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Impacts of different concentrations of cadmium and arsenic on growth, biochemical and physiological properties in lemon balm (*Melissa officinalis* L.).

Efat Fakhr Mohammadi[1], Mostafa Heidari[1*], Mehdi Babaian[2] and Mehdi Baradaran Firoozabadi[1]

[1]Agronomy and Plant Breeding Department, Shahrood University of Technology, Shahrood, Iran
2 Agricultural collage of Shirvan, Bojnord University, Iran

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ABSTRACT

Heavy metal toxicity such as cadmium (Cd) and arsenic (As) are two non-essential elements, toxic, tend to uptake and translocate within plant tissues. In a pot experiment by using factorial design with three replicates, four concentrations (As1 = 0, As2 = 10, As3 = 20, and As4 = 30 mg As kg^{-1} in the soil) of sodium arsenate ($NaH2As4O.7H2O$) were investigated on lemon balm plants. Additionally, four concentrations (Cd1 = 0, Cd2 = 10, Cd3 = 20, and Cd4 = 30 mg Cd kg^{-1} in the soil) of cadmium sulfate ($CdSO4$) were tested. Cd and As each alone, decreased (50.7%) shoot dry weight. By increasing the concentrations from 0 to 30 mg kg^{-1}, Cd (18.5%) and As (32.01%) reduced root dry weight. Both metals significantly reduced the concentrations of chlorophyll “a” (72.2%) and carotenoids (71.6%), while simultaneously increased anthocyanin (77.6%) in the leaves. Cd and As induced considerable oxidative stress, reflected by a 49.4% increase in catalase (CAT) activity and a 49.6% increase in superoxide dismutase (SOD) activity in the leaves. Furthermore, the concentrations of mineral elements such as nitrogen, phosphorus, and potassium were diminished in the leaves due to the toxicity of Cd and As. The concentration of Cd in the roots varied from 2.7 to 53.3 mg kg^{-1} dry weight (19.5 fold increase), while in the shoot, it was increased by 1.5 times. For the concentration of As in the roots, it was elevated by 17.9 times, and in the shoot, it was increased by 15.1 times. In lemon balm, a greater quantity of As compared to Cd was translocated to the aerial parts. As toxicity had a more substantial effect on plant shoot growth relative to Cd.

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* Corresponding author: M. Heidari
E-mail address: m_haydari@shahroodut.ac.ir

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1.Introduction

Human activities, including agricultural practices and municipal waste generation, have led to an increase in the levels of non-essential and toxic metals in the soils. These toxic metal such as heavy metals can have detrimental effects on plants (Osmam et al., 2017). In recent decades, medicinal plants have become increasingly popular as pharmaceutical products, largely due to their perceived natural safety. However, contamination of their growth environments with trace elements, especially heavy metals, can disrupt the biological pathways and production of secondary metabolites (Heidari and Sarani., 2011). Among the heavy metals, arsenic (As) and cadmium (Cd) are of significant concern. Both Cd and As can induce severe toxicity in plants, negatively impacting growth and reproduction, whether individually or in combination (Sadeghi et al., 2016; Fattorini et al., 2017).

In Iran, the soil Cd limit was between 0.34-1.04 mg/kg (Khani and Malekoti, 2000). Cd is particularly hazardous, as it impedes plant development and growth, resulting in decreased morphological traits, disrupted development, and reduced biomass production (Fattahi et al., 2019). Excessive exposure to Cd as a heavy metal can lead to the toxicity in plant. This toxicity is characterized by alterations in chloroplast ultrastructure, degradation of enzymes involved in CO_2 fixation, reduction in photosynthesis rate, and disturbances in sulfur (S) and nitrogen (N) metabolism (Ibrahim et al., 2017). Cd-induced oxidative stress not only inhibits the formation of photosynthetic pigments but also leads to the production of reactive oxygen species (ROS) in plants. In response to ROS, plants activate their antioxidant defense systems such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (Chen et al., 2019; Khan et al., 2020; Zhang et al., 2020).

As, another heavy metal, is absorbed by the phosphate transporter system and inhibits plant growth by disrupting metabolic processes (Zemanová et al., 2020). Similar to Cd, As interacts with various physiological and biochemical processes within plant tissues. In Iran the soil As was between 1.4-4.39 mg kg^{-1} (Safari et al., 2022). It has detrimental effects on physiological processes (e.g. inhibition of growth), morphological characteristics (e.g. chlorosis), and biochemical functions (e.g. oxidative stress) in plants (Chattopadhyay et al., 2021; Li et al., 2021). The toxicity of As leads to a decrease in dry weight, impacting various growth-related mechanisms, including reduced nutrient content and impaired biosynthesis of photosynthetic pigments (Natasha Shahid et al., 2022).

As limits a plant's ability to absorb nutrients by antagonizing the uptake of phosphorus, silicon, zinc, selenium, and magnesium, and decreasing photosynthetic activity by damaging cell membranes through oxidative stress (Carlin et al., 2016). Additionally, As interferes with multiple metabolic processes, resulting in disorders that affect productivity, such as reduced seed germination and poor nutrients absorption, which ultimately inhibit growth of plant root (Vezza et al., 2018). It also decreases the rate of transpiration and photosynthesis by limiting gas exchange and reducing the levels of photosynthetic pigments, leading to premature leaf senescence (Ali et al., 2022). Both As and Cd have been reported to cause growth inhibition, chlorophyll degradation, and diminished photosynthetic activity (Gupta et al., 2013). Furthermore, the closure of stomata due to Cd or As leads to a reduction in CO_2 availability, thereby hindering photosynthetic carbon assimilation (Anjum et al., 2016).

Lemon balm (*Melissa officinalis* L.) is a perennial herbaceous plant from the Lamiaceae family, reaching heights of up to 1.25 cm. It is distributed across Central Asia, Russia, Southern Europe, and the United States. Primarily cultivated for its medicinal properties, lemon balm is also utilized for its essential oil, which offers various health benefits due to its unique chemical composition (Rahimi et al., 2019). In addition to its medicinal uses, lemon balm is consumed as a vegetable, leading to its cultivation in agricultural lands. However, these agricultural areas can be contaminated with various agricultural toxins and heavy metals.

Most researches have focused on the effects of heavy metals on plants in isolation, with limited studies examining their interactions, particularly between As and Cd. Consequently, there is a lack of information regarding the combined effects of these toxic heavy metals on medicinal plants like lemon balm. Medicinal plants can accumulate both essential and toxic metals, posing potential health risks due to the presence of hazardous elements such as lead (Pb), Cd, As, and mercury (Hg). Nevertheless, limited study is available on contamination of soil and organs of medicinal plants, especial lemon balm. There is a little evidence on bioaccumulation of heavy metals in on it as a medicinal plant. Therefore, the aim of this study was to investigate the effects of different concentrations of As and Cd on the growth, photosynthetic pigments, and activities of antioxidant enzymes in lemon balm. Additionally, the study sought to assess the accumulation of Cd and As in the roots and shoots of lemon balm plants.

2. Materials and Methods

2.1. Plant culture

A pot experiment was conducted in 2021 at the Agricultural College of Shirvan, Bojonrd University, Iran. It was conducted as a factorial in a randomized complete block design (4×4 factorial arrangement) and three replicates. The study aimed to investigate the effects of different concentrations of As and Cd on lemon balm (*Melissa officinalis* L.) plants. The seeds of lemon balm were purchased from Pakan Bazr Company in Isfahan, Iran.

