The effect of sulfur-containing compounds and thiobacillus bacteria on corn plants under field soil and soil contaminated by heavy metals lead and zinc conditions

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ABSTRACT

Investigating the effect of various sources of sulfurous compounds along with the inoculation of Thiobacillus bacteria on corn plants under stress conditions of lead and zinc metals, an experiment executed at zanjan uni researching green house. The treatments include elemental sulfur (0.75, 1.25 and 2 g/kg soil), sulfur with Thiobacillus bacteria (1, 2 and 3 g/kg soil) and potassium sulfate (0.5, 1 and 1.5 g/kg soil). The results showed that the traits were significant in all applied treatments except proline. The amount of morphological traits and physiological traits increased in sulfur treatment with thiobacillus bacteria in field soil conditions and heavy metal stress compared to the control. But the properties of soluble sugars and proline decreased in this treatment. On the other hand, with the increase in the concentration of elemental sulfur and potassium sulfate treatments that caused stress in the plant. However, under stress conditions, the amount of absorption of lead and zinc in the root and aerial part of the plant increased to a critical and damaging level, but by applying of treatments, the amount of absorption and damage decreased. it can be concluded that elemental sulfur and potassium sulfate treatments by reducing the absorption of all elements required by the plant caused stress, but sulfur with bacteria treatment reduces the absorption of these heavy elements, and the bacteria in the soil provides the conditions for absorbing more water and nutrients through the roots and improves the growth of the plant.

Keywords: *Lead, Morphological, Physiological, Potassium sulfate, Sulfur, Thiobacillus and Zinc.*

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Highlights of this paper

- Contamination of farms with heavy metals causes stress and reduces the growth characteristics of plants.
- By applying sulfur compounds along with Thiobacillus bacteria, the amount of absorption of these metals by the roots can be reduced and it can reduce the stress in plants and improves the growth of the plant.

1. INTRODUCTION

The problem of contamination with heavy metals in agricultural lands and their products, which is increasing in areas near cities and industrial areas, has caused concern about the risk of consuming contaminated food for human health [1]. Some heavy metals such as cobalt, copper, zinc and chromium are essential elements and others such as lead, cadmium and mercury are unnecessary for plants [2, 3]. Increase in the amount of heavy metals in the soil leads to more absorption of these metals by plants, the presence of heavy metals in plant tissues affects cell metabolism, which causes stress in the plant to reduce growth, photosynthesis, biomass, yield and quality and Plant nutrition [4, 5]. Also reported that; Heavy metals change the size and shape of chloroplasts, photosynthesis and the ratio of transpiration and tissue structure, and also cause lipid peroxidation, damage the function of stomata, and affect the water balance of cells [6, 7]. Also, stated that increasing the concentration of cadmium reduces water absorption, reduces the production of photosynthetic pigments, carbohydrates and soluble sugars, and consequently reduces the height of the tomato plant, the number of leaves, and the dry weight of the plant [8-10].

Like other heavy metals, lead is a toxic metal for plants that is easily absorbed by the plant's root system, and at concentrations higher than 30μgr.grleaf, it prevents the activity of a number of enzymes due to ionic structural similarity of lead with calcium, that leads to a decrease in chlorophyll synthesis and a decrease in vegetative growth [1, 11]. Also, researchers reported that environmental stresses, by affecting the physiological characteristics of plants, cause the inactivation or severe reduction of the activity of the photosynthesizing apparatus [12, 13].

Another heavy metal is zinc, which in low concentrations is an essential element that stimulates plant growth. But in high concentrations and accumulation in soil and plant tissue, depending on the plant species, it causes destruction in the growth and development of plants [7, 14]. High accumulation of zinc in the cytosol of plant cells causes a decrease in the proper growth and development of plants by disrupting cell division and elongation, inhibiting the process of photosynthesis and increasing lipid peroxidation [7, 15, 16]. Heavy metals also cause negative effects on protein synthesis, membrane stability and hormonal balance in plants, which reduce the content of chlorophyll and carotenoids [1, 17].

