



PHYSIOLOGICAL RESPONSE AND HEAVY METALS ACCUMULATION IN CELERY (*APIUM GRAVEOLENS*) AND RADISH (*RAPHANUS SATIVUS*) IRRIGATED WITH WASTEWATER

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SUMMARY

The following study sought to determine the effects of irrigation with treated sewage water on celery (*Apium graveolens*) and radish (*Raphanus sativus*) plants, carried out in Mosul City, Iraq. Additionally, it measured the heavy metals, i.e., cadmium (Cd), lead (Pb), and zinc (Zn), and the percentage of chlorophylls a, b, and a + b and the wet and dry weight of plant parts. By irrigating with water treated by sedimentation, the average concentration of heavy metals (Cd, Pb, and Zn) in those plants reached the highest levels (0.18825, 0.10900, and 0.59775 mg/kg, respectively), as compared with the lowest average concentration of these heavy metals (0.05800, 0.02625, and 0.12450 mg/kg, respectively) irrigated with tap water (control). The maximum average concentration of chlorophyll a, b, and a + b (2.18000, 1.32183, and 1.47150 mg/g, respectively) occurred in celery and radish plants irrigated with untreated water. However, the minimum average concentration of chlorophyll a, b, and a + b (1.21800, 1.08900, and 1.30550 mg/g, respectively) resulted in those two plants being irrigated with tap water. The highest average fresh weight of celery and radish plants (3.03750 g/plant) emerged by irrigating them with water treated with sedimentation.

Keywords: Celery (*A. graveolens*), radish (*R. sativus*), wastewater, heavy metals, physiological and growth traits

Key findings: The sewage water treatment will develop a new water wealth that will benefit humans and living organisms and create a safe environment, eventually improving the agriculture sector in the long term.

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INTRODUCTION

Given the fresh water scarcity and its high cost for use in arable soils, the community requires using sewage water to irrigate different crops, with concentrations of toxins, heavy metals, and the biological requirement of oxygen above the natural limits. These led to severe negative effects on crop plants and their consumers. Therefore, it is necessary to take different measures to protect the plants and ensure sustainable development. Moreover, these prompted researchers to explore the treatment of sewage water before its use for crop irrigation. Additionally, the research progressed on treating wastewater by sedimentation and filtration to determine its effect on plants for heavy metals accumulation and on physiological traits, viz., chlorophyll content.

Several direct and real indications of the amount and extent of pollution prevail, developed by heavy metals and their toxicity (Mahmood *et al.*, 2021). Among these vital indicators, the chlorophyll a and b concentrations play a crucial role, considered a sensitive, critical indicator when pollution occurs in the plant's cellular system with heavy metals. The plants' yellowing due to poisoning by heavy metals emerges, which directly affects crop plants' chlorophyll content. Chlorophyll is an essential component of the plant through which photosynthesis occurs, with the carbon dioxide and water converted in the presence of light energy to produce carbohydrates (Fijalkowski *et al.*, 2017). Therefore, the chlorophyll concentration can be the basis as a biological indicator of poisoning by heavy metals in crop plants (Turek *et al.*, 2019).

It is also a known fact that Iraq suffers from water scarcity despite the presence and passage of the Tigris and Euphrates rivers through its lands. However, it recently began to suffer from the phenomenon of desertification and has resorted to many

technologies to solve the water scarcity problems. These include digging wells and using their water sources to irrigate the crops and water animals. Likewise, the sewage water irrigates crop fields and water plants that provide food for humans.

Climate change also created new problems through various environmental factors, such as severe drought in some areas, uneven and heavy rains, floods, destructive torrents, misuse of water resources, and failure to store them optimally. These caused many environmental concerns, negatively affecting the agriculture sector, leading to increased food prices and, thus, enhancing economic pressures on different countries (Turek *et al.*, 2019).

