinces of Tehran, Gilan, Zanjan, Esfehan and Chaharmahal-o-Bakhtiyari were in the range of 1.9 to 180.5 and 89.4 to 2610.4 mg kg^{-1} soil, respectively [10-12].

The results of investigations conducted by Torabian and Mahjouri [12] show that the range of contamination levels in the fields and farmland to the south of Tehran, for Cadmium, is 0.101 to 7.54 mg kg^{-1} soil and in the crops cultivated in the area, 0.398 to 1.437 mg kg^{-1} dry weight of the crop, which is higher than the permissible limits for human consumption.

Once Cadmium enters the body, it accumulates in soft tissues, primarily the liver and kidneys. Exposure to Cadmium over time can result in accumulation of the metal in the body, which can have significant health significance for children. Human studies have shown that chronic exposure to Cadmium can lead to serious health effects including lung cancer, emphysema and other lung diseases and kidney damage. Cadmium, with normal levels of 0.06 to 1.10 mg kg^{-1} soil, is considered one of the most important and most active heavy metal present in soil. Permissible level of consumption of Cadmium, for human beings, is 70 μg /day [7, 8].

A review of the investigation results shows that the level of concentration of heavy metals depends upon and varies with, the type of metal, soil conditions and type of plant variety, but normally the level of concentration in aerial parts of a plant or crop, such as leaves and stem is significantly higher than other parts and significantly lower in seeds, as compared to leaves and stems [3, 5, 13].

As per standards declared by the United States Environmental Protection Agency (USEPA), the average permissible monthly increase in Cadmium ions in the soil should not be in excess of 39 mg kg^{-1} soil and the total annual accumulation of Cadmium per hectare of soil should not exceed 1.9 kg [12]. The World Health Organization (WHO) has declared that the permissible limits of Cadmium intake in human diet should not be in excess of 0.1 ppm [14]. Standards prescribed for animal consumption, range in quantities between 10-20 ppm [3].

Observation of population growth trends and national industrial development with consequential increase in discharge of waste from factories and mines, it is predicted that the chances of increase in heavy metal contamination levels of soil and their entry into the food chain, is increasing and a cause for alarm. Besides the various studies and investigations regarding the impact of urban and industrial waste water on soil and plant characteristics, more specific information is needed in relation to the absorption and transfer of heavy metals from the root region to various types of leafy and other vegetables, especially the levels of accumulation of heavy metals in different parts of the cultivated crops and plants, under similar conditions; and other related research is required for preserving population health safety.

Considering the importance, this study has been conducted to investigate the levels of absorption and accumulation of Cadmium from the root region to different parts, in a variety of common crops in the country, other conditions being equal and controlled. The most important part of this investigation, which included a study of Cadmium contamination levels in seven types of vegetables commonly consumed in the country, such as onion, leek, parsley, coriander, lettuce, cress and mint; has been reported in this paper.

MATERIALS AND METHODS

The experiments were carried out under laboratory conditions, the test plan using the factorial testing plan in random blocks model, in four treatments and three repetitions, to study the influence of different Cadmium concentration levels in the root zone, on its rate of accumulation in different parts of seven different vegetables; at the Research Institute for Agricultural Engineering and Technology at Karaj. Treatments were prepared by adding Cadmium Nitrate to soil to make homogeneous mixtures. Details of treatments are given in Table 1.

Table 1: Details of different treatments

Treatment No.	Description
1	Control soil (No Cadmium. was added)
2	Soil with 50 mg kg^{-1} Cadmium
3	Soil with 100 mg kg^{-1} Cadmium
4	Soil with 50 mg kg^{-1} Cadmium + 0.5 molar EDTA in irrigation water

Table 2: Physical and chemical properties of the soil sample

Soil characteristics	Values
Sand (%)	35.0
Silt (%)	42.0
Clay (%)	23.0
Organic carbon (%)	1.25
pH	7.40
CEC (meq/100g)	0.11
Cd (mg kg^{-1})	0.03
Na (meq/lit)	2.72
Mg (meq/lit)	3.31
Ca (meq/lit)	4.12
So_4 (meq/lit)	3.80
Cl (meq/lit)	3.32
HCO_3 (meq/lit)	3.20

Seven common vegetables in Iran including onion, leek, parsley, cress, coriander, lettuce and mint were selected. The soil utilized in this study was selected from among various samples and collected from a 400 ha farmland of the Seed and Plant Improvement Institute (S.P.I.I.) in Karaj after being sifted twice with a Mesh (2 mm). Prior to beginning the cultivation test, a sample of the soil was tested and the results are presented in Table 2. To protect the environment from Cadmium contamination, 60 cm high cylindrical plastic vases of 40 cm diameter were used for the experiment.

