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# EFFECTS OF ARSENIC ON THE GROWTH AND OXIDATIVE STRESS IN *MATRICARIA RECUTITA L.*

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**Summary.** The effect of arsenic (As) in chamomile (*Matricaria recutita L.*) was studied by investigating plant growth and oxidative stress. Four levels of As (0, 10, 45, and 80 μM) were applied for 14 days in a greenhouse. The results showed that high concentrations of As decreased growth parameters as well as chlorophyll content and stimulated lipid peroxidation. Treatment dramatically increased As accumulation in both shoot and root, but As content in roots was higher than in shoots. The enhanced content of As significantly increased the concentrations of $H_2O_2$, proline and protein. Carotenoids firstly increased as As concentrations increased and then decreased with further increasing As content. The significant decrease of anthocyanins content was due to the increased As concentration. However, the accumulation of As decreased sugar content in leaves and roots. These results suggest that high concentrations of As may cause oxidative damage in chamomile plants.

**Key words:** arsenic; chamomile; $H_2O_2$; MDA; proline; protein; sugar.

**Abbreviations:** As – Arsenic; $H_2O_2$ – Hydrogen peroxide; MDA – Malondealdehyde; ROS – Reactive oxygen species; TBA – Thiobarbituric acid; PCs – phytochelatins; PAL – Phenylalanine ammonia lyase.

## INTRODUCTION

Pollution with heavy metals received important consideration as a result of the increased environmental pollution from natural geologic activity and manmade sources such as wood preservatives, mining, heavy industry, fertilizers, pesticides, sewage and smelter wastes (Li et al., 2007). The serious harm has become one of the problems causing the attention of researchers in the world. Arsenic (As) is a crystalline metalloid that is usually found in the form of As anions (arsenite and arsenate). These anions are the oxidized state of arsenic, and are rapidly taken up by plants (Schmöger et al., 2000**).** Arsenate affects as a phosphate analogue and is transported across the plasma membrane by phosphate transporter

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systems and can disrupt phosphate metabolism, but it might be reduced to arsenite in the cytoplasm (Stoeva and Bineva, 2003). Arsenite reacts with sulfydryl groups of enzymes and proteins which causes physiological disorders (Gunes et al., 2009). In addition, the high-affinity phosphate/arsenic uptake system is suppressed. Arsenic inhibits mainly the growth (Stoeva and Bineva, 2003), causes a reduction of the photosynthetic rate and decreased chlorophyll content (Miteva and Merak, 2002). The negative As symptoms are closely related to content and exposure time. Stimulation of shoot and root growth was recorded at low concentrations (0-1 mg/kg) in As-treated wheat seedlings (Chun et al., 2007). However, Abedin and Meharg (2002) reported that early seedling growth of rice decreased significantly with increasing concentrations of As (Abedin and Meharg, 2002). There is significant evidence that As treatment leads to the generation of reactive oxygen species (ROS), and these species react with lipids, proteins, pigments and nucleic acids (Khan et al., 2009). Moreover, As stimulated lipid peroxidation and damaged the chloroplast membranes (Stoeva and Bineva, 2003). To conflict these consequences, enzymatic and non-enzymatic (carotenoids, anthocyanins and proline) antioxidants are mobilized. Accumulation of proline can prevent metal-induced lipid peroxidation (Rai, 2002). Chamomile is an annual widespread herb widely used as a medicinal plant that metals in its above-ground biomass (Kováčik et al., 2009). The effects of heavy metals such as Cd, Zn (Kummerová et al., 2010), and Ni (Kováčik et al., 2009) on *M. recutita* were investigated. Thus, the aim of this experiment was to investigate the growth, physiological parameters such as chlorophyll, carotenoids, proteins, proline, sugar, MDA and $H_2O_2$ contents in *M. recutita* exposed to elevated levels of As.

## MATERIALS AND METHODS

Chamomile (*Matricaria recutita L.*) seeds were sown in perlite. After germination, seedlings were supplied with half strength Lang-Ashton nutrient solution three times a week. The pH was maintained close to 6.5. The concentrations of As were 0, 10, 45, 80 µM, prepared freshly as a water solution of $NaASO_2$. The chamomile plants were watered for 14 days with half strength Lang-Ashton solution with or without As (pH 6.5). All analyses were done using fresh or frozen rosettes in liquid nitrogen and stored at -80°C.

