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To cite this article: Zainab Majid Abadi *et al* 2025 *IOP Conf. Ser.: Earth Environ. Sci.* **1487** 012064

Effect of Glutathione Treatment in Reducing the Stress of Heavy Metals and Its Effect on Some Vegetative Characteristics of *Ziziphus mauritiana* Lam. Apple Seedlings

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Abstract. The experiment was conducted in the Department of Horticulture and Landscape Engineering plant canopy, College of Agriculture, University of Basra, during the growing season 2023-2024. The aim is to know the antioxidant glutathione's ability to reduce the effects of pollution of the apple variety Sidr seedlings with heavy elements (lead + cadmium). The experiment was designed as a factorial experiment RCBD for two factors. Two types of pollutants, namely lead and cadmium and three concentrations for each of them represented the first factor: lead (200, 100, 0 mg. L^{-1}, cadmium (6, 3, 0) mg. L^{-1}. The second factor was spraying with the antioxidant glutathione at concentrations (200, 100, 0) mg. L^{-1}. The results were statistically analyzed using the least significant difference (LSD) test at a probability level of 5% and using the statistical program GenStat. The results showed that increasing the concentration of pollutants had a negative effect on most of the traits under study. The greatest effect was for the treatment of 200 mg. L^{-1} lead and 6 mg. L^{-1} cadmium, as the results recorded a decrease in each of the traits of seedling height, number of branches, number of leaves, stem diameter, leaf area, fresh and dry weight, and it reached 0.13. 0.83, 1.99, 16.96, 120.11, 2.00, 124.25 respectively. As for spraying with glutathione, the treatment of spraying with glutathione at a concentration of 200 mg.L^{-1} recorded the best results for each of the traits of seedling height, number of branches, number of leaves, stem diameter, leaf area, and average fresh and dry weight of leaves, and reached 139.56, 4.00, 139.00, 21.46, 3.13, 1.19, 0.21 respectively. The interaction between the two study factors was significant, as the interaction treatment (0 and 200 mg.L^{-1} glutathione) recorded the best results in most of the traits under study.

Keywords. Ziziphus mauritiana, Heavy metals, Glutathione.

1. Introduction

The Sidr plant belongs to the Rhamnaceae family and the genus Ziziphus, which includes more than 100 species, most of which grow in tropical and temperate regions of the world [1,2]. Sidr trees are multipurpose plants, as their fruits are eaten fresh because they contain a high percentage of vitamin C, sugars, proteins, organic and amino acids, fats, and mineral salts, as every 100 grams contains 20.9

calories. Sidr trees are used as fuel or as windbreaks, in addition to the medical benefits of their leaves and bark [3,4]. The fruit of the Sidr is of the Drupe type and is an important source of vitamins, minerals, sugars, antioxidants, and fibers. The agricultural variety is considered to be apple Z. mauritiana Lam, cv. Tufahi is one of the most important and widespread Sidr varieties in the Basra region, as it is one of the excellent varieties with abundant, economically profitable production and the most desired by the consumer, in addition to the large size of its fruits, their special flavor and their high nutritional value [5]. Their spherical, semi-spherical, or elliptical shape characterizes the fruits of this variety, and their yellow color is tinged with green when ripe.
Pollution is defined as an undesirable change in the chemical, physical or biological properties of the environment (air, water, and soil), and is caused by substances called pollutants, which are any solid, liquid, or gaseous substance, as they move from their various sources and in different quantities, causing health and economic damage to humans, other living organisms and the environment [6]. The problem of heavy metal pollution in the air, agricultural soil, and water is one of the important environmental problems worldwide, and despite the presence of these minerals naturally in the Earth's crust, the increase in human activities has led to an increase in environmental pollution levels with these minerals [7]. Heavy metals are divided into essential elements for living organisms that need them in small concentrations and are toxic in high concentrations, such as zinc, iron, copper, and chromium. Non-essential elements, have no known vital role yet and are toxic at any concentration, such as cadmium, lead, and mercury [8]. The accumulation of heavy metals causes toxicity to living organisms, which may have health effects on their consumers, including humans [9].
Glutathione (GSH) is a powerful antioxidant in plants, animals, and microorganisms. Glutathione can prevent damage to important cellular components caused by active oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals. In recent years, glutathione has received the attention of researchers to understand the effect of glutathione and its effect on plants under stress conditions and increase its concentrations and plant tolerance to these stresses [10]. Among its functions is to protect membranes by maintaining reduced levels of both a-tocopherol and zeaxanthin, to prevent oxidative denaturation of proteins under stress conditions by protecting their thiol groups. It works to stabilize or balance oxidation within the plant cell affected by stress. GSH is considered a chelating substance for toxic metals such as metalloids, then transporting and isolating them in the vacuole. Glutathione also enhances plant tolerance to various abiotic stresses including high and low temperatures, salinity, drought, and stress with toxic metals [11,12].