Investing in sewage water has been receiving considerable response recently, and using sewage water after reprocessing it to irrigate crops, forests, and green spaces prevents the phenomenon of desertification and keeps fresh water for human use only (Alwan *et al.*, 2024). Past studies revealed that using treated wastewater to irrigate crops and green spaces eliminates carbon emissions resulting from human activities (Hajihashemi *et al.*, 2020). Therefore, the presented research aimed to determine the effects of irrigation with treated sewage water, particularly on celery (*A. graveolens*) and radish (*R. sativus*) plants.

MATERIALS AND METHODS

Sedimentation method

In polyethylene tanks, wastewater sedimentation transpired for four hours to reduce the percentage of different pollutants. Afterward, the surface layers of water served to irrigate the plants of celery (*A. graveolens*) and radish (*R. sativus*) to evaluate the effects of sedimented wastewater on chlorophyll a and b (Setiawati *et al.*, 2019).

Filtration method

This method used a multi-layer filter to purify and treat the wastewater by passing it through the sedimentation process with a filter. The top consists of 15 cm of sand and four layers of gravel. The size of those elements had a gradual reduction to a depth of 5 cm for each layer. At the bottom of the filter, some holes allowed wastewater to leave.

Treatments used

The use of four types of water irrigated the *A. graveolens* and *R. sativus* plants as follows: a) irrigation with untreated wastewater as the control, b) irrigation with wastewater treated by settling, c) irrigation with wastewater treated by filtration, and d) irrigation with tap water.

Seeds selection and efficiency

The selected seeds of the Iraqi cultivars of *A. graveolens* and *R. sativus* came from the markets of Mosul City, Iraq. Seed efficiency evaluation and selection continued in the laboratory of the University of Mosul. By planting the seeds in plates on two filter papers, 10 seeds per layer and per treatment underwent incubation at 25 °C for seven days, and then measuring the seed germination (Kumar and Prasad, 2018).

$$= \frac{\text{Germination percentage}}{\text{Number of germinated seeds}} \times \frac{\text{Total number of seeds}}{100}$$

The observed efficiency and vitality of celery and radish plants were 95% and 98%, respectively.

Soil selection

Mixed sandy soil succeeded selection from the Rashediia area in Nineveh Province, Iraq, and was brought to the greenhouse successively. The soil samples' washing used distilled water four times before air-drying. Afterward, smoothing the soil employed a sieve with 2-mm diameter holes before placing it in a plastic

anvil of 60-cm length, 20-cm width, and 20-cm depth. Each anvil had a capacity of 14 kg of soil, prepared for the greenhouse (Taher and Saeed, 2022).

Planting and irrigation

The seed planting of celery and radish on August 30, 2023, had the rate of 40 seeds per anvil, considering an equal spacing among the seeds for both types of plants. The plants in anvils obtained conditioning inside the greenhouse, and the plants' water irrigation followed an allocation for each treatment. Specifically, adjusting the water used for irrigation on a daily scale considered the number of watering times and the effect of the greenhouses' climate on the plant. After 10 days for *R. sativus* plants and 30 days for *A. graveolens* plants, the number of seedlings per anvil decreased to 20. After 80 days of planting, the harvest of plants from each anvil and each treatment ensued (Kenenbayev *et al.*, 2024). The experiment layout was in a completely randomized design (CRD) with three replications.

Estimation of leaf chlorophyll

The leaf chlorophyll estimation was according to Kalaji *et al.* (2017) in celery and radish plants. Using the ceramic mortar crushed the wet leaves of both types of plants with the addition of 20 ml of acetone at a concentration of 80%. After separating the filtrate from the sediment using a centrifuge, a spectrophotometer used at a wavelength of 663–645 nm helped read the light absorption of the filtrate to determine the concentration of chlorophyll a and b. The following relationship aided in calculating the concentrations of chlorophyll a, b, and a + b.

$$\text{Chl. a} = (12.7 [\text{D663}] - 2.69 [\text{D645}]) \times V / (1000 \times W)$$

$$\text{Chl. b} = (22.99 [\text{D645}] - 4.68 [\text{D663}]) \times V / (1000 \times W)$$

$$\text{Chl. a+b} = 20.2 (\text{D645}) + 8.02 (\text{D663}) \times V / (1000 \times W)$$

Where: D = Reading of the optical density of chlorophyll extracted at wavelengths of 663 and 645 nm, respectively; V = Final volume (Aston diluted at 80%), and W = Wet weight in grams of plant tissue extracted.

