Agrotechniques in Industrial Crops

Journal Homepage: https://atic.razi.ac.ir

Investigation of the Effect of Arsenic Pollution in the Soil of Some Regions of Kerman on Physiological Characteristics of *Pistacia atlantica* L. and *Medicago sativa* L.

Simin Yazdanpanah-Ravari[1], Hossein Heidari Sharifabad[*2], Hossein Abbaspour[3], Alireza Iranbakhsh[1]

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[3]*Department of Biology, North Tehran Branch, Islamic Azad University, Tehran, Iran*

ARTICLE INFO

Original paper

Article history:
Received: 7 Jul 2022
Revised: 18 Aug 2022
Accepted: 26 Sep 2022

Keywords:
Photosynthetic pigments
Heavy metals
Flavonoids
Soil pollution
Arsenic content

ABSTRACT

Heavy metal poisoning and its accumulation in food chains are one of modern societies' main bioenvironmental and health problems. This study was conducted to investigate the effect of arsenic contamination in soil on the physiological characteristics of pistachio and alfalfa in the form of a completely randomized design. Plant and soil sampling was done in 8 regions of the Kerman province. The results related to the pigments showed that they were influenced in both pistachio and alfalfa by the region. The highest levels of chlorophyll a, chlorophyll b, and total-chl in the alfalfa plant were related to the Shahr-e-Babak region where a similar result was seen for pistachio. chl a (alfalfa (28.50±0.37), pistachio (30.33±0.32)), Chl b ((Alfalfa: 0.13±0.23), pistachio (9.10±0.11)), total-chl (Alfalfa (8.63±0.38 (pistachio: 39.43±0.17)). The results of the regression trend of intra-tissue and peripheral arsenic changes also showed that the amount of peripheral arsenic was able to account for a higher percentage of changes in chl an in both plants and the changes model of chl due to changes in peripheral arsenic was as the second degree. But, the amount of chl b was affected by the amount of intera-tissue arsenic. The highest amount of flavonoid in pistachio was observed in Shahr-e-Babak and Bayaz regions (alfalfa (71.50±0.65), pistachio (74.50±1.32)) and the highest amount of carotenoid was obtained from Shahr-e-Babak, Bayaz, and Anar in alfalfa and pistachio. The amount of alfalfa flavonoid was more affected by intra-tissue arsenic, while in pistachio, the peripheral arsenic had more impact on flavonoids than intra-tissue arsenic. The highest amount of total carbohydrates in alfalfa and pistachios was observed from Shahr-e-Babak and Zarand regions, while the highest amount of protein was observed from Anar, Bayaz, and Kabutar-khan regions. In general, alfalfa is a more arsenic-accumulating plant, shows better resistance to it, and is less affected.

DOI: 10.22126/ATIC.2022.8289.1064

1. Introduction

Increasing soil pollution due to heavy metals because of agricultural and industrial activities has become a serious environmental problem in today's world and caused concern around the globe (Alavi *et al.*, 2022; Khan *et al.*, 2016; Shaban *et al.*, 2015). Heavy metal poisoning and its accumulation in food chains are one of the main environmental and health problems of modern societies (Rasouli *et al.*, 2020; Rizwan *et al.*, 2016). Although arsenic is found in a variety of soils, it usually occurs in a small quantity in most soils (Gupta *et al.*, 2017). The concentration of arsenic in natural soils around the world is about 5 mg/kg, and can vary depending on the source of the soil (Mitra *et al.*, 2017).

According to some reports, the arsenic content in agricultural soils has increased significantly in recent decades (Gupta *et al.*, 2017). Its levels have been significantly increased by industrial activities such as melting heavy metals, coal fuel, glass production, and the current use of arsenic compounds in the production of fertilizers, pesticides, desiccants, and growth enhancers for poultry and other animals (Mitra *et al.*, 2017). Increasing the amount of arsenic is seriously worrying for humans and the environment (Mitra *et al.*,

* Corresponding author.
E-mail address: h.heidari1871@gmail.com

2017). Threats and dangers of arsenic to animal and plant lives, as well as human health, are exacerbated by its long-term presence in the biosphere (Gupta *et al.*, 2017).

The US Environmental Protection Agency (EPA) has classified arsenic as one of the most harmful elements in nature because it can cause countless serious diseases and disorders (Gupta *et al.*, 2017; Mitra *et al.*, 2017). In addition, the transfer of arsenic from soil to crops is a serious issue that causes significant human exposure (Rahman and Hassler, 2014). Researchers have reported that arsenic may be absorbed by plants and eventually passes into human food (Liu *et al.*, 2014). It is obvious that arsenic at high concentrations inhibits the main phenomena of plant metabolism (Gupta *et al.*, 2017; Mitra *et al.*, 2017).

The toxic effects of arsenic cause physiological changes in plants, including chlorophyll depletion, transpiration, and photosynthetic capacity. It also causes chlorosis, dry and yellow leaves, and reduces reproductive capacity and yield (Liu *et al.*, 2014). It has also been reported that major parts of plant roots are exposed to accumulation of arsenic, which their propagation may directly be affected. It is mostly due to displacement in plants, with reduced accumulation from the roots to parts above the ground (Pigna *et al.*, 2010). The most important causes of heavy metal toxicity are oxidative stress, dysfunction of pigments and changes in the activity of proteins (Rastgoo *et al.*, 2014). Arsenic can inhibit plant growth and also cause metabolic and physiological disorders (Kohli *et al.*, 2019). In an experiment, the effect of cadmium on alfalfa was investigated by another researcher (Mahmood *et al.*, 2014). That the results showed that the growth, photosynthesis and content of photosynthetic pigments (chl a, b and total) were remarkably decreased by the presence of cadmium (90 and 120 μML^{-1}).

Pistachio (*Pistacia vera* L.) is a subtropical plant of the Anacardiaceae family and is one of the major non-oil export crops grown in arid and semi-arid regions. Pistachio is an important horticultural product. Iran is considered the main producer of pistachios. Evaluation of heavy metals in this export fruit is necessarily vital to protect public health (Karcı *et al.*, 2022; Malakhov and Islamgulova, 2021).

Alfalfa (*Medicago sativa* L.) is also an important forage crop that is widely cultivated in several parts of the world. In addition, the increasing demand for protein-rich raw materials for livestock increases the tendency to plant forage communities to support small ruminants such as sheep and goats (Kovács *et al.*, 2021). The issue of heavy metals pollution in alfalfa and pistachios can be important because these two plants are widely used in animal and human nutrition. Various studies have been performed on the effect of wastewater, solid waste and sludge on alfalfa growth and the accumulation of heavy metals in soil and their transfer to plants (Elfanssi *et al.*, 2018). However, few researchers have addressed the issue of the accumulation of heavy metals in alfalfa and pistachios in industrial and non-industrial regions (Abid *et al.*, 2016). Kerman province is located in the southeast of Iran and in a region with the development of various industries and mines. This province occupies 11% of Iran's territory. In this regard, the aim of our work was to evaluate the accumulation of the heavy metal arsenic of alfalfa and pistachios in agricultural soils.

2. Materials and methods

This experiment was carried out in 2021 in Kerman province (54º, 21' – 59º, 34' E and 26º, 29' – 31º, 58' N). First of all, surface soil, the soil around the roots and the irrigation site soil were sampled from eight regions of Sirjan, Shahr-e-Babak, Bayaz, Anar, Kabutar-khan, Kazem-Abad, Zarand and Ravar, all located in the south and east of the province. Then, the air-dried samples were passed through a 2 mm sieve for laboratory analysis.

2.1. Sampling of alfalfa and pistachios

Samples of alfalfa (Bam cultivar) and pistachio (Bam cultivar) were collected in the growth stage in July, 2021. Three farms from each region and three samples from each farm were collected. Each farm was divided into three parts. From each section, three $1m^2$ sites were randomly selected. All plants were collected from each location at a distance of 10 cm from the ground alfalfa with a knife and pistachio trees by hand. Then a 3 kg sample was taken from each site. Finally, three samples from each site were mixed and a 3kg sample was isolated from the total mixture (9 kg). Samples were sent to the laboratory to measure physiological traits and other chemical parameters in clean plastic bags.

2.2. Soil sampling

Seventy-two soil samples were taken from alfalfa and pistachio fields. At each stage, three soil samples were collected from each field (Table 1). Soil sampling was performed from a depth of 30 cm. Before that, the soil surface containing fertilizer and 5cm solids was crushed using a steel shovel. Then 10 kg of soil samples from each field was mixed in a plastic bucket. Then a 3 kg sample was isolated from the whole mixture (10 kg). The samples were then placed in a clean plastic bag and sent to the laboratory for further preparation.

Table 1. The details of soil samples were taken from alfalfa and pistachio fields.