Another effect of Salt of heavy metals in the soil is high pH, that affects the absorption of nutrients in the soil by plants. Under conditions of high soil pH, the amount of essential elements and micronutrients in the soil decreases. By acidifying the soil (even locally), the availability of soil elements and micronutrients for the roots can be increased. The sulfur is known as the most abundant and cheapest acidifying substance and also as a required food element, cause increasing the ability to dissolve other elements and adjusting the pH of the soil. The sulfur, as the fourth most important element, is nutritionally as important as phosphorus and also plays a role in the formation of plant proteins as much as nitrogen in plants [18, 19]. Sulfur is an essential nutritional element for cysteine, methionine, glutathione induction, coenzyme A, structure of polysaccharides, vitamins, lipids, chlorophyll synthesis and cofactors. A significant increase in height of canola plant, dry matter production, branches, leaf chlorophyll content and leaf area index is the result of applying 61 kg/ha of sulfur [20].

Therefore, the application of sulfur can improve the growth due to increase in the solubility of nutrients and more nutrients can be absorbed, especially in alkaline and calcareous soils [21-23]. In a pot experiment, the application of sulfur decreased the pH of the soil and increased the availability of micro elements, then the dry matter yield of the plant increased [24]. By applying 40 mg.kg sulfur, the growth and biomass of wheat improved [25]. Kumar and Sidhu reported that lack of sulfur in the soil reduced the number of chloroplasts in the plant [26].

Also, sulfur plays an essential role in tolerance to stresses in plants because the sulfur is a part of many defense compounds such as glutathione, phytochelatins, glycosinolates and vitamins [27]. For example, sulfur has a positive role in mustard seedlings against cadmium toxicity by preventing the transfer of cadmium from the root to the aerial part [28, 29]. In fact, the protective role of sulfur in reducing the risk of heavy metals is related to participation and regulation in the expression of genes involved in the biosynthesis of glutathione and polyclathins, which regulate the oxidative state and chelate metals in the vacuole [30-32].

Sulfur inoculation with bacteria can increase the oxidation of sulfur and decrease the pH of the soil [33]. In the meantimes, Thiobacillus bacteria, which is a gram-negative, acidophilic and chemolithotroph bacteria, has the ability to convert energy from the oxidation of ferrous (Fe^{2+}) to ferric ions (Fe^{3+}) for growth and the reduction of sulfur elements or inorganic compounds of sulfur is converted to sulfate by using oxygen as an electron acceptor [34]. This bacterium has the ability to oxidize the sulfide element and produce sulfide-soluble acid that is useful for the dissolution of food elements. In addition, sulfur with bacteria indirectly increases the amount of antioxidant enzymes and osmolytes under stress conditions, which increase tolerance in plants under stress [35, 36]. Application of sulfur with Thiobacillus bacteria (sulfur oxidizing bacteria) in soil appropriately decreased soil pH and increased the solubility of heavy elements, that causes the elements to be removed from the reach of the roots and its absorption decreases, and on the other hand, It can increase the availability of other essential plant elements such as phosphorus [37-39].

Corn (*Zea mays* L.) is one of the most important cereals in the world due to its high production potential and its ability to adapt to different environments and its high grain nutritional value [40]. This plant is sensitive to heavy metal stress, and its growth and performance are reduced under heavy metal stress [40, 41]. Similar to other parts of the world, areas of Iran such as Zanjan are contaminated with heavy metals and can reduce growth, biomass production, and product quality by affecting the biochemical and physiological characteristics of plants. On the other hand, it is possible that biochemical and physiological characteristics can be changed by using sulfur-containing compounds in different concentrations and with bacteria on plants in field conditions and soil contaminated with heavy metals, which increases the resistance of plants against stress. Considering the importance of corn for seed and fodder production, in this research, the effect of different concentrations of sulfur-containing compounds with bacteria on growth, photosynthesis, and anatomical and physiological characteristics in corn plants under the conditions of field soil and soil contaminated with lead heavy metals and zinc will be investigated and also the possibility of reducing the risk of toxicity of these elements, increasing the growth compared to the control and the rate of absorption of these metals by the plant will be evaluated.

2. MATERIALS AND METHODS

The experiment was conducted in a greenhouse in the form of a factorial and complete random block design under controlled temperature conditions (22-28 degrees Celsius) with 50-60% humidity, 8 hours of darkness and 16 hours of light with natural lighting (10000 -12000lux) and Artificial light (9000 -10000 lux) was performed in the greenhouse of Zanjan University. The experiment was carried out on singlecross 704 corn plants (provided by the breeding research and seedling and seed production department) in field soil and contaminated soil collected from around the lead and zinc factory of Zanjan city. After analyzing the soil and determining the amount of soil elements, especially lead and zinc heavy metals (Table 1), one third of sand was added to the soil. Test treatments include: elemental sulfur (at three levels of 0.75, 1.25 and 2 g/kg of soil), sulfur with bacteria including; It will be elemental sulfur along with thiobacillus bacteria (at three levels of 1, 2 and 3 grams per kilogram of soil) and potassium sulfate (at three levels of 0.5, 1 and 1.5 grams per kilogram of soil). And the desired soils were mixed with the mentioned treatments for cultivation in pots, also, a treatment without the application of treatments was considered as a control and a treatment of contaminated soil without mentioned treatment.