### Determination of As concentration

The samples were dried at 70°C for 3 days in order to determine the mineral element concentration. Air-dried samples (0.5 g) were digested with nitric acid for 24 h. Analysis was performed using an atomic absorption spectrophotometer (Spectra AA 220).

### Photosynthetic pigments

Chlorophyll and carotenoid content was extracted by 80% acetone and assessed spectrophotometrically according to Lichtenthaler (1987).

### Determination of anthocyanin

Anthocyanin content was determined according to the method of Wanger (1979) using a UV-visible spectrophotometer at a wavelength of 550 nm.

### Lipid peroxidation

For the measurement of MDA content, the thiobarbituric acid (TBA) test was applied (Heath and Packer, 1968). The amount of the MDA-TBA complex (red pigment) was measured by its particular absorbance at 532 nm. Non-particular absorbance at 600 nm was also deduced. The content of other aldehydes was determined according to the method of Meirs (1992).

### Proline content

Proline content was determined according to the method of Bates et al. (1973). Samples were extracted with sulfosalicylic acid, then glacial acetic acid and ninhydrine solutions were added. The absorbance of samples was read at 528 nm.

### Protein content

Protein content was determined using the method of Bradford (1976). The absorption was read at 595 nm.

### Sugar content

Sugar content was determined according to Somogy (1952). The absorption was read at 600 nm, and then the values were computed using a standard curve.

### $H_2O_2$ concentration

Hydrogen peroxide concentrations were determined according to Alexieva et al. (2001). $H_2O_2$ content was calculated using the absorbance of the supernatant at 390 nm.

### Statistical analysis

All experiments were done in 3 replications. The data were analyzed using analysis of variance, and means were compared by Duncan's test.

## RESULTS AND DISCUSSION

### Arsenic accumulation and plant growth

In this research, $NaAsO_2$ was applied and accumulation of As in roots was significantly greater than in shoots compared to the control (Fig. 1). The results coincided with previous reports in rice (Shaibur et al. 2006), barely and sorghum plants (Shaibur et al., 2008). Arsenic anions are rapidly absorbed by the root surface and impressively induce the biosynthesis of phytochelatins (PCs) in plants. Arsenite has a high affinity to thiols and complexation of arsenite by PCs

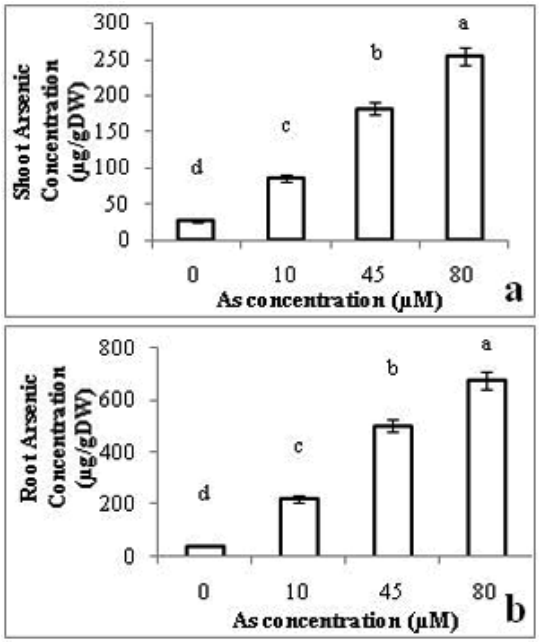


**Figure 1.** Effects of As stress on As content in shoot (a) and root (b) of chamomile plants. Data are means ± SE of three replicates. Values in a group followed by the same letter are not statistically different at $p < 0.05$ level as determined by Duncan's test.

