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**ORIGINAL ARTICLE**

# Effect of Sulfur Application on Growth, Photosynthetic Pigments, Antioxidant Activity and Arsenic Accumulation in Coriander (*Coriandrum sativum*) under Arsenic Stress

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**KEYWORDS**

Ammonium sulfate;
Arsenic nitrate;
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**ABSTRACT:** Soils polluted with toxic elements are one of the major environmental problems in human societies. Sulfur (S), an essential element for the growth and development plants, plays an important role in reducing the toxicity of toxic elements as arsenic. In this study, the role of Sulfur different regimes (0, 50, 100 and 150 mg per kg) in reducing arsenic (As) toxicity in coriander (*Coriandrum sativum*) was investigated. The obtained results indicated that Sulfur application increased the activities of antioxidant enzymes and photosynthetic pigments, but it's decreased the arsenic induced oxidative stress. Reduction of shoot and root biomass occurred in presence of sulfur different regimes and As various concentrations. S supplement under high As concentration increased protein content of shoot. Different S regimes resulted in enhanced both shoot and root As accumulation. Meanwhile, different treatments of sulfur allowed high translocation of As quantities from root to shoot. It is well illustrated that phytoextraction is one of the best methods for toxic metals phytoremediation. Thus from present study it is evident that the phytoremediation ability of plants for accumulates toxic metals may be enhanced through exogenous sulfur application.

## INTRODUCTION

Today, various pollutants such as heavy metals can threaten human life and the environment [1]. Although heavy metals are naturally present in the soil, but due to human activities such as the use of pesticides and fertilizers in agriculture, sediment sludge and the disposal of urban waste, the concentration of these elements has increased in the environment, increasingly; So they have been harmful for various organisms [2, 3]. It has been reported that the decline of plants growth in soils contaminated with heavy metals is the result of a change in the behavior of physiological and biochemical reactions of plants that grow on such soils [4, 5].

Continued decline in plant growth can lead to reduced yield and thus an increase in food shortages worldwide. Heavy metals have metal properties such as flexibility, conductivity and cationic stability. They are known for their relatively high atomic weight and density (3). Some heavy metals such as cobalt, manganese and nickel are required by living organisms in very low quantities, but excessive presence can be detrimental to the organisms. But

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some heavy metals such as lead, mercury and arsenic are even harmful at low levels and do not have any benefits to plants and animals [6].

The effect of heavy metal toxicity on growth of various plants varies with the type of heavy metal (Table 1). For metals such as Pb, Cd, Hg and As, which play no role in plant growth, undesirable effects have been recorded at very low concentrations of these metals in the growth media. The ability of plants to absorb the essential metal elements enables them to absorb unnecessary metal elements. Due to the fact that these metals are not broken, if they continue to accumulate, excessive amounts of them will directly and indirectly damage the plants [7].

**Table 1.** Heavy metal effects on some plants

| Heavy metal | Plant | Toxic effect on plant | Reference |
|---|---|---|---|
| As | Tomato | Reduction in fruit yield; diminution in fresh leaf weight | 8 |
| Cd | Wheat | Reduced seed germination; reduction in content of plant nutrient; diminution in length of shoot and root | 9 |
| Co | Tomato | Reduced plant nutrient content | 10 |
| Cr | Wheat | Reduction in shoot and root growth | 11 |
| Cu | Rhodes grass | Reduction of root growth | 12 |
| Hg | Tomato | Reduced germination percentage and plant height and flowering & fruit weight and chlorosis | 13 |
| Mn | Tomato | Grow slower plant; decline in chlorophyll Concentration | 14 |
| Ni | Wheat | Reduced plant nutrient acquisition | 15 |
| Pb | Oat | Inhibition of enzyme activity that has an effect on the sustainability of $CO_2$ | 16 |
| Zn | Pea | Reduced chlorophyll content; Changes in chloroplast structure decreased photosystem II activity; reduced plant growth | 17 |

Including the direct damages can be pointed out to the inhibitory activity of enzymes and the destruction of the enzymes structure through oxidative stresses [18, 19]. Heavy metals can replace the essential ionic elements in plants, indirectly [20]. Also, by affecting the activity microorganisms in the soil, they can decrease the decomposition of organic matter and thus decreases plant growth [21]. Meanwhile, soil contamination with arsenic has become an important issue to government and industry because there are more health problems with arsenic around the world [22].