Review of similar studies shows that despite the importance of the amount of water used in influencing the rate of metal absorption, few researchers have paid due attention to the fact. The amount of water to be used for irrigating the various crops under study was determined, based on calculating their needs using the Penman-Monteith equation. The amount of water to be used for irrigation was calculated taking into consideration seasonal changes as well as the various stages of plant growth and given to the plants once in two or three days. The necessary information on climatic parameters was collected on a daily basis from the Meshkinabad meteorological station, located in Karaj, close to the experiment site. For determining the amount of water needed during various stages of plant growth, the crop coefficient (k_c) was applied as recommended in the book on calculating amounts of water for irrigation for different plant varieties, by Farshi *et al.* [15]. During the various stages of the experiment from sowing to reaping harvest, no pesticides, fertilizers or weed killers were used.

At the end of the cultivation season, samples were taken from different parts of the plants under study (roots, stems and leaves) to determine the levels of Cadmium accumulation in each part. The samples so collected, were washed and separated by a plastic knife, again washed with distilled water and dried under 70 °C temperature for three days. The dried samples were ground and powdered completely for each repetition after its complete analysis.

Samples (5g) were burnt to ashes in a muffle furnace by gradually increasing temperature from 25° to 450°C over a one and half hour period, followed by 2 hours at 450°C. The ash samples were suspended in 20 ml of aqua regia (HCL: HNO_3, 3:1 v/v) and diluted (1:20) with de-ionized water and analyzed by graphite furnace atomic absorption spectroscopy (GFAA, Perkins-Elmer model 4100ZL, Cupertino, CA) [16].

All the data were subjected to analysis of variance and treatment means were compared by Duncan's Multiple Range test at 1% and 5% probability levels. The SPSS and EXCEL softwares were used for statistical analysis and graphical description, respectively.

RESULTS AND DISCUSSION

Cadmium accumulation in different plants is shown in Table 3. It is obvious from ANOVA table that a high significant difference were observed due the treatments used. Also, the average accumulation of Cadmium in different plant parts on the basis of the Duncan Multiple Range test is demonstrated in Table 4 and Figure 1. Data in Table 4 shows that all plants, except parsley and onion can be categorized in the various statistical groups defined on the basis of Cadmium concentration, except for onion and parsley. In fact lettuce showed the highest Cadmium concentration (1.73 mg kg^{-1}) in its root, while leek had the least (0.59 mg kg^{-1}). The levels of Cadmium concentration in the roots of other plant species, such as cress, mint, coriander, onion and parsley were in that order of sequence.

The results for leaves show that lettuce with 2.43 mg kg^{-1} of Cadmium concentration ranks first and leek with 0.63 mg kg^{-1} ranks last among the tested plant specimens, on the basis of Duncan's classification of statistical groups. Other species such as cress, onion, mint, coriander and parsley are in that order of sequence.

Table 3: Variance analysis of discussed characteristic in studied spices

Source of Variation	df	Mean square (MS) Stem	Leaf	Root
Species	6	2.01**	4.31**	13.2**
Treatment	3	13.2**	26.2**	44.6**
S×T	18	0.33**	0.55**	2.05**
Error	56	0.01	0.03	0.10
C.V.	---	8.57	10.9	15.2

* = Significant at 0.01 probability level

Table 4: Comparison of average Cadmium concentration in different parts of discussed spices (mg kg^{-1})

Species	Root	Leaf	Stem
Onion	0.992 e	1.945 b	1.789 c
Leek	0.587 f	0.629 e	1.001 e
Parsley	0.909 e	1.254 d	1.408 d
Cress	1.575 b	1.949 b	2.319 b
Coriander	1.074 d	1.309 cd	1.893 c
Lettuce	1.738 a	2.436 a	4.180 a
Mint	1.308 c	1.424 c	1.743 c

The results for Cadmium concentration in stems shows that lettuce with 4.18 mg kg^{-1} ranks first among tested plants, based on Duncan's Statistical Grouping and the ranking of other species such as cress, coriander, onion, mint, parsley and leek is in that order of sequence.

