Research Article

Effect of adding Silybum and Licorice Powder on Carbohydrate and Protein Concentration in Beans Grown in Soil Treated with Cobalt and Copper

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Abstract | Heavy metal pollution is one of the major environmental problems affecting all living organisms, both animals and plants. This study aimed to determine the impact of adding powder of silybum and licorice plants at concentrations 4 and 8 g/kg soil each on the concentration of protein and carbohydrates in bean plant seeds grown in soils contaminated with cobalt at concentrations 25 and 50 and copper at concentrations 75 and 90 mg/kg soil. The soil was treated with the two plants powder to reduce the negative influences of heavy elements by increasing the plant resistance. The study showed that treating the soil with silybum powder at a concentration of 4 g/kg soil gave the best result in the concentration of protein and carbohydrates, as it reached 4.88 mg/g for protein and 8.75 mg/g for carbohydrates. While the cobalt treatment at a concentration of 50 mg/kg soil gave the lowest concentration of protein, which amounted to 0.46 mg/g, and for carbohydrates, which amounted to 1.87 mg/g. The addition of silybum Powder at a concentration of 8 g/kg soil to plants grown in soils contaminated with cobalt at a concentration of 25 mg/kg soil gave the best result with a concentration of protein and it amounted to 1.53 mg/g. The addition of silybum powder at the same concentration for plants grown in treatment soils with copper at a concentration of 75 mg/kg, the soil gave the best concentration for carbohydrates, and it reached 3.85 mg/g compared to the control treatment.

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Introduction

Rapid development in industry and manufacturing has caused environmental challenges worldwide, including heavy metals. which is considered a major threat to the environment and human health. The metals include arsenic, chromium, lead, nickel, cadmium, zinc and copper (Jaishankar *et al*., 2014; Kakkalameli *et al*., 2021). And The increase in heavy metal concentrations in the environment can be attributed to soil characteristics or different agricultural practices such as the use of fertilizers and pesticides and other agricultural operations (Foy *et al*., 2005). In addition to mining operations and the significant increase in the population (Emamverdian *et al*., 2015). Many reports show the many damages of heavy metals, which plants are usually the first target for (Arshad *et al*., 2008). Heavy metals are distinguished from other organic pollutants in that they are not degradable or transformed into harmless compounds by biological means, and they persist for a long time in the environment and enter the food chain (Chiban *et al*., 2011). As for copper, it is one of the important minerals in the oxidation and reduction processes in

living systems. Small amounts of it are important in physiological processes, but increasing it has negative effects on different environmental systems. There are many studies concerning the role of copper in living organisms (Syed and Meshari, 2018). Cobalt is a heavy metal that is not necessary for plants that have seven possible oxidation states, and it can be a catalyst for reactions whose importance is clear in that it is involved in the activation of some enzymes, especially those involved in nitrogen fixation. It also has a role in delaying leaf ageing by inhibiting ethylene biosynthesis. and increased drought resistance in seeds (Pilon-Smits *et al*., 2009). As for its toxic effects, it is related to oxidative stress, inhibition of photosynthesis and iron deficiency (Morrissey *et al*., 2009). As for the plant *Silybum marianum*, it is one of the plants that have been used for long periods for medicinal purposes. It is a member of the Asteraceae family. It is a herb that is characterized by its length, with green prickly leaves, and its flowers are purple, tend to be red and end with sharp spines. It is native to the Mediterranean region and has spread to Asia, Europe, Australia, and America. It is grown as a commercial crop in many countries, including Iran and Pakistan, and is a plant that converts to many chemical compounds that are used in the pharmaceutical industries (Porwal *et al*., 2019). The licorice plant is an excellent source of many medicinal compounds. The licorice plant of the Leguminosae family is a herbal plant of medicinal value. It contains many compounds such as isoliquiritin, glycyrrhizin, and glycyrrhizinic acid, which can provide us with medicinal benefits (Hasan *et al*., 2021). The study aimed to find out the effect of the element's cobalt and copper and the effect of adding silybum and licorice plant powder on the concentration of proteins and carbohydrates in the bean's seeds. Also, reduce the negative effect of heavy elements by adding powder from the two plants.

Materials and Methods

Agriculture: The seeds of the bean plant (*Phaseolus vulgaris*) were obtained from the offices of seed and planting materials in Nineveh Governorate/Iraq. The seeds were sown on 25/8/2019 by 4 seeds/pot, considering that the distances between the seeds are equal, and the pots were randomly placed under greenhouse conditions. After (65) days from the date of planting, the bean plants were harvested by (3) replicates per treatment.

