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Effects of Varied Forms of Arsenic Stress on Seedling Growth and Arsenic Distribution in Honeysuckle Plants

Xin Yuan [1], Juntong Zhou [1], Congnan Peng [1], Jiaqi Qiao [1], Shengqi Xia [1], Yilin Fan [1], Li Yao [1], Kailin Qi [1], Dongliang Chen [2], Zhiying Guo [2], Xiaolong Gan [2], Yaxuan Sun [1], Xueling Dai [1], Liangyu Lv [3], Yizhi Ji [1,*] and Qing Huo [1,*]

[1] Biochemical Engineering College, Beijing Union University, Beijing 100023, China; yuanxinxin321@163.com (X.Y.); 18614965860@163.com (J.Z.); pengcongnan@126.com (C.P.); q13213292522@163.com (J.Q.); xiashengqia@126.com (S.X.); 15153293587@163.com (Y.F.); 18601162012@163.com (L.Y.); 18611601319@163.com (K.Q.); sunxx@buu.edu.cn (Y.S.); xueling@buu.edu.cn (X.D.)

[2] Beijing Synchrotron Radiation Facility, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China; dlchen@ihep.ac.cn (D.C.); zyguo@ihep.ac.cn (Z.G.); xlgan@ihep.ac.cn (X.G.)

[3] Biological Science and Medical Engineering College, Donghua University, Shanghai 201620, China; lyuyuri@outlook.com

* Correspondence: jiyizhibuu@163.com (Y.J.); shthuoqing@buu.edu.cn (Q.H.)

Abstract: At present, soil arsenic contamination is one of the prominent environmental problems. The arsenic concentration in honeysuckle exceeds the standard levels, thus affecting the quality of traditional Chinese medicine. In this study, the hydroponic method was employed to explore the effect of organic arsenic (dimethyl arsenic) and trivalent inorganic arsenic ($NaAsO_2$) on the growth of honeysuckle plants. The study demonstrated that low concentrations of inorganic arsenic (10–20 mg/L) and 10–50 mg/L organic arsenic had a stimulating effect on the growth of honeysuckle plants. The activities of antioxidant enzymes (peroxidase and catalase) increased correspondingly. However, the antioxidant system in honeysuckle plants was damaged under high concentrations of inorganic arsenic (20–40 mg/L) and 50–70 mg/L organic arsenic. On exposure to 30 mg/L $NaAsO_2$ or 50 mg/L dimethyl arsenic acid for 10 days, the activities of peroxidase and catalase, as well as the malondialdehyde content, increased with prolonged exposure. The micro X-ray fluorescence analysis revealed that the accumulated arsenic in the roots was transported from the central vascular cylinders to the outer part of the root with the increase in concentration and exposure duration of inorganic arsenic. However, organic arsenic stress did not result in significant variations in the distribution of arsenic with increasing concentrations of arsenic. The arsenic element was predominantly located in the middle woody part of the root. The distributions of arsenic in the stems and leaves, in terms of organic and inorganic arsenic stresses, were similar, with accumulation primarily in the cortex of the stem and veins of the leaves. As a commonly used bulk traditional Chinese medicine, honeysuckle has a wide range of product quality issues. Hence, exploring the absorption, distribution, and transport trends of heavy metals such as arsenic in the plant body is of great significance for scientifically evaluating the impact of heavy metal pollution on the quality of medicinal materials and exploring ways to reduce the accumulation of heavy metals in the medicinal parts of plants.

Keywords: arsenic stress; honeysuckle; micro X-ray fluorescence; physiological indicators

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1. Introduction

As a part of traditional Chinese culture, traditional Chinese medicine (TCM) is one of the few industries in China with significant international competitive advantages. However, heavy metal pollution resulting from rapid industrialization has become a more pressing concern, and Chinese herbal medicines are seriously affected by it. Heavy metal contamination is a major issue affecting the quality and reputation of TCM and obstructing

its global development [1,2]. The World Health Organization estimated that approximately 4 billion people worldwide currently use TCM for disease treatment, accounting for 80% of the global population [3]. The safety of TCM is paramount to the health and well-being of its users. Unlike the simple and clear material basis of Western medicine, the material basis of TCM is extremely complex. Therefore, the morphology, structure, and valence coordination of trace heavy metals in TCM plays a critical role in determining the effects of medicine on patients, including the dynamic metabolism, dose–effect relationship, and toxic effect mechanisms [4].

Arsenic is classified as a heavy metal. It is a highly toxic environmental pollutant, which is not an essential element for plant growth. The 2015 edition of the Chinese Pharmacopoeia prescribes that the concentration of arsenic in Chinese medicinal materials should not exceed 2 mg/kg [5]. However, the International Organization for Standardization stipulates that the concentration of arsenic in Chinese medicinal materials should not exceed 4 mg/kg. The toxicity of arsenic depends largely on its chemical form, as different forms of arsenic exhibit varying toxicity levels. In the environment, arsenic, primarily, is in inorganic forms, such as As_2O_3 and As_2O_5, and organic forms, such as monomethyl arsenic acid, dimethyl arsenic acid (DMA), and trimethylarsenic acid. In sea creatures, arsenobetaine (AsB) and arsenocholine (AsC) are the primary forms of arsenic. The toxicity of arsenic is related to its concentration, form, bioavailability, and ingestion by organisms. In general, inorganic arsenic is more toxic than organic arsenic, and trivalent arsenic is more toxic than pentavalent arsenic [6,7].