There are various biological and non- biological methods for refinement the soils contaminated by heavy metals; the bioremediation method is particular importance. One of the aspects of bioremediation is the use of green plants to clean up and reduce the contamination of heavy metal contaminated areas, which is called a phytoremediation [23, 24]. Phytoremediation the contaminated soils to heavy metal will be done in three ways: phytoextraction, phytostabilization, and phytovolatilization.

In the meantime, the most common form of phytoremediation is phytoextraction. In this method, they use the plants for refinement the contaminated soils that can accumulate heavy metals in both the root and shoot. Finally, they are harvested and burned at the end of the growing season. Plants such as Indian mustard, sunflower, rye, corn, and coriander have the ability to reduce heavy metal pollution. In phytoremediation method bioavailability of heavy metal elements is important. One of the major methods for increasing the bioavailability of heavy metals in the soil and plants is the reduction of soil pH [25].

Considering that pH reduction by organic or inorganic acids or acids produced by chemical fertilizers, including ammonium chloride, has negative effects on the environment; the use of sulfur as a soil pH reducing agent and thus increasing the solubility of heavy metals in the soil has been raised [26, 27]. On the other, the application of sulfur as a biological solution without harmful environmental effects has been considered as a way to improve the nutritional status of plants.

Therefore, the purpose of this study was to investigate the role of sulfur in increasing the absorption of arsenic by coriander (*Coriandrum sativum*) and study the effect of As on dry matter and biochemical parameters as catalase and peroxidase enzymes, protein and photosynthetic pigments

## MATERIALS AND METHODS

### *Preparation of seedlings*

Seeds of coriander (*Coriandrum sativum*) were sterilized in 10% $H_2O_2$ (v/v) for 10 min followed by thorough washing in de-ionized water, and then germinated on transplantation tray for 25 d. The uniforms seedlings at the 3-4 leaf stage were removed from the peat soils. They were then transferred to PVC pots containing 1000 g soil. Each pot contained 6 plants. The seedlings were grown in a greenhouse with 11/13 h light/dark cycles. The temperature was maintained at 25 ± 0. 5ºC.

### *Treatments*

After pre-treatment in soil for compatibility the plants, different concentrations of arsenic nitrate (0, 60 and 120 mg per kg) and ammonium sulfate (0, 50, 100 and 150 mg per kg) was added to soil. Experiment was performed in randomized complete block design with 3 replications. After 30 d of treatment, the necessary measures were assessed. To determine the dry weight of the plant samples, initially they were placed for 72 h in the oven at 70°C, and then the samples were weighed on scales 0. 0001.

### *As content*

The amount of As was determined by fresh ash method [28]. Then, arsenic content in the coriander shoots and roots was determined by atomic absorption spectrometry using ICP device (JVC, Integra-XL, Sequntion). Finally, As concentration expressed by mg per kg of plant dry weight.

### *Photosynthetic pigments measurement*

To measure Photosynthetic pigments, some leaf tissue was homogenized with 80% acetone. After centrifugation, extract absorption read with spectrophotometer (UV.2100 pc) in a wavelength of 646, 663 and 470 nm [29].
The concentration of the pigment was calculated using the following equations:

### *Protein concentration*

In order to measure the protein concentration 100 µl of protein extract and 3 ml of Bradford reagent 25% were added to the test tubes and rapidly vortexed, and then their absorb read by using UV.2100.PC spectrophotometer at 595 nm. For control, a mixture of 100 µl potassium phosphate buffer and 3 ml of Bradford reagent was used [30].

### *Peroxidase (POD) activity*

Peroxidase (POD) activity was assayed according to the method of Plewa *et al*. [31] and based on the amount of tetraguaiacol absorbed after formation by oxidation of guaiacol catalyzed by this enzyme in 1 min at a wavelength of 420 nm.