Treatments: The treatments used in this study included two types of heavy metals: cobalt, with concentrations (25 and 50) and copper at concentrations (75 and 90) mg/kg soil. The soil was treated with two types of wild plant powders: *Silybum marianum* and *Glycyrrhiza glabra*, with concentrations (4 and 8) g/kg of soil for each.

Measuring protein and carbohydrate concentration

The protein was estimated based on (Lowry *et al*., 1951) method, by taking 0.5 g of the plant sample, using TCA, copper reagent, diluted Lowry's reagent. And reading the absorbance at the wavelength of 660 nm. Bovine albumin was used for the standard curve. Estimate carbohydrates in 1 g of dried and crushed plant samples using a spectrophotometer and at a wavelength of 620 nm and the standard curve was used using glucose (Pearson *et al*., 1976).

Statistical analysis

The experiments were designed and statistically analyzed using the factorial experiment according to the Completely Randomized Design (C.R.D) in the factorial experiments, and the significant differences in transaction rates were compared using Duncan's New Multiple Range Test. And rates with similar letters do not differ significantly at the (5%) probability level according to Duncan's polynomial test.

Results and Discussion

Protein concentration

Table 1 shows that soil treatment with cobalt at concentrations 25 and 50 and copper at concentrations 75 and 90 mg/kg soil led to a significant decrease in protein concentration in bean plant seeds compared to the control treatment. It was noted that the lowest concentration of protein appeared in bean plant seeds treated with cobalt at a concentration of 50 mg/kg soil, which amounted to 0.46 mg/gm. As for the treatment with plant powders, it was found that treating the soil with silybum and licorice powder at concentrations 4 and 8 gm/kg soil led to a significant increase in protein concentration. And the highest concentration of protein appeared when treated with silybum powder at a concentration of 4 gm/kg soil, and it reached 4.88 mg / g compared to the control treatment. It was also shown from the table that soil treatment at a concentration of 4 gm/kg soil was better than treatment with a concentration of 8 g/kg soil as the effect of plant powder concentration, and

Table 1: *The effect of soil treatment with Silybum and licorice powder on protein (mg/g) concentration in beans grown in soils contaminated with heavy metals.*

Powder type effect	Powder concentration effect	Cu mg/kg soil		Co mg/kg soil		Control	Powder concentration	Powder type
		90	75	50	25			
1.10 c	1.10 c	0.90 h	1.09 g	0.46 j	0.68 i	2.36 b	0.0	
1.52 a	1.62 a	1.03 g	1.41 de	1.08 g	1.34 e	4.88 a	4	Silybum powder gm/kg
	1.17 b	0.49 j	0.94 gh	1.04 g	1.53 d	1.49 d	8	
1.28 b		0.95 gh	1.09 g	1.01 g	1.16 f	2.29 bc	4	licorice powder gm/kg
		0.65 i	1.28 ef	1.00 g	1.41de	1.91 c	8	
				0.86 b	1.19 a	Element concentration effect		
		0.98 b		1.07 a		Element type effect		

The letters express the significant differences. Different letters are considered to be a significant difference, while the similar letters mean no significant differences between the results.

Table 2: *The effect of soil treatment with Silybum and licorice powder on carbohydrate (mg/g) concentration in beans grown in soils contaminated with heavy metals.*

Powder type effect	Powder concentration effect	Cu mg/kg soil		Co mg/kg soil		Control	Powder concentration	Powder type
		90	75	50	25			
3.36 a	3.36 a	2.24 d	2.24 d	1.87 e	2.64 d	7.81 a	0.0	
3.66 a	3.38 a	3.06 c	3.16 c	2.53 d	2.98 d	8.75 a	4	Silybum powder gm/kg
	3.09 b	2.42 d	3.85 c	2.15de	2.66 d	5.08 b	8	
2.75 b		2.11 de	3.06 c	1.83 e	2.40 d	3.68 c	4	licorice powder gm/kg
		3.01 c	3.05 c	1.99 e	2.76 d	3.61 c	8	
				2.32 b	2.88 a	Element concentration effect		
			2.82 a		2.37 b	Element type effect		

The letters express the significant differences. Different letters are considered to be a significant difference, while the similar letters mean no significant differences between the results.