In this regard Cadmium accumulation in different parts of the tested plant species, as demonstrated in Table 5 and Figure 2 shows that Cadmium concentration in each of the three parts varied with the level of Cadmium concentration in the treatments and the increase of Cadmium concentration resulting from the third treatment was significantly higher at 1%, than from other treatments. In this treatment, the level of Cadmium accumulation in the stem, leaf and root was 3.36, 2.60 and 1.78 mg kg^{-1} dry weight, respectively. The levels of Cadmium accumulation resulting from the fourth, second and first treatments descend in that order. The results also show that Cadmium accumulation was the least in the root and most in the stem of all species.

Data in Table 6 shows that all tested plant species showed a significant increase in Cadmium accumulation resulting from the third treatment, as compared to other

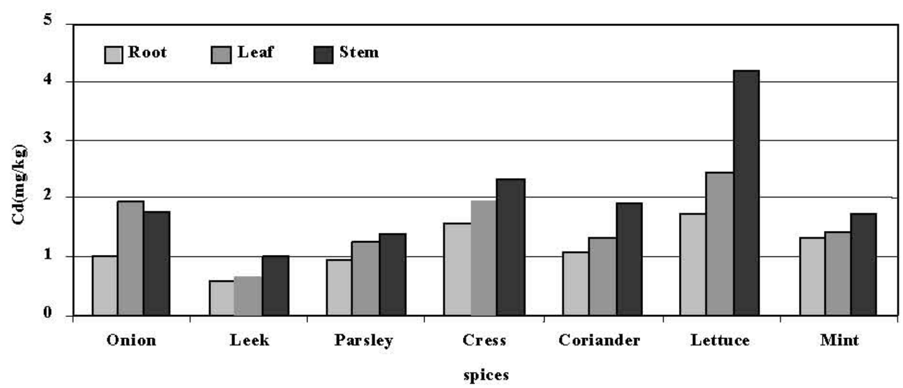

Fig. 1: Comparison of average Cadmium concentration in different parts of discussed spices (mg kg^{-1})

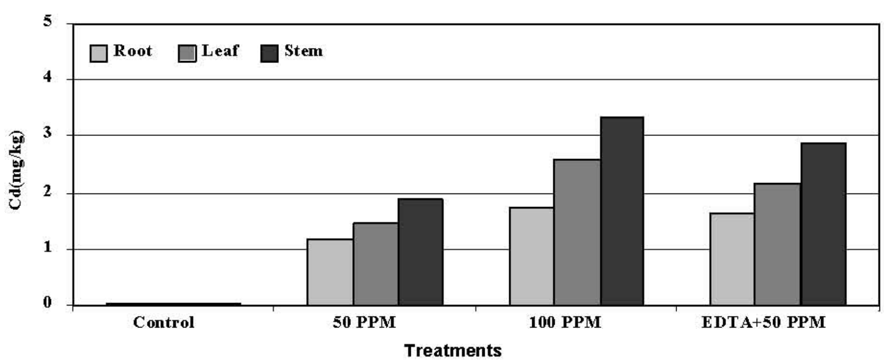

Fig. 2: Comparison of mean Cadmium concentration in different parts of plant species in different treatments (mg kg^{-1})

Table 5: Comparison of mean Cadmium concentration in different parts of plant species in different treatments (mg kg^{-1})

Treatment No.	Root	Leaf	Stem
1	0.043 d	0.050 d	0.058 d
2	1.183 c	1.440 c	1.907 c
3	1.783 a	2.604 a	3.360 a
4	1.636 b	2.161 b	2.870 b

Table 6: Comparison of interaction effect between spices and treatment on Cadmium accumulation amount