Table 1. Physicochemical properties of the soil used in the experiment.

Characteristic	Unite	Value
Sand	%	32
Silt	%	29
Clay	%	39
Texture	–	Clay loam
EC	dsm-1	4
Ph	–	8
Total N	%	0.1
P	mgkg-1	36
K	mgkg-1	287
S	mgkg-1	10
Zn	mgkg-1	600
Pb	mgkg-1	150

Then the seeds were planted in pots with a height of 20 cm and a diameter of 15 cm. Eight seeds were placed in each pot at a depth of three to four centimeters, and after germinating and establishment, four plants were kept in each pot. Plants were sampled 30 days after applying the treatments (at the seven-leaf stage before stem growth) to measure traits.

Attributes to be evaluated include; The height of the plant was measured from the soil surface in the pots to the end of the main branch with a ruler in centimeters. The dry weight of the aerial organs (Biomass) and the dry weight of the root (Root Weight) of each plant, at the time of harvesting and after drying the plants in the oven, at a temperature of 75 degrees Celsius and for 48 hours, according to Gram was obtained. The chlorophyll index was measured with a chlorophyll meter (SPAD; Chlorophyll meter device), four times and every three days. The membrane stability index (Electrical Conductivity) was determined by measuring the electrical conductivity of the materials leaked from the leaf samples into double distilled water at temperatures of 40 and 100 degrees Celsius [42]. Proline concentration was determined using the Bates method and by absorption at a wavelength of 520 nm with a spectrophotometer [43]. The amount of soluble sugars was determined through Anthrone reagent and using the modified method of Sairam, et al. [44]. Absorption of heavy metals in the root tissue and aerial parts of plants treated in soil contaminated with lead and zinc was used separately with an atomic absorption device, Perkin-Elmer model 2380, and it was in terms of mg/kg of dry weight [45]. Transfer factor (TF) is defined as the concentration of metal in the aerial part of the plant divided by the concentration of the same metal in the root of the plant, and the Accumulation factor (AF) includes the concentration of heavy metal in the plant root divided by the concentration of the same metal in the soil. The amount of metals obtained in this part was analyzed separately.

Statistical assays were carried out by SPSS (Statistical Package for the Social Sciences) statistical software. Mean difference comparison between different treatments was done by ANOVA using the Duncan's multiple range test (DMRT) at a 0.05 and 0.01 probability level. Excel 2003 software was used to draw the tables.

3. RESULTS AND DISCUSSION

The results of the analysis of variance showed that the values of the investigated traits in the conditions of field soil and soil contaminated with heavy metals under the applied treatments were significant compared to the control

(Tables 2 and 3). In the absorption of metals by the plant, the results of the average comparison showed that the absorption rate of these metals in contaminated soil without treatment increased to a critical and harmful level in plants, but with the application of treatments, the absorption rate of these metals decreased, which in elemental sulfur treatments and Potassium sulfate decreased absorption more (Table 4).The results of mean comparisons also showed that the assessed traits in field soil conditions increased with sulfur along with bacteria treatment compared to other treatments of elemental sulfur and potassium sulfate as well as the control, Also the assessed traits decreased by applying concentrations of 3 grams of elemental sulfur and 1.5 grams of potassium sulfate and caused stress in plants (Table 5). In the condition of contaminated soil, the examined traits increased in the treatment of sulfur along with bacteria, and in other treatments, elemental sulfur at a concentration of 3 grams and potassium sulfate at a concentration of 1.5 grams and contaminated soil without treatment showed a sharp decrease (Table 5).

