



Configuration of Spinach Heavy Metals Accumulation in Response to Treated Water, Irrigation Systems and Growing Seasons

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Abstract – The aim of the current study was to assess heavy metals accumulation in soil and in leaf, tissue of spinach (*Spinacia oleracea*) treated with wastewater under open-field conditions. Spinach plants were grown in two consecutive growing seasons (summer and fall) and irrigated with two different irrigation systems (sprinkler or drip irrigation). Concentrations of Zn, Cu, Pb, Mn, Ni, Hs, As, Cd and Cr were determined in the irrigation water, soil and spinach leaves in both summer and fall cropping seasons, the concentrations of Zn, Pb and Ni were exceeded the maximum admissible limit 5, 0.5 and 0.5 mg.l⁻¹ in water fresh and treated. The concentrations of heavy metals in the irrigation water sources (Hg (0.0013 mg.kg⁻¹- 0.003 mg.kg⁻¹), Cd (0.041 mg.kg⁻¹- 0.094 mg.kg⁻¹), Pb (0.02 mg.kg⁻¹ -0.065 mg.kg⁻¹)) were lower than the acceptable range recommended for irrigation waters, the contents of heavy metals in soil and leaves in two growing seasons and with drip and sprinkler irrigation methods were below the permissible limits recommended by European Union Standards (EU 2002) guidelines. Nonetheless, all these metals have toxic potential, but the detrimental impact becomes apparent only after decades of exposure. It is therefore suggested that regular monitoring of heavy metals in plant tissues is essential in order to prevent excessive build-up of these metals in the human food chain.

Keywords – Concentrations of Minerals, Drip Irrigation, Human Food Chain, *Spinacia Oleracea*.

I. INTRODUCTION

In many countries, sewage and wastewater from industry are used for irrigating farmland. This practice has two purposes: it disposes of wastewater and adds nutrients and organic matter to soils. However, there is concern about harmful pollutants in wastewater entering the food chain. If consumed in high concentrations, heavy metals can deplete important nutrients and lead to serious health problems [1].

Poor agriculture practices and manufacturing industries have resulted in increased release of a wide range of xenobiotic compounds to the environment. Improper treatment and disposal of industrial wastewaters and solid wastes are the major causes of soil contamination by heavy metals. Heavy metals are absorbed and accumulated by plants thus are absorbed directly or indirectly by human

through the food chain [2], and can be harmful even at quite low concentration [3].

Vegetables may become contaminated with heavy metals if they grow on soils contaminated by natural or anthropogenic sources such as industrial and agricultural activities. Therefore, intake of heavy metal contaminated vegetables may pose a direct threat to the human health and considered as one of the most important aspects of food quality assurance. International and national regulations on food quality have lowered the maximum permissible levels of toxic metals in food items due to an increased awareness of the risk these metals pose to food chain contamination [4].

Heavy Metals can accumulate and migrate in soil environments due to their cumulative effects and long-term interactions; accumulation of heavy metals in soil negatively affects regional eco safety and poses a threat to relevant animals and plants [5].

Vegetables are an important part of human's diet, and constitute important functional food components by contributing protein, vitamins, iron and calcium which have marked health effects. Vegetables, especially those of leafy vegetables grown in heavy metals contaminated soils because of the fact that they absorb these metals through their leaves investigated the concentrations of heavy metals [6], and Spinach was taken as a representative plant for leafy vegetables and has an imperative position in the order due to large and expanded leaves, fast growth and by being a common part of the human diet [7].

Spinach usually does not tolerate high heat temperature, spinach is usually planted in late summer and fall season and irrigated in two different methods of irrigation, drip and sprinkler, and usually in Lebanon, the farmers used sprinkler method to irrigate spinach crop.

Spinacia oleracea is a member of the Caryophyllales order, comprising broad, green and leafy vegetables possessing large surface areas, relatively high growth rates and rather elevated heavy metal absorption rates. Recently, due to these unique characteristics, *S. oleracea* and other members of the Caryophyllales order have been researched in a number of scientific studies to observe their growth and toxicity responses to heavy metal contaminations [7].