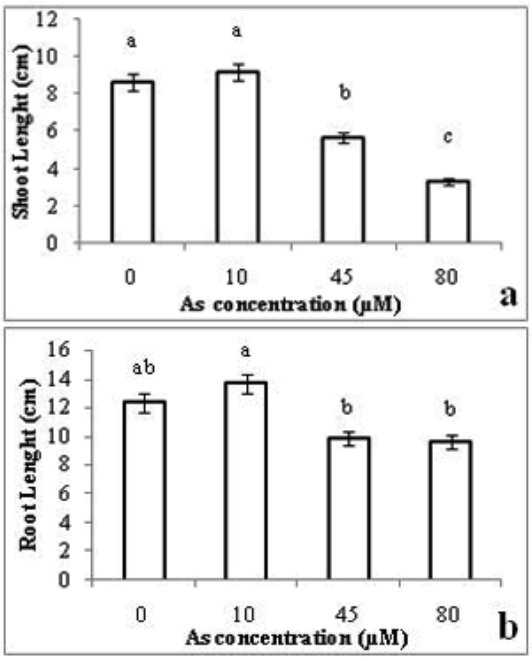


**Figure 2.** Effects of As stress on shoot length (a) and root length (b) of chamomile plants.

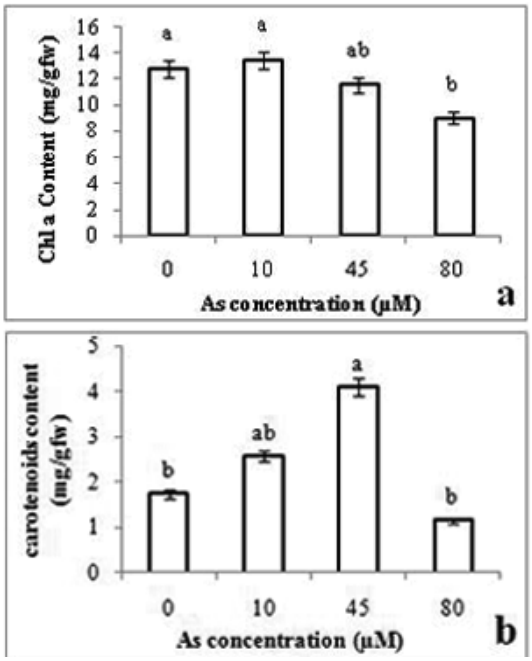


**Figure 3.** Effects of As stress on Chl (a) and carotenoid (b) content in chamomile plants.

may be expected (Schmöger et al., 2000). In addition, chamomile accumulated more As in the roots complexed with thiols, particularly PCs and thus prevented excess As uptake by the shoot. We observed that the length of the shoot and root decreased with increasing As concentration up to 80 µM in commomile (Fig. 2). The toxicity effects of As observed in our study were similar to those previously described for sorghum (Shaibur et al., 2008) and early rice seedling growth (Abedin and Meharg, 2002). We noticed that the shoot was more sensitive to As toxicity than the root. This might be due to the inhibition of nutrient uptake by roots which caused a reduction in plant growth (Shaibur et al., 2008).

**Photosynthetic pigments**

Our results showed that 80 µM As significantly decreased chlorophyll (Chl) content, but 10 and 45 µM As did not significantly affect Chl content compared with the control (Fig. 3A). It is known that the unavoidable production of ROS leads to singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals that affect membrane permeability, enzyme and photosynthetic activity, plant biomass, and cause leaf chlorosis and necrosis (Karabal et al., 2003). Besides, treatment with As affects Chl synthesis and causes Chl degradation. Protochlorophyllide reductase activity is inhibited by heavy metals. Heavy metals link with –SH groups of proteins, thereby destroying their structure and function (Li et al., 2007). Carotenoids (Car) act as a guard pigment in the chloroplast. They have an important antioxidative role in production of lipid peroxidation and exciting chlorophyll, quenching of the singlet oxygen and scavenging free radicals (Jethesh et al.,

2006). The significant increase of Car at 45 µM As may indicate a role of this pigment in preventing chlorophyll destruction. The decrease of Car content at 80 µM As could be related to the enhancement of ROS production. A reduction of pigment content in response to As in oat plants has been reported (Stoeva and Bineva, 2003).