### *Catalase (CAD) activity*

Catalase (CAD) activity was measured according to the method of Dhindsa *et al*. [32] and based on the enzyme's capability for degrading H2O2 in 1 min at a wavelength of 240 nm.

### *Statistical analysis*

Data means were used for Duncan’s multiple range tests after that one ways analysis of variance (ANOVA) with a significance level of 0. 05 were used for analyses of data with data were statistically evaluated by Duncan’s multiple range test. All statistical analyses were carried out using the SPSS 23 statistical software.

## RESULTS AND DISCUSSION

Sulfur can interact with arsenic in various environmental conditions, from biogeochemical level to biological level [33]. As stress hampered coriander plant growth and sulfur could not have rehabilitated the growth in As high

treatment, especially. There was a significant effect of sulfur application on the dry weight of coriander when no As was applied; dry weight significantly decreased in all treatments of S50, S100 and S150 at As0 in both shoot and root (Figure 1). On the other, when As was applied at 60 and 120 mg/kg, sulfur application to the coriander significantly decreased the dry weight in both shoot and root, especially in the S150 treatment. The highest decrease in dry weight of coriander was observed in high As (120 mg/kg) stress and S150 application in both shoot and root. Also, there was 56.5% and 38 % decrease in dry weight of shoot and root, respectively, in the As treatment (120 mg /kg) compared with control when no sulfur was applied.

It was observed that root and shoot biomass decreased in all As treatments [34]. It was shown in studies that the interaction of heavy metals with sulfhydryl groups and the deactivation of plant proteins prevents root and shoot growth [35]. Also, reduced growth through heavy metal toxicity could be due to increased production of reactive oxygen species, including hydrogen peroxide, in photosynthetic optical reactions [36]. In one experiment the biomass yield as well as grain yield of rapeseed was significantly decreased under high As (120 mg/kg) stress; it has been showed that there was no significant effect of sulfur application on the rapeseed dry matter when no As was applied [37]. Reports indicated that the amount of both root and shoot dry weight decreased with increasing cadmium intake; also, increase in sulfur consumption caused a significant decrease in dry weight of shoot and root [38]. A study has reported that greater reduction of root biomass occurred in presence of low sulfur compared of high sulfur under As stress [33].