Honeysuckle, the dried flower bud or first flower of *Lonicera japonica* from the Caprifoliaceae family, is a precious Chinese medicinal material known for its heat-clearing, detoxifying, and wind–heat-expelling properties. Its distinct aroma and health benefits make it a popular herbal tea in some Asian and European countries [8,9].

The last two editions of the Chinese Pharmacopoeia specify the dried flower bud of L. japonica as the medicinal part of honeysuckle. However, different parts of the original plant have been used as medicine in different historical periods, with varying medicinal names. The term "gold-silver flower" was initially used in the "Prescription for Treating Carbuncle" of "Su Shen Liang Fang", where a handful of tender seedlings of honeysuckle and 16 g licorice were directly used. Before the Song Dynasty, only stems and leaves were used, and thus no term such as "gold-silver flower" existed. The use of honeysuckle flowers as medicine began in the Song Dynasty. Before the Qing Dynasty, stems, leaves, and flowers were equally used as medicine. During the Qing Dynasty, the honeysuckle flower was considered more valuable than the other parts of the plant. The quality distinctions were made among honeysuckle plants from different regions only during the period of the Republic of China [10]. Honeysuckle flowers play various roles, such as exerting antipathogenic and antiviral effects, increasing the phagocytosis of inflammatory cells, stimulating the central nervous system, and lowering blood lipid levels. Modern Chinese medicine features honeysuckle flowers in various prescriptions, including Xiaoer Qingre granules, Qingkailing granules, Yinqiao Jiedu tablets, and Lianhua Qingwen capsules [11]. Guo, Lanping, and colleagues, at the Traditional Chinese Medicine Resource Center of the China Academy of Chinese Medical, conducted a literature review on heavy metal contamination in traditional Chinese medicinal materials published between 2000 and 2016. The study was conducted on 1700 samples of 275 different types of traditional Chinese medicinal materials. The results indicated that lead, arsenic, mercury, and cadmium exceeded the acceptable limits for heavy metals. Among these, arsenic had the highest rate of exceeding the standard, with a value of 4.03%. Three batches of seaweeds of unknown origin had the highest arsenic content (81.34–82.55 mg/kg), followed by nine batches of honeysuckle flowers from Shandong, Anhui, and Henan (30.80–73.35 mg/kg) and *Asarum* from Fusong, Jilin (33.82 mg/kg) [12]. Chinese herbal medicines can accumulate arsenic from their growing environment, including soil/plant uptake, irrigation water, and atmospheric deposition. Further, arsenic can also enter TCM during drying, storage, transportation, and manufacturing [13,14]. Studying the chemical structure of arsenic is

crucial due to its poisonous nature and involvement in inflammatory diseases and cancers such as leukemia. Similarly, a detailed analysis of the compound morphology of heavy metals should be conducted, instead of just measuring their overall quantity, to ensure their safety. This approach provides a more scientific and impartial evaluation of heavy metal safety. Under normal conditions, the dynamic balance between the production and the removal of reactive oxygen species (ROS) in plant cells allows the normal growth of plants. However, adverse circumstances can disrupt this balance, leading to an accumulation of a large number of free radicals in plants [15]. Excessive ROS levels in plants can cause oxidative stress, which can damage plant cells. Plants have developed various antioxidant enzymes, such as superoxide dismutase and catalase (CAT), to protect themselves from the harmful effects of ROS. These enzymes help regulate the production and elimination of ROS in plants, thereby restoring balance and reducing the impact of adversity on plant growth and survival [16].

X-ray fluorescence (XRF) spectroscopy is a rapid and nondestructive analytical technique used to determine the elemental composition of materials. XRF uses X-rays to irradiate different atoms; the atoms become excited and emit fluorescence. The wavelength of XRF has a fingerprint effect on atoms. Different substances emit fluorescence of different wavelengths [17]. For example, Pickering et al. used micro synchrotron XRF (μ-SXRF) scanning analysis to examine the distribution of arsenic in the roots, leaf rachis, and leaves of centipede grass. They found that As(V) was in the center of the leaf veins, whereas arsenic $As(SR)_3$ tightly surrounded the arsenate on the veins like a cylindrical shell [18]. Andreas et al. employed micro XRF (μ-XRF) and extended X-ray absorption fine structure (EXAFS) spectra to examine how arsenic was distributed and shaped in contaminated floodplain soil and plant roots. They could visually display the distribution patterns of different elements in the soil [19].

In this study, authentic honeysuckle seedlings were used as test materials to investigate the effects of different concentrations of organic and inorganic arsenic on the growth of honeysuckle and to preliminarily determine the tolerance of honeysuckle to different forms of arsenic. Furthermore, synchrotron μ-XRF analysis was conducted at the 4W1B beamline experimental station of the Beijing Synchrotron Radiation Facility (BSRF) to investigate the micro-distribution characteristics of As in plant roots, stems, and leaves. The aim was to provide a theoretical reference for the safe and rational planting of honeysuckle plants and to regulate the production of traditional Chinese medicinal materials.