	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	pH	EC (ds/m)	Total As (mg/kg)	P (mg/kg)
Sirjan	26.03 ± 2.95^{b}	32.07 ± 2.10^{bc}	39.07 ± 3.00^{c}	0.74 ± 0.03^{cd}	8.23 ± 0.15^{a}	1.02 ± 0.07^{c}	1023 ± 42^{bc}	12.83 ± 0.75^{e}
Shahr-e Babak	44.00 ± 3.50^{a}	39.10 ± 3.85^{a}	17.00 ± 1.70^{d}	0.56 ± 0.02^{f}	8.50 ± 0.21^{a}	0.58 ± 0.04^{e}	1200 ± 62^{a}	21.23 ± 0.70^{a}
Bayaz	28.00 ± 3.00^{b}	36.00 ± 1.51^{ab}	36.00 ± 3.00^{c}	0.78 ± 0.04^{bc}	8.47 ± 0.15^{a}	0.71 ± 0.04^{d}	1055 ± 44^{b}	18.43 ± 0.45^{b}
Anar	26.03 ± 2.45^{b}	37.00 ± 2.03^{a}	36.00 ± 2.60^{c}	0.97 ± 0.05^{a}	8.53 ± 0.25^{a}	0.52 ± 0.03^{e}	998 ± 36^{bc}	19.27 ± 0.65^{b}
Kabootarkhan	24.07 ± 1.90^{b}	29.07 ± 2.91^{c}	46.03 ± 2.93^{b}	0.71 ± 0.04^{de}	8.20 ± 0.21^{a}	0.69 ± 0.04^{d}	989 ± 27^{c}	15.37 ± 0.43^{d}
Kazem Abad	27.03 ± 1.95^{b}	39.03 ± 2.92^{a}	35.07 ± 2.91^{c}	0.81 ± 0.03^{b}	8.27 ± 0.14^{a}	0.77 ± 0.03^{d}	551 ± 27^{d}	17.23 ± 0.57^{c}
Zarand	14.07 ± 1.41^{c}	22.07 ± 1.89^{d}	62.07 ± 2.93^{a}	0.68 ± 0.04^{e}	8.33 ± 0.23^{a}	1.33 ± 0.05^{b}	34 ± 2^{e}	12.27 ± 0.66^{e}
Ravar	16.03 ± 1.93^{c}	20.03 ± 1.94^{d}	61.00 ± 3.00^{a}	0.74 ± 0.05^{cd}	8.30 ± 0.20^{a}	1.72 ± 0.07^{a}	15 ± 2^{e}	9.63 ± 0.46^{f}

Values marked with the same letters are not significantly different (LSD, $p < 0.05$). All the values are means of four replicates ± SD.

2.3. Plant sample preparation in the laboratory

Alfalfa samples were placed in an oven at 105 °C for 24 h to determine their dry matter. After calculating the dry matter, the dried samples were filtered through a 2 mm diameter mesh sieve (Christy and Norris X26 Hammermill). Then the samples were stored in a desiccator to determine nutrients. In order to prepare the ash for arsenic assay, 2 g of the dried sample was placed in a desiccator in a washable material and placed in an electric oven at 550 °C for 24 h. In the next step, acid digestion was performed by transferring the samples to 50 ml Erlenmeyer and then 5 ml of concentrated nitric acid was added. The solution was placed on a hot plate to complete dry digestion. The hot plate temperature was initially set at 25°C and slowly increased to 60, 105 and 120 °C. The samples were heated until the acid evaporated. Next, 10 ml of DDW containing 1% nitric acid was added. Finally, purification of the samples was accomplished by pouring solutions using Whatman paper into previously weighed beakers.

2.4. Soil sample preparation in the laboratory

Soil samples were dried in an oven at 105 °C to homogenize. Stones and other solids were removed via a 2-mm mesh diameter sieve. Next, 50 g of each sieved sample was weighed. In order to remove organic materials from soil samples, they were placed in an electric oven at 550 ° C for 30 min. To evaluate the heavy metals, 2 g of the samples were transferred into 50-ml Erlenmeyer and about 12.5 ml of 4-ml nitric acid was added. The solution was then placed in an oven at 80 °C for 24 h. After the acid evaporated, 10 ml of DDW containing 1% nitric acid was added up. The following steps were similar to the soil preparation steps (Pinto Vilar and Ikuma, 2022).

2.5. Arsenic assay

To measure arsenic, standard solutions (1000 mg/L) were purchased from Merck company, Germany. This solution was diluted sequentially using DDW to obtain the required concentration and standards. All samples were kept in 10% nitric acid solution for 24 h and washed with DDW. After the preparation of the samples, Australian Plasma Light Emission Spectroscopy (ICP-OES Vista-MPX) was used to

determine the concentration of arsenic in alfalfa, pistachio and soil samples.

2.6. Photosynthetic pigments assay

Measurement of photosynthetic pigments was performed with 80% acetone according to the Lichtenthaler method (Lichtenthaler, 1987). The adsorption intensities of the solutions were read at 663.2, 646.8 and 470 wavelengths for chl a, b and carotenoids by spectrophotometer (1240 UV/min model of Shimadzu, Japan), respectively.

2.7. Soluble carbohydrates assay

The amount of carbohydrates was measured using the method by previous research (Irigoyen *et al.*, 1992).

2.8. Total protein assay

To measure the amount of protein, 0.5 g of the powdered sample by liquid nitrogen was taken and then 3 ml of extraction buffer was added. Next, the prepared mixture was centrifuged at 11500 rpm and 4 °C for 21 min. The upper part of the extract was removed and the extract was subsequently centrifuged again at 4,000 rpm for 20 min. After this step, 5 ml of Bradford reagent and 290 µl of extraction buffer were mixed together and then 10 µl of the prepared extracts was added. Afterward, the final mixture was placed in the spectrophotometer at 590 nm to determine the absorption value (Bradford, 1976).

2.9. Flavonoids assay

To measure the total flavonoid content, first 0.5 ml of previously prepared methanolic extract (1 g of dried fruit sample in 10 ml of 80% methanol) was mixed with 1.5 ml of methanol, 0.1 ml/L of 10% aluminum chloride in ethanol (10 g of aluminum chloride in 100 ml of ethanol and DDW), 0.1 ml of 1 M potassium acetate (2.41g per 10 ml of DDW) and 2.8 ml of DDW. Instead of methanolic extract, only pure methanol was used as the control. The mixture was kept in the dark for 30 min and then its absorption was immediately read at 415 nm by spectrophotometer (UV / VIS 2800). Total flavonoid content was determined according to the standard quercetin line (Chang *et al.*, 2002). For this purpose, different concentrations of quercetin standard were made and after reading the adsorption numbers, the amount of flavonoid was calculated using Equation below:

$$Y=0.012x+0.0722 \quad (1)$$

2.10. Statistical analysis

Statistical analysis of data was performed using SAS software version 9.2 and graphs were drawn with Excel. The mean comparison was calculated based on Duncan's test at the level of 5%.

Table 2. Results of variance analysis of photosynthetic pigments in alfalfa and pistachio in different regions of Kerman province.

Sources of Variation	df	Mean Square					
		Chl a(mg/l)		Chl b(mg/l)		Total-chl(mg/l)	
		alfalfa	pistachio	alfalfa	pistachio	alfalfa	pistachio
Region	7	72.65*	23.62**	5.73**	6.37**	101.5**	48.3**
Error	24	25.07	3.51	0.484	0.182	25.62	3.414
CV (%)		20.4	6.6	8	5.3	15.2	5.1

^{ns}, * and ** are non-significant, significant at the 5 and 1% levels respectively.

Table 3. Mean comparison results of photosynthetic pigments in alfalfa and pistachio in different regions of Kerman province.

Region	Chl a (mg/g)		Chl b (mg/g)		Total-chl (mg/g)	
	alfalfa	pistachio	alfalfa	pistachio	alfalfa	pistachio
Sirjan	24.93±.0.32^{a}	29.88±0.32^{a}	9.28±0.34^{ab}	7.15±0.27^{c}	34.20±0.60^{ab}	37.03±0.53^{ab}
Shahr-e-Babak	28.50±0.37^{a}	30.33±0.32^{a}	10.13±0.23^{a}	9.10±0.11^{a}	38.63±0.38^{a}	39.43±0.17^{a}
Bayaz	27.98±0.14^{a}	29.15±0.10^{a}	9.85±0.24^{a}	9.65±0.19^{a}	37.83±0.17^{a}	38.8±0.11^{a}
Anar	28.30±0.58^{a}	29.65±0.16^{a}	9.35±0.47^{a}	9.18±0.15^{a}	37.65±0.30^{a}	38.83±0.28^{a}
Kabootar-Khan	22.13±0.24^{ab}	24.20±0.39^{b}	7.13±0.14^{c}	6.93±0.08^{c}	29.25±0.32^{bc}	31.13±0.43^{c}
Kazem-Abad	16.33±7.02^{b}	27.43±1.34^{a}	8.28±0.198^{b}	7.47±0.21^{c}	24.60±7.06^{c}	34.9±1.21^{b}
Zarand	26.23±0.25^{a}	29.58±0.26^{a}	7.23±0.23^{c}	8.30±0.19^{b}	33.45±0.27^{ab}	37.88±0.43^{a}
Ravar	21.50±0.35^{ab}	24.65±2.20^{b}	7.63±0.60^{c}	6.10±0.37^{d}	29.13±0.77^{bc}	30.75±2.1^{c}

The different letters in each column indicate a significant difference by Duncan's multiple range test at the 5% level.

3. Results and discussion

3.1. Pigments

The results of the main pigment, such as chl a, chl b and total-chl, showed that these pigments in both plants had been affected by the region (Table 2). Means comparison results displayed that the highest level of chl a (28.5 mg/g) in alfalfa belonged to the Shahr-e-Babak region, which was not significantly different from other regions except Kazem-Abad. The trend of changes of this pigment in pistachio was similar to alfalfa and its highest level, with an average of 30.33 mg/g was observed from the same region (Table 3). The results of chl b and total-chl also showed that the highest amount of which in both alfalfa and pistachio related to Shahr-e-Babak region with averages of 10.13 and 9.10 mg/g for chl b, and 38.6 and 39.4 mg/g for total-chl, which were not significantly different in Bayaz and Anar regions and were placed in a similar statistical class. The lowest level of chl b in both plants was related to the Kabutar-Khan region, which was in the same statistical class as the Kazem-Abad region (Table 3).