Based on the above considerations, two open field experiments were conducted to assess the heavy metals content of the fresh and treated water irrigated soil and spinach crop and to correlate the heavy metals concentration of fresh and treated water to that of the soil and spinach crop.

II. MATERIALS & METHODS

2.1 Experimental Site and Climate

The experiment was carried out during two consecutive growing seasons in 2016 at the experimental field of the Litani River Authority (LRA) agricultural extension center in Khirbet Kanafar village (Lebanon, 33.63°N lat, 35.77°E long, 859 m above sea level). Spinach (*Spinacia oleracea* L.) was cultivated in summer season from 20 August 2016 to 6 October 2016, and in autumn season from 14 October 2016 to 8 December 2016. The treated effluent of the Wastewater Treatment Plant (WWTP) that is serving the nearby village of Joubjanine and located very close to the LRA was used in the experiment.

The climate of the area is typically Mediterranean, characterized by a hot and dry season from April to October. In general, the average maximum air temperature was 30.4 °C during the first season and 20.7°C during the second season, whereas the average minimum air temperature was 14.3 °C during season 1 and 7.0°C during season 2. Total rain was 0.6 mm and 201 mm during the summer and autumn growing seasons, respectively.

2.2 Experimental Design, Treatments and Agronomic Management.

The experiment was laid out with eight treatments: T1: Faten variety sprinkler irrigated with fresh water (FW-S-V1); T2: Fayeze variety sprinkler irrigated with fresh water (FW-S-V2); T3: Faten variety drip irrigated with fresh water (FW-D-V1); T4: Fayeze variety drip irrigated with fresh water (FW-D-V2); T5: Faten variety sprinkler irrigated with treated water (TW-S-V1); T6: Fayeze variety sprinkler irrigated with treated water (TW-S-V2); T7: Faten variety drip irrigated with treated water (TW-D-V1); T8: Fayeze variety drip irrigated with treated water (TW-D-V2). Treatments were organized in a randomized complete block design with three replicates. Each experimental unit consisted of a 9 m² plot. The plants in both seasons were placed with 25 cm between the rows and 15 cm within the row living a plant density of 27 plants m². The study is a part of the experimental study based on the Reference of the Materials and Methods of the "Reuse of treated municipal waste water under different growing seasons for the spinach production"

2.3 Water and Soil Sampling and Analysis

Soil samples were taken at the beginning and at the end of each growing season in summer and fall at two different depths: 0-20 cm and 20-40 cm. The samples were analyzed for the presence of trace elements.

Concerning irrigation water, the main physico-chemical, microbial characteristics and trace metals content of both kinds of water (F and TW) were monitored.

The soil of the study area is sandy clay loam (USDA textural soil classification) with 53 % clay, 31% loam and 15% silt. Field slope was less than 0.1% and total available water holding capacity within the top 1 m of soil profile was 109 mm.

2.4 Statistical Analysis

Analysis of variance (ANOVA) of the experimental data was performed using SPSS 10 for Windows, 2001 (SPSS Inc., USA).

Combined analysis of variance was performed using season as a fixed variable [8]. Duncan's multiple range test was performed at P = 0.05 on each of the significant variables measured.

III. RESULTS AND DISCUSSION

3.1. Effect of Wastewater on Water Quality

Table I noted that the trace metal in both fresh and treated water content within the maximum admissible limit proposed by environmental limit values decision of MOE, 2001, while Zn, Pb and Ni concentrations were above the maximum admissible limit 5, 0.5 and 0.5 mg.l⁻¹. Cadmium was higher than the maximum admissible limit 0.2 mg.l⁻¹ in freshwater while it was within the admissible limit in treated water.

The concentrations of heavy metals in the irrigation water sources were lower than the acceptable range recommended for irrigation waters. The presence of low amounts of these metals in irrigation waters may decrease their levels in soils, which in turn may weaken metal uptake by plants ultimately leading to down concentrations in the vegetables.

