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Article

## Response of Vegetables to Cadmium-Enriched Soil

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**Abstract:** Environmental and water pollution through heavy metals is a growing concern. The recycling of untreated wastewater, which is often contaminated with heavy metals, for agricultural applications is becoming more popular. However, information on the amount of absorption and accumulation of cadmium (Cd) at variable concentrations by different crops is limited. This study aims to analyze the impact of various Cd concentrations (0, 30, 60 and 120 mg/kg) in the root zone on the quantity of its absorption as well as accumulation in various parts of seven different types of common vegetables. The experiments were carried out under laboratory-like controlled conditions. Four treatments and three replicates were selected. Cadmium accumulation exceeded the permissible limits for human consumption, and its accumulation in different plant parts followed this order: Leaves: broccoli > spinach > basil > garlic > carrot > tarragon > dill. Stems: broccoli > spinach > basil > garlic > tarragon > carrot > dill. Roots: broccoli > garlic > basil > spinach > carrot > dill > tarragon. Therefore, the authors recommend the reuse of treated wastewater, which should be virtually free of contaminants such as heavy metals, to irrigate farm lands in the future.

**Keywords:** basil; broccoli; cadmium; carrot; garlic; irrigation; metal accumulation; spinach; tarragon; treated wastewater

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## 1. Introduction

An increase in the rate of population growth and changing water usage patterns have led to the production of high volumes of wastewater and depleting freshwater resources. Reusing wastewater can be considered as a potential solution to save freshwater resources for drinking consumption and getting rid of wastewater at the same time. One of the sectors in which non-conventional water resources are recycled is the agricultural sector. However, there are concerns regarding public health and environmental pollution as well as cultural and religious barriers hindering the widespread use of wastewater for irrigation purposes [1,2].

Irrigating agricultural farm land provides food for humans, alleviating poverty [3]. Artificial irrigation is associated with the lion share of water consumption in some regions of the world such as the Middle East and Northern Africa [1,2,4]. Despite intense droughts in some regions, about 67% to 90% of water resources are allocated to agricultural practices [5]. The reuse of wastewater in irrigation systems seems a promising method to provide the essential need for the agricultural industry. In addition to resolving the water shortage problem, irrigating with nutrient-rich wastewater provides free fertilizer to potentially poor soil. However, long-term usage of wastewater, which is mainly prevalent for cultivation of leafy vegetables, has resulted in relatively high heavy metal (e.g., Cd) accumulation in the soil and their subsequent transfer to the crops under cultivation [6,7].

Cadmium is used widely in industry; colour pigment, nickel-cadmium battery, anticorrosive agent, stabilizer in plastics. Unfortunately, the consumption of this potentially dangerous metal is increasing in developing countries such as Iran, because people often discharge this pollutant into the wastewater stream carelessly. Cadmium is often associated with fine clay particles within the raw water. The current concentration of Cd in wastewater received by the wastewater treatment plant of Tehran is comparatively elevated, and varies between 0.05 and 0.018 mg/L [8].

Taking appropriate measures to prevent Cd accumulation in the environment seems vital, because Cd may be easily absorbed by some plants used for human consumption and accumulates in agricultural products in concentrations that can even be considered toxic for some plant species. Excessive accumulation of Cd in soil can reduce the growth of plants [9]. The consumption of vegetables containing accumulated Cd is a known pathway for the entry of toxic metals into humans. Contaminated food can deplete some essential nutrients in the body. This could result in reduced immunological defence and some disabilities associated with malnutrition [10].

Several studies have been undertaken concerning the impact of wastewater containing Cd used for irrigation on plants and particularly vegetables in the south of Tehran (Iran). Table 1 indicates corresponding bioaccumulation findings for different plants [11]. Other vegetables such as broccoli, basil, garlic, tarragon, carrot and dill have not been studied with respect to their tolerance to Cd. Cadmium contamination levels in farm lands are fairly high; for example, between 0.01 and 7.54 mg/kg for soil and between 0.398 and 1.437 mg/kg dry weight for the corresponding crops growing on the contaminated soil south of Tehran [12].