The results of the regression trend of intra-tissue and peripheral arsenic changes with changes in pigments also showed that the level of peripheral arsenic could account for a higher percentage of changes in chl a in both plants and the coefficient of determination of this model was 0.908 and 0.848. While the coefficient of determination of the intra-tissue arsenic model was lower (Fig. 1). The model of changes in chl a due to changes in the level of peripheral arsenic was as the second degree. At low concentrations of arsenic, the amount of chl a decreased, but at concentrations of 600 mg/kg and above that, the amount of Chl a conversely increased, and this change was more severe in alfalfa than in pistachios (Fig. 1).

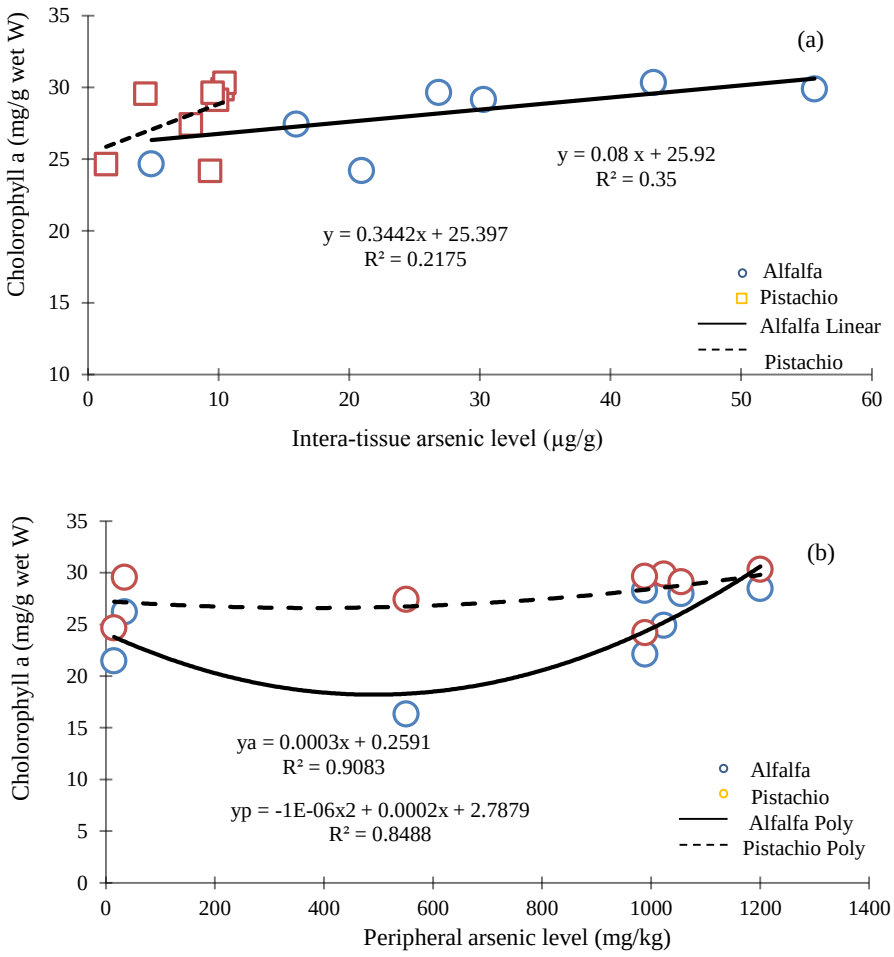


Figure 1. Regression model between the change of Chl a in alfalfa and pistachio due to the effect of intra-tissue arsenic (a) and total soil arsenic (b) in different regions of Kerman.

The results also showed that the amount of chl b in comparison to chl a was more affected by the amount of intra-tissue arsenic than peripheral arsenic. The trend of changes of this pigment due to intra-tissue arsenic in alfalfa was as the second degree and in pistachio was linear, but due to peripheral arsenic in both plants, it was linear. It was also found that in both conditions, the coefficient of determination in alfalfa was higher than that of pistachios (Fig. 2).

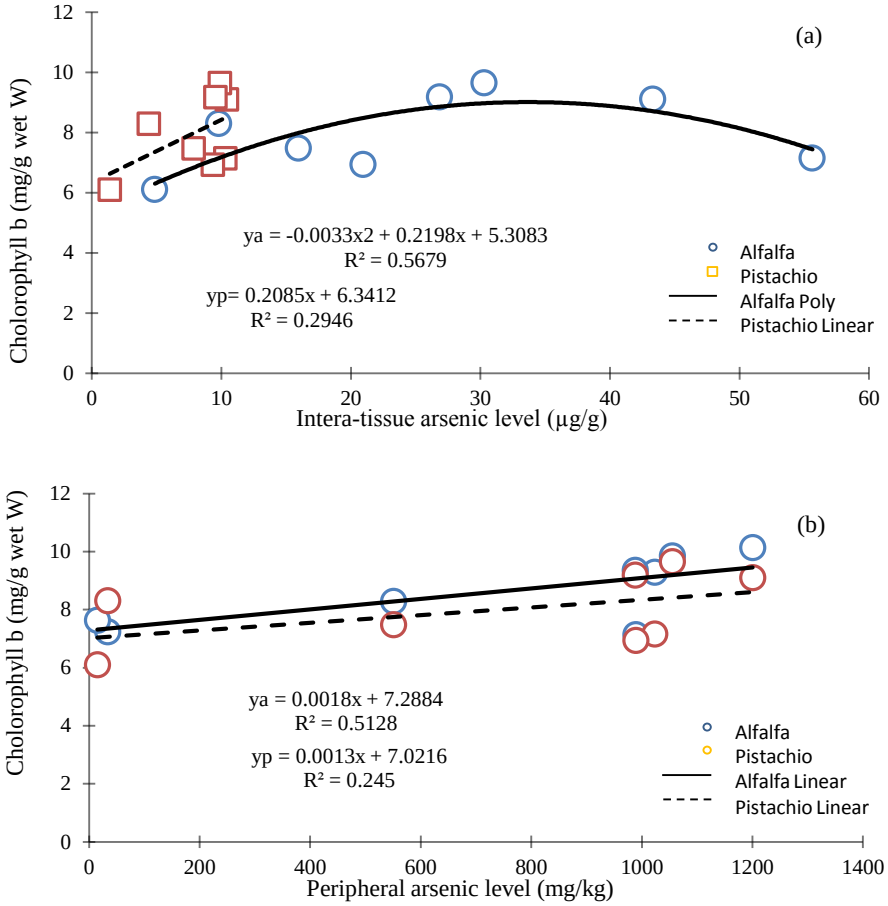
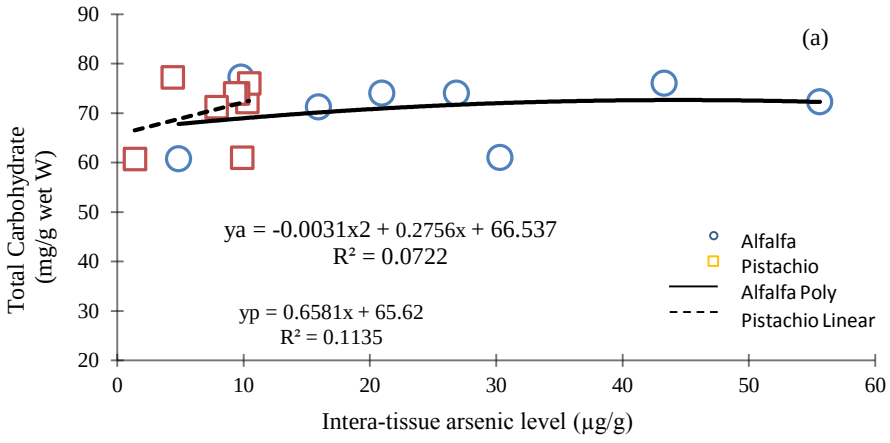


Figure 2. Regression model between changes in the level of chl b in alfalfa and pistachio due to the effect of intra-tissue arsenic (a) and total soil arsenic (b) in different regions of Kerman.

The results also showed that changes in total-chl in alfalfa were more affected by peripheral arsenic, but in pistachios, it was due to intra-tissue arsenic. The trend of changes in total-chl in alfalfa in both conditions was as the second degree, but in pistachios, it was linear (Fig. 3).



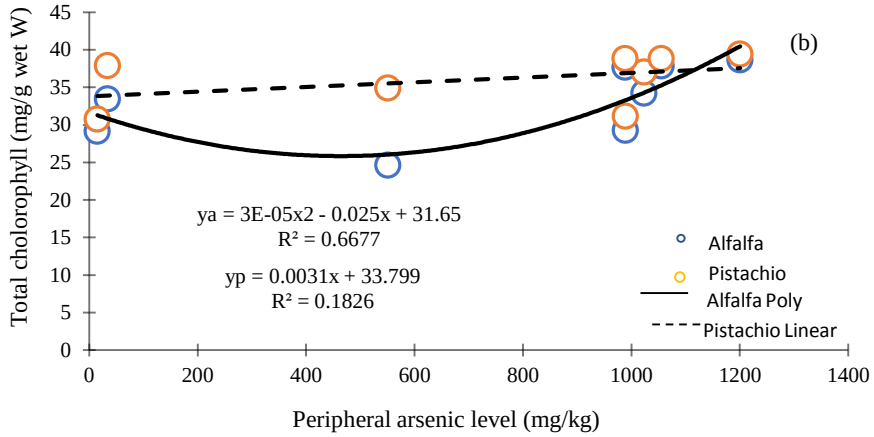


Figure 3. Regression model between changes in the level of total-chl in alfalfa and pistachio due to the effect of intra-tissue arsenic (a) and total soil arsenic (b) in different regions of Kerman.