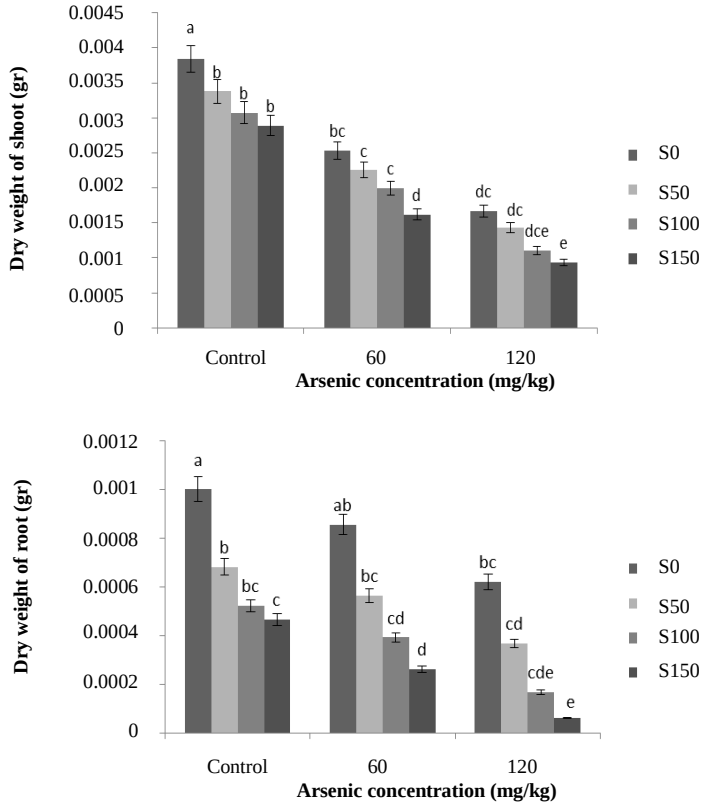
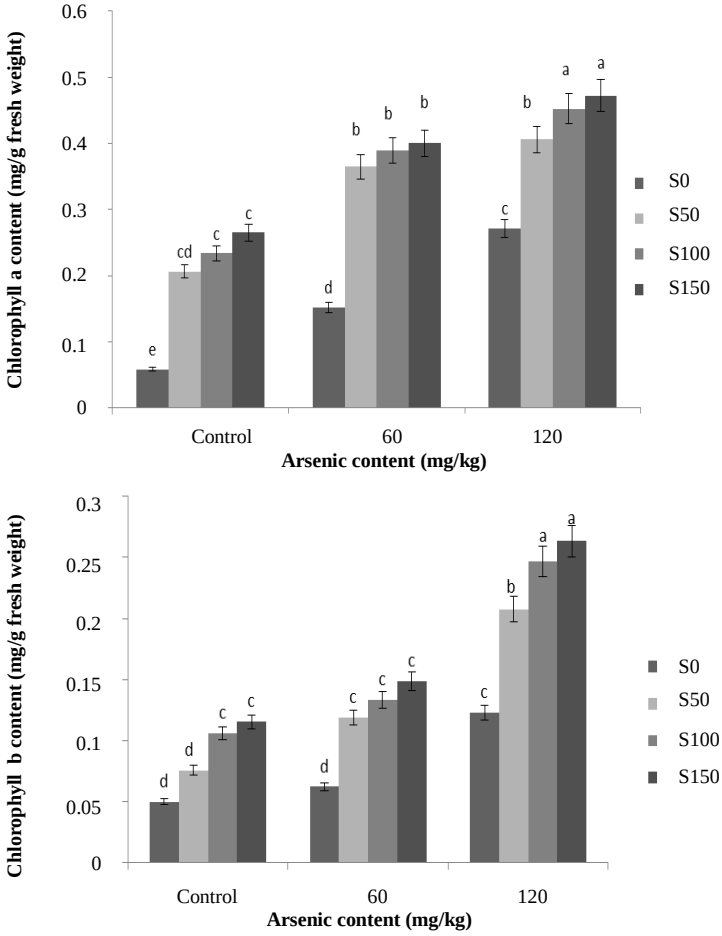



**Figure 1.** The effect of different levels of arsenic and sulfur on the dry weight of shoot and roots. Values are mean of four replicates ± SD. Different letters represent a significant difference between treatments ($P < 0.05$).

The analysis of variance demonstrated that As stress and sulfur application had significant effect on Photosynthetic pigments pigments (Ch-a, Ch-b and carotenoids) of coriander (Figure 2). The most content of photosynthetic pigments was obtained under high As (120 mg/kg) stress and S150 application. There was no significant effect of sulfur application on the carotenoids content when no As was applied. However, when As was applied, sulfur application to the coriander significantly increased the photosynthetic pigments, especially at 150 mg/kg treatment. It was observed that S application increased chlorophyll a, chlorophyll b and total leaf chlorophyll content at all stages of the Mungbean [39]. It has been reported that Chl A and Chl B of *Panax notoginseng* increased at low As concentration; but carotenoid content decreased in all As treatments [34].

The pigment composition and photosynthesis of *Hydrilla verticillata* analyzed under As stress and it was found that the content of chlorophylls and carotenoids decrease [40]. In this study, it seems that increase in chlorophylls and carotenoids may be only correlate to don't effect of As toxicity on the activity of enzymes involved in photosynthetic metabolism of coriander. Hence, it seems that S treatments prevented of destroyed systems of chloroplasts [41]. Exposure to arsenic can cause changes in proteins and chloroplastic enzymes that may affect the photosynthetic efficiency of plants [40].