Wet and dry vegetative and root weight

The drying of vegetative and root parts of the plants under study occurred inside an electric oven at a temperature of 75 °C for 48 h until the weight was stable. Following this was taking the weight of the vegetative and root parts of the celery and radish plants while they were in a soft state with each treatment (Kouki *et al.*, 2021).

Estimation of heavy metals

The accumulated heavy metals estimation in celery and radish plants proceeded in their root and vegetative plant parts by grinding the leaves of the dried samples of both plants under study. Then, taking 0.5 g from each sample, the study used the wet digestion method. The atomic absorption spectrometry (AAS) readings estimated the ions of Cd, Zn, and Pb (Taher and Saeed, 2023).

Statistical analysis

The data analysis according to the completely randomized design confirmed results based on types of water used for irrigation and their effect on chlorophyll content, as well as the concentrations of heavy metals in plant parts. The comparison of differences among the factor means and their interactions used the Duncan multi-range test at the probability level of $p \leq 0.01$ (Weaver *et al.*, 2017). All the statistical analyses performed had the help of the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Heavy metals

The results revealed the lowest concentrations of Pb, Zn, and Cd were evident in the roots of the celery (*A. graveolens*) and radish (*R.*

sativus) plants (0.0995, 0.0930, and 0.1595 mg/kg, respectively). The highest concentrations were in their leaves (0.1185, 0.1055, and 0.1810 mg/kg, respectively) by irrigating with untreated water (Tables 1, 2, and 3). However, the percent accumulation of these three elements in celery and radish plants increased in the roots (0.1760, 0.1030, and 0.5400 mg/kg, respectively) and leaves (0.2005, 0.1150, and 0.6555 mg/kg, respectively) by irrigating with the water treated with sedimentation. By watering these plants with water treated by filtration, the concentration of Pb, Zn, and Cd elements decreased in roots (0.1345, 0.0995, and 0.2140 mg/kg, respectively) and leaves (0.1500, 0.1080, and 0.2715 mg/kg, respectively). However, in celery and radish plants, the lowest concentration of these elements (Pb, Zn, and Cd) was noticeable in roots (0.0515, 0.0240, and 0.0950 mg/kg, respectively) and leaves (0.0645, 0.0285, and 0.1540 mg/kg, respectively) irrigated with tap water. Past studies reported similar accumulation of these heavy metals in different crop plants by irrigating them with wastewater (Hashem and Xuebin, 2021; Rasheed *et al.*, 2024).

Among the reasons causing the increased concentration of heavy metals (Pb, Zn, and Cd) in the leaves and roots of celery and radish plants by irrigating them with water treated by sedimentation was due to the low concentration of nitrates. The concentration of nitrates reduces the accumulation of heavy metal elements (Hussein and Ahmed, 2023). It was also clear that the accumulation of these elements (Pb, Zn, and Cd) was higher in plant leaves than in their roots due to continuous irrigation, which led to the accumulation of heavy elements more in leaf tissues than in roots. With the large surface area of radish plant leaves, the accumulation of these elements was higher than in the celery plant leaves (Nowwar *et al.*, 2023; Younis and Saeed, 2023).

Wet and dry plant weight

The different types of irrigation water treatments revealed significant influence and

Table 1. Effect of water sources, plant types, and plant parts on the Pb accumulation in celery and radish plants.