Total of 48 pots (35×35 cm and height 30 cm) were filled with sandy loam soil, which had a Cd concentration of 1.01 mg kg^{-1}, an As concentration of 0.065 mg kg^{-1}, pH = 7.16, and an electrical conductivity (EC) = 1.7 dS m^{-1}. Four levels of As were applied: $As_1 = 0$, $As_2 = 10$, $As_3 = 20$, and $As_4 = 30$ mg As kg^{-1} of soil, using sodium arsenate ($NaH_2As_4O_7 \cdot 7H_2O$) as the source. Similarly, four levels of Cd were applied: $Cd_1 = 0$, $Cd_2 = 10$, $Cd_3 = 20$, and $Cd_4 = 30$ mg Cd kg^{-1} of soil, using cadmium sulfate ($CdSO_4$). The amounts of As and Cd were calculated based on the treatment and the weight of the soil in each pot, then thoroughly mixed into the soil. To confirm the concentrations of As and Cd in the soil, the pots were subjected to wet and dry conditions for four weeks after the Cd and As were added and mixed with the soil.

Eight seeds were planted in each pot. After germination and establishment, The plants were thinned to four plants per pot. Thirty days after planting, samples were taken to assess growth, physiological traits, and the activity of antioxidant enzymes. At this time, the plants were harvested, and measurements were taken for both root and aerial traits. The data collected at harvest included the fresh and dry weight of the shoot, plant height, and dry weight of the root. The dry weight was determined by drying the plants at 70°C for 48 hours in an air oven (drying and heating oven, UN30, Memmert, Germany).

2.2. Chlorophyll and anthocyanin

The concentration of chlorophyll in the leaves was measured using the method described by Arnon (1967). The c concentration of chlorophyll "a" and "b," and carotenoids were determined by reading absorbance at wavelengths of 663 nm, 647 nm, and 470 nm, respectively, using a UV/Vis spectrophotometer (2150 UNICO). The concentration of anthocyanins in the leaves was measured following the methods outlined by Masukasu et al. (2003).

2.3. Nutrient elements

The Kjeldahl method was employed to measure nitrogen concentration in the leaves. To determine potassium concentration in the leaves, the samples were dry ashed at 500 °C, and the potassium content was measured using a Flame Photometer (Jenway PFP7, Keison Products, UK). The concentration of phosphorus in the leaves was determined according to the methods outlined by Chapman and Pratt (1961). Cd and As concentrations in both shoot and root were analyzed using atomic absorption spectroscopy (Shimadzu AA-6300 Double Beam Atomic Absorption).

Translocation factor (TF) was utilized to assess the accumulation of As and Cd in shoot and root of lemon balm plant. The formula used was TF = concentration in shoot/ concentration in root. A TF value greater than 1 was classified as indicative of a phytoextraction mechanism or high mobility, while a TF value less than 1 was categorized as phytostabilization (Rachmawati et al., 2018).

2.4. Antioxidant enzyme activities in the leaves

To assess antioxidant enzyme activities in the leaves, 0.5 g of the samples were homogenized in a near-zero degree with 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA and then homogenate. The homogenate was transferred to centrifuge at 4 °C for 15 minutes with 15,000 x g. The supernatant was used as the enzyme extract. Total catalase (CAT) activity was assessed by measuring the residual hydrogen peroxide (H_2O_2) using a titanium reagent, following the method described by Taranishi et al. (1974). Superoxide dismutase (SOD) activity was measured according to the protocol established by Dhindsa et al. (1981).

2.5. Statistical analyses

Data were analyzed using SAS (version 9.1). To identify significant treatments at a threshold of $P \leq 0.05$, statistical analysis was conducted using two-way ANOVA. Subsequently, significant differences between individual means were assessed using the Least Significant Difference (LSD) method.

3. Results

3.1. Root and shoot characteristics and photosynthetic pigments

Figure 1 illustrates the interaction effect of Cd and As on shoot dry weight. With the concentrations of Cd and As in the soil were increased, the shoot dry weight was decreased. The highest shoot dry weight recorded was 20.1 g per plant at 0 mg kg^{-1} of Cd and As, while the lowest of it was 9.9 g per plant at 30 mg kg^{-1} of Cd and As, resulting in a reduction of approximately 50.7%. Data in the Table 1 indicate that both Cd and As significantly affected on root dry weight and root length.

Table 1. Root dry weight, root length and nutrient elements in l*emon balm* **plant as affected by arsenic and cadmium**

Treatments	Root dry weight (g plant)	Root length (cm)	Leaf nutrient elements			TF values
			Nitrogen	Phosphorus	Potassium	
				(%)		
Arsenic (mg kg^{-1} soil)						
AS_1=0	12.45a	58.05a	3.14a	0.68a	1.69a	1.10
AS_2=10	11.37b	50.31a	2.66b	0.56b	1.44b	1.09
AS_3=20	10.03b	44.88b	2.63b	0.45c	1.37c	1.02
AS_4=30	8.09c	39.95c	2.38b	0.39d	1.35c	1.01
Cadmium (mg kg^{-1} soil)						
Cd_1=0	11.58a	54.41a	2.98a	0.64a	1.70a	0.26
Cd_2=10	10.64a	49.59b	2.65b	0.56b	1.51b	0.05
Cd_3=20	10.30ab	47.18b	2.35c	0.47c	1.43c	0.03
Cd_4=30	9.43b	42.01c	2.36c	0.45c	1.31d	0.02

Means followed by the same letter are not significantly different within rows and column according to LSD test ($P \leq 0.05$)

However, their interaction did not have a significant effect on these parameters. By Increasing As concentration in the soil (from 0 to 30 mg kg^{-1}) the reductions in root length and root dry weight were 31.1% and 35.01%, respectively. For Cd, these reductions were 22.7% and 18.5% respectively.

Photosynthetic pigments are crucial for photosynthesis and play a significant role in plant growth and production. Figures 2 and 3 demonstrate that by increasing concentrations of Cd and As in the soil the concentration of chlorophyll "a" and carotenoids in the leaves of lemon balm, decreased. The maximum concentration of chlorophyll "a" (1.26 mg g^{-1} fresh weight) was observed at the As_1Cd_1

treatment (absence of Cd and As). While the lowest concentration of it (0.35 $mg\ g^{-1}$ fresh weight) was found at the As_4Cd_4 treatment (30 $mg\ kg^{-1}$ of As and Cd in the soil). Representing a decrease of 72.2%. In contrast, the highest carotenoid concentration (0.67 $mg\ g^{-1}$ fresh weight) was recorded at the As_2Cd_2 treatment (10 $mg\ kg^{-1}$ of As and Cd), and the lowest (0.19 $mg\ g^{-1}$ fresh weight) was noted at the As_4Cd_4 treatment, reflecting a decrease of 71.6% (Figure 3).

Unlike chlorophyll “a” and carotenoids, the concentration of anthocyanins in the leaves increased with higher concentrations of As and Cd in the soil. The lowest anthocyanin concentration (0.17 $mg\ g^{-1}$ fresh weight) was found in the As_1Cd_1 treatment, while the highest (0.76 $mg\ g^{-1}$ fresh weight) was observed at the As_4Cd_4 treatment. Indicating an increase of 77.6% (Figure 4).