Introducing Cd to soil by irrigation with wastewater containing Cd enhances the level of Cd in the corresponding topsoil. It follows that there is a need to assess the responses of different vegetables and their individual parts to Cd bioaccumulation to prevent humans eating vegetable parts that are too contaminated. Furthermore, the most Cd-tolerant vegetable should be promoted for further cultivation.

**Table 1.** Cadmium bioaccumulation in plant parts for a relevant case study south of Tehran, Iran [11].

Plant	Part	Concentration (ppm)
Corn	Seed	3.22
Alfalfa	Shoot	4.9
Lettuce	Leaf	2.8
Radish	Leaf	1.9
Bean	Seed	1.6
Cotton	Root	1.1
Mung bean	Seed	53.5
Sunflower	Seed	0.48
Squash	Root	6.2
Spinach	Leaf	1.3
Turnip	Tuber	1.1

The key aim of this research is to assess the potential of different vegetables and their corresponding parts in absorbing Cd at various concentration levels within the Cd-enriched soils. The paper is concerned with growing edible vegetables in contaminated soil. However, the treatment of the contaminated soil is beyond the scope of this study. The findings will help to identify the most appropriate vegetable parts of selected vegetables for human consumption.

## 2. Materials and Methods

### 2.1. Site Description

The research was conducted in the Varamin Valley near Tehran. This is an agricultural area producing about 40% of Tehran's crop needs according to the authors' estimation. At the first stage of the experiment, an appropriate experimental farm site, which includes essential facilities such as water, electricity and a proper protection to prevent animals and people from entering the site, was selected.

The experiments were carried out at the Abureyhan Campus of Tehran University located in the Varamin Valley (about 40 km south of Tehran). The maximum monthly rainfall in this region is about 40 mm in March compared to and less than 5 mm in July, representing minimum precipitation. The temperature varies between 25 and 37 °C in July, which is known as the hottest month, and between 1 and 8 °C in January, which is usually the coldest month.

Soil with a texture and structure similar to the dominant soil texture and structure of the whole region, was collected by gathering soil samples from different parts of the Abureyhan Campus. In order to avoid contaminating the soil samples utilized in the experiments and the entire experimental site, planting pots with a diameter and height of 80 cm and 100 cm, respectively, were located in a vegetable farm area of about 1000 m<sup>2</sup>. The soil texture comprised silt (42%), sand (35%) and clay (23%). The soil was almost neutral (pH = 7.4) with a background Cd concentration of 0.03 mg/kg (Table 2).

**Table 2.** Physical and chemical characteristics of soil samples (meq/L, milliequivalent of solute per litre).

Variable	Unit	Value
Sand	%	35
Silt	%	42
Clay	%	23
Organic carbon	%	1.25
Phosphorus (available by plants)	mg/kg	3.2
Total nitrogen	%	0.04
Potassium (available by plants)	mg/kg	105
pH	-	7.4
Cation-exchange capacity	meq/100g	11
Cadmium	mg/kg	0.03
Sodium	meq/L	2.72
Magnesium	meq/L	3.31
Calcium	meq/L	4.12
Sulphate	meq/L	3.8
Chloride	meq/L	3.32
Bicarbonate	meq/L	3.2

## 2.2. Cultivation and Measurements

All experiments were carried out under laboratory-like conditions using a factorial testing plan as part of the random block model design with four different treatments and three replicates to assess the influences of dissimilar Cd concentrations within soil samples on three plant parts (root, zone and stem) for seven vegetables (spinach, garlic, carrot, dill, tarragon, basil and broccoli) representative of a wide section of available vegetable plant species. The Cd concentration was the only variable parameter between these treatments to reduce noise in the findings, and to obtain statistically valid results. Details of the four treatments are presented in Table 3.