According to the obtained results (Table 4), the amount of absorption of lead and zinc heavy metals in contaminated soil without treatment in the root tissue and aerial part of the plant compared to the control was critical and harmful, It is consistent with the results of research [5]. In the root tissue, the amount of lead absorption was 22.8 mg/kg dry weight and the metal absorption of zinc was 53 mg/kg dry weight. In the aerial part of the plant, the amount of lead absorption was 13.6 mg/kg dry weight and the absorption of zinc metal was 90.7 mg/kg dry weight was observed. It is consistent with the results of other researchers [46, 47]. By applying the treatments, the amount of absorption of both lead and zinc metals in both the root tissue and the aerial part of the plant decreased compared to the control until normal plant growth conditions. The reduction of lead metal absorption in roots under potassium sulfate treatment was more than other applied treatments, but sulfur treatment along with bacteria decreased the amount of lead metal absorption in the aerial part compared to the control and other treatments by 2 mg/kg dry weight (Table 4). Elemental sulfur decreased the absorption of zinc metal in the root tissue with 11.13 mg/kg dry weight, had the lowest amount of zinc metal absorption compared to the control and other treatments. In the aerial part of the plant, the rate of absorption of zinc metal in potassium sulfate and sulfur treatments with bacteria was observed as 47.47 and 50.9 mg/kg dry weight, respectively, and it decreased compared to the control and other treatments Table 4.

The average comparison results showed (Table 5) that sulfur treatment with Thiobacillus bacteria at the level of three grams increased the amount of all measured chlorophyll indices compared to the control. However, this index decreased with the increase in the concentration of treatments in elemental sulfur treatments at the level of two grams and potassium sulfate at the level of 1.5 grams, as well aschlorophyll index decreased in contaminated soil compared to the control, which is in line with the studies obtained by other researchers [48]. With the application of treatments, the reduction of chlorophyll index in contaminated soil was improved. Sulfur along with bacteria treatment had the highest chlorophyll index at all levels compared to other treatments and even compared to the control (Table 5).

Table 2. Variance analyze effect of Sulfur on corn plant under heavy metals.

S.O.V	Df	MS							
		Plumb shoot	Plumb root	Transfer factor of plumb	Acumlate factor of plumb	Zinc shoot	Zinc root	Transfer factor of zinc	Acumlate factor of zinc
Repat	2	14.4 **	1.48 **	0.26*	0.003 **	278**	23.3 **	0 ns	0.001 **
Treatment	9	44.4 **	12.6 **	1.46 **	0.009 **	406**	263**	0.08 **	0.002 **
Error	18	0.00	0.02	0.06	0.00	0.18	0.00	0.00	0.00

Note: Treatment(so4= solphat kso4= potasium solphat biosolfure= tiobacilos&solphat); ** and ns: Significance at 5% of probability levels and non significant, respectively.

Table 3. Variance analyze effect of Sulfur on corn plant under heavy metals.

S.O.V	Df	MS									
		SPAD1	SPAD2	SPAD3	SPAD4	Height	Biomass	Weigh root	Electrical conductivity	Prolin	Soluble sugars
Repat	2	1.49 ns	3.63 ns	5.01 ns	0.005 ns	2.17 ns	0.17 ns	0.007 ns	25.1 ns	0.00 ns	0.00 ns
Soil	1	116**	171**	1.34 ns	57.9 **	265**	19.8 **	0.82 **	71.2 *	0.00 ns	0.003 **
Treatment	9	59.2 **	76.4 **	117**	76.6 **	100 **	4.13 **	0.13 **	42.6 ns	0.00 ns	0 ns
Soil * treatment	9	16.9 **	8.86 **	31**	26.7 **	58.5 **	1.56 **	0.02 **	42.7 ns	0.00 ns	0.001 *
Error	38	2.11	2.20	5.96	4.50	9.28	0.10	0.004	24.4	0.00	0.00

Note: Treatment(so4= solphat kso4= potasium solphat biosolfure= tiobacilos&solphat); * ,** and ns: Significance at 5% and 1% of probability levels and non significant, respectively.

Table 4. Mean comparison for the effects of Sulfur on corn plant under heavy metals.