2. Materials and Methods

2.1. Material Processing

Two-year-old honeysuckle seedlings were purchased from Linyi, Shandong Province. They were cultivated in pollution-free soil for 60 days, and the seedlings were withdrawn when the leaves had been vigorously grown. The seedlings were washed with 0.3% $KMnO_4$ and then washed thrice with deionized water before they were cultivated in a thermostatic incubator for hydroponics. The plants were successively cultivated with 1/6 Hoagland, 1/2 Hoagland, and Hoagland nutrient solutions [20], and each solution was used for 7 days. The incubator was set to a day–night cycle, and plants were grown under natural light intensity and ventilation. The nutrient solution pH was adjusted to around 5.6 every day. The day and night temperatures were set to be 28 °C and 20 °C, and the day and night humidity was set to be 40% and 60%, respectively. The nutrient solutions were refreshed every 3 days. As was added to the nutrient solutions 21 days later, and the As stress status with different As concentrations was achieved.

2.2. Hydroponic Experimental Method

The whole Hoagland nutrient solution was used as the culture solution, and $NaAsO_2$ was added to the nutrient solution at concentrations of 20, 30, and 50 mg/L. DMA was added to the nutrient solution at a concentration of 70 mg/L. The honeysuckle plants were cultured in Hoagland nutrient solution in the control group. The samples were

analyzed after 3 or 10 days of treatment. Three plants were used per replication, with three replications per treatment.

2.3. Methods for Measuring Physiological Indices

Next, 0.5 g of fresh honeysuckle leaves were weighed and stored at −80 °C after flash freezing with liquid nitrogen to determine the physiological indices. The leaves were then homogenized in 150 mM phosphate-buffered saline (PBS) with a pH of 7.8 and centrifuged at 12,000 rpm and 4 °C for 20 min. The resulting supernatant was used as the enzyme extract to determine the peroxidase (POD), catalase (CAT), and malondialdehyde (MDA) levels [21].

For determining POD levels, 3.0 mL of mixed reaction solution (200 mL of 200 mM PBS with a pH of 6.0, mixed with 76 µL of guaiacol stock solution (2-methoxyphenol) and 112 µL of 30% H_2O_2) was mixed with 30 µL of enzyme extract, and the absorbance was recorded at 470 nm using an ultraviolet (UV) spectrophotometer.

For determining CAT levels, 3.0 mL of mixed reaction solution (200 mL of 0.15 M PBS with a pH of 7.0, mixed with 0.30 mL of 30% H_2O_2) was mixed with 30 µL of the enzyme extract, and the absorbance was read at 240 nm using the UV spectrophotometer.

For determining the MDA content, 0.5 g of honeysuckle leaves were weighed, homogenized in 5 mL of 10% trichloroacetic acid, and centrifuged at 4000 rpm for 10 min. One milliliter of the supernatant was mixed with 1 mL of 0.67% thiobarbituric acid and boiled for 10 min in a water bath at 100 °C. After cooling in ice water, then 4000 rpm for 10 min, the supernatant was taken to record the absorbance values at 450, 532, and 600 nm using the UV spectrophotometer.

The experimental data were analyzed with a one-way analysis of variance using SPSS Statistics 22.0 software ($p \leq 0.05$) and plotted using Origin software.

2.4. Exploring as Distribution in L. japonica through µ-XRF Analysis

Frozen section: During the sampling process, honeysuckle plants were divided into three parts: roots, stems, and leaves. The collected honeysuckle sample was immersed in a 20 mL EDTA-Na2 solution for 15 min to remove As adsorbed on the surface of the honeysuckle sample, which was then rinsed with ultra-pure water. The sample was cut into 30 µm-sized pieces at −20 °C, which were fixed on Kapton tape. Each sample treated with a certain As concentration was measured once [22].

The distribution characteristics of As in the leaves, stems, and roots of honeysuckle plants were explored at the 4W1B beamline station of BSRF. The focusing mode was the primary focus of the toroidal mirror and the secondary focus of the capillary half-lens. The BSRF electronic storage energy level was 2.5 GeV, and the beam intensity was 150–250 mA. The angle between the incident and transmitted beams was set at 45°. The sample platform moved 50 µm in the Y direction and 50 µm in the X direction. XRF emission lines were detected with Si(Li) solid-state detector, and the residence time of each point was 1–3 s. The integrated intensity of arsenic was calculated using the spectral method, and the integrated intensity was normalized to the intensity of the Compton scattering peak. PYMCA 5.5.0-win64 was used for data processing. Origin 2019B software was used to draw the two-dimensional map of As for tissue slices [23].

3. Results and Discussion

3.1. Effects of Arsenic Stress on POD Activity in Honeysuckle Leaves

The concentrations of $NaAsO_2$ stress tested in this study were 0, 10, 20, 30, and 40 mg/L, since honeysuckle withered at higher concentrations.The concentrations of DMA were 0, 10, 30, 50, and 70 mg/L, and the stress was tested for 10 days. Figure 1 shows the effects of $NaAsO_2$ and DMA stress on the enzyme activity of honeysuckle POD. Figure 2 compares the effects of stress on POD enzyme activity in the 3- and 10-day groups under 30 mg/L inorganic arsenic and 50 mg/L organic arsenic stress.