The pH is 7.82 in both treated and fresh water and it is within the limit values for surface water based on MoE Decision 8/1 and specifications for Waste water reuse in irrigation based on proposed Lebanese guidelines FAO, 2011, the normal range for pH in surface water systems is 6.5 to 8.5, The pH is the controlled parameter because it has direct influence on wastewater treatability - regardless of whether treatment is physical/chemical or biological. Because it is such a critical measure of wastewater makeup, it is critically important to treatment.

Table I. Fresh water and treated effluent average quality and limit values for the reuse of TWW in Lebanon

	FW	TW	Environmental limit values for surface water based on MoE Decision 8/1 (MoE, 2001)	Effluent specifications for wastewater reuse in irrigation based on proposed Lebanese guidelines (FAO, 2011)		
				Water Category I	Water Category II	Water Category III
pH	7.85	7.82	6-9	6-9	6-9	6-9
Trace metals (mg.L ⁻¹)						
Zn	789.47	789.47	5			

Cu	<0.001	<0.001	0.5		
Pb	444.44	444.44	0.5		
Mn	0.23	0.52	1		
Ni	372.09	3651.16	0.5		
Hg	0.0100	0.0003	0.05		
As	<0.001	<0.001	0.1		
Cd	111.1100	<0.001	0.2		
Cr	<0.001	<0.001	2		

3.2 Effect of Soil Analysis on Soil and Spinach Product Quality

3.2.1. Chemical Soil Analysis

As showed in table II, the soil is sandy clay loam, the pH of soil has not been significant altered in the treated irrigation water, and a stable value of pH 7.6 as average was noted in soil in both fresh and treated water trials.

Higher electrical conductivity (EC) has been showed as average 0.81 (mS.cm⁻¹) at the beginning and 1 (mS.cm⁻¹) at the end of the summer season and 0.68 (mS.cm⁻¹) at the end of fall season within fresh and treated water trials.

Nitrogen and phosphorus were significantly decrease, the average level of nitrogen in Summer season in both Fresh and water treatment and in both varieties Fayez and Faten was 0.15% and 81.4 ppm for phosphorus, and 0.17 % nitrogen and 78.32 ppm phosphorus as average in both Fresh and Water treatment and in both varieties at the end of the Autumn season, and an application of NPK combined was arranged as 20/20/20 fertilizers in both season the summer and autumn, Reference [9] reported that spinach is greatly responsive to nitrogen and even if increased total amino acid concentration like other *Chenopodiaceous* it not only accumulates nitrates, but is also rich in oxalates, which are hazardous to human health, and had significant effect on the yield fresh and dry matter and chlorophyll content. A significant increase of potassium after planting both season, 454.13 ppm as average at the ending of Summer season and 600.7 ppm as average at the end of autumn season and had not any significant effect on yield, Potassium plays significant roles in enhancing crop quality, high levels of available K improve the physical quality, disease resistance, and shelf

life of fruits and vegetables used for human consumption [10].

Table II showed a slight increase of organic matter after planting of two seasons, the level as average is 1.92 % and 2 %, and the increasing of organic matter in soil is to improve soil properties by increasing the water and nutrient holding capacity, improving aeration and drainage, feeding microorganisms and providing some nutrients [11].

Ca concentrations were significantly increase before and after planting in both treatments fresh and treated water, the average level of CaO at the beginning of the season was 694.28 ppm and at the end of the summer season the level was 15557.46 ppm and 15183.59 ppm at the end of the fall season and also Magnesium accumulation was also observed in treated and fresh water irrigated soils, the level of MgO was at the beginning of the season was 430.39 ppm and after the end of the summer season the level was 962.10 ppm and 823.26 ppm at the end of the fall season, and that is indicating that the absorption of the elements on the exchangeable complex was higher than plant root uptake, and high MgO and CaO concentrations lead to poor plant growth and poor physical soil, and desirable high concentration conditions of nickel may also occur in these soil [12].