Soil	Treatment	Plumb shoot(mg kg^{-1}Dw)	Plumb root(mg kg^{-1}Dw)	Transfer factor of plumb	Acumlate factor of plumb	Zinc shoot(mg kg^{-1}Dw)	Zinc root(mg kg^{-1}Dw)	Transfer factor of Zinc	Acumlate factor of Zinc
S1	0	13.6 a	22.8 a	0.59 e	0.32 a	90.7 a	53 a	0.58 a	0.20 a
S1	bs1	2.92 c	4.18 c	1.52 b	0.04 c	50.9 d	26 ab	0.52 a	0.11 e
S1	bs2	2.07 c	4.88 c	2.35 a	0.02 c	82 a	28 a	0.34 d	0.18 b
S1	bs3	3.38 c	4.17 c	1.27 bc	0.04 c	64.1 c	24 bc	0.38 c	0.14 d
S1	s1	9.28b	2.30 d	0.23 e	0.13 b	77.1 ab	11.1 f	0.14 g	0.174 bc
S1	s2	13 a	2.01 de	0.13 e	0.18 b	80.4 a	13.9 e	0.17 f	0.178 b
S1	s3	9.79 b	2.30 d	0.22 e	0.13 b	68.2 bc	14.4 d	0.21 e	0.15 cd
S1	ks1	4.02 c	1.33 e	0.31 e	0.05 c	62.3 c	14.2 d	0.06 h	0.13 d
S1	ks2	9.18 b	6.12 b	0.65 de	0.13 b	47.4 d	22 c	0.46 b	0.10 e
S1	ks3	5.12 c	4.86 c	0.91 cd	0.07 c	62.1 c	15.5 d	0.02 i	0.13 d

Note: S1= polluted soil, bs=biosolfour, s=sulfur, ks=potasiumsulfate, 0= Conteroled. letters; indicate differences between treatments.

Table 5. Mean comparison for the effects of Sulfur on corn plant under heavy metals.

Soil	Treatment	SPAD1	SPAD2	SPAD3	SPAD4	Height(cm)	Biomass(gr)	Weigh root(gr)	Electrical conductivity (%)	prolin (µm gr⁻¹Fw)	Soluble sugars (mg glucose gr⁻¹Fw)
S0	0	19.8 cd	21.2 d	12.9 cdef	15.6 bc	59.6 bc	2.45 de	0.55 d	83.9 abc	0.001 b	0.076 bcde
S0	bs1	21.1 cd	23.4 bcd	13.6 cd	14 cd	59.5 bc	2.86 bcde	0.65 bcd	82.6 abcd	0.001 b	0.072 cde
S0	bs2	22.2 ab	24.5 bc	14.7 c	15.1 bc	61.1 ab	3.21 b	0.76 b	85 ab	0.001 b	0.068 de
S0	Sbs3	26.8 a	29.1 a	19.3 b	19.7 a	65.8 a	4.46 a	0.98 a	87.1 a	0.001 b	0.05 e
S0	s1	20.3 cd	22.6 cd	12.8 cdef	13.2 cde	58.1 bcde	2.55 cde	0.61 cd	81.6 abcd	0.001 b	0.08 bcde
S0	s2	21.1 cd	23.4 bcd	13.6 cd	14 cd	58.8 bcd	3.06 bc	0.66 bcd	81.6 abcd	0.004 a	0.069 cde
S0	s3	14.1 e	16.4 fgh	6.64 hi	7.04 ghi	47.5 ij	0.36 i	0.35 e	75.6 bcd	0.001 b	0.07 bcde
S0	ks1	20.6 cd	22.9 bcd	13.1 cde	13.5 cd	58.5 bcd	2.59 cde	0.63 cd	82 abcd	0.001 b	0.07 bcde
S0	ks2	20.8 cd	23bcd	13.3 cde	13.7 cd	59.6 bc	3 bcd	0.66 bcd	81.7 abcd	0.001 b	0.067 de
S0	ks3	13.4 e	15.6 gh	5.91 i	6.31 i	43.3 j	0.39 i	0.35 e	75 cd	0.001 b	0.07 bcde
S1	0	14.7 e	14.7 h	8.95 defghi	8.46 fghi	52 fghi	0.83 ghi	0.34 e	73.4 d	0.001 b	0.071 cde
S1	bs1	20.1 cd	21 de	9.7 defghi	11.6 cdef	54.6 cdefg	1.03 fgh	0.31 e	77.2 abcd	0.001 b	0.08 bcde
S1	bs2	19.4 d	24.2 bc	21.2 ab	19.6 a	54.8 cdef	2.32 e	0.6 cd	82 abcd	0.001 b	0.073 bcde
S1	bs3	24.3 b	25.5 b	24.9 a	18.5 ab	59.1 bcd	2.37 e	0.67 bc	85.2 ab	0.001 b	0.101 ab
S1	s1	15.7 e	18 fg	8.41 fghi	8.5 fghi	53.3 defgh	1.41 fg	0.35 e	78.4 abcd	0.001 b	0.084 bcd
S1	s2	14.6 e	17.5 fgh	11.6 cdefg	9.6 efghi	53.6 defgh	1.48 f	0.34 e	73.5 d	0.001 b	0.11 a
S1	s3	18.4 d	16.6 fgh	10.7 cdefgh	10.8 defg	52.5 efghi	1.17 fgh	0.34 e	81.9 abcd	0.001 b	0.07 bcde
S1	ks1	15.8 e	17.4 fgh	7.61 ghi	6.7 hi	49 ghi	0.7 hi	0.28 e	80.8 abcd	0.001 b	0.07 bcde
S1	ks2	15.2 e	18.5 ef	11.3 cdefg	10.3 defgh	48.8 hi	1.2 fgh	0.34 e	80.7 abcd	0.001 b	0.09 abc
S1	ks3	14.3 e	14.8 h	8.7 efghi	8.63 fghi	52.1 fghi	0.91 fghi	0.28 e	81 abcd	0.001 b	0.07 bcde