2. Materials and Methods

The study was conducted in the plant canopy of the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Basrah during the growing season 2023-2024. During the period extending from October to April, where seedlings of the jujube plant, the apple variety Ziziphus mauritiana Lam. Cv. Tufahi from one of the private nurseries, two years old, was as homogeneous as possible in terms of the strength of vegetative growth and length, placed in 10 kg plastic anvils in a growing medium (mixture: peat moss) Table 1. The experiment began by creating an environment polluted with heavy elements by irrigating with water polluted with heavy elements containing lead at a concentration of (0, 100, 200), mg. L^{-1} and cadmium at a concentration of (0, 3, 6) mg. L^{-1} in addition to joint treatments between the two pollutants, and the seedlings were sprayed with three concentrations of glutathione, which are (0, 100, 200) mg. L^{-1}. Digital marks were placed on them according to the treatment and the repetition, with all service operations carried out from regular irrigation, pruning, pest control, and NPK fertilization in an integrated manner until the end of the experiment.

2.1. Physical and Chemical Properties of the Study Soil

The agricultural medium was used, consisting of a mixture of sandy soil (mixture: peat moss) at a ratio of 1:1. The agricultural medium was analyzed by taking random samples and the elements and components of the agricultural medium were estimated in the central laboratory of the College of Agriculture\University of Basra, as shown in Table (1).

IOP Conf. Series: Earth and Environmental Science **1487** (2025) 012064 doi:10.1088/1755-1315/1487/1/012064

2.2. Study Parameters

The experiment included the following parameters:

2.2.1. Soil Pollutants Factor, which Includes the Following Parameters

- Comparison treatment (without addition)
- Lead treatment Pb(NO3)2 at a concentration of 100 and 200 mg.L^{-1}
- Cadmium treatment CdCl2 at a concentration of 3 and 6 mg.L^{-1}

2.2.2. Antioxidant Concentration

- Comparison treatment (without addition)
- Glutathione treatment at a concentration of 100 and 200 mg.L^{-1}

2.3. Experimental Measurements

2.3.1. Main Stem Height (cm)

The height of the main stem was measured at the end of the experimental season using a metric ruler starting from the area of its contact with the soil until the end of the growing tip of the stem and for all experimental units and the average was extracted for each treatment.

2.3.2. Stem Diameter (mm)

The diameter of the main stem was measured for each seedling of the plants using a Vernier caliper.

2.3.3. Number of Branches (branch. seedling^{-1})

The number of branches was calculated manually at the end of the experiment and the average was extracted for each experimental unit.

2.3.4. Leaf Area (cm^2)

The leaf area was calculated using the weight method by taking a leaf from each plant and recording its weight. A square with an area of 2 cm was cut, the fresh weight of this cut was recorded, and the area ofone leaf was calculated according to the following equation:

Leaf area (cm^2) = (Average leaf weight (g) × Area of the cut square (cm^2) / Average weight of the cut square (g)) × 100

2.3.5. Number of Leaves (leaf. plant^{-1})

The number of leaves was calculated at the end of the experiment and for all experimental units.

2.3.6. Fresh Weight of the Leaf (g)

The fresh weight of five leaves from each experimental unit was calculated using a sensitive balance of the Sartorius type, then the average fresh weight was extracted.

2.3.7. Dry Weight of the Leaf (g)

After calculating the fresh weight of the leaves, they were dried in an electric oven (Vacum Oven) at a temperature of 70°C for a period until the weight stabilized, then they were weighed using a sensitive balance, and the average dry weight was extracted for each experimental unit.

Table 1. Some physical and chemical properties of the culture medium.

