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THE EFFECT OF SOIL TREATMENT WITH HEAVY METALS ON THE CONCENTRATION OF CALCIUM, MAGNESIUM, CADMIUM, AND COPPER IN THE FENUGREEK AND SPINACH PLANTS

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ABSTRACT

This research was conducted with the aim of studying the effect of treating soil contaminated with cadmium metals with concentrations (3, 6, 9) and copper metals with concentrations (80, 150 and 180) mg/kg soil on Fenugreek (*Trigonella foenum-graecum*) and Spinach (*Spinacia oleracea* L.) plants. The results showed that treating the soil with Cd and Cu led to a significant decrease in the concentration of calcium and magnesium in the Spinach shoot , as it was found that treating the soil with cadmium at a concentration of 9 mg / kg soil led to a decrease in the concentration of calcium which reached (2,400) mg / g and that the soil treatment with copper at the concentration of (180) mg / g, the concentration of magnesium decreased to (1.440) mg / g. While there was a significant increase in the concentration of copper in the shoot and root of the Fenugreek plant when treating the soil with copper at the concentration (180) mg/kg soil, it reached (23,000) and (31,000) mg/kg. respectively, and there was an increase in the concentration of cadmium in the shoot of Spinach plants and Fenugreek when treating the soil with cadmium at the concentration (9) mg/kg soil, it reached (0,532) mg /kg in spinach and (0.662) mg /kg in the fenugreek plant compared to the comparison treatment.

KEY WORD : Spinach, Fenugreek, Cd, Cu, Ca, Mg.

INTRODUCTION

There are different types of heavy elements, but we can make them in three forms, which are harmful elements such as cadmium, nickel, chromium, cobalt, zinc, copper, mercury and others, and the elements with a radioactive effect such as uranium, radium, americium, thorium, etc., and expensive or precious metals such as platinum, gold, silver, etc. There are some elements that take first place in toxicity and are always a vital subject, and studies are conducted on them constantly due to the environmental reasons (Sandeep *et al.,* 2019). Soil contamination with heavy elements through both natural and human sources has caused great damage to society in recent decades as heavy elements are easily absorbed by the surfaces of cellular membranes and cause many diseases in plants (Demey *et al.,* 2018). The toxicity of heavy elements depends on a set of factors, including the dose to which the organism is exposed, the diseases it suffers from and the period of exposure. The heavy component can enter the body of the organism in different ways. Once inside the body, the elements quickly bind to the cell and work to disrupt important biological functions, as they may bind to enzymes, which have a large role in many interactions, especially metabolic reactions, by forming complexes of enzymes with the heavy element, which is called the mineral enzyme complex (Alessio and Luigi, 2020). Contamination of agricultural lands with heavy metals has become a matter of great environmental importance due to the expansion of their spread in large areas as well as because of their large, long-term and severe damage to plants that grow in soils that suffer from pollution with heavy elements.These heavy metals not only accumulate in the plant but also enter into the food chain, thus making heavy metal pollution quite significant both from nutritional as well as environmental point of view. Plants respond to various types of stresses depending upon their genetic makeup. Heavy metals show unconstructive effects on the physiological and metabolic processes of plants like photosynthesis, water relations, protein synthesis, nutrient absorption, etc. (Wani *et al.,* 2012; Upadhyay and Panda, 2009). In addition to the aquatic ecosystem and the environmental system ground when contaminated with high proportions of heavy metals, these metals are working to cause disturbances in these systems (Salt *et al.*, 1995). And since we took the effect of cadmium and copper on plants in this research, it is important to talk in a simple way about these two elements, as cadmium is one of the dangerous elements and has a toxic effect for the living organisms present on the surface of the earth, including humans, animals, plants or other living organisms, and it is also a widespread environmental pollutant and there are many sources. It works to increase the concentration of this element in various environments, including mining, smelting, plastic industries, battery manufacturing and other sources. Cadmium can easily enter the plant through watering, as cadmium is one of the elements that dissolve quickly in water and can be transmitted to humans and animals through the feeding process through the food chain (McLaughlin *et al.,* 1999). As for the copper element, it is a highly toxic element, especially when it is in high proportions, as it causes many unwanted symptoms for the plant, such as shortening the plant, fading the colors of the plant's leaves, and preventing the increase in the length and weight of the roots. When it is in low concentrations, it improves the growth and development of plants through its role in the exchange of oxygen and carbon dioxide in the processes of photosynthesis and respiration, it plays a clear role in the formation of one of the components of the plant cell wall, which is lignin (Borghi *et al.,* 2008; Cuypers *et al*., 2000). In the presence of copper excess, plants undergo oxidative stress due to the overproduction of reactive oxygen species (Miotto *et al.,* 2014; LaTorre *et al*., 2018). Which thus leads to a negative impact on many important processes that occur inside the plant, especially related to the process of making carbohydrates and energy stores in the plant (Ambrosini *et al.,* 2018; Pietrini *et al.,* 2017) .