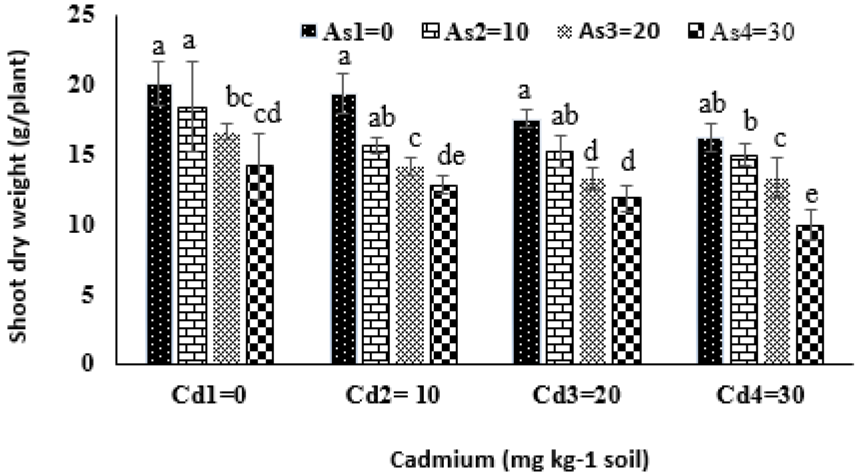

Figure 1. Interaction between arsenic and cadmium on shoot dry weight (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium in the soil)

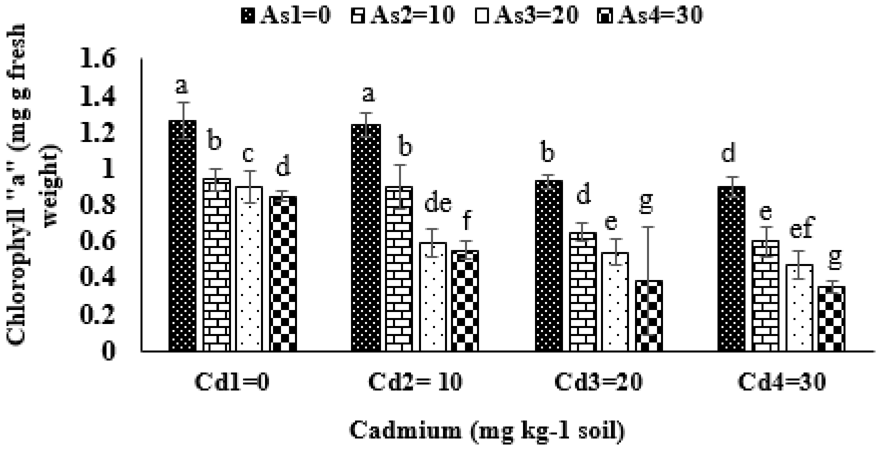

Figure 2. Interaction between arsenic and cadmium on chlorophyll “a” in leaf (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium in the soil)

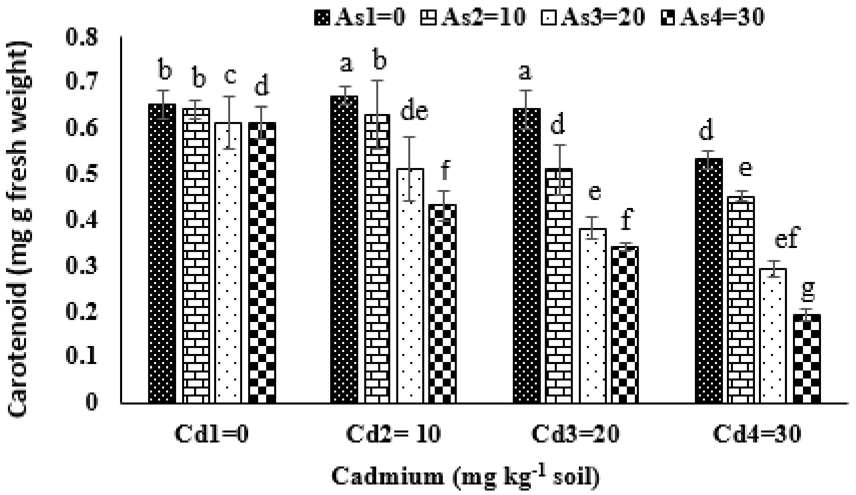

Figure 3. Interaction between arsenic and cadmium on carotenoid in leaf (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium in the soil)

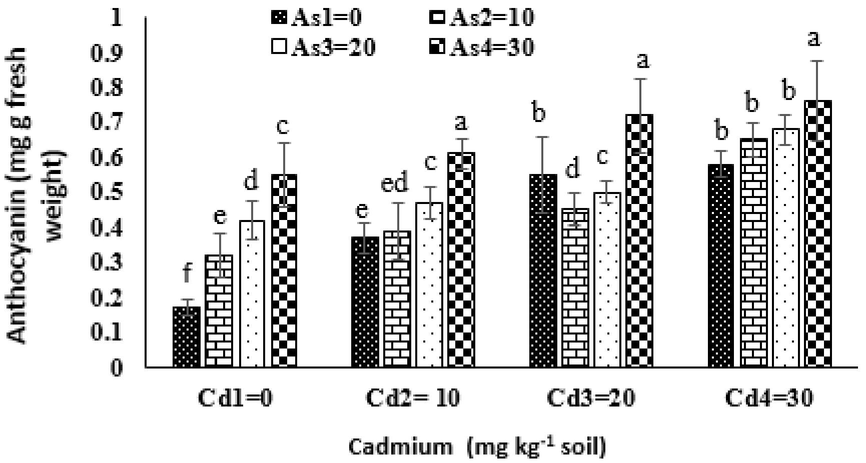

Figure 4. Interaction between arsenic and cadmium on *anthocyanin* in leaf (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium in the soil)

3.2. Antioxidant enzyme activity

One consequence of heavy metal stress on plants is the production of reactive oxygen species (ROS), which can lead to oxidative stress. To counteract ROS, plant cells have developed a complex network of both enzymatic and non-enzymatic defense mechanisms. The enzymatic components include catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and guaiacol peroxidase (POD). In this experiment, we measured the activities of only two antioxidant enzymes, SOD and CAT. The results indicated that the toxicity of Cd and As, increased the activity of these two enzymes in the leaves of lemon balm plants.

Figure 5 illustrates that by increasing the concentrations of Cd and As in the soil, the activity of SOD increased. ***This increase continued up to a level of 30 mg kg^{-1} Cd and As in the soil.*** The highest SOD activity was recorded 1.33 µmol H_2 O_2 min^{-1} mg^{-1} protein in the As_4Cd_4 treatment. The lowest SOD activity was 0.67 µmol H_2 O_2 min^{-1} mg^{-1} protein in the As_1Cd_1 treatment. It indicating an increase of 49.6%.

For CAT, enzyme activity increased with rising Cd concentrations in the soil up to 30 mg kg^{-1}. The highest CAT activity was observed at a concentration of 20 mg kg^{-1} of As. Beyond this point, further increases in As concentration led to a decrease in CAT activity. The maximum CAT activity was recorded (28.9 µmol H_2O_2 min^{-1} mg^{-1} protein) at the As_3Cd_4 treatment. Compared to the lowest activity of 14.6 µmol H_2O_2 min^{-1} mg^{-1} protein in the As_1Cd_1 treatment, reflecting an increase of 49.4% (Figure 6).

