Effect of Lead on Some Phenotypic Characteristics, Protein Changes and Productivity of *Beta vulgaris var. cicle*

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Abstract

This study was conducted at the College of Education for women, Tikrit University / department of biology, and aims to find out the effect of lead in different concentrations (125, 250 and 500) mg / kg on the phenotypic, physiological and genetic characteristics (amino acids) of chard *Beta vulgaris var. cicle*.

The results showed that soil pollution with lead leads to inhibition of vegetative growth through its effect on the processes of division, cellular expansion and hormonal regulation, as well as negatively affects various vital activities and different metabolic processes (photosynthesis, respiration, building of nucleic acids, proteins, enzymatic activities, and other vital activities necessary for plant growth and reduces productivity).

Key words*: lead, amino acids, chard plant, genetic characteristics, phenotypes.*

Introduction

In recent times, environmental and genetic studies are of interest to many researchers, especially after the high rate of environmental pollution (soil, water and air) in the world with heavy metals, and the emergence of many problems resulting from high concentrations of heavy metals as a result of their wrong uses in industry, agriculture and energy, in addition to the high local waste [(1,2)]. Heavy metals have the ability to transmit to humans and animals fed on edible plants through their accumulation in their tissues and at high levels that lead to toxicity [(3)]. One organic complex to another, and thus this environmental problem raised concerns and worries of many researchers [(4)]. The two elements of lead and cadmium are among the most heavy elements affecting human life even when they are present in a small percentage and although their physiological role is not known, they do not pose a danger to humans [(5)]. The human ingestion of plants contaminated with cadmium or lead leads to damage to the lung, kidney and nervous system and great damage to it [(6)(31)]. The presence of heavy metals such as lead, cadmium and nickel also affects the plant's growth and physiological activity, and its high concentrations reduce the expansion and division of cells and thus affects plant growth and productivity [(7)]. Chard plant Beta vulgaris var. cicle is a species of the Beta genus that belongs to the Betaea clan of the Chenopodiaceae family, this plant belongs to the field crops cultivated in winter and from the leaves have large bright leaves that are used as food and fodder in Europe, Asia, Iraq, Jordan, Lebanon and Syria [(8)].

The plant of the Rumatica family contains a high percentage of vitamins such as A, B, and C, and from here it acquires its nutritional value, and the difference in amino acids content is observed in the types of plants of this family. Amino acids gain their importance from their ability to help the plant to withstand the stresses resulting from environmental conditions and also help in the synthesis process, it is a bio-enzymatic stimulant.

There are many studies that have confirmed the toxicity of these minerals and their negative impact on productivity and human health, including the study [(9-11)] studied the potential of halophytic plants to eliminate the influence of heavy metals like lead and nickel in the soil. While Yongsheng *et al*. [(12)] studied the effect of lead accumulation on the components and growth of the tea plant, and noted its negative effect, and the decrease in the yield and quality of the tea plant. Alamari *et al*. [(13)] studied the efficacy of ascorbic acid in improving the

tolerance of wheat plants to toxicity to lead. Despite the presence of many recent studies of the effect of heavy metals on the growth and effectiveness of plants, there is a dearth of knowledge of the effect of these metals on the protein activity of the various plants spread in the Iraqi lands. That is why the current study aims to know the extent to which genetic factors (amino acids) are affected by soil pollution with different concentrations of lead and its reflection on some phenotypic characteristics and the concentration of amino acids of the chard plant for different growth stages, and to shed light on the most important protein variables as a result of high pollution in Iraqi lands.

Materials and Methods

Initialization and examination of samples

The current study was conducted in the laboratories of the department of Biology/ College of Education for women/ University of Tikrit - Iraq for a period between 2-5 months of 2020 to find out the effect of lead on the phenotypic and genetic characteristics of the chard plant and its reflection on the amino content of plant leaves in the vegetative growth and harvest stages and seeds.