MATERIALS AND METHODS

The soil of the al-shalalate area in the Mosul city was selected for research experiments in which the topsoil was taken from a depth of (0-30) cm and the air was dried, then smoothed and passed through the opening diameters of the sieve (2) mm. The experiments were then carried out with the intention of testing the effect of pollutants with heavy metals on growth and some physiological characteristics. The treatments used in this study included fenugreek (*Trigonella foenum-graecum*) and spinach (*Spinacia oleracea* L.) plants, two heavy metals (Cu, Cd) and three different concentration for each metal, as well as three replicates for each treatment. The soil was fertilized with urea fertilizer at a concentration of (40) ppm/kg soil and superphosphate fertilizer at a concentration of (40) ppm/kg soil for each pot, the metals (each component separately according to treatment) and the fertilizers were applied to the soil together as they were mixed outside the pot and then put in the pot to ensure that they were well mixed and used Plastic pots with a diameter of 23 cm and a height of 20 cm of each pot 5 kg of soil as heavy metals were added to the soil as follows , adding cadmium to the potting soil by concentration (3,6,9) mg / kg soil and adding copper to the potted soil by concentration (80,150, 180) mg/kg soil. Spinach and Fenugreek seeds were obtained from local markets in Mosul. The seeds were planted on 1/10/2019. After (60) days from the date of agriculture, the plants are harvested by 3 replicates per procedure. The pot was washed with water to remove the root aggregates from the soil using a special water mist, taking into account the use of the sieve to prevent the loss of any part of the roots, and the roots were well cleaned. The concentration of calcium, magnesium in the shoot and Cd and Cu in the shoot and root of the two plants was measured by drying and grinding the plant sample and then followed (Jackson, 1985) by taking 0.5 g. of dried plant samples were taken in digestion tubes. Nitric acid and perchloric acid in the ratio of 2:1 were taken for digestion. Digested samples were followed by filtration and diluted up to the volume of 100 ml. Standard solutions of CdCl2 and $CuSO_4$ were prepared before the estimation of the copper and cadmium by Atomic Absorption Spectrophotometer (Allen *et al.,* 1976). As for calcium and magnesium, they were measured according to the method of Cheng and Bray (1951) by correction with Na-EDTA.

RESULTS AND DISCUSSION

Concentration of calcium in the shoot

Table 1 shows the treatment of soil with cadmium and copper at concentrations (3, 6, 9) and (80,150,180) mg/kg soil, respectively, led to a decrease in the concentration of calcium in the shoot of fenugreek and spinach plants, although it did not reach the moral limits, and the highest effect was when treating the soil with copper at a concentration of (180) mg/kg in Spinach plant reached (2,800) mg/g compared to the comparison treatment.

This may be due to excess heavy metals cause impairing the uptake of essential elements such as calcium (Piotrowska *et al.,* 2009) and the accumulation of heavy component in the soil prevents seed germination and retards the growth of seedlings, reduces germination percentage, germination index, root length/buds, index of tolerance and dry mass of roots and buds (Farouk and Muhammad, 2018). These results are consistent with Sani *et al.,* (2017) findings regarding a decrease of all macro essential elements in the Lettuce plant compared with the control. It was noted that the spinach plant was superior to the concentration of magnesium in the shoot on the spinach plant, as it reached (3.943) mg/g as the effect of the plant species. No significant differences were observed with the effect of cadmium and copper on the concentration of magnesium in the shoot of fenugreek and spinach plants compared to the comparison treatment as the effect of the heavy metals type.