Properties	**Values**	**Units of Measurement**
Organic matter	7.53	(g.kg^{-1})
Electrical conductivity	1.62	(dSiemens m^{-1})

Properties	Values	Units of Measurement
PH	8.1	-
Phosphorus	2.8	(g.kg^{-1})
Nitrogen	28.6	(g.kg^{-1})
Potassium	65.2	(mg.L^{-1})
Cadmium	0.0097	mg.L^{-1}
Lead	0.5	(g.kg^{-1})
Sand	81.36	%
Gilt	11.32	%
Clay	7.32	%
Soil texture	Sandy Mixture	

3. Results and Discussion

3.1. Effect of Pollution with Heavy Elements and Spraying with the Antioxidant Glutathione and Their Interaction on Seedling Height (cm), Number of Branches (branch.seedling^{-1}), Number of Leaves (leaf.plant^{-1}), and Stem Diameter (mm)

The results in Table (2) indicate that the treatment with heavy elements led to a decrease in plant height, as the concentration (cadmium) + (6 lead 200) mg.L^{-1} gave the lowest rate of 124.49 cm compared to the comparison treatment, which gave the highest rate of seedling length, reaching 139.33 cm. The results of the same table indicate that spraying with the antioxidant glutathione at a concentration of 200 mg.L^{-1} gave the highest rate in the plant height trait, reaching 139.56 cm, while the comparison treatment recorded the lowest rate, reaching 122.33 cm. As for the effect of the interaction between the pollution and antioxidant factors, the results below indicate the presence of significant differences, as the interaction treatment between (0) and (200 mg.L^{-1} glutathione) outperformed, as it recorded the highest rate of 155.67 cm, while the interaction treatment (0) and (6 Cadmium + lead 200 mg.L^{-1}) and the comparison treatment had the lowest rate of 111.25 cm.

The results in Table (2) also indicate that the treatment with heavy elements caused a decrease in the number of branches, as the comparison treatment recorded the highest rate, reaching 3.99 branches. seedling^{-1}, while the concentration (cadmium)) + (6 lead 200) mg.L^{-1} recorded the lowest rate, reaching 2.00 branches. seedling^{-1}. As for glutathione, it had a significant effect on the number of branches, as the concentration 200 mg.L^{-1} recorded the highest rate, reaching 4.00 branches. seedling^{-1}, while the comparison treatment recorded the lowest rate, reaching 2.88 branches. seedling^{-1}. As for the effect of the interaction between the pollution and antioxidant factors, the results below indicate the presence of significant differences, as the interaction treatment between ((0 and 200 mg.L^{-1} glutathione) outperformed, as it recorded the highest rate of 5.33 branches. seedling^{-1}, with a significant difference over the rest of the interaction treatments, while the interaction treatment between ((0 and 6) cadmium + lead 200 mg.L^{-1}) recorded the lowest rate of 1.66 branches. seedling^{-1}. The results of Table (2) also show that high concentrations of heavy elements caused a significant decrease in the number of leaves, as the rate of concentration of heavy elements affected the number of leaves and led to a significant decrease in the pollution treatment with a concentration of (cadmium)) + (6 lead 200) mg.L^{-1}, as it reached 120.11 leaves. plant^{-1}, compared to the comparison treatment, which recorded the highest rate of 130.78 leaves. plant^{-1}.

Spraying with glutathione had a positive effect in reducing heavy metal stress, as the number of leaves increased with increasing glutathione concentration. The 200 mg.L^{-1} glutathione treatment obtained the highest rate, reaching 139.00 leaves per plant-1, compared to the comparison treatment, which recorded the lowest rate, reaching 124.78 leaves per plant-1. As for the effect of the interaction between the pollution treatment and the antioxidant treatment, the table below indicates the presence of significant differences, as the interaction treatment between ((0 and 200 mg.L^{-1} glutathione) was superior and recorded the highest rate of the number of leaves, reaching 145.00 leaves.plant^{-1}. While the interaction treatment between ((0 and 6) cadmium + lead 200 mg.L^{-1}) recorded the lowest rate, reaching 113.33 leaves.plant^{-1}. The results in Table (2) also indicate that the treatment with heavy elements led to a significant decrease in the diameter of the stem, as the comparison treatment was superior and recorded the highest rate, reaching 20.30 mm, while the pollution treatment recorded a concentration of (cadmium 6 + lead 200 $mg.L^{-1}$) the lowest rate, reaching 16.96 mm, followed by the pollution treatment, a concentration of (cadmium 3 + lead 100 $mg.L^{-1}$), and reached 18.13 mm. The effect of the antioxidant also had a significant effect on the stem diameter, as the spray treatment with a concentration of (Glutathione 200 $mg.L^{-1}$) was superior and recorded the highest rate of 21.46 mm, while the comparison treatment recorded the lowest rate of 16.58 mm. As for the effect of the interaction between the pollution and antioxidant factors, the results below indicate the presence of significant differences, as the interaction treatment between ((0 and 200 $mg.L^{-1}$ Glutathione) had the highest rate of stem diameter, reaching 27.70 mm, while the interaction treatment between ((0 and 6) Cadmium + Lead 200 $mg.L^{-1}$) recorded the lowest rate, reaching 11.71 mm. The results in Table (2) show the negative effect caused by heavy elements (lead + cadmium) on most of the vegetative characteristics of the seedlings under study. This may be due to the interaction effect of lead and cadmium in reducing the absorption of nutrients beneficial to the plant and reducing their readiness for absorption [13,14].