Because of the pH dependency of the cation exchange capacity of amorphous minerals, and their exchange capacity must be measures at a particular soil pH, amorphous minerals may have different effects on the distribution of nutrients ions between the soil solution and hence a different effect on nutrients availability [13].

Table II. Soil Analysis before& after planting.

Treatment	pH	EC (mS.cm ⁻¹)	N %	P2O5 ppm	K2O ppm	CaO ppm	MgO ppm	M.O. %	Clay %	Silt%	Sand%	Texture
T1	7.47	0.856	0.243	126.33	390.44	6258.48	430.09	1.45	31.32	15.45	53.23	Sandy Clay Loam
T2	7.58	0.698	0.233	67.44	283.79	6538.42	410.36	1.05	31.13	15.91	52.96	Sandy Clay Loam
T3	7.48	0.86	0.217	111.59	480.98	6685.48	445.38	1.63	30.65	15.13	54.22	Sandy Clay Loam
T4	7.51	0.89	0.205	112.85	465.14	6582.39	441.04	1.58	28.02	16.15	55.83	Sandy Clay Loam
T5	7.32	0.876	0.221	132.11	401.10	6245.18	421.34	1.34	28.51	14.55	56.94	Sandy Clay Loam
T6	7.51	0.765	0.245	145.67	314.90	6643.21	408.77	1.05	28.02	15.59	56.39	Sandy Clay Loam
T7	7.43	0.809	0.209	125.67	477.09	6456.54	448.54	1.36	30.06	14.83	55.11	Sandy Clay Loam
T8	7.49	0.789	0.244	170.43	462.12	6568.53	437.56	1.55	30.34	13.93	55.73	Sandy Clay Loam

Treatment	pH	EC (mcm)	N %	P2O5 ppm	K2O ppm	CaO ppm	MgO ppm	M.O. %
Summer season								
T1	7.66	0.864	0.112	37.69	520.95	15936.28	889.54	1.44
T2	7.63	0.76	0.128	58.00	422.90	15694.70	1003.90	2.94
T3	7.53	1.552	0.121	66.82	334.96	16809.68	1033.29	1.65
T4	7.72	0.844	0.19	53.43	469.70	15717.14	1137.86	1.86
T5	7.81	0.88	0.174	44.35	352.04	13380.09	791.33	1.96
T6	8.07	0.564	0.165	214.18	381.32	15326.90	802.27	2.01
T7	7.58	1.348	0.2	131.91	506.18	16437.02	958.39	1.57

Treatment	pH	EC (mcm)	N %	P2O5 ppm	K2O ppm	CaO ppm	MgO ppm	M.O. %
T8	7.46	1.228	0.168	45.41	644.99	15157.88	1080.22	1.97
Fall season								
T1	7.33	0.939	0.17	79.20	552.94	18044.96	863.77	1.82
T2	7.46	0.627	0.146	114.31	785.21	19329.06	951.20	2.36
T3	7.52	0.423	0.187	36.74	572.01	15703.09	860.35	1.87
T4	7.68	0.699	0.202	83.73	712.00	13861.75	799.99	1.58
T5	7.86	0.627	0.194	54.46	671.22	13984.64	742.64	2.11
T6	7.69	0.549	0.169	63.62	456.37	13375.64	691.35	1.83
T7	7.7	0.744	0.152	107.77	478.77	13924.64	866.56	2.33
T8	7.7	0.852	0.19	86.00	577.65	13244.99	810.24	2.17

3.2.2. Heavy Metals Soil Analysis

Table III reported the contents of heavy metals (Hg, Cd, Pb, As & Cu) studied in soil before planting and within the Summer and Fall season of Spinach crop, the Hg (0.0013 mg.kg⁻¹ - 0.003 mg.kg⁻¹), Cd (0.041 mg.kg⁻¹ - 0.094 mg.kg⁻¹), Pb (0.02 mg.kg⁻¹ - 0.065 mg.kg⁻¹) were below the permissible limits recommended by Indian Standard [14] and European Union Standards [15] guidelines for safe limits of heavy metals in soil, and (As & Cu) are not detected in soil samples in fresh and treated waste water and in both growing seasons.