3.2. Accessory pigments

The results of flavonoid and carotenoid content in the studied plants also showed that flavonoids were affected only in pistachio by the region. In contrast, the content of carotenoids in both plants was affected by the region (Table 4). Mean comparison showed that the highest content of flavonoids in pistachio was related to Shahr-e-Babak and Bayaz regions which were not statistically different from each other and pistachio leaves of the mentioned regions had about 74.5 and 76 µg/g of flavonoids. The range of flavonoid fluctuations in alfalfa was 61.2 to 71.5 µg / g (Table 5). The results of carotenoid also showed that Shahr-e-Babak, Bayaz and Anar regions had the highest level of carotenoid in both alfalfa and pistachio. The lowest amount of this pigment was related to the Ravar region (Table 5).

Table 4. Results of variance analysis of accessory pigments in alfalfa and pistachio in different regions of Kerman province

Sources of Variation	Degree of Freedom (df)	Mean Square			
		Flavonoids(µg/l)		Carotenoids(µg/l)	
		alfalfa	pistachio	alfalfa	pistachio
Region	7	66.138	131.14	8.411	9.79
Error	24	36.51	1.83	0.077	0.165
Coefficient of Variation (CV) (%)		9.1	1.97	5.4	5.71

^{ns}, * and ** are non-significant, significant at the 5 and 1% levels, respectively.

Table 5. Mean comparison results of accessory pigments in alfalfa and pistachio in different regions of Kerman province.

Region	Flavonoids (µg/g)		Carotenoids (µg/g)	
	alfalfa	pistachio	alfalfa	pistachio
Sirjan	65.50±0.29^{ab}	70.00±0.41^{b}	5.40±0.16^{b}	7.23±0.11^{b}
Shahr-e-Babak	71.50±0.65^{a}	74.50±1.32^{a}	6.75±0.09^{a}	8.78±0.22^{a}
Bayaz	63.25±8.44^{ab}	76.00±0.71^{a}	6.73±0.16^{a}	8.70±0.09^{a}
Anar	69.25±0.48^{ab}	69.77±0.63^{b}	6.33±0.17^{a}	8.43±0.28^{a}
Kabootar-Khan	61.25±0.48^{b}	60.75±0.48^{b}	3.35±0.12^{d}	6.03±0.11^{c}
Kazem-Abad	65.00±0.41^{ab}	64.00±0.41^{c}	3.83±0.13^{c}	6.28±0.28^{c}
Zarand	69.50±0.50^{ab}	71.25±0.48^{b}	5.45±0.13^{b}	7.33±0.09^{b}
Ravar	61.50±0.65^{ab}	61.75±0.48^{d}	3.38±0.16^{d}	4.25±0.29^{d}

The different letters in each column indicate a significant difference by Duncan's multiple range test at the 5% level.

Table 6. Results of variance analysis of protein and total carbohydrate in alfalfa and pistachio in different regions of Kerman province.

Sources of Variation	Degree of Freedom (df)	Mean Square			
		Protein(mg/l)		Carbohydrate(mg/l)	
		alfalfa	pistachio	alfalfa	pistachio
Region	7	52.35	164.83	4.73	4.32
Error	24	1.208	1.791	1.505	0.501
Coefficient of Variation (CV) (%)		2.01	1.8	8.3	13.05

^{ns}, * and ** are non-significant, significant at the 5 and 1% levels, respectively.

The results of the regression model of these traits in the amount of intra-tissue arsenic and peripheral arsenic were also determined. The amount of alfalfa flavonoids were more affected by intra-tissue arsenic while the content of pistachio flavonoids was more affected by peripheral arsenic. Changes in this pigment in both plants were the second degree in both conditions (Fig. 4). It was also found that carotenoids in both plants were more affected by the level of intra-tissue arsenic and the coefficient of determination of their equations was higher than that of peripheral arsenic. Changes of this pigment in alfalfa were as the second degree, but in pistachio in intra-tissue arsenic conditions and peripheral arsenic were linear and as the second degree, respectively (Fig. 5).

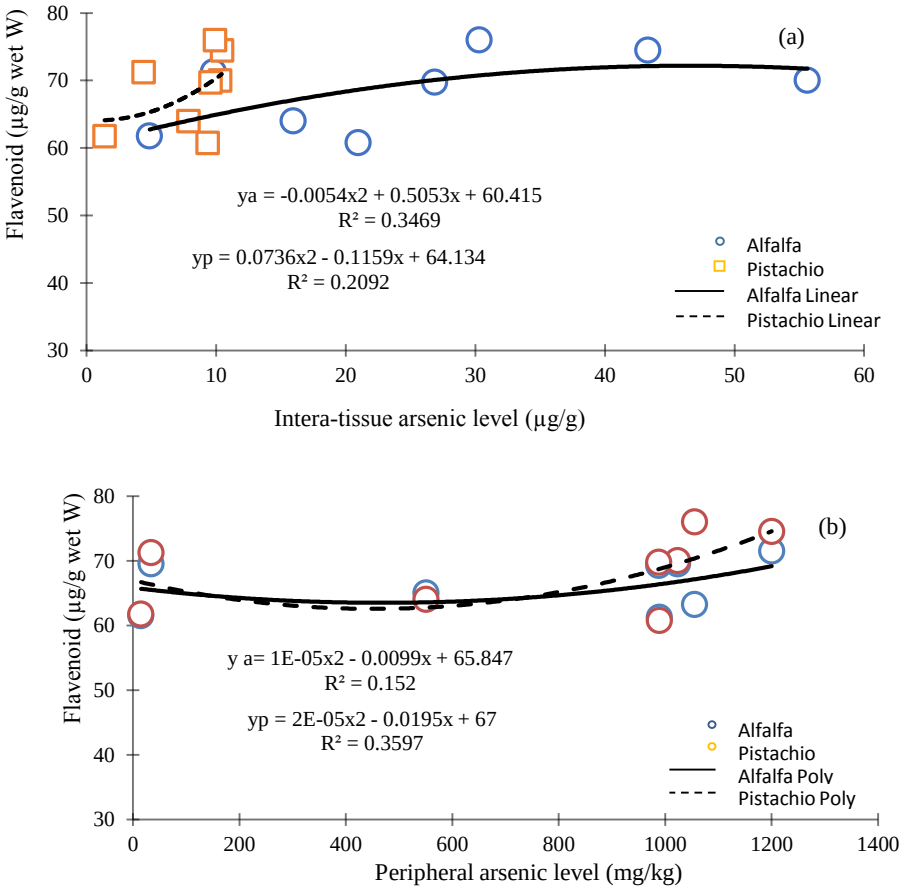
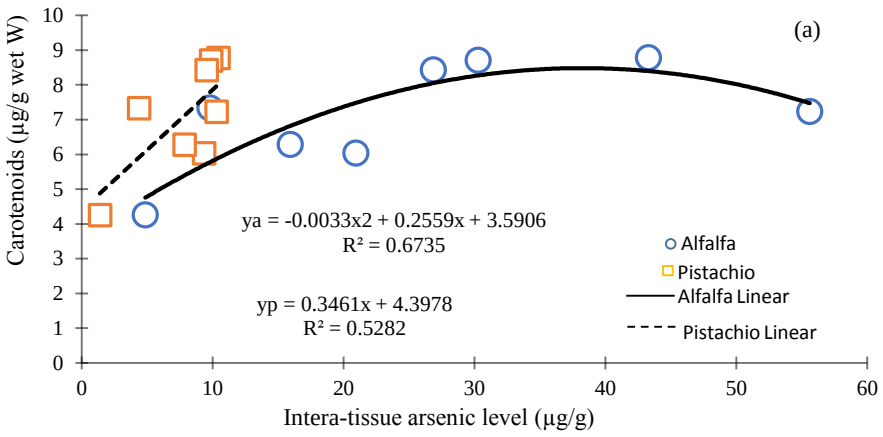


Figure 4. Regression model between changes in flavonoid content in alfalfa and pistachio due to the effect of intra-tissue arsenic (a) and total soil arsenic (b) in different regions of Kerman.



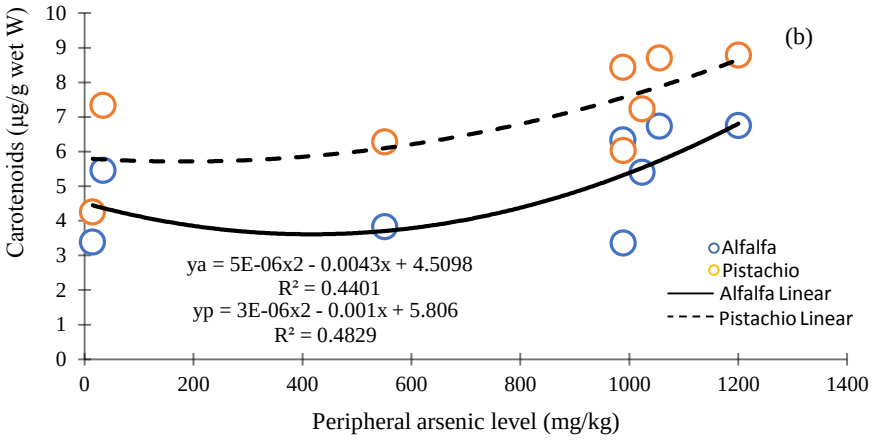


Figure 5. Regression model between changes in carotenoid content in alfalfa and pistachio due to the effect of intra-tissue arsenic (a) and total soil arsenic (b) in different regions of Kerman.