Plant types	Plant parts	Water sources				B x C means	Plant type means	Plant part means
		a ₁	a ₂	a ₃	a ₄			
Celery	Leaves	0.72k	0.145e	0.117g	0.034l	0.092c		
	Roots	0.067k	0.112hg	0.106h	0.023m	0.077d		
Radish	Leaves	0.165d	0.256a	0.183c	0.095i	0.17475a		
	Roots	0.132f	0.240b	0.163d	0.08j	0.15375b		
Celery		0.0695g	0.1285d	0.1115e	0.0285h		0.0845b	
Radish		0.1485c	0.248a	0.173b	0.0875f		0.16425a	
Leaves		0.1185e	0.2005a	0.150c	0.0645g			0.133375a
Roots		0.0995f	0.176b	0.1345d	0.0515h			0.115375b
Water sources means		0.109c	0.18825a	0.14225b	0.058d			

Values followed by the same letter for each factor and interactions between them are not significantly different from each other. a₁ = untreated wastewater, a₂ = wastewater treated by sedimentation, a₃ = wastewater treated by filtration, a₄ = tap water.

Table 2. Effect of water sources, plant types, and plant parts on the Zn accumulation in celery and radish plants.

Plant types	Plant parts	Water sources				B x C means	Plant type means	Plant part means
		a ₁	a ₂	a ₃	a ₄			
Celery	Leaves	0.082c	0.090c	0.085c	0.040f	0.07425b		
	Roots	0.061e	0.072d	0.69de	0.034f	0.0590c		
Radish	Leaves	0.129b	0.140a	0.310b	0.017g	0.10425a		
	Roots	0.125b	0.134ab	0.130b	0.014g	0.10075a		
Celery		0.0715d	0.0810c	0.077dc	0.037e		0.066625b	
Radish		0.1270b	0.1370a	0.1305b	0.0155f		0.066625a	
Leaves		0.1055bc	0.1150a	0.1080b	0.0285e			0.089250a
Roots		0.093d	0.103bc	0.0995c	0.0240e			0.079875b
Water sources means		0.09925c	0.1090a	0.10375b	0.02625d			

Values followed by the same letter for each factor and interactions between them are not significantly different from each other. a₁ = untreated wastewater, a₂ = wastewater treated by sedimentation, a₃ = wastewater treated by filtration, a₄ = tap water.

Table 3. Effect of water sources, plant types, and plant parts on the Cd accumulation in celery and radish plants.

Plant types	Plant parts	Water sources				B x C means	Plant type means	Plant part means
		a ₁	a ₂	a ₃	a ₄			
Celery	Leaves	0.2010h	0.3730c	0.3220d	0.1870i	0.27075c		
	Roots	0.1740k	0.269e	0.2520f	0.0710p	0.19150d		
Radish	Leaves	0.1610l	0.9380a	0.2210g	0.1210n	0.36025a		
	Roots	0.1450m	0.8110b	0.1760j	0.1190o	0.31275b		
Celery		0.1875e	0.3210b	0.2870c	0.1290g		0.231125b	
Radish		0.1530f	0.8745a	0.1985d	0.120h		0.336500a	
Leaves		0.1810e	0.65550a	0.27150c	0.1540g			0.315500a
Roots		0.15950f	0.540b	0.2140d	0.0950h			0.252125b
Water sources means		0.17025c	0.59775a	0.24275b	0.1245d			

Values followed by the same letter for each factor and interactions between them are not significantly different from each other. a₁ = untreated wastewater, a₂ = wastewater treated by sedimentation, a₃ = wastewater treated by filtration, a₄ = tap water.

Table 4. Effect of water sources, plant types, and plant parts on the plant fresh weight in celery and radish plants.

Plant types	Plant parts	Water sources				B x C means	Plant type means	Plant part means
		a ₁	a ₂	a ₃	a ₄			
Celery	Leaves	1.850cd	1.570d	1.240d	1.1100d	1.4425c		
	Roots	1.3100d	1.240d	1.100d	1.100d	1.1875c		
Radish	Leaves	6.270a	3.620b	2.760c	1.2300d	3.470a		
	Roots	2.2720c	1.9200cd	1.7900d	1.1300d	1.890b		
Celery		1.580c	1.4050c	1.170c	1.105c		1.3150b	
Radish		4.4950a	2.770b	2.275b	1.180c		2.680a	
Leaves		4.060a	2.5950b	2.000bc	1.170d			2.4562a
Roots		2.0150bc	1.580cd	1.4450cd	1.1150d			1.5388b
Water sources means		3.0375a	2.0875b	1.7225b	1.1425c			

Values followed by the same letter for each factor and interactions between them are not significantly different from each other.

a₁ = untreated wastewater, a₂ = wastewater treated by sedimentation, a₃ = wastewater treated by filtration, a₄ = tap water.