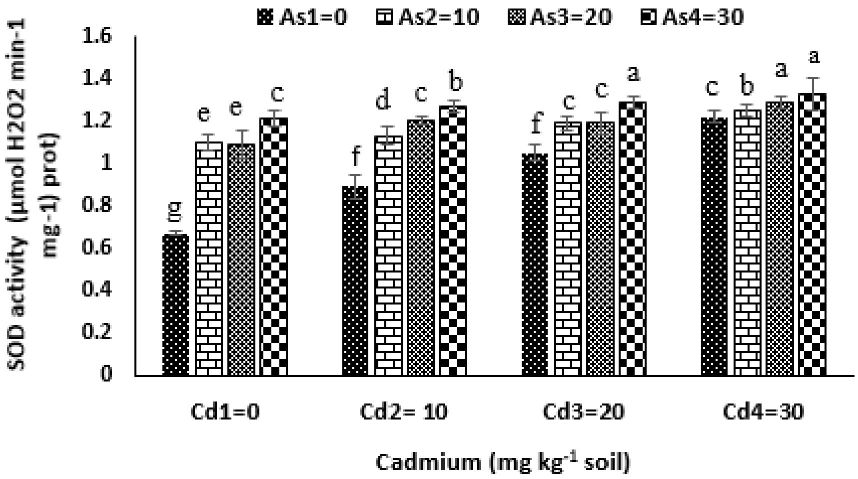

Figure 5. Interaction between arsenic and cadmium on SOD activity in leaf (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium in the soil)

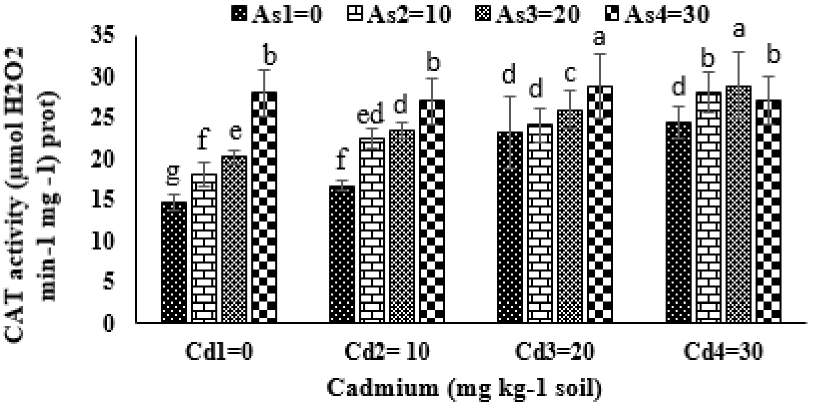

Figure 6. Interaction between arsenic and cadmium on CAT activity in leaf (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium

3.3. Ion concentration in root and leaf

The analysis of the data indicated that only the main effects of Cd and As treatments had a significantly influence on the concentrations of nitrogen, phosphorus, and potassium in the leaves of lemon balm plants. The interaction between Cd and As treatments did not have a significant effect on these nutrient concentrations (Table 1). As shown in Table 1, by increasing the concentration of As from 0 to 30 mg kg^{-1} in the soil, the concentrations of all three elements in the leaves decreased. The highest concentrations recorded for nitrogen (3.14 mg kg dry weight), phosphorus (0.68 mg kg dry weight), and potassium (1.69 mg kg dry weight) at the As_1 (control). While at As_4 the concentration of nitrogen, phosphorus and potassium were 2.38, 0.39 and 1.35 mg kg dry weight respectively. This reduction was 24.2% for nitrogen, 42.6% for phosphorus, and 20.1% for potassium.

The changes observed with Cd treatment were similar to those with As. By increasing the concentration of As and Cd in the soil, the concentrations of nitrogen, phosphorus and potassium in the leaves also decreased. The highest concentrations of nitrogen (2.98 mg kg dry weight), phosphorus (0.64 mg kg dry weight), and potassium (1.70 mg kg dry weight) were found in the Cd$_1$, while the lowest concentrations (2.36 mg kg dry weight for nitrogen, 0.45 mg kg dry weight for phosphorus, and 1.31 mg kg dry weight for potassium) were observed in the Cd$_4$ treatment. The reductions were 20.8% for nitrogen, 29.6% for phosphorus, and 22.9% for potassium (Table 1).

Regarding the effects of Cd and As on the concentrations of these elements in both roots and leaves, the results indicated that the interaction between Cd and As significantly affected the concentrations in both plant parts. Figures 7 and 8 illustrate the concentrations of Cd in the aerial and root parts of the lemon balm plant. The concentration of Cd in the roots increased from 2.72 mg kg^{-1} dry weight to 53.3 mg kg^{-1} dry weight, representing an increase of approximately 19.5 times. In contrast, the concentration in the aerial parts rose from 0.63 mg kg^{-1} dry weight to 0.96 µg g dry weight, reflecting a 1.5-fold increase. As shown in Figures 7 and 8, increasing the concentration of Cd from 0 to 30 mg kg^{-1} in the soil led to greater accumulation of Cd in both the roots and aerial parts, with the majority of the accumulation occurring in the roots. The presence of As in conjunction with Cd partially inhibited Cd accumulation in the roots while partially increasing its concentration in the aerial parts.

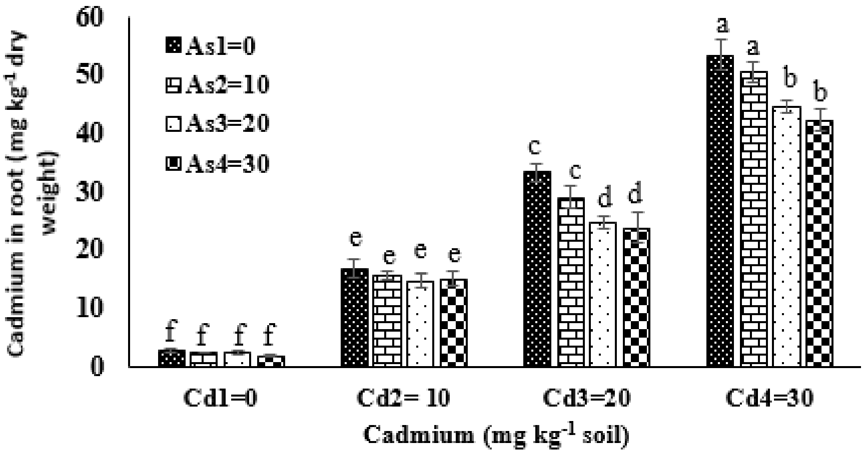

Figure 7. Interaction between arsenic and cadmium on cadmium in root (As$_1$=0, As$_2$=10, As$_3$=20 and As$_4$= 30 mg kg arsenic in the soil. Cd$_1$=0, Cd$_2$=10, Cd$_3$=30 and Cd$_4$= 30 mg kg cadmium in the soil)