Due to the high variability of Cd in real wastewater and the presence of other contaminants, the effect of wastewater contaminated with Cd used for irrigation of soil was simulated by using soil already contaminated by Cd. Therefore, cadmium nitrate was powdered and mixed entirely to gain a homogenous mixture, which was used to prepare soil samples with three different Cd concentrations (30, 60 and 120 mg/kg; Table 3). Finally, to check for the uniformity of Cd concentrations at different levels of the prepared soil sample, various samples were taken and their Cd concentrations were analysed in the laboratory. It should be mentioned that the soil samples were collected from different levels of the soil column using stainless steel sampling tools and clean plastic buckets. Samples were immediately sent to the laboratory in clean standard soil containers.

Considering that real wastewater is highly variable in its chemical composition, groundwater, which is relatively stable in its composition and which is the dominant water resource in the Varamin Valley, was used as a substitute to irrigate the example vegetable species during the growing season. The groundwater had an electrical conductivity of 635 dS/m. The chemical traits of the groundwater are shown in Table 4. The Penman-Monteith equation [13] was utilized to accurately determine the crops' water demand during different growth stages.

**Table 3.** Characteristics of the studied treatments.

Treatment number	Description	Comment
1	Soil with 0 mg/kg cadmium (Cd)	Control
2	Soil with 30 mg/kg Cd	None
3	Soil with 60 mg/kg Cd	None
4	Soil with 120 mg/kg Cd	None

**Table 4.** Chemical configurations of Varamin Valley groundwater (meq/L, milliequivalent of solute per litre).

Variable	Unit	Value
Electric conductivity	dS/m	635
pH	-	7.3
Calcium	meq/L	3.2
Magnesium	meq/L	3.8
Sodium	meq/L	1.69
Potassium	meq/L	0
Carbonate	meq/L	0
Bicarbonate	meq/L	3.64
Sulphate	meq/L	2.87
Chloride	meq/L	2.05
Cadmium	meq/L	0

Cultivation of the investigated species was done according to the local cropping schedule using pots. No pesticides and fertilizers were used during the cultivation season to eliminate their potentially problematic impact on the outcome of the research. Any weeds were removed manually if and when appropriate.

At the end of the cultivation season, different sections of the investigated crops (root, stem and leaf) were used to assess Cd accumulation within these plant parts. After cutting the samples, they were washed with distilled water. They were subsequently dried at 100 °C and then burnt to gain their ashes, which were put in 100 mL of aqua regia (3 parts in volume of 12 M HCl to 1 part of 16 M HNO<sub>3</sub>) and diluted (1 in 20) with distilled water. Finally, the Cd concentrations were measured by graphite furnace atomic absorption spectroscopy (Perkins-Elmer model 4100ZL, Cupertino, CA, USA) for the prepared crop samples [12].

### 2.3. Statistical Analysis

An analysis of variance (ANOVA) was carried out on the data obtained from the measurements to analyse the differences between group means and their associated procedures such as the variation among and between groups. The observed variance in a particular variable was partitioned into components that can be attributed to various sources of variation. The ANOVA was used to assess whether or not the means of several groups were equal, and to compare at least three means of group or variables for statistical significance.

The common software package IBM SPSS Statistics was used. Moreover, the results obtained from the data analysis were then statistically compared with each other using the Duncan's Multiple Range test at 1% and 5% probably levels. This multi-comparison procedure provides significance levels for

the difference between any pair of means. For graphical representation, common Microsoft Excel software was used.

### 3. Results and Discussion

#### 3.1. Effect of Species on Cadmium Accumulation

The ANOVA table for Cd accumulation levels in different parts of the vegetables is presented in Table 5. The findings were significant at 0.01 probability level for all plant parts. It follows that it would make sense to distinguish between different vegetables and associated plant parts when regulating the agricultural market regarding contaminated products in the future.