**Anthocyanin content**

In this study, anthocyanin content increased up to 10 µM As, and then gradually decresed with increasing As content compared with the control (Fig. 4). It was shown that in As treated plants, PAL activity increased and phenyl-propanoid derivatives accumulated in the stress-affected tissues, thus protecting plants against environmental stresses (Dixon and Paiva, 1995). The decreased anthocyanin content up to 80 µM As may be due to the fact that oxidative stress probably destroyed PAL and/or activated phenylpropanoid pathway toward lignification, increased $H_2O_2$ content and reduced growth.

**$H_2O_2$ content and lipid peroxidation**

Arsenic is known to induce oxidative stress. Damage to leaf membranes was assessed by evaluating the content of MDA and other aldehydes (Fig. 5). The data indicated that the level of As supply had a positive effect on MDA content and it increased compared with the control. It has been reported that ROS could induce chain-like peroxidation by disorganizing the membrane structure which could make the production of lipid peroxide and MDA. Heavy metals such as lead and mercury caused loss of membrane integrity in rice (Mishra and Choudhuri, 1999). In this study, the

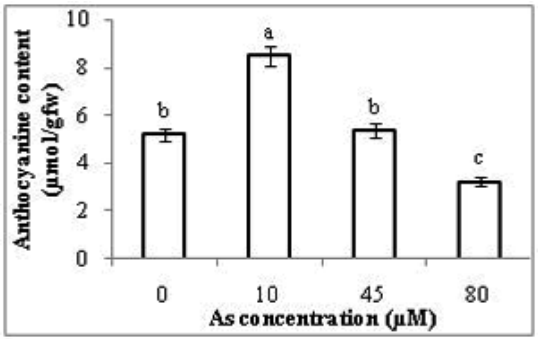


**Figure 4.** Effect of As stress on anthocyanin content in chamomile plants.

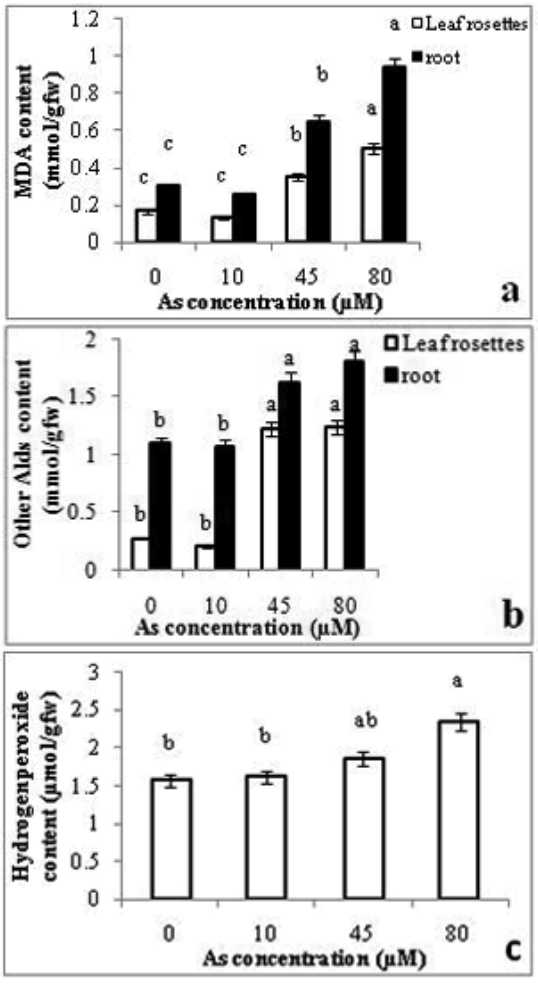


**Figure 5.** Effects of As stress on the content of MDA (a), other aldehydes (b) and H2O2 (c) in chamomile plants.

contents of MDA and other aldehydes were not significantly different compared with control at low concentration of As, whereas toxic concentrations of As (45-80 µM) increased lipid peroxidation. These results indicated that antioxidant system of chamomile could eliminate free radicals and prevent from oxidative destruction, while accumulation of ROS at high concentrations of As increased membrane lipid peroxidation. Our results showed that $H_2O_2$ content gradually increased with increasing As concentration (Fig. 5).