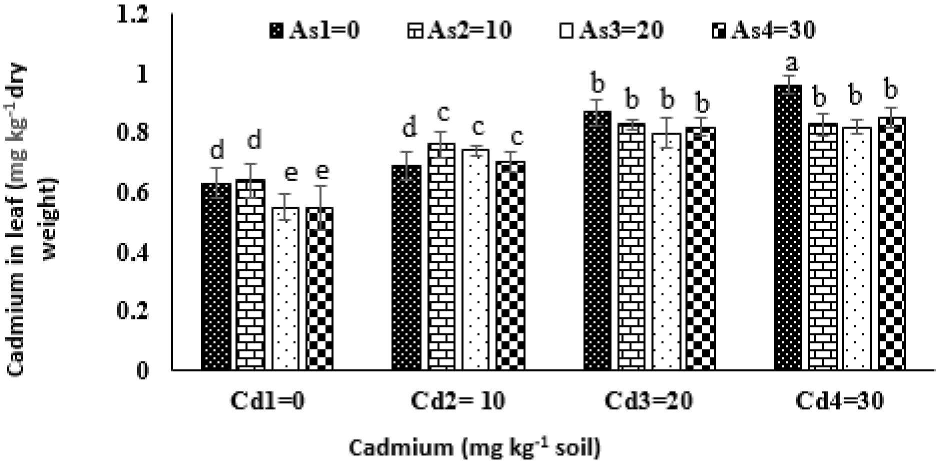

Figure 8. Interaction between arsenic and cadmium on cadmium in leaf (As$_1$=0, As$_2$=10, As$_3$=20 and As$_4$= 30 mg kg arsenic in the soil. Cd$_1$=0, Cd$_2$=10, Cd$_3$=30 and Cd$_4$= 30 mg kg cadmium in the soil)

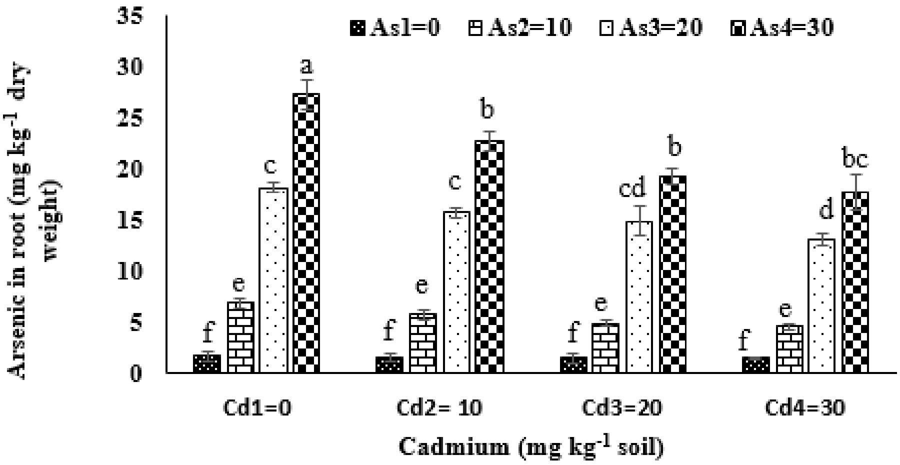

Figure 9. Interaction between arsenic and cadmium on arsenic in root (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium in the soil)

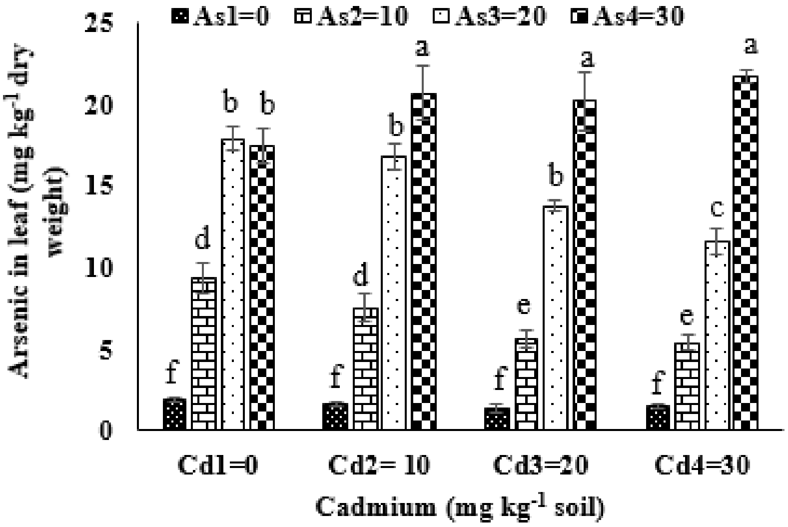

Figure 10. Interaction between arsenic and cadmium on arsenic in leaf (As_1=0, As_2=10, As_3=20 and As_4= 30 mg kg arsenic in the soil. Cd_1=0, Cd_2=10, Cd_3=30 and Cd_4= 30 mg kg cadmium in the soil)

Figures 9 and 10 present the concentrations of As in both the aerial and root parts. The concentration of As in the roots increased from 1.52 mg kg^{-1} dry weight to 27.3 mg kg^{-1} dry weight, an increase of 17.9 times. In the aerial parts, the concentration of As rose from 1.44 µg g dry weight to 21.7 mg kg^{-1} dry weight, resulting in a 15.1-fold increase. Figures 9 and 10 further demonstrate that as the concentration of As in the soil increased (from 0 to 30 mg kg^{-1}), and with the concurrent increase of Cd concentration (also from 0 to 30 mg kg^{-1}), the accumulation of As in the roots decreased, with a significant amount being transferred to the aerial parts.

4. Discussion

Cadmium and arsenic are non-essential elements, today their concentration in the soil have risen significantly due to industrial and agricultural activities (Armendariz et al., 2016; Saidi et al., 2013) and can disrupt various enzymatic functions, leading to inhibition of essential processes in plants (Zhao et al., 2021). The uptake and accumulation of As and Cd can adversely effect on plant growth, resulting in decreased photosynthetic rates and biomass production. Additionally, these elements can cause imbalances in nutrient uptake (Zou et al., 2017).

In this experiment, the results indicated that both Cd and As negatively impacted on growth of lemon balm (*Melissa officinalis*) and reduced dry weight in both shoot and root parts. This reduction was observed up to a concentration of 30 mg kg^{-1} of As and Cd in the soil. Specifically, the reduction in dry weight for the shoot at the As_4Cd_4 treatment compared to the As_1Cd_1 was 50.7% (Figure 1). For the root dry weight, a 35.01% reduction was noted at 30 mg kg^{-1} of As in the soil, while for Cd, the reduction was 18.5% (Table 1).

Gharebaghi et al. (2017) demonstrated that the germination of basil seeds (*Ocimum basilicum* L.) was inhibited by Cd when compared to the control group. Furthermore, plant height, leaf number, and root length were significantly reduced when Cd concentrations exceeded certain levels. This study confirmed that Cd is toxic to *Ocimum basilicum*. The observed changes in growth are closely related to the amount of organic matter produced during photosynthesis. Cd can directly or indirectly inhibit physiological processes such as water movement, gas exchange, respiration, and photosynthesis, ultimately leading to disruptions in plant metabolism (Tang et al., 2018).

Sugars play a crucial role in mitigating the effects of free radicals generated during heavy metal stress in plants. An increase in soluble and reducing sugars has been observed in *Satureja hortensis* as a response to Cd treatment (Azizollahi et al., 2019). Carbohydrates are closely linked to photosynthesis, and the photosynthetic pigments, primarily carotenoids, chlorophyll “a”, and chlorophyll “b” are essential for this process. Carotenoids are important for regulating plant growth and development, as well as for facilitating interactions between plants and their environment. Chlorophyll “a” is vital for oxygen production during photosynthesis, while chlorophyll “b” absorbs blue light energy (Xie et al., 2019).