**Table 5.** Variance analysis of the investigated characteristics in studied species.

Sources of variation	Degrees of freedom	Mean square (MS)		
		Stem	Leaf	Root
Species (S)	6	15.14**	4.98**	4.13**
Treatment (T)	3	51.32**	30.143**	15.16**
S × T	18	2.36**	0.627**	0.373**
Error	56	0.07	0.021	0.007
Coefficient of variation	not applicable	15.72	10.68	8.85

Note: \*\*Significant at 0.01 probability level.

Cadmium pollution in water and soil has been a great challenge for a long time, and several suggestions regarding the maximum allowable concentrations have been recommended in the literature [9,14,15]. In addition to these recommendations, some standards have been presented concerning permissible Cd accumulations in various herbs, concentrations in sewage used for irrigation of farm land and human diet. A few representative and relevant maximum threshold recommendations are tabulated in Table 6.

**Table 6.** Maximum allowable concentrations of cadmium (Cd).

Organization	Reference	Maximum Cd	Application threshold
World Health Organization	[16]	0.1 mg/L	Drinking water
American Food Industries	[17]	92 µg/day	Solid food intake
United States Environmental Protection Agency	[17]	85 ppm	Ooze deposition onto farm land
United Nations	[18]	7 µg/kg of body weight	Solid food intake

Cadmium concentrations for the tested vegetable species are presented in Table 7. The maximum concentration of Cd within leaves, stems and roots were always associated with broccoli. The corresponding minimum concentrations were linked to dill, dill and tarragon, respectively. However, the root concentration for dill was also relatively low.

#### 3.2. Effect of Treatment on Cadmium Accumulation

The effect of different Cd concentrations in soil samples on the corresponding Cd accumulation level of the selected vegetables is presented in Figure 1. The highest amount of Cd concentration

corresponds usually to leaves, while the lowest amount of Cd concentration has been determined for the roots. This poses a problem for those vegetables such as broccoli, spinach and basil where the leaves are consumed by humans and not the roots. On the other hand, a relatively low root contamination is advantageous for vegetables such as carrots where the roots are eaten by consumers.

**Table 7.** Average cadmium (Cd) concentrations for the studied dried plants.

Species	Cd concentration (mg/kg)		
	Leaf	Stem	Root
Carrot	2.67	1.28	1.83
Tarragon	2.51	1.68	0.71
Basil	3.23	1.96	1.98
Garlic	2.82	1.94	2.16
Broccoli	4.25	3.21	2.52
Spinach	3.54	2.47	1.86
Dill	1.83	1.03	0.84

**Figure 1.** Comparison of the mean total cadmium (Cd) concentrations for different parts of the vegetables and for different treatments (1: 0 mg/kg Cd; 2: 30 mg/kg Cd; 3: 60 mg/kg Cd; 4: 120 mg/kg Cd).