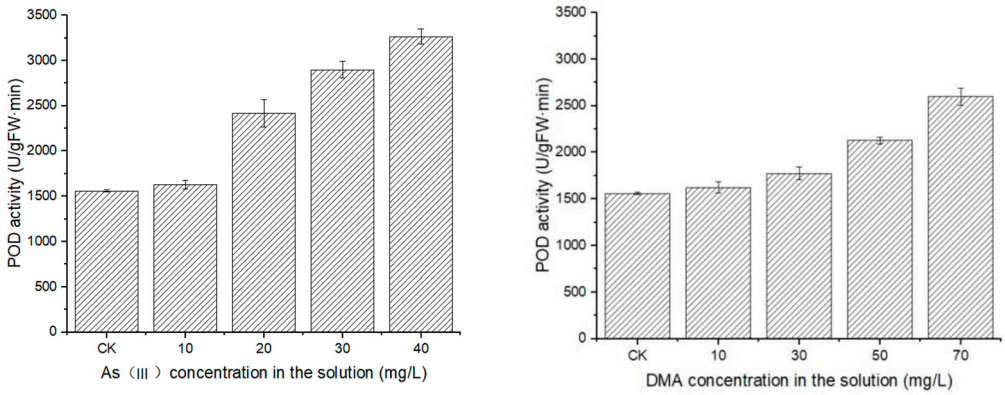

Figure 1. POD activity of honeysuckle leaves under different concentrations of DMA and As (III).

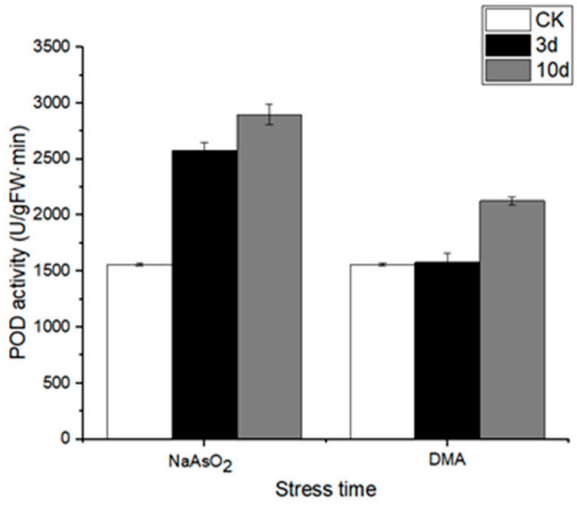

Figure 2. Effects of 30 mg/L $NaAsO_2$ and 50 mg/L DMA on POD activity of honeysuckle leaves with different exposure durations.

POD is one of the key enzymes to protect plants against adversity and is most activated under stress. While oxidizing other substances, POD also reduces H_2O_2 to H_2O to eliminate the H_2O_2 produced in the plant body. H_2O_2 is a cytotoxic substance produced in the redox reaction catalyzed by oxidase. Both oxidase and catalase exist in peroxisomes, which can protect cells [24]. As shown in Figure 1, the activity of POD also increased with increasing concentrations of inorganic and organic arsenic. The enzyme activities under the stress of 20 mg/L inorganic arsenic and 70 mg/L organic arsenic were extremely close, indicating that inorganic arsenic $NaAsO_2$ caused more oxidative damage to honeysuckle compared with organic arsenic DMA. As shown in Figure 2, the comparison between the 3- and 10-day groups under 30 mg/L inorganic arsenic and 50 mg/L organic arsenic stress revealed that the longer the stress duration, the higher the POD enzyme activity, which helped the plant resist stress.

3.2. Effects of Arsenic Stress on CAT Activity in Honeysuckle Leaves

CAT decomposes H_2O_2 into H_2O and O_2. As the reactive oxygen metabolic ability of plants became stronger under adverse conditions, a large amount of H_2O_2 was accumulated. Also, CAT can decompose H_2O_2 to protect cells from its harmful effects, thus reducing plant damage. The effects of $NaAsO_2$ and DMA stress on the enzyme activity of honeysuckle CAT are shown in Figure 3. Figure 4 compares the effects of stress on the CAT enzyme activity in honeysuckle leaves in the 3- and 10-day groups under 30 mg/L inorganic arsenic and 50 mg/L organic arsenic stress.

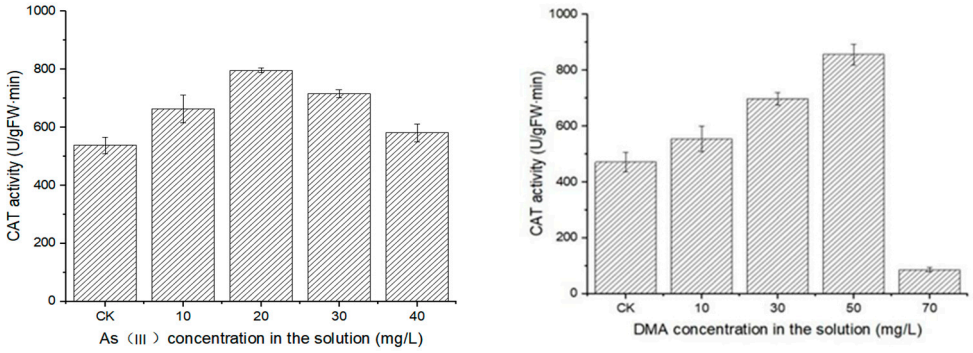

Figure 3. CAT activity of honeysuckle leaves under different concentrations of DMA and As(III).