Table 5. Effect of water sources, plant types, and plant parts on the plant dry weight in celery and radish plants.

Plant types	Plant parts	Water sources				B x C means	Plant type means	Plant part means
		a ₁	a ₂	a ₃	a ₄			
Celery	Leaves	1.0600a	1.0400a	1.0200a	1.0100a	1.0325a		
	Roots	1.0400a	1.0300a	1.1000a	1.0100a	1.0450a		
Radish	Leaves	1.3400a	1.2400a	1.1300a	1.0200a	1.1825a		
	Roots	1.2000a	1.1400a	1.1000a	1.0100a	1.1125a		
Celery		1.050a	1.0350a	1.060a	1.010a		1.03875a	
Radish		1.270a	1.190a	1.1150a	1.0150a		1.14750a	
Leaves		1.200a	1.140a	1.0750a	1.0150a			1.10750a
Roots		1.120a	1.0850a	1.1000a	1.0100a			1.07875a
Water sources means		1.160a	1.1125a	1.0875a	1.0125a			

Values followed by the same letter for each factor and interactions between them are not significantly different from each other. a₁ = untreated wastewater, a₂ = wastewater treated by sedimentation, a₃ = wastewater treated by filtration, a₄ = tap water.

differences in wet and dry weight of celery and radish plants (Tables 4 and 5). The results showed the average wet and dry weight in both plants reached the highest value by irrigating with untreated wastewater (3.0375 and 1.1600 g/plant, respectively). Meanwhile, the lowest average wet and dry weights prevailed by irrigating the celery and radish with tap water (1.1425 and 1.0125 g/plant, respectively). This could refer to the rich culture of sewage water with nitrates and nutrients that increase the mass of proteins and plant tissues. Additionally, heavy metal elements in water treated by sedimentation and filtration lead to the poisoning and

deterioration of plant cells and health with heavy metals and eventually reduce the average fresh and dry weight (Hermanto *et al.*, 2021).

The decrease in the wet and dry weights of the vegetative and root systems of the celery and radish is attributable to irrigation water treated by sedimentation and filtration compared with untreated water. The decline in the concentration of nitrates as a source of nitrogen in the irrigation water leads to a decrease in nitrogen and nutrients, playing an essential role in plant growth (Chaoua *et al.*, 2019; Hussain, 2024). The reduction in fresh and dry weights of the green and root

systems of plants irrigated with treated water was due to the lessened concentrations of other nutrients, such as phosphorus, zinc, magnesium, nitrogen, and calcium, in the water. Moreover, the decrease in nitrate concentrations reduces the negative effect of lead, cadmium, and zinc (Kausar *et al.*, 2017; Lewis *et al.*, 2024).

Leaf chlorophyll a, b, and a+b

The results of estimating the chlorophyll a, b, and a + b content in celery and radish plants under different types of water influences indicated effects on these concentrations. Findings came from comparing the quality of irrigation water with the differences in

concentration means (Tables 6, 7, and 8). The results showed the highest concentration of chlorophyll a, b, and a + b in *A. graveolens* plants (2.82, 1.40, and 1.30 mg/g wet weight, respectively) occurred by irrigating them with untreated water. However, these physiological parameters were decreased by irrigating those plants with water treated by sedimentation and filtration (1.740 and 2.139 mg/g wet weight in Ch. a, 1.145 and 1.072 mg/g wet weight in Ch. b, and 1.280 and 1.117 mg/g wet weight in Ch. a + b) compared with irrigating with untreated water. The concentrations of chlorophyll a, b, and a + b were the lowest (1.1150, 1.0610, and 1.0102 mg/g wet weight, respectively) by irrigating the plants with tap water.