After developing the plant by following the scientific environmental conditions (preparing soil, determining the type of seeds, analyzing the soil, estimating the field capacity of the soil and preparing the pots used for planting the plant) according to the method of Black and Hartge [14] and determining the treatments for lead by three replicates and at three concentrations (125, 250 and 500 mg / kg).) And estimating the percentage of germination, method of cultivation and method of irrigation, and after 45 days of planting, three replications were taken (treatment) and the phenotype of the plant was measured and observed with the control plant, where the phenotypes of the plant height, the length of the root system, and the leaf area were recorded and the relative water content in the leaves was estimated using the equation below.

$$\text{Relative water content (\%)} = \frac{\text{Soft Weight - Dry Weight}}{\text{Bulging Weight - Dry Weight}} \times 100$$

The degree of damage (the degree of stability of the membranes) according to the method [15]. The root and vegetative total were weighed and the dry weight was measured. Chlorophyll [16] and carbohydrates were estimated. After 125 days of planting, the mature plants were uprooted in the final growth stage, washed, and performed some chemical and phenotypic tests again, such as plant height, root system length, dry weight, shoot weight, number of branches, number of heads, number of seeds, seed weight, and the amount of the concentration of nutrients in the plant in the dried parts. For each of the shoots, the root system and the seeds, as samples were taken after being dried and milled with a special mill, 0.5 gm of each sample was taken and digested by the method of wet digestion [17] and the nutrients potassium, sodium, chloride, calcium and magnesium were estimated to see the extent to which proteins in the plant and affected cell membranes were affected. Amino acids affected in turn by the heavy metal lead then the heavy metals were estimated using the Atomic Absorption device and the lead element was estimated in the root and vegetable group in the vegetative growth and seed stages by the method [18], taking 0.5 g of the powder of the previously digested dried plant samples using sulfuric acid and perchloric acid at a ratio of 3: 1. [18]. After that, the last and basic step of the research was done in the detection of the type and concentration of amino acids in plant leaves and seeds.

Detection of type and concentration of amino acids in plant leaves and seeds:

The leaves and seeds of the chard plant were dried in the vegetative and reproductive growth stages in order to detect the type and concentration of amino acids. Leaves and dried seeds were ground and 10 g was taken from them, 100 ml of ethyl alcohol, 80% concentration, were added to them and left in the refrigerator for 5 minutes. Then the sample was placed in a Sixhlet for an hour and 100 ml of ethyl alcohol was added to it. The temperature of 50 ° C and the dry residue at the bottom of the flask were allowed to dissolve with 2 ml of methanol, after which the sample was mixed with the Orthophthalaldehyde (OPA) amino acid derivative, and injected with an HPLC device.

According to the method Carpena *et al*. [19].

Statistical Analysis

The experiments were designed and analyzed statistically using a Completely Randomized Design [20]. The significant differences between the transaction rates were compared using the lowest significant difference at the 5% probability level according to Test Duncan New Multiple Round.

Results and Discussion

The results of soil treatment with lead in three concentrations (125, 250 and 500 mg / g) showed that there was a significant decrease in plant height compared to the control sample that reached 30 cm, while the lowest plant height was 17 cm at a concentration of 500 mg / g, and a significant decrease was observed at the two concentrations (125 and 250 mg / g). It reached 18 and 20 cm (Table 1). The high concentration of lead has a toxic effect, which in turn reduced the plant height and the occurrence of significant reduction in vegetative characteristics as a result of inhibiting the hormonal regulation of plant growth or inhibiting the process of cell division and expansion and thus the accumulation of lead leads to inhibit the processes and metabolic activities such as photosynthesis, respiration, amino acid synthesis, and enzyme activity [7, 21,22].

The current study showed a significant increase in the length of the root group when increasing the concentration of the element of lead compared to the control sample, so the length of the root group reached 19 cm at the concentration of 125 and 250 and reached 20 cm at the concentration of 500 mg compared to the control treatment and the presence of this significant difference may be due to the fact that heavy elements increase the radical secretions and reduce the pH Soil and the increase in the readiness of heavy elements for absorption into the soil by the root system. It was also found that there was a significant decrease in the leaf area of the chard plant when treated with different concentrations of lead, the highest decrease was 84 cm at a concentration of 500 mg / g compared to the control treatment as in Table 1. The reason is the decrease in the leaf area leads to the accumulation of lead and its toxicity, which inhibit cell division and reduce the paper space. It was observed that there was a significant decrease in the relative water content of the chard plant when it was treated with different concentrations of lead, indicating a significant decrease at the concentration (500 mg / g) compared to the control sample in which the percentage of water content reached 92%, and a significant increase was found at the concentrations of 125 and 250 mg / g for the element. Lead reached 92.5% and 92.5%. The reason for the difference is due to the change in the movement of water inside the plant through the lack of transport vessels (their area and number). Thus, the results of the current study are in agreement with the results of the study of Vassileu *et al*.[23] and Maruthi *et al*.[24]. While the degree of stability of the membranes was affected. Cytokinesis and the percentage of damage (MSI) according to different concentrations of lead, and it was found that there was a significant increase compared to the control sample, so the damage percentage reached (33, 32 and 45), respectively, Table 1. Here it should be noted that the cause of the damage is due to the occurrence of changes in the composition and functions of cellular membranes, and that the increased damage occurred as a result of the reduction of the ATPase enzyme present in the cytoplasmic membranes, and thus the results of the study agree with the results of the study of Bnavide *et al*.[25]. It was also found that there was a significant decrease in the dry and soft weight of the shoots and the percentage of carbohydrates when treating the chard plant with different concentrations of lead compared to the control sample, while there was no significant decrease in the dry and fresh weight of the root total of the chard plant treated with different concentrations of lead compared to the control sample, as shown in Table 1. These results are consistent with the results of the study [7].