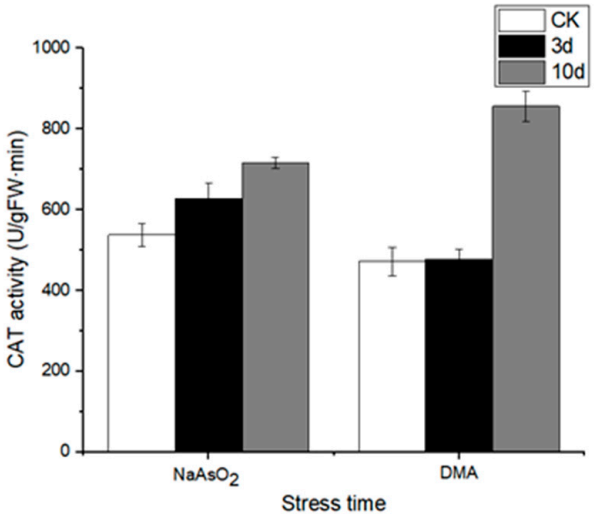

Figure 4. Effects of 30 mg/L $NaAsO_2$ and 50 mg/L DMA on the CAT activity of honeysuckle leaves with different exposure durations.

Figure 3 demonstrates that under stress conditions of 10–20 mg/L of inorganic arsenic, the increasing concentrations of arsenic stimulated the metabolic movement of reactive oxygen in honeysuckle plants, leading to increased accumulation of H_2O_2. In response, plant cells produced more CAT to decompose H_2O_2 and improve the defense system. However, when the concentration of $NaAsO_2$ was 20–40 mg/L, it exceeded the capacity of the cells and affected the synthesis of substances such as proteins in plant cells, resulting in decreased activity of antioxidant enzymes. When the phytotoxicity defense system eventually went beyond the limit of self-regulation, the process became irreversible and led to decreased CAT activity. The CAT activity gradually decreased under the stress of 10–70 mg/L organic arsenic. The CAT activity was extremely low at a concentration of 70 mg/L. This might be because the excessively high concentration of produced H_2O_2 destructed the plants, inhibiting the activity or synthesis of the enzyme. Figure 4 compares the effects of stress on the CAT enzyme activity in honeysuckle leaves in the 3- and 20-day groups under 30 mg/L inorganic arsenic and 50 mg/L organic arsenic stress. The results indicated that the longer the exposure duration, the higher the CAT activity.

3.3. Effects of Arsenic Stress on the MDA Content in Honeysuckle Leaves

MDA is a key product of cell membrane lipid peroxidation, and its concentration can indicate the extent of membrane lipid peroxidation. It is an important indicator of the strength of membrane lipid peroxidation and indirectly reflects plant antioxidant capacity. The effects of $NaAsO_2$ and DMA stress on the MDA content in honeysuckle leaves are shown in Figure 5. Figure 6 shows a comparison of the effects of stress on the MDA content

in honeysuckle leaves between the 3- and 10-day groups exposed to 30 mg/L inorganic arsenic and 50 mg/L organic arsenic stress.

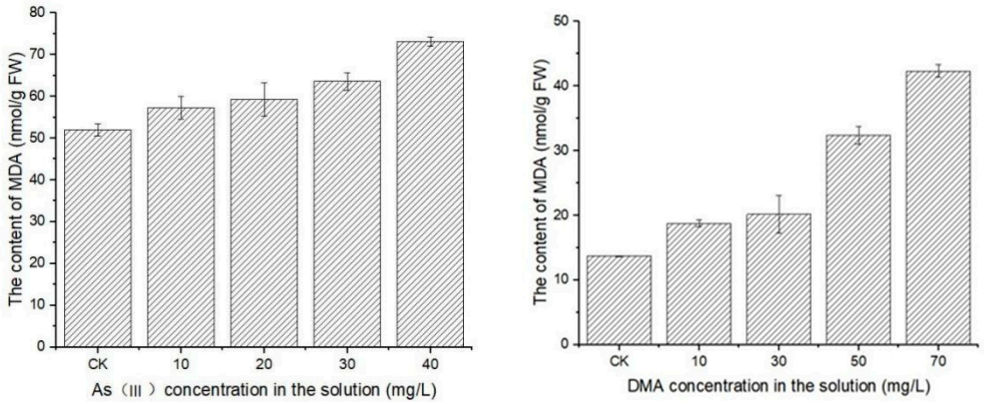

Figure 5. MDA content of honeysuckle leaves under different concentrations of DMA and As(III).

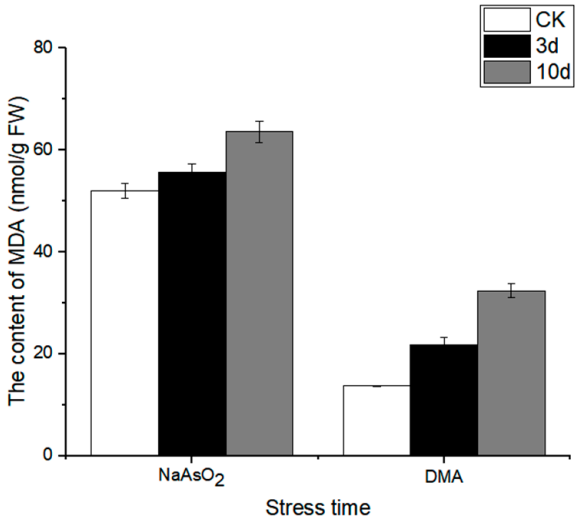

Figure 6. Effects of 30 mg/L $NaAsO_2$ and 50 mg/L DMA on the MDA content of honeysuckle leaves with different exposure durations.