Table 6. Effect of water sources and plant types on the leaf chlorophyll-a content in celery and radish plants.

Plant types	Water sources				Means
	a ₁	a ₂	a ₃	a ₄	
Celery	2.820a	1.74bc	2.1390b	1.1150c	1.9535a
Radish	1.540bc	1.50bc	1.440c	1.3220c	1.4505b
Means	2.180a	1.620bc	1.7895ab	1.2185c	

Values followed by the same letter for each factor and interactions between them are not significantly different from each other. a1 = untreated wastewater, a2= wastewater treated by sedimentation, a3 = wastewater treated by filtration, a4 = tap water.

Table 7. Effect of water sources and plant types on the leaf chlorophyll-b content in celery and radish plants.

Plant types	Water sources				Means
	a ₁	a ₂	a ₃	a ₄	
Celery	1.4007a	1.145ab	1.072b	1.061b	1.16967a
Radish	1.243ab	1.210ab	1.172ab	1.117ab	1.18550a
Means	1.32183a	1.17750ab	1.1220b	1.0890b	

Values followed by the same letter for each factor and interactions between them are not significantly different from each other. a1 = untreated wastewater, a2 = wastewater treated by sedimentation, a3 = wastewater treated by filtration, a4 = tap water.

Table 8. Effect of water sources and plant types on the leaf chlorophyll a+b content in celery and radish plants.

Plant types	Water sources				Means
	a ₁	a ₂	a ₃	a ₄	
Celery	1.300c	1.280c	1.1170d	1.1020d	1.19975b
Radish	1.6430a	1.601ab	1.559ab	1.509b	1.5780a
Means	1.47150a	1.44050a	1.3380b	1.3055b	

Values followed by the same letter for each factor and interactions between them are not significantly different from each other. a1 = untreated wastewater, a2 = wastewater treated by sedimentation, a3 = wastewater treated by filtration, a4 = tap water.

However, the radish plants showed differences in chlorophyll a, b, and a + b concentrations according to the four irrigation water treatments. By irrigating the radish plants with untreated water, the values for the said physiological traits were 1.540, 1.243, and 1.643 mg/g wet weight, respectively. By irrigating with water treated by sedimentation and filtration, the said values were 1.500 and 1.440 mg/g wet weight, 1.210 and 1.172 mg/g wet weight, and 1.600 and 1.559 mg/g wet weight, respectively, versus irrigating with untreated water. The concentrations of chlorophyll a, b, and a + b were the lowest by irrigating the radish plants with tap water (1.322, 1.117, and 1.509 mg/g wet weight, respectively). Irrigation with treated wastewater leads to physiological variations in plants, as the heavy metals inhibit the biosynthesis of chlorophyll and the enzymes involved in its construction, and eventually affecting negatively the leaf chlorophyll content (Younas et al., 2020; Sanwal, 2021).

Irrigation with water treated by filtration and sedimentation leads to a decrease in the concentration of nutrients, such as magnesium, which negatively affects the physiological activities of crop plants and reduces the leaf chlorophyll content. Heavy metals may replace magnesium in the chlorophyll molecule; thus, the chlorophyll molecule loses its important function in crop plants (Valivand and Amooaghaie, 2021). Past studies also authenticated the decrease in leaf chlorophyll b content in plants by irrigating them with wastewater treated by filtration (Paleologos et al., 2019).

CONCLUSIONS

Irrigation with sewage water treated by filtration and sedimentation loses some water properties, such as its nutrient content, especially the nitrates, which negatively affect the plant's biomass and leaf chlorophyll content. Heavy elements may replace the magnesium in the chlorophyll and adversely influence the photosynthetic enzymes. In such conditions, the plants will suffer from yellowing due to lack of food production instead of the

dwarfing of plants being exposed due to irrigation with treated water. Note that the sedimentation treatment method was more positive than other methods.

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