Concentration of magnesium in the shoot

Table 2 shows the treatment of soil with cadmium and copper at concentrations (3,6,9) and (80, 150 180) mg/kg soil, respectively, resulted in a decrease in the concentration of magnesium in the shoot of fenugreek and spinach plants, although it did not reach the moral limits, and the highest effect was when treating the soil with copper at a concentration of (180) mg/kg in Spinach plant

Table 1. Effect of soil treatment with heavy metals on calcium concentration (mg/g) in shoot fenugreek and spinach

Transactions		Plant type		Effect of element Type
		Fenugreek	Spinach	
Control		4.000 a	6.000 a	5.000a
Cd Mg/kg	3	3.800 a	3.600 a	3.167b
	6	3.400 a	2.600 a	4.100ab
	9	3.200 a	2.400 a	
Cu Mg/kg	80	4.600 a	5.800 a	
	150	4.000 a	4.400 a	
	180	3.000 a	2.800 a	
Effect of plant type		3.714 a	3.943 a	

Equations with similar characters do not differ significantly at the probability level (5%) according to the Duncan test

Table 2. Effect of soil treatment with heavy metals on the Magnesium concentration in shoot (mg/kg) of fenugreek and spinach plants

Transactions		Plant type		Effect of element Type
		Fenugreek	Spinach	
Control		6.000 a	3.000abcd	4.500a
Cd Mg/Kg	3	5.780 ab	2.520 bcd	3.137a
	6	3.490abcd	1.960 d	3.167a
	9	3.390abcd	1.680 d	
Cu Mg/Kg	80	5.530 abc	2.160 cd	
	150	4.580abcd	1.560 d	
	180	3.730abcd	1.440 d	
Effect of plant type		4.643 a	2.046 b	

Equations with similar characters do not differ significantly at the probability level (5%) according to the Duncan test.

reached (1,440) mg/g compared to the comparison treatment. This is similar to what was found by (Naz *et al.*, 2015), where he observed that treating *S. oleracea* plant with high levels of cadmium led to a deterioration in the concentration of the magnesium component, which reached 24% compared to the control treatment, and it was significant at ($p < 0.05$). was noted that the fenugreek plant significantly outperformed the concentration of magnesium in the shoot over spinach, as it reached (4.643) mg/g as the effect of the plant type. When treating the soil with cadmium, it reached (3.137) mg/g compared to the comparison treatment.

Concentration of copper in the shoot

Table 3 shows that the treatment of soil with heavy metals Cd and Cu at concentrations (3, 6, 9) and (80, 150, 200) mg/kg of soil led to a significant excessed in the copper concentration in the shoot of fenugreek when treating the soil In copper and the three concentrations, the highest effect was at the third concentration, as the copper concentration in the shoot up of fenugreek plant reached (23,000) mg/kg compared to the comparison treatment.

This is in agreement with (Wassim *et al.*, 2017) that the treatment of soil with cadmium and copper with high concentrations led to an increase in the concentration of the two elements in the parts of the Two Legumes (Bean and Faba Bean) compared to the comparison treatment. As for the effect of the plant type, it was observed that the fenugreek plant significantly out performed the spinach plant by concentrating copper in the shoot as it reached (14.486). Mg/kg. With regard to the effect of the heavy metals type, a significant accretion in the concentration of copper was observed in the shoot of fenugreek and spinach plants when treating the soil with copper, as it reached (12,917) mg/kg compared to the comparison treatment.

Concentration of copper in the root

Table 4 shows an increase in copper concentration in the root of fenugreek and spinach plants, despite the absence of significant differences in copper concentration in the root of spinach, either in the fenugreek plant. It was observed that a significant increase in copper concentration in the root was observed and the highest effect was when treating

Table 3. Effect of soil treatment with heavy metals on the Copper concentration in shoot (mg/Kg) of fenugreek and spinach plants

Transactions	Plant type	Effect of element Type		
		Fenugreek	Spinach	
Control	8.000cdef	4.000g	6.000c	
Cd Mg/Kg	3	10.000cd	4.900fg	8.717b
	6	10.500cd	7.000defg	12.917a
	9	10.900c	9.000cde	
Cu Mg/Kg	80	17.000b	4.500fg	
	150	22.000a	5.000fg	
	180	23.000a	6.000efg	
Effect of plant type		14.486 a	5.771 b	

Equations with similar characters do not differ significantly at the probability level (5%) according to the Duncan test.

Table 4. Effect of soil treatment with heavy metals on the Copper concentration in Root (mg/Kg) of fenugreek and spinach plants

Transactions		Plant type		Effect of element Type
		Fenugreek	Spinach	
Control	10.000ef	5.000g	7.500c	
Cd Mg/Kg	3	12.000d	5.400g	10.667b
	6	15.000d	5.700g	15.917a
	9	20.000c	5.900g	
Cu Mg/Kg	80	19.000c	5.500g	
	150	26.000b	6.000g	
	180	31.000a	8.000fg	
Effect of plant type		19.00 a	5.929 b	

Equations with similar characters do not differ significantly at the probability level (5%) according to the Duncan test.