The current study showed its agreement with the study of Zhou [26], which emphasized the existence of a relationship between the stability of the membranes and the relative water content. The more proline accumulation, the higher the relative water content, and this leads to a water stress and a reaction in the plant cell and an increase in the accumulation of proline and thus it is an opposite relationship or negative.

Table 1. The effect of lead on the general characteristics of chard plants during the vegetative growth stage.

Treatment / Plant Parts	Control	Pb mg/kg		
		500	**250**	**125**
Plant length (cm)		17c	18c	20c
Root total length (cm)	30a	20b	19b	19b
Leaf space (cm2)	16c	84f	93d	216b
Relative water content %	273a	88b	92.5a	93a
Degree of stability of the films (damage ratio)	92a	45b	32c	33c
Vegetable dry weight (g)	22e	0.44b	0.65b	0.32b
Root dry weight (g)	5.1a	0.25a	0.46a	0.41a
Fresh weight of vegetative total (g)	0.9a	2.2.4c	4.5bc	2.1c
Fresh Weight of Root total (g)	26.1a	1.6b	1.9b	1.4b
Carbohydrate percentage (mg / g)	3.0a	4.3cd	6.1b	6.9b
Percentage of chlorophyll a(mg/g)	8.7a	19.2a	10.5b	4.8c
Percentage of chlorophyll b(mg/g)	11.96a	9.9a	1.2b	3.7d
Percentage of chlorophyll a+b(mg/g)	8.1cd	9d	6.7a	8.5d

* Similar letters indicate no significant differences

The results of the current study showed that there was a significant decrease in plant height in the harvest phase compared to the control sample, as the highest decrease in the concentration of 250 mg / g was found, while it was found that there was a significant increase in the length of the root system in the harvest phase compared to the control sample and the vegetative growth phase at the concentration of 125 mg / g while it was not recorded. The concentration is 500 mg / g, no significant difference compared to the control sample, and there was a significant difference in the number of branches and fruiting heads and the number of seeds, so 500 mg / g was one of the most effective concentrations on the number of fruiting heads and the number of seeds, while the concentration of 500 mg / g was more influential on the decrease in the number of branches as in Table 2. The results of the present study are in agreement with the findings of the Alamri study [13] which indicated a decrease in growth in roots and stems, decrease in fresh and dry weight, lack of relative water content, deterioration of chlorophyll, and low nutrients in wheat plants treated with lead concentrations.

Table 2. The effect of lead on the general characteristics of chard plants during the harvest stage.

Treatment / Plant Parts	Control	Pb mg/kg		
		500	250	125
Plant length (cm)	104 a	80b	71c	100a
Root total length (cm)	35 b	33b	22e	45a
Vegetable dry weight (g)	15 a	10.4 c	7.1d	13.7b
Root dry weight (g)	2.60 b	2.4b	2.3b	4.0a
Number of branches plants	12 a	11a	7c	9bc
Number heads fruity	207 a	67b	79b	77b
Number of seeds	355 a	102e	200c	250f

* Similar letters indicate no significant differences.

The results of the current study (Table 3) showed a significant decrease in the concentrations of all the nutrients that were measured, such as potassium (K^+) and sodium in the leaves and roots of the chard plant. The higher the concentration of lead, the greater the decrease in the absorption of potassium from the soil, and the reason for the lack of absorption of potassium and sodium and chloride (Cl^-) by the roots compared to the control sample. The cause of the disturbance of nutrients in plant tissues may be due to the imbalance caused by heavy metals and their negative effect on the osmotic pressure as well as lead to an imbalance in the ionic balance.

It was also found that there was a significant increase in the concentration of magnesium in the roots of the chard plant, whenever the concentration of lead was high in the soil compared to the control sample. The reason for this is due to an imbalance in the ionic balance, and thus the results of the present study agree with the results of Vassilev[23].