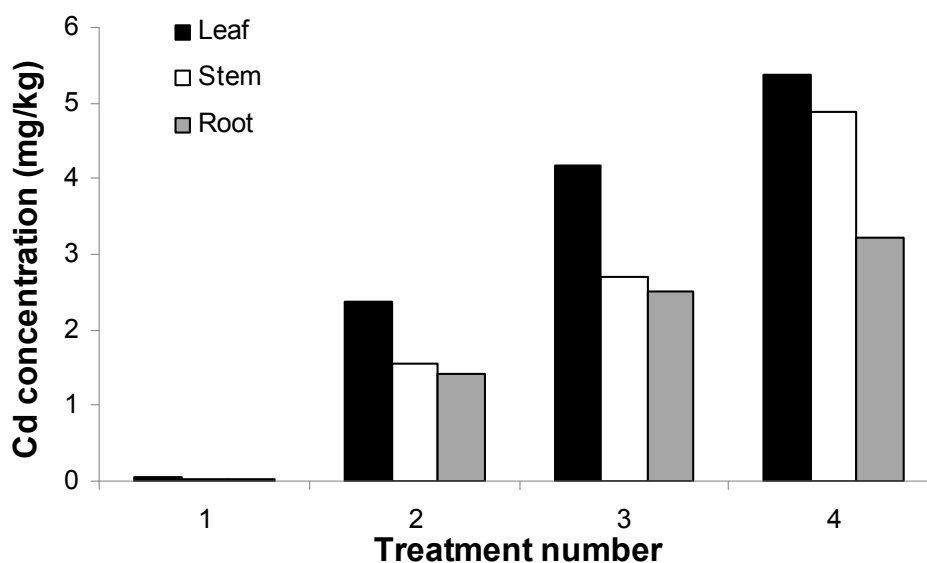


Table 8 compares the responses of different plant parts to various Cd treatments, indicating that the accumulated Cd is the highest for the fourth treatment (Table 3). Moreover, there is a direct positive correlation relationship between Cd concentration levels in all plant organs of the tested vegetables and the Cd concentrations in soil, simulating previously irrigated land with wastewater containing high levels of Cd. It follows that a continuation of the historic practice of irrigation with wastewater needs to be regulated to prevent further permanent soil contamination.

The Cd accumulation results can be summarized as follow: Leaves; broccoli > spinach > basil > garlic > carrot > tarragon > dill. Stems: broccoli > spinach > basil > garlic > tarragon > carrot > dill. Roots: broccoli > garlic > basil > spinach > carrot > dill > tarragon. These relationships are useful when deciding on what vegetables to grow in the future.



**Table 8.** Comparison of Cd concentrations (mg/kg) within different plant parts as a response to various cadmium (Cd) treatments (Table 3). Sample number n = 3.

Species	Treatment	Mean			Standard deviation		
		Leaf	Stem	Root	Leaf	Stem	Root
Carrot	Treatment 1	0.053	0.026	0.037	0.020	0.004	0.006
	Treatment 2	2.136	1.024	1.464	0.942	0.295	0.451
	Treatment 3	3.738	1.792	2.562	0.939	0.625	0.955
	Treatment 4	4.806	2.304	3.294	0.961	0.406	0.403
Tarragon	Treatment 1	0.050	0.034	0.014	0.010	0.012	0.002
	Treatment 2	2.008	1.344	0.566	0.230	0.44	0.130
	Treatment 3	3.514	2.352	0.990	0.606	0.901	0.056
	Treatment 4	4.518	3.024	1.273	1.106	1.563	0.055
Basil	Treatment 1	0.065	0.039	0.042	0.013	0.009	0.025
	Treatment 2	2.584	1.568	1.586	0.198	0.254	0.225
	Treatment 3	4.522	2.744	2.776	1.161	0.829	0.731
	Treatment 4	5.814	3.528	3.569	0.796	1.098	1.190
Garlic	Treatment 1	0.056	0.039	0.043	0.010	0.011	0.011
	Treatment 2	2.256	1.552	1.728	0.568	0.350	0.585
	Treatment 3	3.948	2.716	3.024	1.063	1.631	0.225
	Treatment 4	5.076	3.492	3.888	1.844	0.747	0.982
Broccoli	Treatment 1	0.085	0.064	0.051	0.020	0.021	0.013
	Treatment 2	3.400	2.568	2.568	1.198	0.601	0.759
	Treatment 3	5.950	4.494	4.494	1.805	1.103	0.630
	Treatment 4	7.650	5.778	5.778	2.141	0.799	1.264
Spinach	Treatment 1	0.071	0.049	0.037	0.011	0.013	0.006
	Treatment 2	2.832	1.976	1.488	0.699	0.871	0.358
	Treatment 3	4.956	3.458	2.604	1.888	0.777	0.438
	Treatment 4	6.372	4.446	3.348	0.808	0.518	0.936
Dill	Treatment 1	0.030	0.021	0.017	0.011	0.008	0.002
	Treatment 2	1.464	0.824	0.672	0.477	0.226	0.338
	Treatment 3	2.562	1.442	1.176	0.723	0.568	0.375
	Treatment 4	3.294	1.854	1.512	0.891	0.911	0.198

The simulated treatments are representative of future soil conditions if the practice of using irrigation wastewater containing Cd is not discontinued. The evaluation of vegetable responses to each treatment may lead to an informed decision about future plant selection. Table 8 indicates that dill and tarragon are possibly the best planting options in contrast to broccoli, which planting should not be encouraged anymore.

