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Physiological changes and phytoremediation ability of *Moringa oleifera* growing in polluted soil with Zn and Co heavy metals

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ABSTRACT

Extensive environment pollution by explosives and heavy metals caused bymanufacturing, disposal and testing of munitions becomes an issue of increasing concern. Accordingly, this study attempted to examine the effect of phytoremediation of *Moringa oleifera* on soil polluted with heavy metals. It was performedat College of Agriculture, Tikrit University, from Autumn 2018 to Summer 2019. One type of soil was used, taken from Al-Mahzam city in Salah Al-Din governorate. It concluded that *Moringa oleifera* showed a great tolerance to the heavy metals (Zn and Co) found in soil and had a good phytoremediation effect. The physiological parameters were represented by (N, P, K, C, protein) and Chlorophyll A+B to obtain better results.

Key words : *Moringa oleifera, Phytoremediation, Physiological changes, Zn and Co heavy metals.*

Introduction

Moringa oleifera (MO) is a tropical plant, belonging to the family Moringaceae, which is a single family of shrubs. It is a multipurpose tree, naturally growing in India, South Saharan Africa, South America and Malaysia's climate. It is often cultivated in home gardens and as living fences. MO contains an active bio-coagulating element. Additionally, almost all of its parts, namely, owers, leaves, seeds, bark and roots can be consumed as food or used for therapeutic purposes (Ramachandran *et al.,* 1980; Teixeira *et al*., 2012). The leaves paste is applied externally for the treatment of wounds. Furthermore, the leaves represent a source that is rich with essential amino acids like methionine, cystine, tryptophan and lysine with a high protein content (Makkar and Becker, 1997; Anwar *et al*., 2007). There are various applications for decoctions and extracts made from the plant leaves in medicine (Morton, 1991; Razis *et al*., 2014). Pal *et al*. (1995) reported that the methanol fraction of MO extract has antiulcer effecton induced gastric ulcers in rats.

Moreover, juice made from the fresh leaves hasa strong antibacterial effecton *Micro-coccus pyogenes* var. *aureus*, *Escherichia coli*, and *Bacillus subtilis*. The MO flowers also have a therapeutic value as a stimulant, aphrodisiac, diuretic and cholagogue. They contain flavonoid pigments like quercetin, kaempferol, rhamnetin, isoquercitrin and kaempferitrin (Nair and Subramanian, 1962; Mbikay, 2012). Ghasi *et al.* (2000) found that the treatment with the MO crude leaf extract and a high-fat diet has reduced the high-fat diet-induced

increases in the levels of serum (by 14.4%,), liver (by 6.4 %,) and kidney cholesterol (by 11.1%) in Wistar rats.

Currently, Estrella *et al*. (2000) stated that the production of breast milk can be increased by consuming the MO leaves from the third to the fifth postpartum day among mothers who delivered preterm infants. Consequently, the Philippine women consume the MO leaves mixed in chicken or shellfish soups to increasethe production of breast milk. In southern India, the fresh leaves are used by villagerswhen preparing cow and buffalo ghee from butterfat for their effect on increasing the shelf life of ghee as well as being a good source of natural antioxidants. The MO leaves contain antioxidant components like ascorbic acid, carotenoids and phenolic substances that work on enhancing the shelf life of ghee. Consequently, it is necessary to have alternative effective antioxidants made from natural sources for preventing foods deterioration. Hence, natural materials could be more effective than synthetic compounds due to being safer for humans. In addition, spices and herbs are the main sources of natural antioxidants,used throughout history not only as flavors but also as preservative materials. It is also noteworthy not to consume food phytochemicals as isolated or purified formula, but in combination with other phytochemicals and food components. Only then, the consumption of such nutraceuticals of plant origins could be effective as dietary disease-preventive food components (Dillard and German, 2000).

Materials and Methods

Soil Sample

The soil wastaken from Al-Mahzam city in Salah Al-Din governorate. Hence, 46 containers werefilled with this soil, and then MO was cultivated in it.

Samples Collection from Containers

The study lasted for four seasons. The first samples were collected in Autumn 2018 and the last ones were collected in Summer 2019. The leaves samples were collected from the lowest branches of the trees (the nearest ones to the soil surface of the containers). Leaves were sampled uniformly around the foliage of the trees. While the soil samples were taken from (5 cm) depth from (4) different directions in the container (+ shape) and then were mixedtogether as one soil sample.