The low quantity of heavy metals in soil may be due to leaching of metals to lower soil layers and to sandy clay loam texture soil [16], soil testing for metal contaminants is a continually evolving process aimed at improving the assessment of environmental and human health hazards associated with heavy metals in soils and plants. A number of challenges present themselves before accurate, reliable and precise contaminant hazard assessment criteria for soils and plants can be made, while a significant advance on the use of total metal concentrations, the concept raises

difficulties in providing an adequate assessment of potential risk, due to changes in environmental conditions which affect bioavailability, e.g. soil pH, soil organic matter content [17] and high soil alkalinity stimulated certain heavy metals as (Cd, Cr, Ni, Pb and Zn) uptake by plants, heavy metals uptake by plants is a complex soil plan process, influenced by many factors such as plant species, genotype, availability, and mobility of metals in soil, soil properties, and all the biogeochemical processes and the microbial activity and there is a common understanding reported in the literature that soil pH is the major factor influencing the mobility and bioavailability of heavy metals to plants, generally the mobility of metals decrease in alkaline soils [18].

Reference [19] noted that the lettuce, radish and

Spinach was found to be more responsible than other vegetables for the accumulation of heavy metals in humans through the edible portion.

The concentration of heavy metals in all the samples indicating that there was no source of contamination of heavy metals in soil.

Table III. Heavy metals analysis in soil.

	Hg (mg.kg ⁻¹)	Cd (mg.kg ⁻¹)	Pb (mg.kg ⁻¹)	As (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)
Before Planting					
M1	0.0012	0.071	0.036	not detected	not detected
M2	0.0012	0.055	0.039	not detected	not detected
M3	0.001	0.066	0.022	not detected	not detected
M4	0.001	0.062	0.02	not detected	not detected
M5	0.0013	0.061	0.031	not detected	not detected
M6	0.002	0.063	0.033	not detected	not detected
M7	0.002	0.058	0.065	not detected	not detected
M8	0.0013	0.041	0.033	not detected	not detected
Summer Sesaon					
M1	0.002	0.066	0.033	not detected	not detected
M2	0.002	0.063	0.031	not detected	not detected
M3	0.002	0.059	0.033	not detected	not detected
M4	0.003	0.055	0.031	not detected	not detected
M5	0.002	0.052	0.05	not detected	not detected
M6	0.003	0.057	0.032	not detected	not detected
M7	0.002	0.056	0.032	not detected	not detected
M8	0.002	0.053	0.036	not detected	not detected
Fall Season					
M1	0.002	0.055	0.021	not detected	not detected
M2	0.002	0.052	0.036	not detected	not detected
M3	0.002	0.052	0.033	not detected	not detected
M4	0.003	0.078	0.033	not detected	not detected
M5	0.002	0.053	0.031	not detected	not detected
M6	0.002	0.069	0.033	not detected	not detected
M7	0.002	0.094	0.031	not detected	not detected
M8	0.002	0.062	0.033	not detected	not detected

3.3. Effect of Trace Metals on Spinach Leaves

The metal uptake by spinach leaves of the two studied varieties grown under different water quality regimes, irrigation methods and growing seasons was provided in Table IV.

Mercury (Hg) was not detected in spinach leaves although the metal was present in both irrigation waters, but at levels that do not exceed the Environmental limit values for surface water based on MoE Decision 8/1 [20].

Marked differences, at $P \leq 0.001$, were observed for Pb uptake among seasons, with the highest mean concentration of Pb found in summer ($0.0634 \text{ mg.kg}^{-1}$) than in fall ($0.0312 \text{ mg.kg}^{-1}$). However, lead concentration did not exceed the safe limit as given by Reference [21] who reported that the normal range of Pb in edible vegetables range between 0.20 and 20 mg.kg^{-1} . It should be noted that lead is a highly toxic metal that can lead to number of disorders. The minute amount of Pb results in collapse of nervous system, anemia and heart problems

[22].