In this experiment, the presence of Cd and As significantly reduced the concentration of photosynthetic pigments in lemon balm (*Melissa officinalis*). Both chlorophyll “a” and carotenoids exhibited high sensitivity to As and Cd levels in the soil. Specifically, the reduction in chlorophyll “a” in the As_4Cd_4 treatment compared to the As_1Cd_1 was 72.2%, while the reduction in carotenoids was 71.6% (Figures 2 and 3). Cd can adversely effect on plant metabolism by inhibiting photosynthesis, chlorophyll synthesis, and antioxidant enzyme activity, which ultimately leads to decreased crop yield and biomass (Li et al., 2015). As binds to proteins and enzymes, disrupting cellular biochemistry and impairing physiological processes such as respiration, photosynthesis, and transpiration in plants (Finnegan et al., 2015). As toxicity results in decreased synthesis of photosynthetic pigments, distortion of chloroplast structure, and reduced activity of photosystem II (PSII) and photosystem I (PSI) (Duman et al., 2010). In the context of As stress, the decline in plant dry weight can be attributed to toxic effects on various growth-related processes, including reduced biosynthesis of photosynthetic pigments and decreased water and nutrient content (Wang et al., 2020). As can negatively impact photosynthetic pigments, the chloroplast membrane system, and chlorophyll fluorescence, thereby diminishing overall photosynthetic activity (Singh et al., 2013). Oxidative stress is a significant factor contributing to the reduction of photosynthesis due to Cd and As exposure (Ahmad et al., 2020; Kaya et al., 2020). Cd toxicity is particularly notorious for inducing oxidative damage, leading to reduced growth by altering membrane permeability and promoting the production of reactive oxygen species at the organelle level (Anjum et al., 2015).

In this experiment, it was observed that Cd and As increased the activity of CAT and SOD enzymes in lemon balm (*Melissa officinalis*) plants. The highest enzyme activity was recorded at a concentration of 30 mg kg^{-1} of As and Cd in the soil, with an approximate increase of 49% (Figures 5 and 6). Studies have demonstrated that As induces the generation of ROS, such as hydroxyl radicals ($OH^{\bullet}$), superoxide ($O2^{\bullet-}$), and H_2O_2 (Siddiqui et al., 2020). To counteract the detrimental effects of oxidative stress, plants activate various enzymatic and non-enzymatic antioxidant molecules, including SOD, APX, CAT, GPX, monodehydroascorbate reductase (MDHAR), and peroxidase (POD) (Rajput et al., 2021).

Azizi et al. (2020) reported that Cd stress led to decreased growth and photosynthetic pigments, including chlorophyll “a”, chlorophyll “b”, total chlorophyll, and carotenoids in savory (*Satureja hortensis*), while simultaneously increasing cell membrane leakage, proline levels, CAT, and POD activity in response to rising Cd concentrations in the soil. Hassan et al. (2020) investigated the effects of different Cd concentrations on antioxidant enzyme activities in two sorghum cultivars, finding that varying levels of Cd significantly elevated H_2O_2 concentrations in the leaves of both cultivars up to 100 µM Cd, with maximum POD and CAT activity recorded at 25 µM Cd. CAT protects cells through an energy-efficient mechanism that removes H_2O_2. Higher CAT activity was also observed in mung bean and *Taxithelium nepalense* during As exposure (Singh et al., 2007). The presence of antioxidant compounds and enzymes can partially mitigate the adverse effects of heavy metal stress on plants; without these protective mechanisms, the extent of damage to the plants would increase.

As induces harmful effects on roots, which can impair the uptake of water and ions, consequently reducing transpiration and photosynthetic rates while inhibiting stomatal regulation (Heidari and Sarani, 2011; Carlin et al., 2016). Severe toxicity from As and Cd alters the concentration, accumulation, and translocation of nutrient elements in plants (Shaibur et al., 2009). Both Cd and As in the soil interfere with nutrient absorption in plants. Some plants can reduce physiological and biochemical damage by limiting the translocation of these metals to aerial parts (Shaibur et al., 2009). The results of this experiment indicated that As and Cd reduced the concentrations of phosphorus, nitrogen, and potassium in the leaves of lemon balm (Table 1). Notably, the lemon balm plant was able to partially prevent the transfer of absorbed Cd and As from the roots to the aerial parts. As illustrated in Figures 9 and 10, a significant portion of the Cd absorbed by the roots accumulated in the roots without translocating to the aerial parts. However, the amount of As accumulated in the roots was comparable to that in the aerial parts.

Roots serve as the first line of defense, protecting other parts of the plant from metal toxicity. The root system establishes the rhizosphere in the soil, allowing roots to accumulate and translocate specific heavy metals to shoots across cellular membranes (DalCorso et al., 2019). The translocation factors for As and Cd are presented in Table 1. It was observed that translocation factors greater than 1 were predominantly associated with As, with the highest translocation factors recorded at 1.10, 1.09, 1.02, and 1.01. This finding suggests that lemon balm plants are capable of translocating As from the roots to the leaves. In contrast, the translocation factors for Cd were significantly lower, at 0.26, 0.05, 0.03, and 0.02, indicating that lemon balm primarily retained Cd in the roots rather than transferring it to the leaves.

Phosphorus is an analogue of As phosphate (Pi), and it can enter plant cells via Pi transporters, potentially disrupting plant metabolism. Phosphate competes with arsenate for uptake by roots. An increase in phosphorus concentration is associated with a decrease in arsenate uptake. Research conducted on various soil types with differing phosphorus and iron levels has shown a positive correlation between available iron and arsenate concentrations in grains, whereas this correlation becomes negative when soil phosphorus availability is high (DalCorso et al., 2014). Additionally, it is believed that arsenate mobility is diminished in organic acid-rich soils, as these acids may act as binding agents or contribute to the formation of insoluble compounds, hindering arsenate absorption by roots (DalCorso et al., 2014).

Conclusions

Soil pollution with heavy metals can hinder plant growth, reduce crop yields, and pose significant risks to human health through the food chain. Medicinal plants, which are vital for treating various diseases, must be free from heavy metal contaminants. Among heavy metals, As and Cd are particularly concerning as they can be readily absorbed by plant roots and transported to the aerial parts. Our data indicate that soil concentrations of As (10, 20, and 30 mg kg^{-1}) and Cd (10, 20, and 30 mg kg^{-1}) limit photosynthesis and growth in lemon balm plants by increasing oxidative stress due to the generation of free radicals within the cells. The combination of As and Cd in the soil has a more pronounced effect on reducing plant growth and decreasing the concentrations of chlorophyll "a" and carotenoids, two essential photosynthetic pigments. Additionally, the presence of both As and Cd enhances the activity of antioxidant enzymes, such as SOD and CAT, in the leaves. This increase is attributed to the absorption and subsequent transfer of Cd and As from the roots to the aerial parts of the plant. In lemon balm plants, a significant concentration of Cd is retained in the roots, with only a small fraction moving to the aerial parts, whereas a larger proportion of absorbed As is transferred to the aerial parts. Therefore, it can be concluded that As and Cd negatively impact the growth of lemon balm, making it unsuitable for cultivation in soils contaminated with heavy metals, particularly As, due to the high transfer of As to the edible portions of the plant.