Figure 5 reveals that the MDA content in honeysuckle leaves increased under the stress of 10–40 mg/L inorganic arsenic and 10–70 mg/L organic arsenic. It indicated that when the plant was exposed to adversity stress, it produced reactive oxygen, caused membrane lipid peroxidation, and damaged the membrane system [25]. The resulting ROS may affect the cell and protein structures. The larger the stress, the greater the degree of membrane lipid peroxidation and the extent of damage. As shown in Figure 6, the comparison between the 3- and 10-day groups of 30 mg/L inorganic arsenic and 50 mg/L organic arsenic stress demonstrated that the longer the stress exposure duration, the higher the MDA content. Long-term heavy metal stress was detrimental to plant growth. A comparison of the MDA content revealed that the inorganic arsenic stress caused more severe damage to the cell membrane than the organic arsenic stress. The MDA content is an important indicator to measure the accumulation of ROS and membrane lipid peroxidation. While POD is an important enzyme for removing ROS in cells, it can also effectively reduce the accumulation of MDA by removing ROS [26,27]. Although the activity of POD increased with the increasing concentrations of arsenic stress in plants, the accumulation of MDA in plants could not be reduced (Figure 1).

3.4. μ-XRF Fluorescence Scanning

Synchrotron μ-XRF at the 4W1B beamline station of BSRF was used to perform fluorescence scanning on the roots, stems, and leaves of honeysuckle plants to investigate the distribution of arsenic in honeysuckle plants under different forms of arsenic stress. Figure 7 shows the distribution of arsenic in the roots, stems, and leaves of honeysuckle plants in the control group (blank) as revealed by μ-XRF scanning.

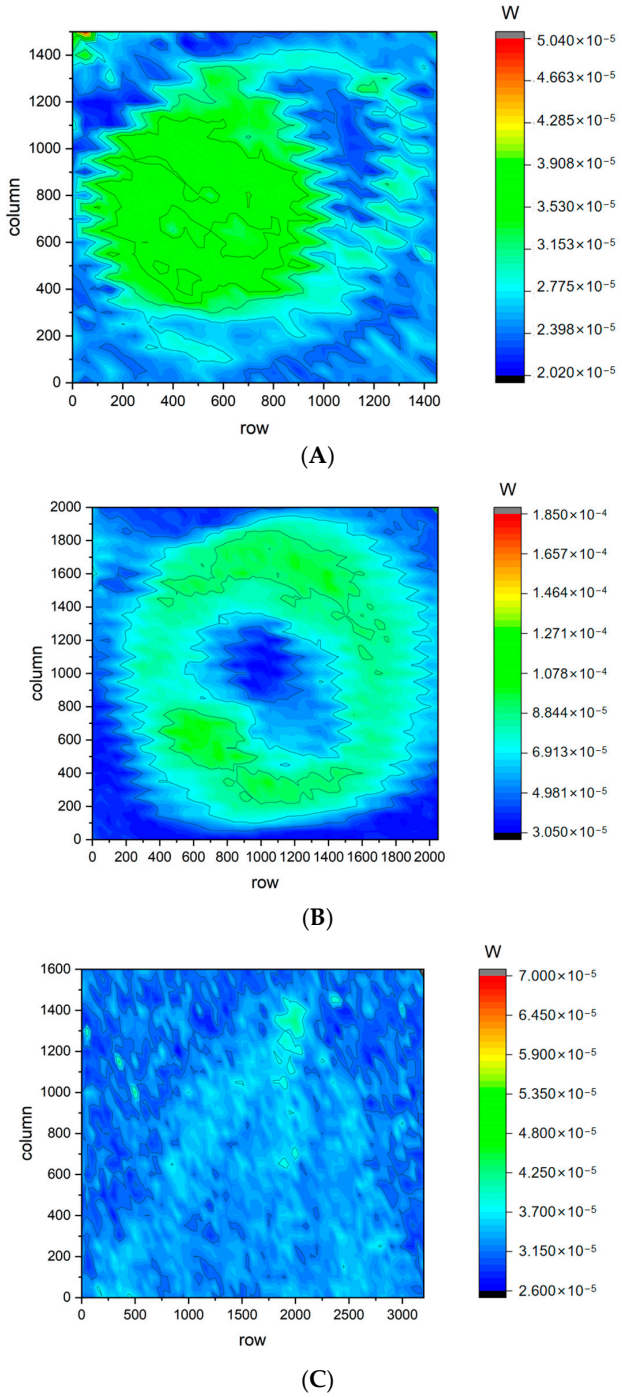

Figure 7. Distribution of arsenic in the control group: (**A**) root; (**B**) stem; and (**C**) leaf.

3.4.1. Distribution of Arsenic in Honeysuckle Plants under Inorganic Arsenic Stress

Figure 8 exhibits the distribution of arsenic in the honeysuckle roots under various concentrations of inorganic arsenic for various exposure durations using μ-XRF scanning.