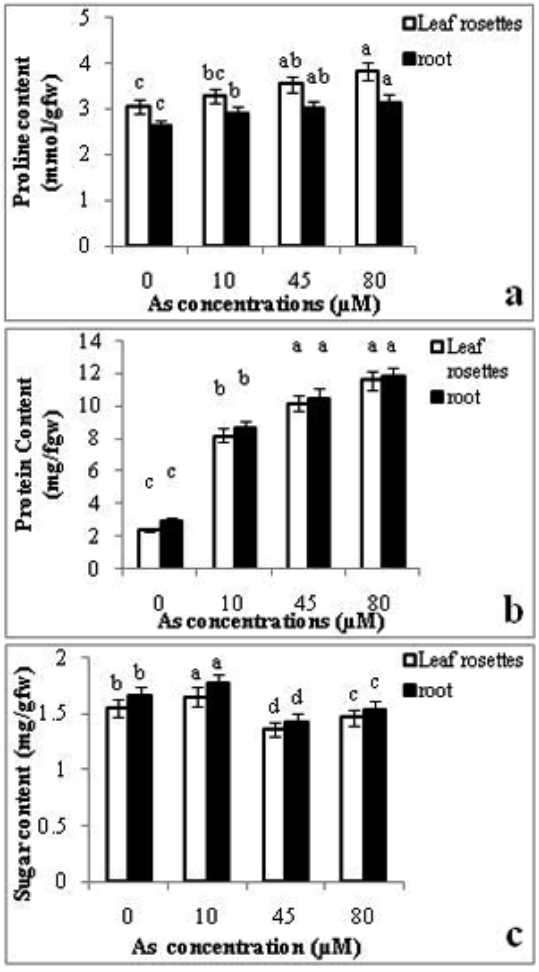


**Figure 6.** Effects of As stress on proline (a), protein (b) and sugar (c) content in chamomile plants.

$H_2O_2$ is also toxic to cells and detoxified via catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD). Since the growth of the plants was not affected at low As concentrations, this could suggest that $H_2O_2$ level in the leaves of plants treated with low As concentrations did not cause any harmful oxidative damage to the plants. Low levels of ROS ($H_2O_2$) is important for its operation in signal- transduction pathways in plants (Van Breusegem et al., 2001). Oxidative damage may occur either by unqualified or non-effective scavenging of ROS (Edreva, 2005).

**Proline, protein and sugar content**

The application of As had an obvious effect on the accumulation of proline (Fig. 6A). Proline content significantly increased with increasing As concentration. Many plants have been shown to accumulate proline when exposed to heavy metals (Rai, 2002). Proline can act as a non-toxic osmolyte but free proline plays only a minor role, while reducing sugars and $K^+$ are major contributors to osmoregulaion (Rai, 2002). The possibility that proline may protect plants against heavy metals by forming chelates has also been examined. Proline has also been proposed as a protector of enzymes against environmental stress. Sharma and Lakhvir (1988) showed that proline protected the activity of glucose-6-phosphate dehydrogenase and nitrate reductase *in vitro* against inhibition by Cd and Zn due to formation of a proline-metal complex (Sharma and Lakhvi, 1988). The increased protein content in chamomile plants treated with As (Fig. 6B) was a consequence either of accelerated biosynthesis or reduced catabolic

processes. The induction of protein synthesis under toxicity of different metals has been reported, which play a considerable role in the maintenance of metal homeostasis and/or detoxification within the cells. Therefore, proteins that were not used in cells accumulated, thus increasing soluble protein content (Mishra and Dubey, 2006).

Sugar content increased at a concentration of 10 µM As and gradually decreased with increasing As concentrations (Fig. 6C). When chamomile plants were exposed to high As concentrations (45 and 80 µM) probably the respiration was speeded up and needed more substrate (soluble sugar) to provide more energy for the plant. The high carbohydrate concentration with its role to reduce water potential helps to prevent oxidative losses and maintain protein structure during water shortage (Koch, 1996).

## CONCLUSIONS

Our results showed that *Matricaria recutita* L. induced synthesis of antioxidant compounds such as anthocyanine, carotenoids and proline at low concentrations of As (10 µM), whereas high concentrations of As (45 and 80 µM) increased the content of reactive radicals and caused oxidative stress including a decrease in plant growth and chlorophyll content, and an enhancement in $H_2O_2$ content and lipid peroxidation.

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