Spices	Treatment No.	Root	Leaf	Stem
Onion	1	0.026 y	0.066 tu	0.030 u
	2	0.886 o	2.017 th	1.750m
	3	1.210 k	3.197 d	2.753 h
	4	1.563 h	2.507 f	2.653 i
Leek	1	0.030 y	0.050 tuw	0.037 u
	2	0.573 q	0.427 s	1.133 r
	3	0.770 p	1.118 p	1.587 n
	4	0.973 n	0.860 r	1.250 q
Parsley	1	0.028 y	0.041 uw	0.080 s
	2	1.113 m	1.120 q	1.273 q
	3	1.333 g	2.113 h	2.747 h
	4	1.160 i	1.740 i	1.527 o
Cress	1	0.041 y	0.051 tuv	0.060stu
	2	1.370 i	1.500 m	2.190 k
	3	2.640 a	3.400 b	3.850 d
	4	2.250 c	2.843 e	3.177 e
Coriander	1	0.030 y	0.041 uw	0.060stu
	2	1.203 k	1.213 o	2.160 j
	3	1.703 g	2.130 h	2.807 g
	4	1.360 i	1.853 k	2.547 i
Lettuce	1	0.049 y	0.070 t	0.080 st
	2	1.893 e	2.455 e	3.440 c
	3	2.630 a	3.870 a	6.800 a
	4	2.380 b	3.350 c	6.400 b
Mint	1	0.034 y	0.310 w	0.050tu
	2	1.240 j	1.350 n	1.405 p
	3	2.193 d	2.337 g	2.973 f
	4	1.763 f	1.980 i	2.540 i

treatments. The order of the group ranking for the other soil treatments was fourth, second and first. Accumulation of Cadmium in the root of tested plant species showed that leek and onion were exceptional, in that the fourth soil treatment resulted in the highest Cadmium accumulation in these two plant species. Cadmium accumulation in the root of other species ranked in the following descending order; treatment 3, treatment 4, treatment 2 and treatment 1.

Data also showed that Cadmium contamination of soil in the different treatments resulted in a significant increase in Cadmium accumulation in some plant specimen, while showing no significant change in other types of plant specimens (Table 6).

The results of this study show that among the different plant species tested, the amount of Cadmium were more accumulated in stem, leaves and root of lettuce compared to other plant specimens. Similar results are in line with those obtained by Doyle [5], Torabian and Mahjouri [12], Giordano and Mays [13] and Fazeli [17].

The findings may be summarized as follows for the accumulation of Cadmium in different parts of tested plant species:

Root: leek < parsley < onion < coriander < mint < cress < lettuce

Stem: leek < parsley < mint < onion < coriander < cress < lettuce

Leaf: leek < parsley < coriander < mint < onion < cress < lettuce

Giordano and Mays [13] showed that among different plant specimen types, the highest amount of Cadmium accumulation is in lettuce, spinach and radish in that order. Shariat and Farshi [18] showed that the levels of Cadmium accumulation are in a descending order in lettuce, radish, beet and spinach, respectively. Torabian & Mahjouri [12] show that Cadmium accumulation in plants irrigated with wastewaters in South Tehran is in the following order of ranking.

spinach<cress<radish<coriander<lettuce<parsley<mint

The amount of Cadmium accumulation in the aerial parts (Stem and Leaf) of a plant is higher than in the parts below the ground (root). This finding is comparable with the findings of other earlier studies [5, 17, 19]. They reported that Cadmium has been reported as a highly mobile metal, easily absorbed by the plant specimens tested, through the root surface and moves to wood tissue and transfers to upper parts of plants.

Gardiner *et al.* [20] and Ramos *et al.* [21] showed that there is a direct relation between the level of presence of Cadmium in the root zone and its absorption by plant specimens and increase in levels of Cadmium contamination directly results in an increase in the amount absorbed and accumulated in different plant parts. Also, adding EDTA in irrigation water (Fourth Treatment) effectively increased the amount of Cadmium absorption and accumulation in plants. In fact, all plant specimens tested, showed a significant increase in the rate of absorption and amount of Cadmium accumulation in all treatments, as compared to second treatment. As shown in Table 5, this increase was 38% in root, 50% in leaf and stem. This increase is probably due to the increased solubility of Cadmium, which in turn increases its availability for studied plants. Li and Shuman [22], Lee *et al.* [23] and Robinson [24] stated that Zinc and Cadmium are highly solvent in their natural state and addition of solvents such as organic acids or EDTA has no effect on its degree of solvency. This is in contrast to other studies which state that EDTA is increasing the solvency and absorption of Cadmium in plants [3,25,26].

Absorption and accumulation of Cadmium in stem and leaf was higher for third treatment, for all plant species tested. Treatment 4, 2 and 1 ranked in that descending order in their impact on the level of Cadmium absorption and accumulation in plant species. Results for Cadmium absorption in roots, shows that except for leek and onion which show the highest Cadmium absorption and accumulation in batch 4 soil fourth treatment; however, other species showed decreasing levels of Cadmium concentration in treatment 3, 4, 2 & 1, in that order of ranking. The findings are conclusive evidences of the effect of EDTA in increasing levels of Cadmium absorption and accumulation in lettuce and in the root of leek and onion, as compared with other plant species tested.