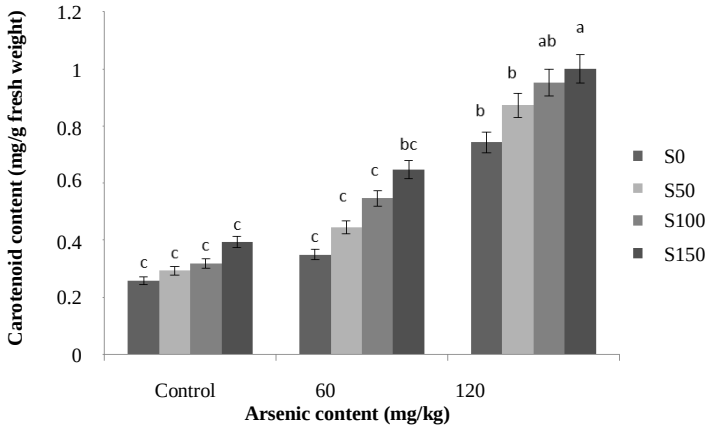



**Figure 2.** The effect of different levels of arsenic and sulfur on the amount of chlorophyll a, chlorophyll b and carotenoids. Values are mean of four replicates ± SD. Different letters represent a significant difference between treatments ($P < 0.05$).

The soluble protein amounts in the coriander decreased by 28% and 8% at As 60 and As 120, respectively, when no S applied. Different S regimes induced increase in soluble protein amount at As 120 (Figure 3). In As 60 and in the present of different concentrations of sulfur, the protein amount decreased significantly; so that in treatment of As60 and S150, we obtained the lowest amount of protein (0.7 mg/g fresh weight of plant). By increasing the amount of arsenic to 120 mg per kg of soil (As120), the protein amount was increased, as in the presence of S150 the highest protein content was measured (2.4 mg/g fresh weight of plant). It has been reported of negative effects of arsenic concentration on soluble protein [42]. According to previous studies [43], protein degradation is in fact the correlation of cells with sugar deficiency. The literature demonstrated that arsenic treatment could induce changes in various Protein and AA composition because of its role for chelation of metal and cellular homeostasis [44].

In similar study in leaves of rice plant, a number of proteins were differentially expressed among different S treatments [45].Similarly, interaction of high sulfur+As treatment resulted in higher Cys and Met levels and related enzymes in aquatic plant Hydrilla [46]. Also, it has been reported that higher level of Glutathione (GSH) could be resulted in present of high sulfur+As treatment [47, 48]. GSH is requiring in processes of detoxification, and it can be play a role as an enzyme cofactor [49]. GSH can bond with metals or metalloid that would be helpful in preventing oxidative stress [44].

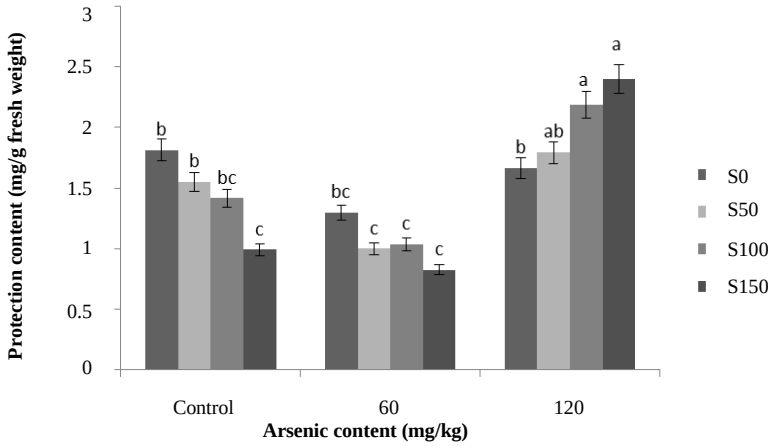



**Figure 3.** Effects of various levels of arsenic and sulfur on the amount of protein. Values are mean of four replicates ± SD. Different letters represent a significant difference between treatments ($P < 0.05$).