3.3. Total carbohydrates and protein

Leaf protein and carbohydrate levels in alfalfa and pistachios, like most other traits, were significantly different in the studied reigns (Table 6). According to the results shown in Table 7, Shahr-e-Babak and Zarand regions had the highest amount of TCARB in alfalfa and pistachio, but the highest amount of protein was observed from Anar, Bayaz and Kabutar-khan regions. The lowest amount of TCARB in both plants was relevant to the Ravar region. While the lowest amount of protein was obtained from the Zarand region.

Table 6. Results of variance analysis of protein and total carbohydrate in alfalfa and pistachio in different regions of Kerman province.

Sources of Variation	Degree of Freedom (df)	Mean Square			
		Protein(mg/l)		Carbohydrate(mg/l)	
		alfalfa	pistachio	alfalfa	pistachio
Region	7	52.35	164.83	4.73	4.32
Error	24	1.208	1.791	1.505	0.501
Coefficient of Variation (CV) (%)		2.01	1.8	8.3	13.05

^{ns}, * and ** are non-significant, significant at the 5 and 1% levels, respectively.

Table 7. Mean comparison results of protein and total carbohydrate in alfalfa and pistachio in different regions of Kerman province.

Region	Protein (mg/g)		Total carbohydrate (mg/g)	
	alfalfa	pistachio	alfalfa	pistachio
Sirjan	55.00±0.71^{c}	72.25±0.48^{cd}	13.88±0.18^{b}	4.68±0.20^{c}
Shahr-e-Babak	58.75±0.48^{a}	76.00±0.71^{ab}	13.45±0.23^{b}	5.23±0.28^{bc}
Bayaz	51.50±0.65^{e}	61.00±0.71^{e}	15.4±0.56^{ab}	4.95±0.74^{bc}
Anar	57.00±0.41^{b}	74.00±0.41^{bc}	15.9±0.78^{a}	6.03±0.36^{ab}
Kabootar-Khan	53.50±0.65^{cd}	74.00±0.91^{bc}	15.08±1.07^{ab}	5.95±0.25^{ab}
Kazem-Abad	53.25±0.48^{d}	71.25±0.63^{d}	13.95±0.32^{b}	6.83±0.38^{a}
Zarand	59.25±0.48^{a}	77.25±0.48^{a}	13.83±0.43^{b}	3.43±0.05^{d}
Ravar	48.75±0.48^{f}	60.75±0.85^{a}	16.33±0.75^{a}	4.75±0.09^{c}

The different letters in each column indicate a significant difference by Duncan's multiple range test at the 5% level.

The results of the regression model of changes in protein and total carbohydrate in intra-tissue and peripheral arsenic also showed that changes in total carbohydrate had no significant and acceptable relationship with intra-tissue and peripheral arsenic changes, but this model was able to predict changes in pistachio protein better than in carbohydrates. In a way, the coefficient of determination between changes in pistachio protein and peripheral arsenic was 0.630 (Fig. 6 and 7).

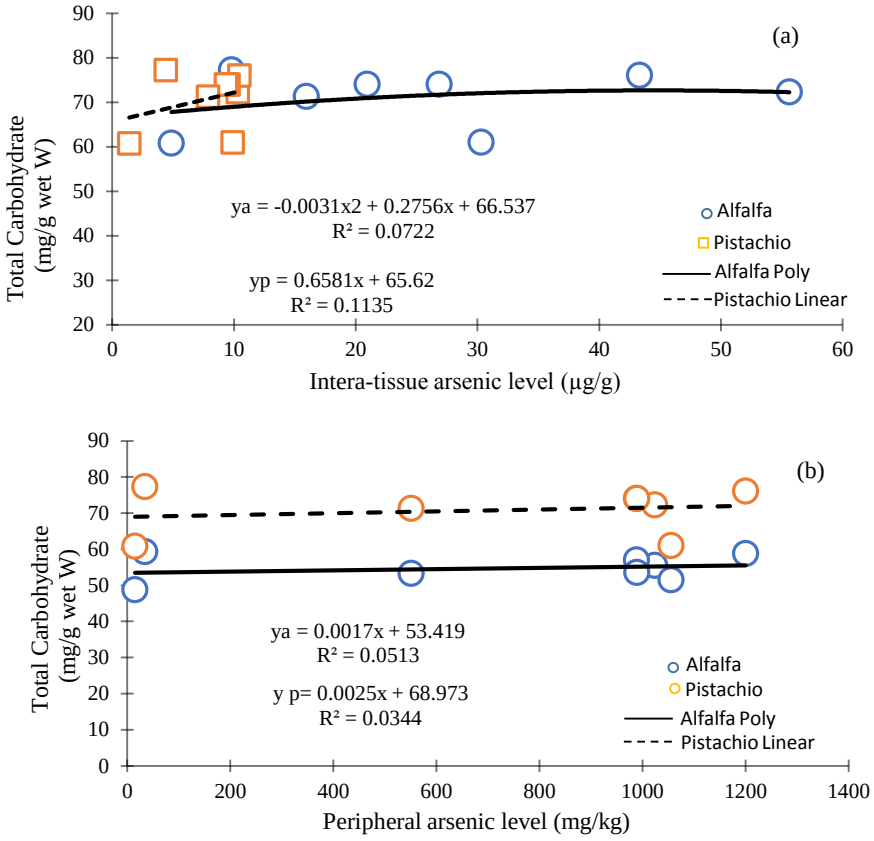


Figure 6. Regression model between changes in total carbohydrate content in alfalfa and pistachio due to the effect of intra-tissue arsenic (a) and total soil arsenic (b) in different regions of Kerman.

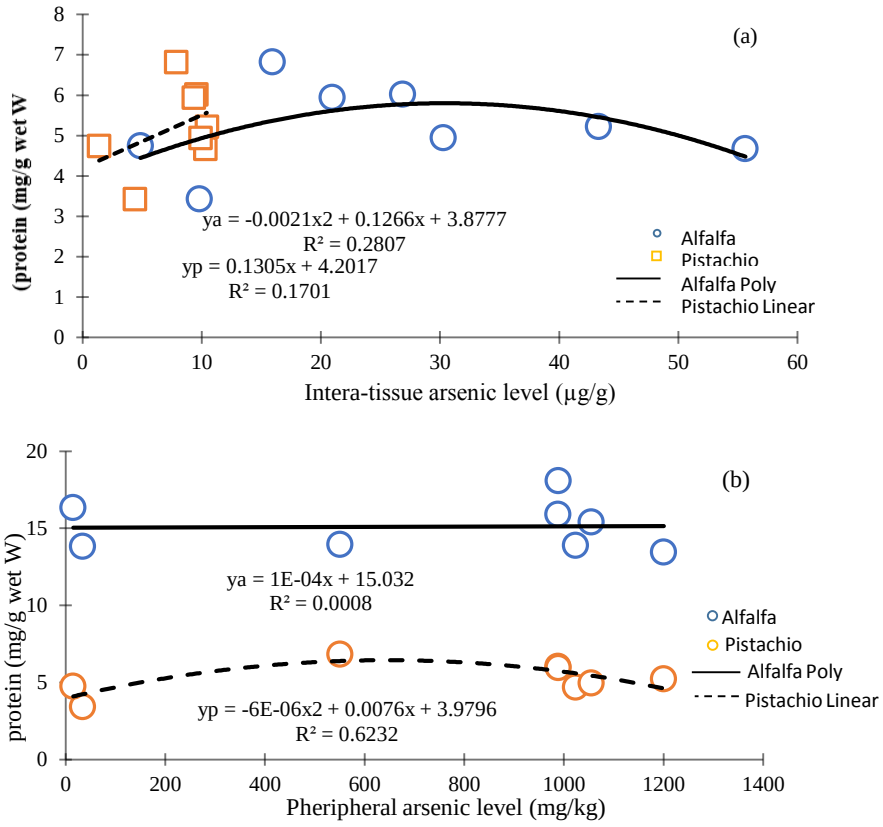


Figure 7. Regression model between changes in protein content in alfalfa and pistachio due to the effect of intra-tissue arsenic (a) and total soil arsenic (b) in different regions of Kerman.

Among the processes that are affected by stress caused by heavy metals are photosynthesis and photosynthetic pigments. Photosynthesis is one of the most sensitive metabolic processes to heavy metal toxicity and several studies have reported photosynthetic inhibition in various plants grown under heavy metal stress (Reddy *et al.*, 2005b). Heavy metals cause iron deficiency in shoots by disordering metal homeostasis (Fodor *et al.*, 2005). Subsequently, the biological synthesis of chlorophylls, the formation of protein-chlorophyll complexes, and the formation of thylakoid membranes would be disrupted (Basa *et al.*, 2014). Arsenic may reduce photosynthesis by opening stigmas, damaging the ultrastructural organization of chloroplasts, altering photosynthetic metabolites, replacing ions such as magnesium and manganese, etc. with heavy metals such as arsenic in chloroplasts, and preventing inductive photosynthetic pigments formation or degradation.