it amounted to 1.62 mg/g. It was found that treating the soil with silybum powder led to a significant increase in protein concentration, which amounted to 1.52 mg/gm, compared to treating the soil with licorice powder and the control treatment. As for the effect of heavy element concentration, it was noted that soil treatment with the second concentration of each heavy element had the most significant and significant effect on the protein concentration, reaching 0.86 mg/g. The copper element had the most significant effect on the protein concentration compared to the effect of the cobalt element, and it was 0.98 mg/g as the effect of the element type on the protein concentration in the bean plant seeds. This may be because proteins are among the main targets of heavy elements, as they form side complexes with them or replace the main ions involved in the formation of proteins, which negatively affects their functions (Tamás *et al*., 2014). And those plants were exposed to different concentrations of heavy metals, which leads to oxidative stress in plants, which leads to an increase or decrease in the level of enzymes and disruption of their work, which affects protein production (Oladele *et al*., 2019). Heavy metal may affect plants by causing oxidation of proteins and fats in the plant cell and the occurrence of damage to nucleic acids, as well as inhibiting enzymes, which affects the production of proteins (Adrees *et al*., 2015). Furthermore, the treatment with Co and Cu elements negatively affected the absorption of zinc, potassium and manganese and led to a significant decrease in plant growth and an increase in oxidative stress (ROS and MDA), but at the same time, there was an increase in the content of phytochelatin (PC) and glutathione (GSH and GSSG) (Jonas, 2020). This may affect the synthesis and concentration of proteins is similar to what was found by (Kakkalameli *et al*., 2021) that the exposure of Water lettuce plants to high concentrations of chromium led to a decrease in the concentration of both carbohydrates and protein.

Carbohydrate concentration

It was found from Table 2 that soil treatment with cobalt and copper and with the concentrations fixed

in the above table showed a significant decrease in the carbohydrate concentration in bean plant seeds, and the treatment with cobalt at the concentration of 50 mg/kg soil was the most effective and amounted to 1.87 mg/gm compared to the control treatment. As for the treatment with plant powders, it was found that treating the soil with silybum and licorice plant powder at concentrations 4 and 8 gm/kg soil led to a significant increase in carbohydrate concentration. And the treatment with silybum powder at a concentration of 4 gm/kg soil was significantly superior to the rest of the treatments and it reached 8.75 mg / g. It was noted from the table that the treatment of soil with plant powders at a concentration of 4 gm/kg soil was better than treatment with a concentration of 8 gm/kg, as it was observed that the concentration of carbohydrates in the seeds was 3.38 mg/g as the effect of the concentration of the plant powder.

And as the effect of the type of powder, treatment with silybum powder led to a significant increase in carbohydrate concentration compared to treating the soil with licorice powder, and it amounted to 3.66 mg/g as for the effect of the concentration of the element, the second concentration of the two heavy elements was significantly more effective compared to the first concentration, and the concentration of carbohydrates was 2.32 mg/g. And treatment with cobalt element significantly affected the concentration of carbohydrates in seeds compared to copper element as the effect of the element type, and it amounted to 2.37 mg/g. The decrease in carbohydrate content when treated with heavy metals may be attributed to the fact that heavy metals impeded metabolic activities that may reduce carbohydrate content, and a decrease in photosynthetic pigments, which leads to inhibition of photosynthesis activity, which reduces the proportion of carbohydrates (Hussain *et al*., 2013).The reason for the low content of carbohydrates may also be because heavy metals affect the photosynthesis of plants by reducing the content of chlorophyll and the absorption of the necessary elements for chlorophyll such as magnesium and iron, which affects the chloroplasts, and changes the basic enzymatic processes of the photosynthesis process, which negatively affects the content of carbohydrates in plant leaves (Alia *et al*., 2015). And possibly a decrease in photosynthetic pigments as a result of the negative effects of heavy elements in the plant (AL-Rashedy, 2020). And Plants treated with heavy metals had a decrease in the rate of photosynthesis, stomata conduction and transpiration rate (Alkhatib *et al*., 2019) This is one of the reasons that reduce the process of carbohydrate formation. This is similar to what was found (Selvaraj, 2018) in that the exposure of black gram plants to toxic concentrations of nickel led to a decrease in the concentration of photosynthetic pigments such as chlorophyll and carotenoids due to an imbalance in cellular systems, and this negatively affects the concentration of carbohydrates, which is the main product of the photosynthesis optical.

Conclusions and Recommendations

It was found from the research that the treatment of soil with heavy metals led to a significant decrease in the concentration of carbohydrates and protein in bean plants. The effect was greater with high concentrations of these minerals. Whereas, treating the soil with powder of wild plants improved and increased the concentration of protein and carbohydrates. Therefore, we recommend the use of other plant powders to treat the effect of heavy metals.

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Novelty Statement

The study is novel as it shows increase in the tolerance of plants and reduction of heavy metals when the soil is contaminated by using wild plant powders.

Author's Contribution

Hussein Saber Mohammaed Ali Al-Rashedy: Sample handling, laboratory testing, performed statistical analyses and research writing.
Wadullah Asaad Abdullah Al-Mtewti: Cultivation, sample handling, and search for sources.

Conflict of interest
The authors have declared no conflict of interests.

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