In study present, antioxidant enzymes activities of catalase (CAT) and guaiacol Peroxidase (POD) increased by 60 and 120 mg $kg^{-1}$ As concentrations compared with the control. Compared to the control, POD and CAT activities were a significant 179.16 % and 254.85% higher, respectively, in the 120 mg / $kg^{-1}$ As treatment. Different substances act as protective systems against reactive oxygen species, including peroxidases, catalase and superoxide dismutase [50]. On the other hand, peroxidases protect cells from heavy metal stress [51]. Different regimes of sulfur could increase, significantly, the activity of enzymes in the presence of arsenic concentrations (Figure 4). The highest amount of both CAT and POD enzymes was obtained in As120 and S150 treatment. Therefore, it can be concluded that the activity of enzymes has been excited by S treatment. It has been observed that sulfur application at the higher doses had a significant positive effect on activities of catalase, ascorbate peroxidase, guaiacol peroxidase of *vigna radiata* [39]. In a report, it has been shown that catalase and glutathione- S-transition in corn were all triggered by exposure to arsenic [52]. The literature demonstrated that activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) of *Panax notoginseng* increased under As stress [53]. We noted that arsenic causes oxidative damage to plants and increases the content of peroxidase and catalase. These observations agree with reports of previous studies [50, 53] proposing an increase in peroxidase and catalase content were correlated with As concentrations.

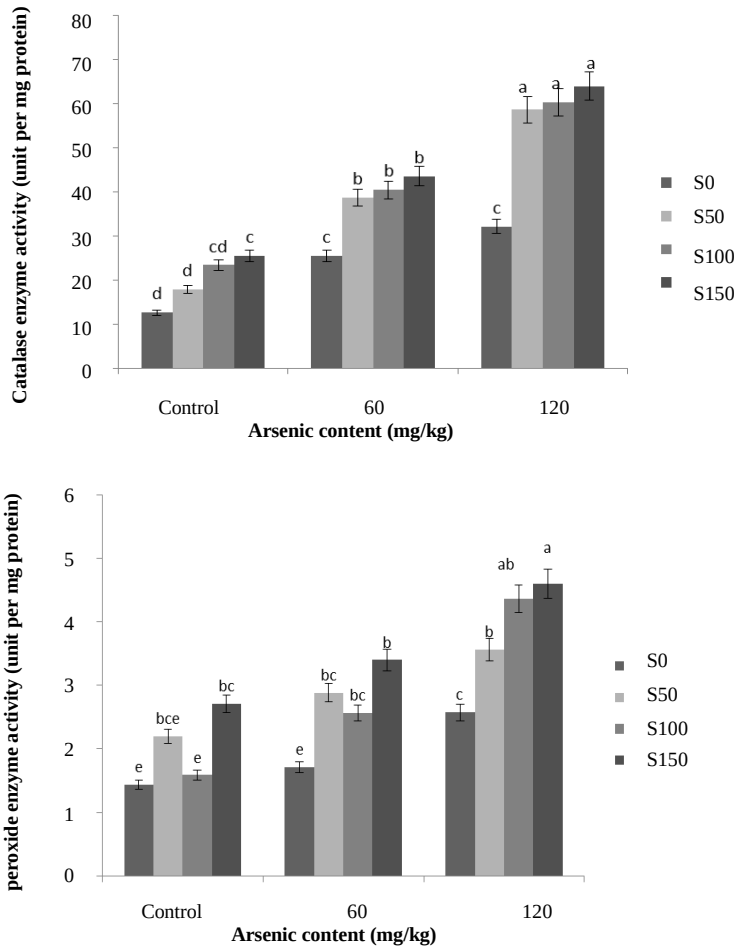



**Figure 4.** Effect of different levels of arsenic and sulfur on the amount of guaiac peroxidase and catalase enzymes. Values are mean of four replicates ± SD. Different letters represent a significant difference between treatments ($P < 0.05$).

The results presented here show that by increasing the concentration of arsenic, the amount of As increased in both shoot and root of coriander plant, significantly (Figure 5). It has been showed that arsenic accumulation in the *Hydrilla verticillata* plants increased by increment of arsenic concentration [40]. Sulfur treatment could increase the arsenic absorption in plant. So the highest amount of arsenic was obtained in S150 treatment for both root and shoot. Similarly, increasing sulfur concentration resulted in increased root As accumulation of rice, but As content of shoot decreased [33]. Also, in the present study was showed that the ratio of arsenic accumulation in the shoot was higher than the root, perhaps due to lesser chelation of As by thiols in root which cause more As quantities to be transferred to shoot [33]. Several other studies also demonstrated differential transport of metals from soil to plant tissues [54, 55].