Note: S0=farm soil, S1= polluted soil, bs=biosolfour, s=solfour, ks=potasium solfate, 0=Conteroled. letters; indicate differences between treatments.

The height of the plant also increased with the application of the treatments compared to the control (59.6 cm), that sulfur treatment with bacteria(65.8 cm) caused the most increase in height compared to the control and other treatments. Kumar, et al. [20] reported that sulfur causes a significant increase in plant height [20]. With increasing concentration of elemental sulfur and potassium sulfate, as well as in contaminated soil, the measured plant height decreased compared to the control. According to the researchers' report, the height of the plants decreased under environmental stress and heavy metals, which is consistent with the obtained results [10]. By applying sulfur treatment along with bacteria, the height reduction of plants grown in contaminated soil was compensated, The highest plant height in the contaminated soil compared to other treatments was in the treatment of sulfur along with bacteria at the level of three grams(59.1 cm) (Table 5).

According to Table 5, the amount of biomass and root weight of the control plant was 2.45 and 0.55 grams, respectively. By applying sulfur treatments, these traits increased by more than 90%. It increased more in sulfur treatment with bacteria, especially at the level of three grams, the amount of biomass and root weight of the plant was 4.46 and 0.98 grams, respectively. Observations by other researchers showed that sulfur increases the growth and accumulation of dry matter [25]. In the presence of heavy metals as well as, by increasing the concentration of elemental sulfur and sulfate-potassium treatments applied, root weight and biomass showed a decreased significantly. By applaying treatments under contaminated soil Conditions, the amount of plant biomass and root weight increased compared to the contaminated soil treatment, which was more in the sulfur treatment along with thiobacillus bacteria and was consistent with control treatment and normal conditions (Table 5).

The results of the mean comparison showed (Table 5) that the membrane stability index increased under the treatment of sulfur with inoculated bacteria compared to the control, at the level of three grams of this applied treatment (87.1%), it had the highest stability index compared to the control (83.9%). However, with increasing levels of elemental sulfur compounds and potassium sulfate, the stability of the membrane decreased compared to the control. Under contaminated soil conditions, membrane stability decreased by 73.4% compared to control. Amari, et al. [1] reported that heavy metals cause a decrease in water absorption, negative effects on transpiration and membrane stability in plants [1]. On the other hand, the application of treatments in contaminated soil increased the stability of the membrane compared to the control. The application of sulfur treatment along with bacteria at the level of three grams, caused increase in membrane stability index by 85.2%. The results obtained from other researchers also showed that sulfur improves plant growth, reduces the effects of stress, detoxification, and electrolyte leakage in the plant [49-51].

The amount of soluble sugars showed a decrease by applaying treatments of sulfur with bacteria, elemental sulfur at the level of 1.25 grams and potassium sulfate at the level of one gram compared to the control (Table 5). But application of elemental sulfur at the level of 0.75 grams had the highest amount of soluble sugars . In contaminated soil, the amount of soluble sugars decreased by 0.071 mg/g fresh weight compared to the control. The results obtained from researchers showed that heavy metal stress reduces the synthesis of carbohydrates and soluble sugars [9]. By applying treatments on plants under contaminated soil conditions the amount of soluble sugars increased compared to the control. By applying treatments of sulfur with bacteria at the level of three grams, elemental sulfur at the level of 1.25 grams, and potassium sulfate at the level of one gram, the amount of soluble sugars increased by 0.1, 0.11, and 0.09 mg/g of fresh weight, respectively (Table 5). According to the results of analysis of variance and the mean comparison there is no significant difference between the amount of proline of the applied treatments and the contaminated soil compared to the control (Tables 3 and 5).