### 3.3. Consequences of Findings

The Cd accumulations in the selected plant species exceeded the permissible limit (0.5 mg/kg as discussed by [9]) for human food intake. It follows that the studied example vegetables should not be consumed anymore. This could be regulated for the agricultural industry. However, small farmers and

poor families who support their diet with food grown in their garden are likely to continue producing and eating contaminated vegetables due to economic pressure.

Cultivating vegetables on farms where the Cd concentration in the corresponding soil is more than 30 mg/kg leads to Cd accumulations in vegetables likely to exceed existing permissible thresholds recommended for human consumption. This is the case for large areas of the case study region, and representative for other countries in North Africa and the Middle East.

In areas where the soil is heavily contaminated due to decades of wastewater application and where wastewater is the only available and economic water source, farmers have either the option to continue farming and to produce contaminated crops or to stop farming. A political decision needs to be made by the responsible authorities to come to a realistic solution that does not harm public health and agricultural exports. Subsidies for the installation of (better) wastewater treatment systems and soil remediation programmes might be an option for oil-rich countries such as Iran. In contrast, economically poor countries in the same region such as Yemen and Jordan might not be able to afford potentially expensive treatment systems [1,2]. However, there is well-established guidance on how to irrigate farms with treated wastewater in a sustainable manner without undertaking expensive land remediation programmes [19].

### 3.4. Limitations

Uncontaminated soil to which increasing concentrations of Cd was added was used in this experiment. Alternatively, freshwater spiked with different Cd concentrations could have been used. This would have resulted in long-term accumulation of Cd. However, it would have taken many years to reach the desired concentrations within the soil before the experiment could have been started. Moreover, Cd is easily adsorbed by clay located within the soil matrix, which makes the argument for long-term adsorption less relevant.

The usefulness of the findings is limited to arid countries where irrigation with heavy metal-enriched wastewater has been practiced for many years or even decades. However, the study is also relevant for countries wishing to recycle wastewater for irrigation in the future. They could learn from the mistakes of those countries with contaminated soil due to poor irrigation strategies.

A further limitation is the lack of a high number of replicates. Three replicates were assessed in Table 8. The findings would have been more significant if at least 30 replicates would have been used. However, there were resource restrictions in terms of work force and funding.

## 4. Conclusions and Recommendations

Wastewater recycling for the irrigation of crops is a possibility for countries with low freshwater availability. However, studies have shown that relatively high concentrations of Cd in soil samples resulted in high Cd accumulations in plant parts. The maximum average Cd concentrations of 4.25, 3.12 and 2.52 mg/kg were observed in leaves, stems and roots of broccoli, respectively. There is a positive correlation between the amount of Cd in soil samples and its accumulation in plant parts. Considering that most of the selected plants such as broccoli and spinach absorbed and accumulated relatively high Cd quantities in their stems and leaves, which are consumed by humans, it is recommended to prevent cultivation of these vegetables on farm lands, which are irrigated with

essentially untreated urban and industrial wastewater containing high levels of heavy metals such as Cd. Moreover, vegetables should not be grown on farm land, which has already a Cd concentration of more than 30 mg/kg.

The authors recommend the reuse of treated wastewater to irrigate farm lands in the future. This should result in edible vegetables. Treatment technology such as integrated vertical-flow constructed wetlands might offer a cost-effective and sustainable solution.

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### Author Contributions

Babak Bakhshayesh and Madjid Delkash undertook the experimental work. Miklas Scholz wrote the article based on a first draft paper provided by Madjid Delkash.

### Conflicts of Interest

The authors declare no conflict of interest.

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