The use of sulfur element can increase the sulfur concentration in plant tissues [56]. Positive application of sulfur on N content of shoots in many legumes and non-legume crops has already been demonstrated [57, 58]. In another study, the active content of iron in leaves significantly increases with different levels of sulfur [39]. One report indicated that high sulfur has enhanced the As accumulation in root while decreased in shoot of *Oryza sativa* [59]. In the present study, arsenic increased the accumulation of sulfur in both root and shoot compared to plants that are not exposed to As. The accumulation of sulfur in root and shoot was related to the amount of sulfur in the soil. The accumulation of sulfur at the root and shoot was positively associated with the exposure of arsenic. This result which is in agreement with previous studies [59].

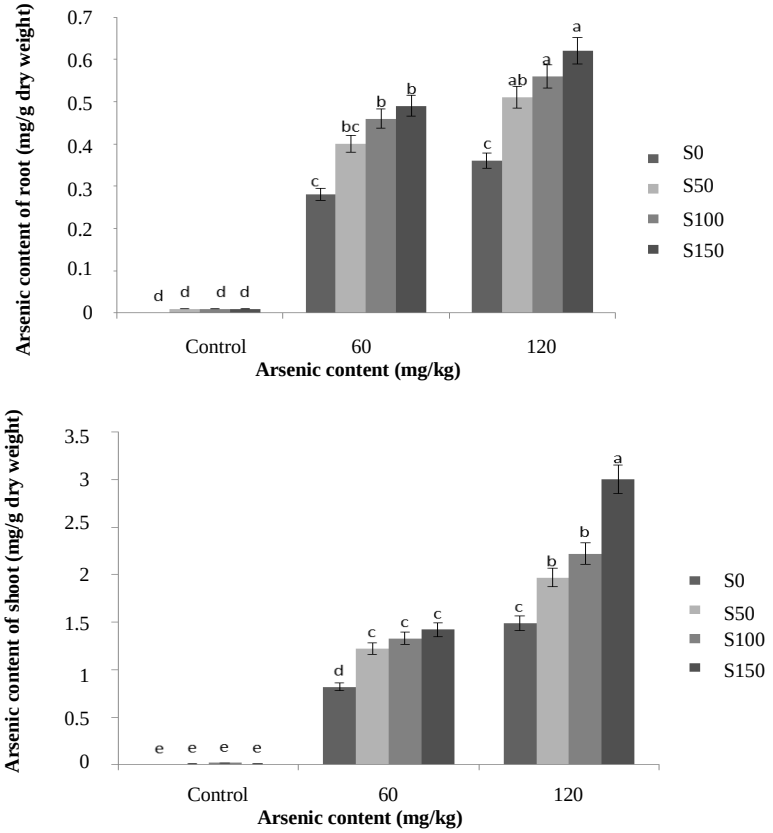



**Figure 5.** Effect of different levels of arsenic and sulfur on the amount of arsenic accumulation in both shoot and root. Values are mean of four replicates ± SD. Different letters represent a significant difference between treatments ($P < 0.05$).

## CONCLUSIONS

Parameters of biochemical and vegetative in coriander specie were influenced by increasing arsenic and sulfur concentrations in the growth medium. However, shoot and root dry weight appeared to be more susceptible in the studied coriander specie in response to As contamination. These results suggest that the adverse effects of As stress can induce antioxidant defense activity in plants to remove the possible toxic effects of free radicals, making the plants more resistant to toxic elements stress. Present study clearly showed that sulfur supplement resulted in As accumulation in coriander plant. But sulfur treatment allowed high translocation of As from root to shoot. Hence, in coriander plant, exposure to sulfur was found to be effective in increasing their tolerance to oxidative stress and accumulation ability for As. This study is well illustrated phytoextraction is one of the best methods for toxic metal phytoremediation. Therefore, the phytoremediation ability of plants for accumulates toxic metals may be enhanced through exogenous sulfur application.

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