Determination of Heavy Metals in the Soils

Soil samples were softened to pass through a sieve with a holes diameter of (6.0 mm). Then, (2 g) of soil samples were taken to be digested using Perchloric acid ($HClO_4$), Nitric acid (HNO) and Sulfuric acid (H_2SO_4) with percentage of 3: 1: 1, respectively. After that, the samples wereplaced in a sand bathat (200-225 °C). After filtration, the leachate was taken and Atomic Absorption Spectrophotometer (AAS) device was used to determine heavy metals (Jackson, 1958).

Determination of the Dry Weight of the Leaves

The dry weight (g) of the leaves was estimated by placing the samples inside paper envelopes to absorb moisture. Then, they were placed in the forced-air electric oven at 70 °C for 72 hours. After that, they were weighed by a sensitive balance and the measurements were recorded for each sample (Awal *et al*., 2004).

Determination of Heavy Metals in the Leaves

After drying, (0.1g) of the leaves samples was taken to be digested using Sulfuric acid (H_2SO_4), Nitric acid (HNO) and Perchloric acid ($HClO_4$) with percentage of 3: 1: 1, respectively. Then, the samples were placed in a sand bathat (200-225) °C. Atomic Absorption Spectrophotometer (AAS) device was used to determine heavy metals (Jackson, 1958).

Statistical Analysis, Results and Discussion

The results were analyzed by statistical program (Minitap) according to F-test analysis using completely randomized design in factorial treatment. The treatments means compared by Duncan's multiple range at the significant levels (0.05 % and 0.01%) (Al-Rawi, 2000).

* The same letters mean that there are no significant differences between them.

Table (1) showed the concentration of heavy metals in the soil in Autumn. The concentration of (Zn and Co) in the soil was (0.687 and 0.488) ppm, re-

Table 1. Concentration of heavy metals in the soil in Autumn

Soil type	Zn	Co	Unit
Control soil	0.687a	0.488b	ppm

Table 2. Concentration of heavy metals in the leaves of the plants in Autumn

Plant type	Zn	Co	Unit
Moringa oleifera	0.033a	0.021a	ppm

spectively. Autumn was the first season in this study.

* The same letters mean that there are no significant differences between them.

* This is columns comparison.

Table 2 showed the concentration of heavy metals in the leaves of the plant in Autumn. The concentration of (Zn and Co) in the soil was (0.033 and 0.021) ppm, respectively.

Table 3 showed that the highest value of Zn concentration in the MO soil in Summer was (0.244 ppm) in the pot3, replication3. While the lowest value was (0.011 ppm) in the pot1, replication2. There were no significant differences in Zn concentration in MO soil in the four pots. While the mean of replication had a significant effect on Zn concentration in MO soil. This result indicated that the MO soil in the pots of the replications 3 and 4 had more Zn concentration than the pots of replications 1 and 2.

Table 4 showed that the highest value of Zn concentration in the MO leaves in Winter was (0.089 ppm) in the pot 2, replication 2. While the lowest value was (0.015 ppm) in the pot 2, replication 3. There were no significant differences in Zn accumulation in MO leaves in the four pots. While the mean of replication had a significant effect on Zn accumulation in the MO leaves. This result revealed that MO in the pots of the replication 1 and 2 accumulated more Zn in its leaves than the pots of replication 3 and 4. Medicinal plants can accumulate heavy metals through the uptake from the roots (Dzomba *et al*., 2012; Olowoyo *et al*., 2011).

Table 5 revealed that the highest value of Zn concentration in the MO leaves in Spring was (0.098 ppm) in the pot2, replication2. While the lowest value was (0.016 ppm) in the pot1, replication2. There were no significant differences in Zn accumulation in the MO leaves in the four pots. While the mean of replication had a significant effect on Zn accumulation in MO leaves. This result revealed that MO in the pots of replication 1 accumulated more Zn in its leaves than the pots of replication 2, 3 and 4.

Table 6 showed that the highest value of Zn con-

Table 3. Concentration of Zn heavy metal (ppm) in the soil in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.183	0.015	0.084	0.101	0.096B
	Replication 2	0.011	0.083	0.159	0.107	0.090B
	Replication 3	0.122	0.106	0.244	0.131	0.151A
	Replication 4	0.187	0.169	0.108	0.118	0.146A
Means of Concentrations of *Moringa oleifera*		0.126a	0.093a	0.149a	0.114a	

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* The capital letters refer to columns comparison, while the small letters refer to rows comparison.