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References

Ali, S., Tyagi,A., Mushtaq, M., Al-Mahmoudi, H. and Bae, H.H., 2022. Harnessing plant microbiome for mitigating arsenic toxicity in sustainable agriculture. *Environmental Pollution*, 300, 118940.

Ahmad, P., Alam, P., Balawi, T.H., Altalayan, F.H., Ahanger, M.A. and Ashraf, M., 2020. Sodium nitroprusside (SNP) improves tolerance to arsenic (As) toxicity in *Vicia faba* through the modifications of biochemical attributes, antioxidants, ascorbate-glutathione cycle and glyoxalase cycle. *Chemosphere*, 244, 125480.

Anjum, S.A., Tanveer, M., Hussain, S., Bao, M., Wang, L., Khan, I., Ullah, E., Tung, S.A., Samad, R.A. and Shahzad, B. 2015. Cadmium toxicity in Maize (*Zea mays* L.): Consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. *Environmental Science and Pollution Research*, 22, 17022-17030.

Anjum, S.A., Tanveer, M., Hussain, S., Shahzad, B., Ashraf, U., Fahad, S., Hassan, W., Jan, S., Khan, I., Saleem, M.F., Bajwa, A.A., Wang, L., Mahmood, A., Samad, R.A. and Tung, S.A., 2016. Osmoregulation and antioxidant production in maize under combined cadmium and arsenic stress. *Environmental Science and Pollution Research*, 23, 11864-11875.

Armendariz, A.L., Talano, M.A., Travaglia, C., Reinoso, H., Oller, A.L.W. and Agostini, E. 2016. Arsenic toxicity in soybean seedlings and their attenuation mechanisms. *Plant Physiology and Biochemistry*, 98, 119-127.

Arnon, A.N. 1967. Method of extraction of chlorophyll in the plants. *Agronomy Journal*, 23, 112-121.

Aziz, I., Esmaielpour, B. and Fatemi, H. 2020. Effect of foliar application of selenium on morphological and physiological indices of savory (*Satureja hortensis*) under cadmium stress. *Food Science and Nutrition*, 8, 6539–6549.

Azizollahi, Z., Ghaderian, S.M. and Ghotbi-Ravandi, A. 2019. Cadmium accumulation and its effects on physiological and biochemical characters of summer savory (*Satureja hortensis* L.). *International Journal of Phytoremediation*, 21, 1241-1253.

Carlin, D.J., Naujokas, M.F., Bradham, K.D., Cowden, J., Heacock, M., Henry, H.F., Lee, J.S., Thomas, D.J., Thompson, C., Tokar, E.J. and Waalkes, M.P. 2016. Arsenic and environmental health: State of the science and future research opportunities. *Environmental Health Perspectives*, 124, 890-899.

Chapaman, H.D. and Pratt, P.E. 1961. Method of analysis for soil, plant and water. University of California, River Side, USA.

Chattopadhyay, A., Singh, A.P., Kasote, D., Sen, I. and Regina, A. 2021. Effect of phosphorus application on arsenic species accumulation and co-deposition of polyphenols in rice grain: Phyto and food safety evaluation. *Plants*, 10, 281.

Chen, H.C., Zhang, S. L., Wu, K.J., Li, R., He, W.R., He, D.N., Huang, C. and Wei, H. 2020. The effects of exogenous organic acids on the growth, photosynthesis and cellular ultrastructure of *Salix variegata* Franch Under Cd stress. *Ecotoxicology and Environmental Safety*, 18, 1–10.

DalCorso, G., Fasani, E., Manara, A., Visioli, G. and Furini, A. 2019. Heavy metal pollutions: State of the art and innovation in phytoremediation. *International Journal of Molecular Sciences*, 20 (14), 3412.

Dhindsa, R.S., Plumb-Dhindsa, P. and Thorpe, T.A. 1981. Leaf senescent: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, 32 (1), 93–101.

Duman, F., Ozturk, F. and Aydin, Z. 2010. Biological responses of duckweed (*Lemna minor* L.) exposed to the inorganic arsenic species As (III) and As(V): Effects of concentration and duration of exposure. *Ecotoxicology*, 19, 983-993.

Fattahi, B., Arzani, K., Souri, M.K. and Barzegar, M. 2019. Effects of cadmium and lead on seed germination, morphological traits, and essential oil composition of sweet basil (*Ocimum basilicum* L.). *Industrial Crops and Products*, 138, 111584.

Fattorini, L., Ronzan, M., Piacentini, D., Della Rovere, F., De Virgilio, C., Sofo, A., Altamura, M.M. and Falasca, G. 2017. Cadmium and arsenic affect quiescent centre formation and maintenance in

Arabidopsis thaliana post-embryonic roots disrupting auxin biosynthesis and transport. *Environmental and Experimental Botany*, 144, 37-48.

Finnegan, P. and Chen, W. 2012. Arsenic toxicity: The effects on plant metabolism. *Frontiers in physiology*, 3, 182.

Gharebaghi, A., Haghighi, M.H.A. and Arouiee, H. 2017. Effect of cadmium on seed germination and earlier basil (*Ocimum basilicum* L. and *Ocimum basilicum* var. purpurascens) seedling growth. *Trakia Journal of Sciences*, 1(1), 1-4.

Gupta, D.K., Huang, H.G., Nicoloso, F.T., Schetinger, M.R., Farias, J.G., Li, T.Q., Razafindrabe, B.H.N., Aryal, N. and Inouhe, M. 2013. Effect of Hg, As and Pb on biomass production, photosynthetic rate, nutrients uptake and phytochelatin induction in *Pfaffia glomerata*. *Ecotoxicology*, 9, 1403-1412.

Hassan, M.J., Raza, M.A., Rehman, S.U., Ansar, M., Gitari, H., Khan, I., Wajid, M., Ahmed, M., Shah, G.A., Peng, Y. and Li, Z. 2020. Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two sorghum cultivars. *Plants*, 9, 1575.

Heidari, M. and Sarani, S. 2011. Effects of lead and cadmium on seed germination, seedling growth and antioxidant enzymes activities of mustard (Sinapis arvensis L.). *ARPN Journal of Agricultural and Biological Science*, 6(1), 44-47.

Ibrahim, M.H., Kong, Y.C. and Zain, N.A.M. 2017. Effect of Cadmium and Copper Exposure on Growth, Secondary Metabolites and Antioxidant Activity in the Medicinal Plant Sambung Nyawa (*Gynura procumbens* (Lour.) Merr). *Molecules*, 22, 1623.

Jiang, W., Hou, Q., Yang, Z., Zhong, C., Zheng, G., Yang, Z. and Li, J. 2014. Evaluation of potential effects of soil available phosphorus on soil arsenic availability and paddy rice inorganic arsenic content. *Environmental Pollution*, 188, 159-165.

Kaya, C., Ashraf, M., Alyemeni, M.N. and Ahmad, P. 2020. Responses of nitric oxide and hydrogen sulfide in regulating oxidative defense system in wheat plants grown under cadmium stress. *Plant Physiology*, 345-360.

Khani, M.R. and Malekoti, M.J. 2000. Survey of cadmium changes in soils and rice of rice fields in north of Iran. *Journal of Soil and Water*, 12(9), 19-26.