the soil with copper at The concentration (180) mg/kg of soil reached (31,000) mg/kg compared to the comparison treatment. The increased concentration of heavy elements in plant parts may be due to the high concentrations of these elements in the soil (Esawy and Adel, 2016) This is consistent with (Marisa and John, 2005). that soils contaminated with heavy elements such as cadmium and copper have led to an increase in focus of these elements in parts of lettuce, spinach, carrots, and radish plants. As for the effect of the plant type, it was observed that the fenugreek plant significantly outperformed the spinach plant by accumulating copper in its root total as it reached (19.00) mg/kg. For the effect of the heavy metals type, the soil is treated with cadmium and copper led to a significant increase in the concentration of copper in the root system of fenugreek and spinach plants, and the highest effect was when treating the soil with copper, as it reached (15,917) mg/kg compared to the comparison treatment.

Concentration of cadmium in the shoot

We note from Table (5) that the treatment of soil with heavy metals Cd and Cu at concentrations (3, 6, 9) and (80, 150, 200) mg/kg, respectively, resulted in a significant increase in the concentration of cadmium in the shoot of fenugreek and spinach plants. The highest effect when treating the soil with cadmium was at a concentration (9) mg/kg soil, as it reached (0.532) mg/kg in spinach compared to the comparison treatment.

These results are consistent with (Nafees *et al.*, 2015) findings regarding an increase in the cadmium Concentration in the sorghum plant with the exposure to increased concentration of cadmium compared with the control. That may be easily taken by the plant and grouped into its various parts (Farouk and Muhammad, 2018). It was noted that the spinach plant was significantly superior to the concentration of cadmium in the shoot on the fenugreek plant as it reached (0,959) mg/kg as the effect of the plant species. There was also a significant difference in the concentration of cadmium in the shoot of fenugreek and spinach plants when treating with cadmium as the effect of the heavy metals type compared to the comparison treatment.

Table 5. Effect of soil treatment with heavy metals on the Cadmium concentration in shoot (mg/Kg) of fenugreek and spinach plants

Transactions		Plant type		Effect of element Type
		Fenugreek	Spinach	
Control		0.041 d	0.051 d	0.046 c
Cd Mg/Kg	3	0.221 bc	0.305 bc	0.323 a
	6	0.231 bc	0.379 ab	0.169 b
	9	0.270 bc	0.532 a	
Cu Mg/Kg	80	0.122 bc	0.167 bc	
	150	0.162 bc	0.171 bc	
	180	0.187 bc	0.206 bc	
Effect of plant type		0.176 b	0.259 a	

Equations with similar characters do not differ significantly at the probability level (5%) according to the Duncan test

Table 6. Effect of soil treatment with heavy metals on the Cadmium concentration in Root (mg/Kg) of fenugreek and spinach plants

Transactions		Plant type		Effect of element Type
		Fenugreek	Spinach	
Control		0.042 n	0.057 m	0.050 c
Cd Mg/Kg	3	0.196 j	0.186 k	0.333 a
	6	0.447 b	0.250 d	0.207 b
	9	0.662 a	0.255 c	
Cu Mg/Kg	80	0.211 h	0.162 l	
	150	0.221 f	0.206 i	
	180	0.226 e	0.216 g	
Effect of plant type		0.286 a	0.190 b	

Equations with similar characters do not differ significantly at the probability level (5%) according to the Duncan test.

Concentration of cadmium in the root

Table 6 shows a significant increase in the concentration of cadmium in the root of fenugreek and spinach plants, and when treating the soil with the two heavy metals and the three concentrations for each element. The highest effect when treating soil with cadmium was at concentration (9) mg/kg soil, as it reached (0.666) mg/kg in fenugreek plant compared to the comparison treatment. These results are consistent with Haipeng *et al.,* (2016), it was found cadmium Concentration in the root of the plant increased with the exposure to increased concentration of cadmium and increasing the metal accumulation in plants species grown on the contaminated soil (Gupta *et al.,* 2008). Calcium, zinc and iron, for example, are basic cations and have special channels and vectors for entering plant cells, so they are used by cadmium to enter the plant cell (Clemens, 2006) and At the whole plant level, symptoms of heavy element toxicity include delayed growth, leaf dysfunction, photosynthesis and respiration inhibition (Navarro *et al.,* 2019). It was noted that the fenugreek plant was significantly superior to the concentration of cadmium in the root of spinach, as it reached (0.286) mg/kg as the effect of the plant type. It was noted that the treatment of soil with cadmium and copper led to a significant increase in the concentration of cadmium in the root system and the effect of cadmium was higher than copper, as it reached (0.333) mg/kg compared to the comparison treatment as the effect of the type of heavy metals.

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