Table 4. Concentration of Zn heavy metal (ppm) in the leaves of the plants in Winter

Plant Type	Replication number	Pots number				Means of Replications
		Pot1	Pot2	Pot3	Pot4	
Moringa oleifera	Replication 1	0.075	0.055	0.050	0.082	0.066A
	Replication 2	0.016	0.089	0.042	0.068	0.054AB
	Replication 3	0.053	0.015	0.051	0.019	0.035C
	Replication 4	0.030	0.058	0.041	0.031	0.040BC
Means of Concentrations of *Moringa oleifera*		0.044a	0.054a	0.046a	0.050a	

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centration in the MO leaves in Summer was (0.112 ppm) in the pot2, replication3. While the lowest value was (0.025 ppm) in the pot2, replication3. There were no significant differences in Zn accumulation in the MO leaves in the four pots. While the mean of replication had a significant effect on Zn accumulation in MO leaves. This result revealed that MO in the pots of replication1 and 3 accumulated more Zn in its leaves than the pots of replication 2 and 4.

Table 7 showed that the highest value of Co concentration in the MO soil in Summer was (0.085 ppm) in the pot1, replication1. While the lowest value was (0.008 ppm) in the pot4, replication2. There were significant differences in Co accumulation in MO leaves. While the mean of replication had no significant effect on Co accumulation in MO leaves.

Table 8 showed that the highest value of Co concentration in the MO soil in Winter was (0.087 ppm) in the pot4, replication4. While the lowest value was (0.011 ppm) in the pot2, replication1. There were no significant differences in Co accumulation in MO leaves in the mean of pots number. While the mean of replication had a significant effect on Co accumulation in MO leaves. The highest value was in the

Table 5. Concentration of Zn heavy metal (ppm) in the leaves of the plants in Spring

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Morina oleifera	Replication 1	0.087	0.068	0.071	0.093	0.080A
	Replication 2	0.016	0.098	0.051	0.071	0.059B
	Replication 3	0.069	0.017	0.062	0.021	0.042B
	Replication 4	0.032	0.061	0.047	0.033	0.043B
Means of Concentrations of *Moringa oleifera*		0.051a	0.061a	0.058a	0.055a	

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* The capital letters refer to columns comparison, while the small letters refer to rows comparison.

Table 6. Concentration of Zn heavy metal (ppm) in the leaves of the plants in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Morina oleifera	Replication 1	0.088	0.069	0.082	0.089	0.082A
	Replication 2	0.082	0.025	0.036	0.035	0.045B
	Replication 3	0.039	0.112	0.031	0.105	0.072A
	Replication 4	0.028	0.041	0.059	0.051	0.045B
Means of Concentrations of *Moringa oleifera*		0.059a	0.062a	0.052a	0.070a	

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Table 7. Concentration of Co heavy metal (ppm) in the soils in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.085	0.015	0.039	0.021	0.040A
	Replication 2	0.032	0.031	0.046	0.008	0.030A
	Replication 3	0.049	0.018	0.038	0.058	0.041A
	Replication 4	0.056	0.043	0.027	0.040	0.042A
Means of Concentrations of *Moringa oleifera*		0.056a	0.027b	0.038ab	0.032b	

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replications 2, 3 and 4 while the lowest value was in replication1.

Table 9 showed that the highest value of Co concentration in the MO soil in Spring was (0.092 ppm) in the pot 2, replication 3. While the lowest value was (0.013 ppm) in the pot 2, replication1. There were no significant differences in Co accumulation in MO leaves in the mean of pots number. While the mean of replication had a significant effect on Co accumulation in MO leaves. This result revealed that MO in the replications 3 and 4 accumulated more Co in its leaves than in replication 1.

Table 10 revealed that the highest value of Co concentration in the MO leaves in Summer was (0.048 ppm) in the pot1, replication1. While the lowest value was (0 ppm) in almost all of the pots. There were significant differences in Co accumulation in MO leaves in the mean of pots number. The highest value was in pot1. While the mean of replication had no significant effect on Co accumulation in MO leaves.