Table. 3 The effect of lead on the percentage of nutrients in the leaves and roots of the chard plant, measured in mg / kg

Treatment / Nutrients	**Control**		**Pb mg/kg**					
	leaves	**roots**	**500**		**250**		**125**	
			leaves	**roots**	**leaves**	**roots**	**leaves**	**roots**
Potassium	20a	13a	1.1e	2.5ef	1.8e	2f	1.2e	3e
Sodium	80a	9a	3.6e	3d	2.5e	4.2c	1.7e	4c
Magnesium	30a	7c	8.8d	8.8a	6.3d	9a	1.6e	10a
Calcium	18a	3c	3d	2d	3.2d	2.5d	3.2d	4b
Chloride	10c	25a	8d	7d	4e	8.6bc	9.3cd	9d

* Similar letters indicate no significant differences.

The results of the current study (Table 4) showed that the treatment of soil with different concentrations of the element lead led to the emergence of a significant increase in the concentration of the element lead in the roots, reaching (3.6, 6.8 and 8 mg / kg), respectively, compared to the control sample as it reached 5.1, 8.1 and 8.8 mg / kg. In leaves, compared to control sample, which reached 2 mg / kg, while the concentration was 12, 12.9 and 13.1 mg / kg of seeds compared to control sample.

The accumulation of heavy metals in the different plant parts has a negative effect on human and animal health due to its link with the food chain, and the accumulation of lead depends on the efficiency of the plant in absorbing the metal from the soil, the type of plant and its ability to neutralize the metal in different parts of its body. It was found that the chard plant has the ability to absorb the lead element from the soil in very low concentrations, and this indicates the danger of growing it in soils contaminated with lead. The biological assembly of the element was observed to increase with increasing concentration, and this is consistent with the results of Saeed [27], who indicated that the concentration of lead in the wheat plant increased when its concentration increased in the soil. The results of the current study are also consistent with the study of Siddhu [28], which confirms that despite the high salts in the soil, its negative effect on plants may be related to many factors, including the acidic function and the solubility of salts and carbonates in the soil.

Table 4. Concentration of the element lead in the different parts of the chard plant under study.

Treatment / Parts	Control	Pb mg/kg		
		500	250	125
Roots	3.3d	8.0a	6.8b	3.6c
Leaves	2.0a	8.8b	8.1b	5.1c
Seeds	1.0d	13.1a	12.9b	12c

* The values in the table represent ranges.

The effect of lead on amino acids: It was found through the results of the current study that the accumulation of lead occurs as a decrease of aspartic acid in the vegetative growth stage of all concentrations, and the accumulation of lead also affects the accumulation of amino acid in the roots, which led to the appearance of an increase in the proportion of amino acid than the control sample and its increase was not confirmed in the seeds compared to the control sample at the concentration of 125,500 mg / kg, however, an increase in the concentration of histidine was observed at the concentration of 250 mg / kg of lead, while the glutamic amino acid decreased compared to the control sample with regard to the concentration of 125 mg / kg and it increased at the concentration of 250 mg / kg. Kg and the percentage varied at the concentration 500 mg / kg. Also, a clear decrease in serine acid was observed compared to the control sample in the two concentrations 125 and 250 mg / kg for vegetative and root growth, while the results showed a clear rise in amino acid (serine) for all concentrations and for all plant parts, and it was found that there was a decrease in the values of the amino acid glutamine. In vegetative and root growth for all concentrations except for the concentration of 500 mg / kg, an increase was observed in the vegetative growth stage, and the values of this acid increased for all concentrations in seeds. While a decrease in the concentration of the amino acid threonine was observed in the stage of vegetative and root growth and for all the studied concentrations except for the concentration of 500 mg / kg for vegetative growth, it was also found that there was an increase in the seeds. It was observed

through the study that there was a significant decrease, not a predominance of amino acids, in the stage of vegetative growth and sometimes the root whereas, a significant increase was observed at the concentration of 500 mg / kg for glycine, histidine, alanine, tyrosine, arginine, methionine, valine, tryptophan, phenylalanine, iso-lysine and eosin, while it was found that there was a significant increase in amino acid concentrations for seeds and for all concentrations. The results of the study agree with the results of the study of Gioseffi *et al*. [29], which indicated the importance and effect of the element of nitrogen on amino acids such as glycine and glutamine in the roots of the wheat plant. and this study dot agree with [30].

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

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