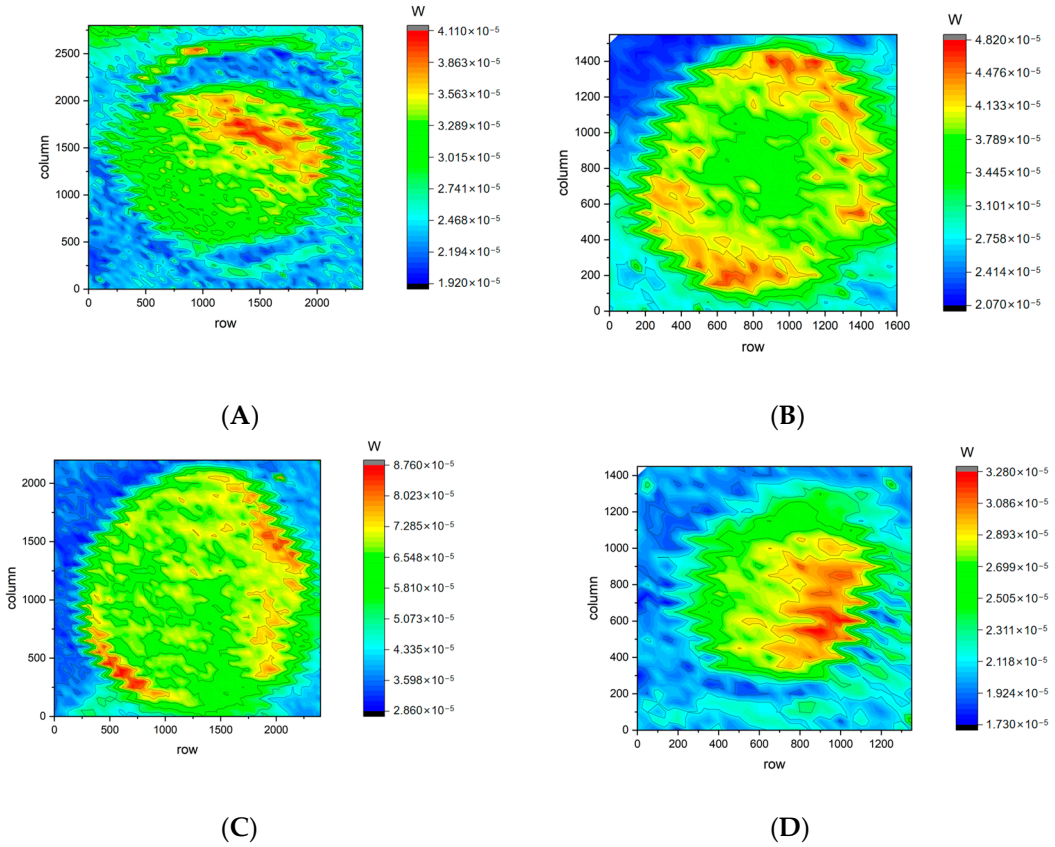

Figure 8. Distribution of arsenic in the root under $NaAsO_2$ stress: (**A**) 10 mg/L (10 days); (**B**) 20 mg/L (10 days); (**C**) 30 mg/L (10 days); and (**D**) 30 mg/L (3 days).

As shown in Figure 8, the distribution of arsenic in the honeysuckle roots became closer to the phloem with the increasing concentration of $NaAsO_2$. Under high concentrations of inorganic arsenic, most of the arsenic accumulated in the outer epidermis to prevent the transport of arsenic to the aerial parts of the honeysuckle plant. As the exposure duration increased under 30 mg/L $NaAsO_2$ stress, the distribution of arsenic in the roots gradually spread out to the phloem (Figure 8C,D).

The distribution of arsenic in honeysuckle stems and leaves under 30 mg/L inorganic arsenic stress for 10 days is shown in Figure 9.

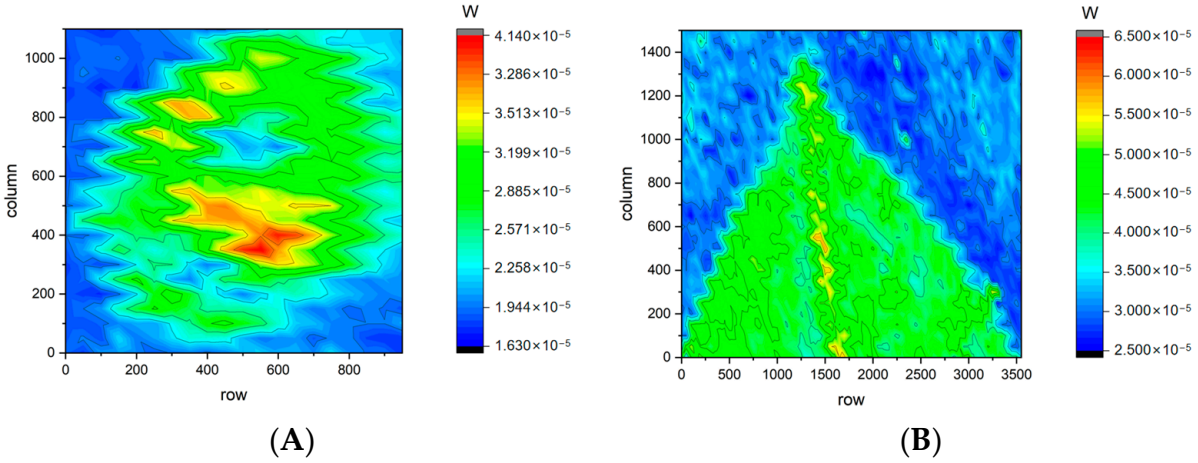

Figure 9. Distribution of arsenic in the honeysuckle stems and leaves under 30 mg/L $NaAsO_2$ stress for 10 days: (**A**) stem; (**B**) leaf.