Khan, Z.S., Rizwan, M., Hafeez, M., Ali, S., Adrees, M., Qayyum, M.F., Khalid, S., Rehman, M.Z.U. and Sarwar, M.A. 2020. Effects of silicon nanoparticles on growth and physiology of wheat in cadmium contaminated soil under different soil moisture levels. *Environmental Science and Pollution Research*, 27, 4958–4968.

Li, S., Yang, W., Yang, T., Chen, Y. and Ni, W.Z. 2015. Effects of cadmium stress on leaf chlorophyll fluorescence and photosynthesis of *Elsholtzia argyi*: a cadmium accumulating plant. *International Journal of Phytoremediation*, 17(1-6), 85-92.

Li, X., Ahammed, G.J., Zhang, X.Z., Zhang, L., Yan, P., Zhang, L.P., Fu, J.Y. and Han, W.Y. 2021. Melatonin-mediated regulation of anthocyanin biosynthesis and antioxidant defense confer tolerance to arsenic stress in *Camellia sinensis* L. *Journal of Hazardous Materials*, 403, 123922.

Masukasu, H., Karin, O. and Kyoto, H. 2003. Enhancement of anthocyanin biosynthesis by sugar in radish (*Raphanus sativus*) hypocotyls. *Plant Science*, 164 (2), 259-265.

Natasha Shahid, M., Khalid, S. and Saleem, M. 2022. Unrevealing arsenic and lead toxicity and antioxidant response in spinach: A human health perspective. *Environmental Geochemistry and Health*, 44(2), 487-496.

Osmam, E., Elveren, M. and Karakoyun, G. 2017. Heavy metal accumulation affects growth of Scots pine by causing oxidative damage. *Air Quality, Atmosphere and Health*, 10 (1), 85–92.

Rachmawati, R., Yona, D. and Kasitowati, R.D. 2018. Potential of *Avicennia alba* as an Agent of phytoremediation heavy metal (Pb and Cu) in Wonorejo, Surabaya. *Depik*, 7(3), 227-236.

Rahimi, G., Kolahchi, Z. and Bayat, S. 2019. Heavy metals' bio-accumulation and transfer in `lemon balm (*Melissa officinalis L.*) irrigated with industrial wastewater. *International Journal of Environment and Waste Management*, 23 (3), 238.

Rajput, V.D., Harish Singh, R.K., Verma, K.K., Sharma, L., Quiroz-Figueroa, F.R., Meena, M., Gour, V.S., Minkina, T., Sushkova, S. and Gour. S. 2021. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. *Biology*, 10, 267.

Sadeghi S., Oustan, S., Najafi, N., Valizadeh, M. and Monirifar, H. 2016. Interaction effects of zinc and cadmium on growth and chemical composition of canola (*Brassica napus* cv. Hyola) in a loamy sand soil. *Journal of Water and Soil Science*, 26(4), 237–254.

Sarai, A., Nazari, H., Neshli, H.F., Rahimi, E., Madihi, M., Afshari, H. and Heydari, N. 2022. An analysis of environmental evalution in the area of Qalae Bon, Babol city, Mazandaran province. *Quaternary Journal of Iran*, 4(3,4), 937-966.

Saidi, I., Ayouni, M., Dhieb, A., Chtourou, Y., Chaïbi, W. and Djebali, W. 2013. Oxidative damages induced by short-term exposure to cadmium in bean plants: protective role of salicylic acid. *South African Journal of Botany,* 85, 32–38.

Shaibur, M.R., Kitajima, N., Sugawara, R., Kondo, T., Imamul Huq, S.M. and Kawai, S. 2009. Effect of arsenic on phytosiderophores and mineral nutrition of barley seedlings grown in iron-depleted medium. *Journal of Soil Science and Plant Nutrition,* 55, 283-293.

Siddiqui, M.H., Alamri, S., Khan, M.N., Corpas, F. J., Al-Amri, A.A., Alsubaie, Q.D., Ali, H.M., Kalaji, H.M. and Ahmad, P. 2020. Melatonin and calcium function synergistically to promote the resilience through ROS metabolism under arsenic-induced stress. *Journal of Hazardous Materials,* 398, 122882.

Singh, H.P., Batish, D.R., Kohali, R.K. and Arora, K. 2007. Arsenicinduced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation. *Plant Growth Regulation,* 53, 65–73.

Singh, V.P., Srivastava, P.K. and Prasad, S.M. 2013. Nitric oxide alleviates arsenic-induced toxic effects in ridged Luffa seedlings. *Plant Physiology and Biochemistry,* 71, 155–163.

Tang, Y., Xie, Y., Sun, G., Tan, H., Lin, L., Li, H., Liao, M., Wang, Z., Lv, X., Liang, D., Xia, H., Wang, X., Wang, J., Xiong, B., Zheng, Y., He, Z. and Tu, L. 2018. Cadmium-accumulator straw application alleviates cadmium stress of lettuce (*Lactuca sativa*) by promoting pgotosynthetic activity and antioxidative enzyme activities. *Environmental Science and Pollution Research*, 25, 30671–30679.

Taranishi, Y., Tanaka, A., Osumi, N. and Fukui, S. 1974. Catalase activity of hydrocarbon utilizing Candida yeast. *Agricultural and Biological Chemistry*, 38: 1213-1216.

Vezza, M.E., Llanes, A., Travaglia, C., Agostini, E. and Talano, M.A. 2018. Arsenic stress effects on root water absorption in soybean plants: physiological and morphological aspects. *Plant Physiology and Biochemistry,* 1–35.

Wang, Q., Wen, J., Zheng, J., Zhao, J., Qiu, C., Xiao, D., Mu, L. and Liu, X. 2021. Arsenate phytotoxicity regulation by humic acid and related metabolic mechanisms. *Ecotoxicology and Environmental Safety*, 207, 111379.

Xie, J., Yao, S., Ming, J., Deng, L. and Zeng, K. 2019. Variations in chlorophyll and carotenoid contents and expression of genes involved in pigment metabolism response to oleocellosis in citrus fruits. *Food Chemistry,* 272, 49–57.

Zemanová, V., Pavlíková, D., Hniliˇcka, F. and Pavlík, M..2021. Arsenic Toxicity-Induced Physiological and Metabolic Changes in the Shoots of *Pteris cretica* and *Spinacia oleracea. Plants,* 10, 1-19.

Zemanová, V., Pavlíková, D. and Pavlík, M. 2020. Free amino acid regulation in fronds and roots of two *Pteris cretica* L. ferns under arsenic stress. *Plant, Soil and Environment,* 66(10), 483-492.

Zhang, Y., Wolosker, M.B., Zhao, Y., Ren, H. and Lemos, B. 2020. Exposure to microplastics cause gut damage, locomotor dysfunction, epigenetic silencing, and aggravate cadmium (Cd) toxicity in drosophila. *Science of the Total Environment*, 744, 140979.

Zhao, H., Guan, J., Liang, Q., Zhang, X., Hu, H. and Zhang, J. 2021. Effects of cadmium stress on growth and physiological characteristics of *sassafras* seedlings. *Scientific Reports,* 11, 9913.

Zou, J., Wang, G., Ji, J., Wang, J., Wu, H., Qu, Y. and Li, B. 2017. Transcriptional, physiological and cytological analysis validated the roles of some key genes linked Cd stress in *Salix matsudana* Koidz. *Environmental and Experimental Botany,* 4, 116–129.