Heavy metals stresses such as arsenic also induce oxidative stress through the overproduction of reactive oxygen species, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide (Hamidi *et al.*, 2020; Reddy *et al.*, 2005a). Heavy metal stresses have a direct effect on photosynthesis, mainly by reducing chlorophyll synthesis (Boddu *et al.*, 2008). The reduction of pigments is due to the increase in the production of oxygen-free radicals, which cause peroxidation and, consequently, the decomposition of chlorophylls (Schutz and Fangmier, 2001). In an experiment (Amini and Amirjani, 2012), the results showed that total-chl content was significantly reduced by nickel and lead treatments (4replications) in alfalfa compared to the control plant.

In a study of the stress of different concentrations of nickel, lead, and zinc in maize, net photosynthesis decreased and the rate of reduction was higher at high concentrations and long treatment times (Heckathorn *et al.*, 2004). Also, the reduction of chlorophyll content by another heavy metal, such as cadmium in *Riccia* sp. has been reported by previous research (Prasad *et al.*, 2004). The results showed (Babaakbari-Sari *et al.*, 2013) that the chlorophyll index of leaves in plants grown in arsenic-contaminated soil, compared to non-contaminated soil, resulted in a significant decrease at the level of 1%. Research (Shaibur *et al.*, 2009) by planting rice in an aqueous culture medium showed that by increasing the concentration of arsenic in the nutrient solution from 0 to 26.8 μML^{-1}, the leaf chlorophyll index decreased from 30 to less than 10.

Arsenic and iron showed a negative interaction. With increasing the concentration of dissolved arsenic, the concentration of iron in the aerial part of the plant decreased, but it was the opposite in the roots. Also, the plants became yellow and chlorinated. The stress of heavy metals such as arsenic has destructive effects on photosynthetic rate and intracellular CO2 concentration and can interfere by replacing the manganese ions with cadmium ions in chlorophyll molecules, production of fluorescent dyes in magnesium chlorophylls, with photosynthetic pigments (Jing *et al.*, 2005). The toxic effects of heavy metals can reduce chlorophyll and, consequently, photosynthesis, which can lead to cell aging and death (El-Mahrouk *et al.*, 2019; Fatima *et al.*, 2021). In an experiment, arsenic toxicity led to a decrease in relative water content, photosynthesis rate, stomatal conductance, transpiration, and chlorophyll content in wheat (Maghsoudi *et al.*, 2020). It was reported (Gill *et al.*, 2012) that higher concentrations of cadmium resulted in a reduction of photosynthesis and nitrogen metabolism in *Lepidium sativum* L. Similarly, in tomato (*Lycopersicon esculentum*), low cadmium concentrations (10 mM) had no effect on photosynthesis, but higher concentrations (100 µM) in nutrient solution reduced photosynthesis and photosynthetic pigment concentrations (López-Millán *et al.*, 2009).

The biological synthesis of several cellular biomolecules is the main mechanism for tolerating or neutralizing metal toxicity which involves the induction of many components such as amino acids, organic acids, hormones and phenolic compounds (Viehweger, 2014). It was related (Bizzo *et al.*, 2014) that *Salvinia auriculata* Aubl electrolyte leakage ranged from 14 to 82% and lipid peroxidation ranged from 7 to 46 nmol / m^2, while soluble flavonoids and carbohydrates decreased with increasing copper concentration. Also, anthocyanins, phenolic compounds and proline were increased to 0.1 mm of copper, indicating the adaptation of plants to stress caused directly by reactive oxygen species. In an experiment on bean, the main and accessory pigments in seedlings treated with lead metal gradually decreased with increasing the lead concentration.

The decrease in chlorophyll and carotenoid content in seedlings under lead stress may be due to the inhibitory effect of lead on an enzyme involved in pigment biosynthesis (Singh *et al.*, 2017). It was shown (Tegelberg *et al.*, 2004) that there is a relationship between the amount of phenol and flavonoid in plants and the activity of the polyphenol oxidase enzyme. So, by increasing the concentration of total soluble phenol, the activity of the polyphenol oxidase enzyme increases and makes the plant resistant to stress. Carotenoids, flavonoids and anthocyanins are protective mechanisms that protect the plant from oxidative stress by scavenging free radicals (Woodson and Lawton, 1988). Carotenoids generally play an important role in inactivating reactive oxygen species and inhibiting free radicals due to their ability to transfer energy to photosynthesis and their role in light protection (Jithesh *et al.*, 2006). The results of treatment with heavy elements in common beans (*Phaseolus vulgaris*) showed a significant increase in flavonoids (Sakihama and Yamasaki, 2002). In general, flavonoids are believed to prevent oxidative damage by scavenging several types of reactive oxygen species and disrupting radical chain reactions during lipid peroxidation.

Heavy metals such as arsenic have a detrimental effect on the physiological processes of plants. Therefore, they have an impact on growth and productivity. It is evident that heavy metal affects the content of photosynthetic pigments, sugars and proteins (Bhardwaj *et al.*, 2009). Various enzymes can be inactivated by connecting the heavy metals with the enzymes (Pinho and Ladeiro, 2012). Sugars and proteins are energy-rich compounds that provide the necessary energy for the biosynthesis of compounds required by plants. The sugar and protein content in plants that are exposed to adverse conditions can be different. Thus, membrane permeability would be influenced and changes (Singh *et al.*, 2017). Heavy metal reduces protein content which may be due to the inhibition of protein synthesis or protein degradation (Palma *et al.*, 2002). It was reported (Costa and Spitz, 1997) a decrease in protein levels in *Lupinus albus* under heavy metal stress.

Heavy metal oxidative stress, such as arsenic, may cause a decrease in protein content (Bharwana *et al.*, 2013). Heavy metals in plants disrupt metabolic activities and gradually reduce the sugar content. The reduction of photosynthetic pigments inhibits photosynthetic activity, which directly reduces the sugar content (Hussain *et al.*, 2013). In a study (Maghsoudi *et al.*, 2020), the toxicity of arsenic increased the accumulation of osmotic active molecules such as proline, soluble proteins and soluble sugars in wheat. There is no question that increasing the amount of proline and soluble sugars in plants due to their active role in membrane stabilization, protection of enzyme structure, osmotic regulation and defense against hydroxyl radicals, is necessary to withstand the stress of heavy metals (Ghodke *et al.*, 2018). It was conducted (Stiborová *et al.*, 1987), heavy metals interact with the ribulose-bis-phosphate carboxylase reaction center and have a negative effect by inhibiting carboxylation.

It was conducted (Singh *et al.*, 2017) that photosynthetic pigments, soluble sugar and protein were reduced in the presence of arsenic, which was in line with the findings of previous research (Bhardwaj *et al.*, 2009). It has also been reported that the amount of reducing sugars in rice seedlings under cadmium stress increased from 5 to 20 days, while the amount of non-reducing sugars decreased (Ramos *et al.*, 2002). Furthermore, proteins, as one of the dominant constituents in the cell, can be attacked by free radicals. They play a variety of structural, catalytic, and functional roles in the cell. And the oxidation of them influences their function (Valko *et al.*, 2016). Oxidative damage due to heavy metals, including cadmium, has many destructive consequences in plants.

Due to the abundance of proteins in living systems, they undergo a wide range of changes by the influence of stress. Oxidation of proteins and their sensitivity to protease are from the changes and kind of oxidative stress caused by heavy metals (Romero-Puertas *et al.*, 2002). It was conducted (Bafeel, 2010; Pourrut *et al.*, 2011) that increasing the concentration of lead stress resulted in a reduction of the protein content in the plant. Protein biosynthesis reduction in plants due to oxidative stress caused by lead may cause changes in cell membranes and their effect on protein and sugar metabolism in the total plant metabolism, which can be a justification for the reduced growth observed in plants (Bafeel, 2010). In another study, the treatment of cadmium chloride increased the amount of total protein and soluble sugar. Also, with increasing the cadmium concentration, the activity of catalase, peroxidase and polyphenol oxidase enzymes increased (Raeesi Sadati *et al.*, 2016).

Moreover, an increase in the amount of soluble sugar due to the treatment with cadmium chloride in canola, safflower and lentil plants has been reported by other researchers (Nooranizadeh and Kafilzadeh, 2011). They reported that the total leaf protein content of the bean plant decreased with increasing the cadmium concentration (6 and 8 g/kg soil) compared to the control plants (Bhardwaj *et al*., 2009). Heavy metal stresses with the production of oxygen-free radicals have a high affinity for protein and cause them to oxidize (Khudsar *et al*., 2001).

4. Conclusion

The results of the present study illustrated that the main and accessory pigments, total protein and carbohydrates, in both plants were affected by the region. In a way that the highest main pigments in alfalfa and pistachio were related to Shahr-e-Babak region. The amount of peripheral arsenic could allocate more proportion of changes in Chl a in both plants. The trend of changes in alfalfa was as the second degree and it was linear in pistachios. The level of alfalfa flavonoids was more affected by intra-tissue arsenic, while the level of pistachio flavonoids was more influenced by peripheral arsenic. It was also found that the values of carotenoids in both plants were more influenced by the amount of intra-tissue arsenic. In general, the best region from the point of view of adaptation to arsenic and in terms of main and accessory pigments was Shahr-e-Babak which most of the traits were found to be affected by intra-tissue arsenic.