The amount of absorption and critical accumulation of heavy elements lead and zinc in plants is in the range of 10-20 and 100-400 mg/kg dry weight, respectively [47, 52]. In this experiment, the absorption of these metals in the root and aerial part of the plant were in this range and caused a crisis and reduced growth in the plant (Table 4). Research results have shown that these metals are absorbed by the roots of the plant and connectted to the outer parts of the root and cellular organs, due to this, its transmission is limited to aerial organs, as a result, the highest amount of accumulation is observed in the roots and the lowest amount is in the aerial part [46, 51, 53]. The results of this experiment showed the accumulation of lead metal was in the roots.

Plants that grow in the presence of heavy metals, by absorbing these unnecessary elements (heavy metals), their physiological traits such as membrane stability index and chlorophyll index are affected, and it causes the activation of the plant's enzymatic and non-enzymatic defense system and also causes the increase of plant osmolytes such as proline and soluble sugars and due to this, morphological traits such as height, biomass and root dry weight are also affected. And finally, the rate of growth and product will decrease. In this research, the presence of heavy metals in the plant growth environment caused a decrease in the physiological and morphological characteristics of the plant.

Oxidative stress created by heavy metals in plant cells by destroying the cell membrane and photosynthetic apparatus and preventing photosynthetic activity and ATP production as well as damage to DNA causes a decrease in height, biomass, dry weight and plant growth [11, 54].

In stressful conditions, plants increase balancing osmolytes such as proline and soluble sugars. The increase of soluble carbohydrates during stress can inhibit growth, destroy insoluble sugars and also produce these compounds from the non-photosynthetic pathway (increasing the activity of invertase and sucrose synthetase enzymes) which causes an increase in soluble sugars [55].

In order to improve the growth of plants and especially to reduce the effects of the created stress, the use of sulfur is one of the essential and nutritional elements of the plant and also a part of the essential compounds of the plant such as cysteine, which plays a role in the induction of glutathione, coenzyme A and chlorophyll synthesis. On the other hand, with the inoculation of soil by thiobacillus bacteria, the amount of sulfur oxidation, pH decrease and the amount of sulfate produced in the inoculated soil increases compared to the uninoculated soil. The oxidation and reduction of soil pH will cause absorption of elements such as phosphorus and micro elements by root increases and improves nutrition and physiological indicators in the plant and finally increases the amount of chlorophyll, nodules in the roots and yield. which is consistent with the results of this experiment [23, 56, 57].

Also, sulfur supports plants against stress and as a part of plant defense compounds includes glutathione, phytochelatins, glycosinolates and vitamins. which, by participating and regulating the expression of genes involved in the biosynthesis of glutathione and polyclathins, regulate the oxidative state and chelate metals in the vacuole, and as a result improve membrane stability and other physiological indicators in the plants. On the other hand, sulfur reduces the transfer of heavy elements through the roots to the aerial part of the plant, which reduces the toxicity of these metals in the plant. Sulfur along with the inoculation of thiobacillus bacteria under contaminated soil conditions by creating oxidizing conditions and reducing the pH, increases the solubility of elements in the soil and causes the leaching of heavy elements in the soil and removes the heavy metals from the accessible of the roots and also by dissolving most of the essential elements will increase the absorption of these elements. In addition, it has been reported that plant roots reduced absorption of heavy metals due to sensitivity of the roots to lead and cadmium heavy metals [58]. Here also with more solubility of elements in the soil, heavy metals increase in the root environment, and as a result, it seems that it causes the roots to be more sensitive to these elements, and finally, their absorption rate decreases. In this experiment, the sulfur element along with bacteria improved the physiological indicators in the plant, increased the height, biomass index and improved plant growth. And also under contaminated soil conditions, the sulfur element along with bacteria improved the growth indicators.

4. CONCLUSION

Sulfur is one of the important compounds of the plant and also in appropriate concentrations can improve the availability of water and minerals for the plant, which improve the plant growth. In stressful conditions, Sulfur with bacteria by dissolving elements in the soil causes the leaching of heavy metals and increases the absorption of essential plant elements, and ultimately causes the plant to bear the stress and increase the growth of the plant.

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