Arsenic (As) was not detected in spinach leaves for both growing seasons. Concentration of Cadmium (Cd) was below the standard limits for leafy vegetables proposed by Reference [23] of 0.2 mg.kg^{-1} . In addition, the concentrations were below the range given by Reference [24] who has reported that the Cd concentration in contaminated plants ranges between 5 and 30 mg.kg^{-1} . The metal was detected only during the fall season. It should be noted that physiological attributes of spinach plants could be severely affected by increased metal accumulation in soils [25].

Finally, it has been observed that concentration of copper in spinach leaves for both seasons was below the maximum safe limit of 0.20 mg.kg^{-1} given by Reference [26].

No significant difference in the treatments has been noted within the difference irrigation methods.

Table IV. Metal contamination of two spinach varieties grown under different water quality regimes, irrigation methods and growing seasons.

Source of variance	Hg	Pb	As	Cd	Cu
	($\text{mg kg}^{-1} \text{ d.wt.}$)	($\text{mg kg}^{-1} \text{ d.wt.}$)	($\text{mg kg}^{-1} \text{ d.wt.}$)	($\text{mg kg}^{-1} \text{ d.wt.}$)	($\text{mg kg}^{-1} \text{ d.wt.}$)
Treatment (T)	-	NS	-	NS	NS
Season (S)	-	***	-	***	***
T x S	-	NS	-	NS	NS
Treatment					
T1	nd	0.0062	nd	0.0020	0.0109
T2	nd	0.0558	nd	0.0023	0.0144
T3	nd	0.0515	nd	0.0027	0.0100
T4	nd	0.0570	nd	0.0018	0.0095
T5	nd	0.0657	nd	0.0025	0.0123
T6	nd	0.0440	nd	0.0030	0.0238
T7	nd	0.0477	nd	0.0024	0.0095
T8	nd	0.0505	nd	0.0040	0.0103
Season					
Autumn	nd	0.0312b	nd	0.0051a	0.0061b
Spring	nd	0.0634a	nd	0.0000b	0.0191a
T x S					
T1 Autumn	nd	0.0000	nd	0.0039	0.0053
T1 Spring	nd	0.0123	nd	0.0000	0.0164
T2 Autumn	nd	0.0387	nd	0.0046	0.0070
T2 Spring	nd	0.0730	nd	0.0000	0.0217
T3 Autumn	nd	0.0310	nd	0.0054	0.0071
T3 Spring	nd	0.0720	nd	0.0000	0.0128
T4 Autumn	nd	0.0327	nd	0.0035	0.0070
T4 Spring	nd	0.0813	nd	0.0000	0.0121
T5 Autumn	nd	0.0510	nd	0.0049	0.0038
T5 Spring	nd	0.0803	nd	0.0000	0.0208
T6 Autumn	nd	0.0253	nd	0.0059	0.0078
T6 Spring	nd	0.0627	nd	0.0000	0.0397
T7 Autumn	nd	0.0360	nd	0.0048	0.0039
T7 Spring	nd	0.0593	nd	0.0000	0.0152
T8 Autumn	nd	0.0350	nd	0.0079	0.0066
T8 Spring	nd	0.0660	nd	0.0000	0.0140

ns, *, **, *** Non-significant or significant at $P \leq 0.05$, 0.01 , and 0.001 , respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P = 0.05$). nd, not detected.

IV. CONCLUSION

S. oleracea exposure to heavy metals contamination resulted in severe toxicity even with low concentrations, and the challenge is to determine the minimum amount of each source of contamination that will result in the accumulation of heavy metals in soils beyond acceptable limits.

The long-term wastewater irrigation led to the accumulation of heavy metals in soil and consequently into the vegetables. Heavy metal concentration varied among the test vegetables, which reflect the differences in their uptake capabilities and their further translocation to edible portion of the plants.

We recommend that the government enforce strict rules and legislation on adequate treatment of wastewater and effluents before discharge to the environment. Proper washing and disinfection of vegetables before consumption is strongly advised.

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