Abbreviation

Chl a: Chlorophyll a
Chl b: Chlorophyll b
Total chl: total chlorophyll
DDW: Double Distilled Water
TCARB: Total carbohydrates

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had equal roles in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

Funding/Support

This study was supported by Islamic Azad University, Tehran, Iran.

Acknowledgement

This article was achieved based on the material and equipment of Islamic Azad University.

References

Abid M., Mansour E., Yahia L. B., Bachar K., Ben Khaled A., Ferchichi A. 2016. Alfalfa nutritive quality as influenced by drought in South-Eastern Oasis of Tunisia. Ital. J. Anim. Sci 15(2): 334-342. https://doi.org/10.1080/1828051x.2016.1175916

Alavi M., Rai M., Martinez F., Kahrizi D., Khan H., Rose Alencar de Menezes I., Douglas Melo Coutinho H., Costa J. G. M. 2022. The efficiency of metal, metal oxide, and metalloid nanoparticles against cancer cells and bacterial pathogens: different mechanisms of action. Cell. Mol. Biomed. Rep 2(1): 10-21. 10.55705/cmbr.2022.147090.1023

Amini F., Amirjani M. R. 2012. Effect of nickel and lead treatment on chlorophyll content and accumulation of these metals in alfalfa (*Medicago sativa*). Journal of Production and Processing of Crops and Horticulture 2: 11-19.

Babaakbari-Sari M., Farahbakhsh M., Savaghebi G. R., Najafi N. 2013. Investigation of Arsenic Concentration in Some of the Calcareous Soils of Ghorveh and Arsenic Uptake by Maize, Wheat and Rapeseed in a Natural Contaminated Soil. Water and Soil Science 23(4): 116.

Bafeel S. 2010. Physiological and biochemical aspects of tolerance in *Lepidium sativum* (cress) to lead toxicity. Catrina Int J Environ Sci 5(1): 1-7.

Basa B., Lattanzio G., Solti Á., Tóth B., Abadía J., Fodor F., Sárvári É. 2014. Changes induced by cadmium stress and iron deficiency in the composition and organization of thylakoid complexes in sugar beet (*Beta vulgaris* L.). Environ. Exp. Bot 101: 1-11. https://doi.org/10.1016/j.envexpbot.2013.12.026

Bhardwaj P., Chaturvedi A. K., Prasad P. 2009. Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L. Nat. sci 7(8): 63-75.

Bharwana S., Ali S., Farooq M., Iqbal N., Abbas F., Ahmad M. 2013. Alleviation of lead toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. J. Bioremed. Biodeg 4(4): 10.4172. http://dx.doi.org/10.4172/2155-6199.1000187

Bizzo A. L. T., Intorne A. C., Gomes P. H., Suzuki M. S., Esteves B. d. S. 2014. Short-term physiological responses to copper stress in *Salvinia auriculata* Aubl. Acta Limnol 26: 268-277. https://doi.org/10.1590/S2179-975X2014000300006

Boddu V. M., Abburi K., Talbott J. L., Smith E. D., Haasch R. 2008. Removal of arsenic (III) and arsenic (V) from aqueous medium using chitosan-coated biosorbent. Water Res 42(3): 633-642. https://doi.org/10.1016/j.watres.2007.08.014

Bradford M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72(1-2): 248-254.

Chang C.-C., Yang M.-H., Wen H.-M., Chern J.-C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food. Drug. Anal 10(3): 178-182.

Costa G., Spitz E. 1997. Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured Lupinus albus. Plant Sci 128(2): 131-140. https://doi.org/10.1016/S0168-9452(97)00148-9

El-Mahrouk E.-S. M., Eisa E. A.-H., Hegazi M. A., Abdel-Gayed M. E.-S., Dewir Y. H., El-Mahrouk M. E., Naidoo Y. 2019. Phytoremediation of cadmium-, copper-, and lead-contaminated soil by Salix mucronata *(Synonym Salix* safsaf). HortScience 54(7): 1249-1257. https://doi.org/10.21273/HORTSCI14018-19

Elfanssi S., Ouazzani N., Mandi L. 2018. Soil properties and agrophysiological responses of alfalfa (*Medicago sativa* L.) irrigated by treated domestic wastewater. Agric. Water Manag 202: 231-240. https://doi.org/10.1016/j.agwat.2018.02.003

Fatima A., Farid M., Farid S., Ishaq H. K., Iftikhar U., Rizwan M., Zubair M., Ali S. 2021. Heavy Metals Induced Physiological and Biochemical Changes in Fenugreek (*Trigonella foenum-graceum* L.). In Hasanuzzaman M (Ed.), Approaches to the Remediation of Inorganic Pollutants (pp. 239-258). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-15-6221-1_12

Fodor F., Gáspár L., Morales F., Gogorcena Y., Lucena J. J., Cseh E., Kröpfl K., Abadía J., Sárvári É. 2005. Effects of two iron sources on iron and cadmium allocation in poplar (*Populus alba*) plants exposed to cadmium. Tree Physiol 25(9): 1173-1180. https://doi.org/10.1093/treephys/25.9.1173

Ghodke P., Andhale P., Gijare U., Thangasamy A., Khade Y., Mahajan V., Singh M. 2018. Physiological and biochemical responses in onion crop to drought stress. Int J Curr Microbiol App Sci 7(1): 2054-2062. https://doi.org/10.20546/ijcmas.2018.701.247

Gill S. S., Khan N. A., Tuteja N. 2012. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). Plant Sci 182: 112-120. https://doi.org/10.1016/j.plantsci.2011.04.018

Gupta D. K., Tiwari S., Razafindrabe B. H. N., Chatterjee S. 2017. Arsenic Contamination from Historical Aspects to the Present. In Gupta D K, Chatterjee S (Eds.), Arsenic Contamination in the Environment: The Issues and Solutions (pp. 1-12). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-54356-7_1

Hamidi H., Masoudian N., Ebadi M., Roudi B. 2020. Relationship of Sodium Nitroprusside with Growth and Antioxidant Enzymes of Canola under Lead Stress. J. Chem. Health Risks 10(1): 57-65. https://dx.doi.org/10.22034/jchr.2020.1863574.1020

Heckathorn S. A., Mueller J. K., LaGuidice S., Zhu B., Barrett T., Blair B., Dong Y. 2004. Chloroplast small heat-shock proteins protect photosynthesis during heavy metal stress. Am. J. Bot 91(9): 1312-1318. https://doi.org/10.3732/ajb.91.9.1312

Hussain A., Abbas N., Arshad F., Akram M., Khan Z. I., Ahmad K., Mansha M., Mirzaei F. 2013. Effects of diverse doses of Lead (Pb) on different growth attributes of *Zea-Mays* L. Agricultural Sci 4(5): Article ID:32311. http://www.scirp.org/journal/PaperInformation.aspx?PaperID=32311

Irigoyen J., Einerich D., Sánchez-Díaz M. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativd*) plants. Physiol. Plant 84(1): 55-60. https://doiorg/101111/j1399-30541992tb08764x

Jing D., Fei-bo W., Guo-ping Z. 2005. Effect of cadmium on growth and photosynthesis of tomato seedlings. J. Zhejiang Univ. Sci. B 6(10): 974-980. https;//doi.org/10.1007/bf02888488

Jithesh M., Prashanth S., Sivaprakash K., Parida A. K. 2006. Antioxidative response mechanisms in halophytes: their role in stress defense. J. Genet 85(3): 237-254. https://doi.org/10.1007/BF02935340

Karcı H., Paizila A., Güney M., Zhaanbaev M., Kafkas S. 2022. Revealing genetic diversity and population structure in Pistachio (*Pistacia vera* L.) by SSR markers. Genet. Resour. Crop Evol 1-15. https://doi.org/10.1007/s10722-022-01410-w

Khan M. U., Shahbaz N., Waheed S., Mahmood A., Shinwari Z. K., Malik R. N. 2016. Comparative health risk surveillance of heavy metals via dietary foodstuff consumption in different land-use types of Pakistan. Hum Ecol Risk Assess 22(1): 168-186. https://doi.org/10.1080/10807039.2015.1056294

Khudsar T., Mahmooduzzafar, Iqbal M. 2001. Cadmium-Induced Changes in Leaf Epidermes, Photosynthetic Rate and Pigment Concentrations in Cajanus Cajan. Biol. Plant 44(1): 59-64. https://doi.org/10.1023/A:1017918320697

Kohli S. K., Khanna K., Bhardwaj R., Abd_Allah E. F., Ahmad P., Corpas F. J. 2019. Assessment of Subcellular ROS and NO Metabolism in Higher Plants: Multifunctional Signaling Molecules. Antioxidants 8(12): 641. https://doi.org/10.3390/antiox8120641

Kovács Z., Soós Á., Kovács B., Kaszás L., Elhawat N., Bákonyi N., Razem M., Fári M. G., Prokisch J., Domokos-Szabolcsy É., Alshaal T. 2021. Uptake Dynamics of Ionic and Elemental Selenium Forms and Their Metabolism in Multiple-Harvested

Alfalfa (*Medicago sativa* L.). Plants 10 (7): 1277. https://doi.org/10.3390/plants10071277

Lichtenthaler H. K. 1987. [34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes Methods in enzymology (Vol. 148, pp. 350-382): Elsevier. https://doi.org/10.1016/0076-6879(87)48036-1