Perhaps it is due to the fact that heavy metal stress has caused damage to the cell division process and caused changes in sugar metabolism. It is known that lead hinders cell division, differentiation and elongation [15]. Heavy metals have also caused chromosomal abnormalities and abnormal divisions, as well as a reduction in the rates of photosynthesis or inhibition of the work of most enzymes responsible for growth. In addition, heavy metals reduce respiration rates and thus reduce the rate of production of the energy compound ATP, which is necessary for the formation of new tissues and branches [16]. While the mechanism of the effect of cadmium is represented by its effect on the readiness and absorption of nutrients, as it interferes with the absorption and transport of several elements such as calcium, magnesium and potassium, in addition to its negative effect on reducing the number of microorganisms in the soil [17].Cadmium also reduces the absorption of nitrates and their transfer from the roots to the vegetative group [18]. The results are consistent with what was reached by [19], in a plant treatment experiment for soil contaminated with four concentrations of crude oil, 4, 3, 2, 1, and 0) (% weight / weight by Eucalyptus Eucalyptus camaldulensis, who noticed a decrease in plant height with increasing crude oil concentration, and the largest decrease was at a crude oil concentration of 4%, as the decrease rate reached 25% compared to the control treatment. The reason may also be due to the plant absorbing these toxic substances and accumulating them in the intermediate tissue, and with increasing absorption and accumulation, damage occurs in the leaf tissues, especially the peripheral ones, forming the phenomenon of mottling and premature aging of the leaves, which leads to their falling [20]. It was also noted that the plants sprayed with glutathione were superior in vegetative growth characteristics, and the reason for this is that glutathione consists of three amino acids, glycine acids, glutamic acids, and cysteine, and that amino acids work to change the osmotic potential and lead to its decrease, which leads to reducing the water potential For the cell, thus increasing the cell's ability to take water and dissolved nutrients, and thus leading to increased vegetative growth of the plant [21].

Table 2. Effect of heavy metal pollution, glutathione spraying, and the interaction between them on seedling height, number of branches, number of leaves, and stem diameter of the Sidr plant, Apple variety.

Pollutants	Plant height (cm)	Number of branches ($branch.seedling^{-1}$)	Number of leaves ($leaf.plant^{-1}$)	Stem diameter (mm)
0	139.33	3.99	130.78	20.30
Cadmium3+Lead100	130.00	3.11	123.23	18.13
Cadmium6+Lead200	124.25	2.00	120.11	16.96
LSD	2.59	0.50	1.89	0.82
Antioxidant				
0	122.33	2.88	124.78	16.58
100	130.22	3.33	130.44	18.36
200	139.56	4.00	139.00	21.46

Pollutants		**Plant height (cm)**	**Number of branches (branch.seedling^{-1})**	**Number of leaves (leaf.plant^{-1})**	**Stem diameter (mm)**
LSD		3.59	0.50	1.89	0.82
Interaction between pollutants and antioxidants					
0	0	115.33	3.66	124.39	20.68
	100	139.40	4.66	137.00	23.46
	200	155.67	5.33	145.00	27.70
Cadmium3+Lead100	0	116.50	2.33	119.00	15.47
	100	125.67	3.00	124.67	17.99
	200	134.67	3.55	130.33	19.96
Cadmium6+Lead200	0	111.25	1.66	124.78	11.71
	100	122.00	2.33	130.44	14.47
	200	128.33	3.00	139.00	18.77
LSD		4.49	0.87	2.25	1.42

3.2. Effect of Heavy Metal Pollution, Antioxidant Spraying, and Their Interaction on Leaf Area (cm^2), Fresh Weight (g), and Dry Weight (g)