This study also provides evidence as to the presence of Cadmium concentration in plant species, except the control treatment, in levels exceeding permissible limits for human consumption.

CONCLUSSION

The conclusions that may be drawn from the results of this study are that the levels of Cadmium accumulation in the plant species tested are in direct proportion of the level of Cadmium concentration in root zone. The exceptions being onion which showed higher concentration in the stem as compared to leaves and in the leaves higher than in the root. Lettuce and leek ranked first and last for Cadmium accumulation in the leaves, with Cadmium concentrations of 2.44 and 0.63 mg kg^{-1} dry weight, respectively, on the basis of Duncan's group classification. Cadmium absorption and accumulation in cress, onion, mint, coriander and parsley showed decreasing levels of concentration, ranking in the order listed. Cadmium accumulation in the stem is highest for lettuce, among plant species tested, with 4.18 mg kg^{-1} followed by cress, coriander, onion, mint, parsley and leek in that order. The study also shows that the level of Cadmium concentration in different plant parts was the highest for lettuce as compared to the others.

The amount of Cadmium accumulation was the highest for third treatment. In this treatment, the amount if Cadmium accumulation in stem, leaf and root was 3.36 mg kg^{-1}, 2.60 mg kg^{-1} and 1.78 mg kg^{-1} dry weight, respectively and treatment 4, 2 and 1, in that order of ranking.

In view of the fact that among the various parts of plants, leaves are consumed the most as human and animal diet, the accumulation of Cadmium in this plant part is special concern. The results obtained are useful as a guide for the selection and priority rating of the types of plants that should be cultivated in farmlands irrigated by waste water contaminated with Cadmium, taking into consideration the parts of the vegetables consumed. The study provides evidence that Cadmium accumulation in leaves was the highest in lettuce, followed by cress, onion, mint, coriander, parsley and leek, in the order listed.

The increase in Cadmium accumulation in different species and their different parts is not constant and is not in proportion to the increase in Cadmium concentration in treatments. This increase is significant for Cadmium accumulation levels in different plant species when the treatments with Cadmium concentrations was applied at a rate of 50 and 100 mg kg^{-1} soil, where it may be clearly observed. The results obtained in relation to the trends in Cadmium absorption and accumulation in leaves, shows that in onion, lettuce, coriander, mint and parsley, the increase in accumulation is lesser than the increase in concentration in treatments; whereas for leek and cress, it is the reverse, with higher rates of absorption and accumulation in leaves than in the root region.

Similarly, based on the evidence provided by the study, besides the high levels of solvency and absorption of Cadmium, in comparison with other heavy metals and its accumulation in the upper parts of plant species tested, it was observed that EDTA has a significant impact on the increase in accessibility and absorption of Cadmium in the plant species tested.

Perhaps the most important conclusion that may be drawn from the findings of the study, is that since vegetables tend to absorb and accumulate Cadmium in the stem and leaves, the most consumed parts of the plants and in view of their important role in the food chain, it is recommended that these type of plants should not be cultivated in farms and fields irrigated by urban and industrial waste water or water contaminated by Cadmium. Especially in the case of lettuce, it is strongly advised that their irrigation with water contaminated by Cadmium be stopped.

The findings of this study show that plant species cultivated in soil treatment batches contaminated with 50 mg Cadmium per kg soil accumulated the higher Cadmium concentrations than permissible limits for human consumption. It is recommended that for a more comprehensive understanding of the relation between soil contamination levels and Cadmium accumulation in plant species, more number of treatments, in a stepped up order are required with Cadmium treatments in the range of 0-150 mg kg^{-1} soil, to determine the permissible limits of soil contamination for cultivation of crops and edible plants, as well as for a frame work for limiting the plant variety permissible for cultivation in Cadmium contaminated farmland.

ACKNOWLEDGEMENT

The research was supported by the Department of Environmental Engineering, University of Tehran and Iranian Agricultural Engineering Research Institute and directed by Dr. A.A. Azimi, Dr. A. Baghvand, Dr. A.M. Liaghat and Dr. G.A. Fardi.

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