Liu C.-W., Chen Y.-Y., Kao Y.-H., Maji S.-K. 2014. Bioaccumulation and translocation of arsenic in the ecosystem of the Guandu Wetland, Taiwan. Wetlands 34(1): 129-140. https://doi.org/10.1007/s13157-013-0491-0

López-Millán A.-F., Sagardoy R., Solanas M., Abadía A., Abadía J. 2009. Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. Environ. Exp. Bot 65(2-3): 376-385. https://doi.org/10.1016/j.envexpbot.2008.11.010

Maghsoudi K., Arvin M. J., Ashraf M. 2020. Mitigation of Arsenic Toxicity in Wheat by the Exogenously Applied Salicylic Acid, 24-Epi-Brassinolide and Silicon. J. Soil Sci. Plant Nutr 20(2): 577-588. https://doi.org/10.1007/s42729-019-00147-3

Mahmood S., Malik S. A., Tabassum A., Younis U., Athar M. 2014. Biometric and biochemical attributes of alfalfa seedlings as indicators of stress induced by excessive cadmium. J. Soil Sci. Plant Nutr 14(3): 546-553. https://doi.org/10.1016/j.chemosphere.2018.12.101

Malakhov D., Islamgulova A. 2021. The Ecological Niche of *Pistacia Vera* L. (Anacardiaceae) in Central Asia: A Comprehensive Tool for Agromeliorative Planning. Biosis: Biological Systems 2(1): 209-216. https://doi.org/10.37819/biosis.002.01.0089

Mitra A., Chatterjee S., Moogouei R., Gupta D. K. 2017. Arsenic accumulation in rice and probable mitigation approaches: a review. Agronomy 7(4): 67. https://doi.org/10.3390/agronomy7040067

Nooranizadeh H., Kafilzadeh F. 2011. The effect of cadmium toxicity on growth, soluble sugars, photosynthetic pigments and some of enzymes in safflower (*Carthamus tinctorius* L.). Iranian Journal of Biology 24(6): 850-868.

Palma J. M., Sandalio L. M., Corpas F. J., Romero-Puertas M. C., McCarthy I., Luis A. 2002. Plant proteases, protein degradation, and oxidative stress: role of peroxisomes. Plant Physiol. biochem 40(6-8): 521-530. https://doi.org/10.1016/S0981-9428(02)01404-3

Pigna M., Cozzolino V., Caporale A. G., Mora M. L., Meo V. D., Jara A. A., Violante A. 2010. Effect of phosphurs fertilization on arsenic uptake by while grown in polluted soils. J. Soil Sci. Plant Nutr 10(4): 428-442. http://dx.doi.org/10.4067/S0718-95162010000200004

Pinho S., Ladeiro B. 2012. Phytotoxicity by Lead as Heavy Metal Focus on Oxidative Stress. J. Bot., 2012: Article ID: 369572. https://doi.org/10.1155/2012/369572

Pinto Vilar R., Ikuma K. 2022. Effects of Soil Surface Chemistry on Adsorption and Activity of Urease from a Crude Protein Extract: Implications for Biocementation Applications. Catalysts 12(2): 230. https://doi.org/10.3390/catal12020230

Pourrut B., Shahid M., Dumat C., Winterton P., Pinelli E. 2011. Lead uptake, toxicity, and detoxification in plants. Rev Environ Contam Toxicol 213: 113-136. https://doi.org/10.1007/978-1-4419-9860-6_4

Prasad S.M., Dwivedi R., Zeeshan M., Singh R. 2004. UV-B and cadmium induced changes in pigments, photosynthetic electron transport activity, antioxidant levels and antioxidative enzyme activities of Riccia sp. Acta Physiologiae Plantarum 26(4): 423-430. https://doi.org/10.1007/s11738-004-0033-8

Raeesi Sadati S. Y., Jahanbakhsh Godekahriz S., Sedghi M. 2016. The effect of cadmium and mercuric chlorides on some physiological traits in two cultivars of wheat. Iran. J. Plant Physiol 6(3): 1761-1770. https://doi.org/10.30495/ijpp.2016.532692

Rahman M. A., Hassler C. 2014. Is arsenic biotransformation a detoxification mechanism for microorganisms? Aquat.Toxicol 146: 212-219. https://doi.org/10.1016/j.aquatox.2013.11.009

Ramos I., Esteban E., Lucena J. J., Gárate A. n. 2002. Cadmium uptake and subcellular distribution in plants of Lactuca sp. Cd–Mn interaction. Plant Sci 162(5): 761-767. https://doi.org/10.1016/S0168-9452(02)00017-1

Rasouli H., Popović-Djordjević J., Sayyed R. Z., Zarayneh S., Jafari M., Fazeli-Nasab B. 2020. Nanoparticles: A New Threat to Crop Plants and Soil Rhizobia? In Hayat S Pichtel J Faizan M, Fariduddin Q (Eds.), Sustainable Agriculture Reviews 41: Nanotechnology for Plant Growth and Development (pp. 201-214). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-33996-8_11

Rastgoo L., Alemzadeh A., Tale A. M., Tazangi S. E., Eslamzadeh T. 2014. Effects of copper, nickel and zinc on biochemical parameters and metal accumulation in gouan, "*Aeluropus littoralis*". [Other Journal Article]. Plant Knowledge J 3(1): 42-49.

Reddy A. M., Kumar S. G., Jyothsnakumari G., S.Thimmanaik, ChintaSudhakar. 2005a. Lead induced changes in antioxidant metabolism of horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengal gram (*Cicer arietinum* L.). 60(1): 97-104. https://doi.org/10.1016/j.chemosphere.2004.11.092

Reddy V., Urooj A., Kumar A. 2005b. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. Food chem., 90(1-2): 317-321. https://doi.org/10.1016/j.foodchem.2004.05.038

Rizwan M., Ali S., Qayyum M. F., Ibrahim M., Zia-ur-Rehman M., Abbas T., Ok Y. S. 2016. Mechanisms of biochar-mediated alleviation of toxicity of trace elements in plants: a critical review. Environ. Sci. Pollut. Res 23(3): 2230-2248. https://doi.org/10.1007/s11356-015-56977

Romero-Puertas M., Palma J., Gómez M., Del Rio L., Sandalio L. 2002. Cadmium causes the oxidative modification of proteins in pea plants. Plant Cell Environ 25(5): 677-686. https://doi.org/10.1046/j.1365-3040.2002.00850.x

Sakihama Y., Yamasaki H. 2002. Lipid Peroxidation Induced by Phenolics in Conjunction with Aluminum Ions. Biol. Plant 45(2): 249-254. https://doi.org/10.1023/A:1015152908241

Schutz H., Fangmier E. 2001. Growth and yield responses of spring Wheat (*Triticum aestivum* L.) to elevated CO2 and water limitation. Environ. Pollut 14(2): 187-194. https://doi.org/10.1016/S0269-7491(00)00215-3

Shaban H., Fazeli-Nasab B., Alahyari H., Alizadeh G., Shahpesandi S. 2015. An Overview of the Benefits of Compost tea on Plant and Soil Structure. Advances in Bioresearch 6(61): 154-158. https://doi.org/10.15515/abr.0976-4585.6.1.154158

Shaibur M. R., Kitajima N., Huq S. I., Kawai S. 2009. Arsenic–iron interaction: Effect of additional iron on arsenic-induced chlorosis in barley grown in water culture. J. Soil Sci. Plant Nutr 55(6): 739-746. https://doi.org/10.1111/j.1747-0765.2009.00414.x

Singh H., Singh A., Hussain I., Yadav V. 2017. Oxidative stress induced by lead in *Vigna radiata* L. seedling attenuated by exogenous nitric oxide. Trop Plant Res 4(2): 225-234. https://doi.org/10.22271/tpr.2017.v4.i2.031

Stiborová M., Ditrichová M., BŘEzinová A. 1987. Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley and maize seedlings. Biol. Plant 29(6): 453-467. https://doi.org/10.1007/BF02882221

Tegelberg R., Julkunen-Tiitto R., Aphalo P. 2004. Red: far-red light ratio and UV-B radiation: their effects on leaf phenolics and growth of silver birch seedlings. Plant Cell Environ 27(8): 1005-1013. https://doi.org/10.1111/j.1365-3040.2004.01205.x

Valko M., Jomova K., Rhodes C. J., Kuča K., Musílek K. 2016. Redox-and non-redox-metal-induced formation of free radicals and their role in human disease. Arch. Toxicol 90(1): 1-37. https://doi.org/10.1007/s00204-015-1579-5

Viehweger K. 2014. How plants cope with heavy metals. Bot Stud 55(1): 35. https://doi.org/10.1186/1999-3110-55-35

Woodson W. R., Lawton K. A. 1988. Ethylene-induced gene expression in carnation petals: relationship to autocatalytic ethylene production and senescence. Plant Physiol 87(2): 498-503. https://doi.org/10.1104/pp.87.2.498

HOW TO CITE THIS ARTICLE

Yazdanpanah-Ravari S., Heidari Sharifabad H., Abbaspour H., Iranbakhsh A. 2022. Investigation of the Effect of Arsenic Pollution in the Soil of Some Regions of Kerman on Physiological Characteristics of *Pistacia atlantica* L. and *Medicago sativa* L. *Agrotechniques in Industrial Crops* 2(3): 140-155. 10.22126/ATIC.2022.8289.1064