Concerning the concentrations of nitrogen (N), phosphorus (P), potassium (K), C, protein and chlorophyll A+B, large amounts of these mineral elements are required for plants as they have an important role in assuring their growth (Epstein and Bloom, 2005; Marschner, 2012). It is reported that N has an important role in the life cycle of plant for being the main mineral nutrient required for the production of chlorophyll and other components of plant cell (proteins, nucleic acids and amino acids) (Sinfield, *et al*., 2010). The growth and survival of plant requires several nutrient elements with sufficient concentrations in tissues of plant (Mengel and Kirkby, 2001). However, it is generally recognized that C, N and P are the main components limiting the growth rate of all plants (Ägren 2008; Han *et al*., 2011). In some plants, RUBISCO even crystallizes inside the leaf because of its high concentration (Willison and Davey, 1976). Many chloroplast proteins, including RUBISCO, are highly conserved at the levels of gene and protein (Sane and Amla, 1991). Therefore, RUBISCO is fairly much the same protein in all green leafy plants, with only a few amino acids changes from one species to another. In photosynthesis, a blue/green substance (chlorophyll A) and a yellow/green substance (chlorophyll B) use light energy (normally sunlight but sometimes artificial) to convert carbon dioxide and water into sugars (carbohydrates) and oxygen in the green parts of the plant (Finch *et al.,* 2014).

Hence, Tables 11 and 12 showed the concentra-

Table 8. Concentration of Co heavy metal (ppm) in the leaves of the plants in Winter

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringaoleifera	Replication 1	0.046	0.011	0.051	0.042	0.038B
	Replication 2	0.027	0.061	0.079	0.037	**0.051A**
	Replication 3	0.067	0.083	0.064	0.044	0.065A
	Replication 4	0.047	0.078	0.028	0.087	0.060A
Means of Concentrations of *Moringa oleifera*		0.047a	0.058a	0.056a	0.053a	

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* The capital letters refer to columns comparison, while the small letters refer to rows comparison.

Table 9. Concentration of Co heavy metal (ppm) in the leaves of the plants in Spring

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.066	0.013	0.054	0.053	0.047C
	Replication 2	0.031	0.071	0.085	0.042	0.057BC
	Replication 3	0.082	0.092	0.078	0.054	0.077A
	Replication4	0.049	0.089	0.031	0.091	0.065AB
Means of Concentrations of *Moringa oleifera*		0.057a	0.066a	0.062a	0.060a	

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Table 10. Concentration of Co heavy metal (ppm) in the leaves of the plants in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot3	Pot 4	
Moringa oleifera	Replication 1	0.048	0	0	0	0.012A
	Replication 2	0.025	0	0	0	0.006A
	Replication 3	0	0	0	0	0.00A
	Replication 4	0	0	0	0	0.00A
Means of Concentrations of *Moringa oleifera*		0.018a	0.00b	0.00b	0.00b	

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Table 11. Concentration of N, P, K, C and protein in the leaves of the plant grows in control soil in autumn

Control soil	N%	P%	K%	C%	Protein%
Moringa oleifera	0.980a	0.035d	0.550b	0.016d	0.068c

* The same letters mean that there are no significant differences between them.

tion of N, P, K, C, protein and chlorophyll A+B (mg/g) in the MO leaves in Autumn represented by (0.980%, 0.035%, %0.550, 0.016%, %0.068 and 0.346 mg/g), respectively.

Table 13 showed that the highest value of N concentration in the MO leaves in Summer was (2.352 %) in the pot1, replication3. While the lowest value was (0.392 %) in the pot2, concentration2. In the mean of pots, there were significant differences in N concentration in MO leaves. The highest value was in the pots 1, 3 and 4, while the lowest value was in the pot 2. The mean of replication had a significant effect on N concentration in MO leaves. The study showed that MO in the replications 1,3 and 4 accumulated more Co in its leaves than in replication 2.

Table 12. Concentration of chlorophyll A+B (mg/g) in the leaves of the plant grows in control soil in autumn

Control soil	Estimation
Moringa oleifera	0.346a
Nerium oleander	0.362a
Myrtus communis	0.311a

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Table 14 showed that the highest value of P concentration in the MO leaves in Summer was (0.141 %) in the pot3, replication2. While the lowest value was (0.010 %) in the pot3, replication3. In the mean of pots, there were no significant differences in P concentration in MO leaves. The study showed that MO in the replication 2 had more P in its leaves than the replications 1, 3 and 4. While comparing these results with the concentration of P in the leaves of the plants in Autumn, it was observed that the concentrations of P were very good. Perhaps this may be because MO had a great adaptation to the concentration of heavy metals found in soil.