The results in Table (3) indicate that treatment with heavy elements led to a significant decrease in leaf area, as the concentration (cadmium +6 lead 200) mg.L^{-1} gave the lowest rate of 1.99 cm^2.leaf^{-1}, while the comparison treatment recorded the highest concentration of 2.94 cm^2.leaf^{-1}. The effect of the antioxidant also had a significant effect on leaf area, as the spray treatment with a concentration of 200 mg.L^{-1} was significantly superior and recorded the highest rate of 3.13 cm^2.leaf^{-1}, while the comparison treatment recorded the lowest rate of 2.49 cm^2.leaf^{-1}. As for the effect of the interaction between the pollution factor and the antioxidant spray factor, the interaction between ((0 + (200 mg.L^{-1} glutathione) recorded the highest rate of leaf area, reaching 3.88 cm^2.leaf^{-1}, while the interaction treatment between ((0 and 6) cadmium + lead 200 mg.L^{-1}) recorded the lowest rate, reaching 1.93 cm^2.leaf^{-1}. The results shown in Table (3) showed the effect of pollution with heavy metals and the addition of glutathione and the interaction between them on the fresh and dry weight of the leaves of the apple variety of Sidr. The treatment with heavy elements led to a decrease in the fresh and dry weight, as the pollutants had a significant effect in the decrease in the fresh and dry weight of the leaves, as the comparison treatment recorded the highest rate for the fresh weight, reaching 1.10 g and the dry weight 0.19 g, respectively, while the concentration recorded (cadmium)) + (6 lead 200) mg.L^{-1} The lowest fresh weight rate was 0.83 g and dry weight was 0.13 g, respectively. As for glutathione, it had a significant effect on the fresh and dry weight of the leaves, as the concentration of 200 mg.L^{-1} recorded the highest fresh weight rate of 1.19 g and dry weight of 0.21 g, respectively, while the comparison treatment recorded the lowest fresh weight rate of 0.72 g and dry weight of 0.12 g. As for the effect of the interaction between the pollution and antioxidant factors, the results below indicate the presence of significant differences, as the interaction treatment between ((0 + (200 mg.L^{-1} glutathione) outperformed and recorded the highest rate of fresh weight, reaching 1.86 g and dry weight, 0.27 g, with a significant difference over the rest of the interactions, while the interaction treatment between ((0 and 6) cadmium + lead 200 mg.L^{-1}) recorded the lowest rate of fresh weight, reaching 0.45 g and dry weight, 0.04 g.

The harmful effect of heavy elements (lead + cadmium) on leaf area and fresh and dry weights of the leaf may be due to the fact that heavy element stress causes disturbance in various metabolic activities such as photosynthesis, respiration and protein synthesis in the leaf and thus a negative decrease in leaf area and fresh and dry weight [22,23]. The increase in pollution also caused a decrease in all indicators of vegetative growth and reduced water absorption from the soil due to its pollution with heavy elements by plant roots, which is a major factor in the elongation and growth of plant cells [24]. On the other hand, spraying with the antioxidant glutathione reduced the harmful effect of pollutants and improved the characteristics of vegetative and dry growth. This is due to its containing small molecules that enter into the formation of salicylic acid, which improves plant growth and increases the efficiency of the photosynthesis process [25,26]. Or perhaps the reason is that glutathione is an antioxidant that reduces the effects of oxidative stress, as hydrogen peroxide H2O2 causes damage to cell components, which leads to accelerated leaf aging and oxidizes cell membranes, which leads to a decrease in the total chlorophyll content and leaf area [27].

Table 3. Effect of heavy metal pollution, glutathione spraying, and the interaction between them on leaf area, fresh weight and dry weight of the apple variety of Sidr plan.

Pollutants		**Leaf area (cm^2)**	**Fresh weight (g)**	**Dry weight (g)**
0		2.94	1.10	0.19
Cadmium3+Lead100		2.95	0.96	0.16
Cadmium6+Lead200		1.99	0.83	0.13
LSD		0.10	0.02	0.01
Antioxidant				
0		2.49	0.72	0.12
100		2.74	0.97	0.16
200		3.13	1.19	0.21
LSD		0.10	0.02	0.01
Interaction between pollutants and antioxidants				
0	0	2.82	0.89	0.19
	100	2.95	1.61	0.24
	200	3.88	1.86	0.27
Cadmium3+Lead100	0	2.11	0.59	0.08
	100	2.55	0.84	0.15
	200	2.74	0.87	0.18
Cadmium6+Lead200	0	1.93	0.45	0.04
	100	2.30	0.72	0.13
	200	2.43	0.83	0.16
LSD		0.17	0.03	0.02

Conclusions

In light of the current study, can conclude the following:

- The effect of high levels of heavy element pollution on vegetative growth traits in Sidr seedlings.
- The antioxidant glutathione can be used to reduce the effects of heavy element stress on jujube seedlings according to the concentrations used in the research (0, 100, 200).

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