Table 15 showed that the highest value of K con-

Table 13. Concentration of N (%) in the leaves of the plant in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.833	0.490	1.078	1.372	0.943AB
	Replication 2	0.637	0.392	0.833	1.225	0.772B
	Replication 3	2.352	0.637	0.539	0.735	1.066A
	Replication 4	0.833	0.637	1.960	0.882	1.078A
Means of Concentrations of *Moringa oleifera*		1.164a	0.539b	1.103a	1.054a	

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centration in the MO leaves in Summer was (1.400 %) in the pot 2, replication 2. While the lowest value was (0.575 %) in the pot 1, replication 1. In the mean of pots, there were significant differences in K concentration. The highest value were in the pots 2, 3 and 4, while the lowest value was in the pot 1. MO in the replications 2 and 3 had more K in its leaves than replication 1 and 4. MO had a great adaptation to the concentration of heavy metals found in soil.

Table 16 showed that the highest value of C concentration in the MO leaves in Summer was (0.104 %) in the pot 3, replication 4. While the lowest value was (0.005 %) in the pot 2, replication 2. In the mean of pots, there were significant differences in C concentration. The highest value was in the pots 1, 3 and 4, while the lowest value was in the pot 2. MO in the replications 4 had more C in its leaves than replications 1, 2 and 3.

Table 17 showed that the highest value of protein concentration in the MO leaves in Summer was (0.546 %) in the pot 3, replication 4. While the lowest value was (0.026 %) in the pot 2, replication 2. In the mean of pots, there were no significant differences in protein concentration. MO in the replication 4 had more protein in its leaves than replications 1, 2 and 3.

Table 14. Concentration of P (%) in the leaves of the plant in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.042	0.015	0.059	0.067	0.0458BC
	Replication 2	0.054	0.051	0.141	0.035	0.0702A
	Replication 3	0.067	0.026	0.010	0.026	0.0323C
	Replication 4	0.088	0.061	0.029	0.027	0.0512B
Means of Concentrations of *Moringa oleifera*		0.0628a	0.0382a	0.0597a	0.0388a	

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Table 15. Concentration of K (%) in the leaves of the plant in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.575	0.650	0.800	1.025	0.7625B
	Replication 2	1.075	1.400	1.125	0.575	1.0440A
	Replication 3	0.675	1.000	1.050	1.000	0.9312A
	Replication 4	0.727	0.850	1.075	0.650	0.8255AB
Means of Concentrations of *Moringa oleifera*		0.763b	0.975a	1.013a	0.812ab	

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Table 16. Concentration of C (%) in the leaves of the plant in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.021	0.037	0.064	0.012	0.0335B
	Replication 2	0.053	0.005	0.010	0.016	0.0210BC
	Replication 3	0.032	0.009	0.013	0.011	0.0163C
	Replication 4	0.047	0.020	0.104	0.090	0.0653A
Means of Concentrations of *Moringa oleifera*			0.0383a	0.0178b	0.0478a	0.0323a

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Table 17. Concentration of protein (%) in the leaves of the plant in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.110	0.194	0.336	0.063	0.1757B
	Replication 2	0.278	0.026	0.052	0.084	0.1100B
	Replication 3	0.168	0.047	0.068	0.115	0.0995B
	Replication 4	0.246	0.105	0.546	0.472	0.3420A
Means of Concentrations of *Moringa oleifera*		0.2005a	0.0930a	0.2510a	0.1835a	

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* The capital letters refer to columns comparison, while the small letters refer to rows comparison.

Table 18. Concentration of chlorophyll A+B (mg/g) in the leaves of the plant in Summer

Plant Type	Replication number	Pots number				Means of Replications
		Pot 1	Pot 2	Pot 3	Pot 4	
Moringa oleifera	Replication 1	0.493	0.110	0.294	0.133	0.2575B
	Replication 2	0.311	0.352	0.165	0.180	0.2520B
	Replication 3	0.096	0.259	0.455	0.372	0.2955B
	Replication 4	0.206	0.809	0.353	0.500	0.4670A
Means of Concentrations of *Moringa oleifera*		0.2765a	0.3830a	0.3167a	0.2963a	

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* The capital letters refer to columns comparison, while the small letters refer to rows comparison.

Table 18 showed that the highest value of chlorophyll A+B concentration in the MOleaves in Summer was (0.809 mg/g) in the pot2, replication4. While the lowest value was (0.096 mg/g) in the pot1, replication3. In the mean of pots, there were no significant differences in protein concentration. MO in the replication 4 had more protein in its leaves than replications 1,2 and 3.

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