Honeysuckle is a dicotyledonous plant. Its stem comprises three main parts: the epidermis, the cortex, and the vascular cylinder. The epidermis is the outermost cell layer of the stem, which is composed of small, tightly arranged trichomes and stomata. The cortex is located between the epidermis and vascular cylinder and comprises the collenchyma and parenchyma layers. The vascular cylinder is located inside the cortex and consists of vascular bundles, medullary rays, and pith. As shown in Figure 9A, arsenic primarily accumulated in the cortex in the stem. Figure 9B shows that arsenic was mostly distributed in the veins in the leaves, which are primarily composed of vascular bundles and act as the supporting structure of the leaves. The mesophyll, which is responsible for photosynthesis, had a minimal distribution of arsenic, resulting in less damage to the plant. A comparison of Figure 8C with Figure 9A,B revealed that inorganic arsenic was more toxic to plants than organic arsenic. Therefore, after exposure to high concentrations of inorganic arsenic stress, the arsenic contents in the stems and leaves were lower than those in the roots because the roots effectively trapped arsenic to minimize the damage caused by arsenic to plant growth.

3.4.2. Distribution of Arsenic in Honeysuckle Plants under Organic Arsenic Stress

The stems, roots, and leaves of honeysuckle under the DMA stress of 50 mg/L (for 3 days), 50 mg/L (for 10 days), and 70 mg/L (for 10 days) were scanned using XRF. The distribution of arsenic is shown in Figure 10.

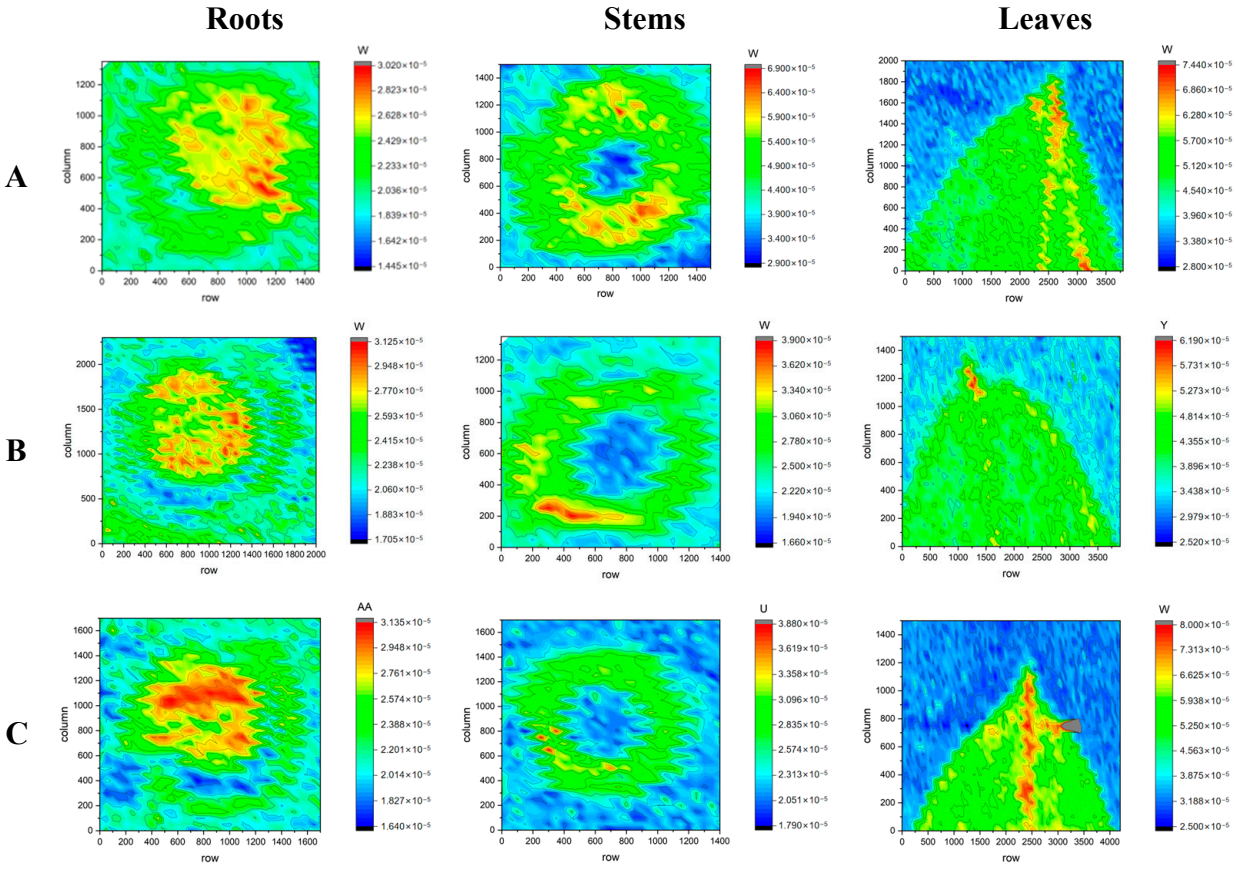

Figure 10. Distribution of arsenic in the roots, stems, and leaves of honeysuckle plants after organic arsenic stress: (**A**) 50 mg/L DMA group (3 days); (**B**) 50 mg/L DMA group (10 days); and (**C**) 70 mg/L DMA group (10 days).

Figure 10A shows that arsenic primarily accumulated in the vascular cylinder in the central part of the root and migrated to the upper part of the plant via this channel in the 50 mg/L DMA stress group. Upon entering the stem, arsenic was primarily distributed

in the cortex. When it reached the leaves, it was distributed in the veins. A significantly elevated amount of arsenic accumulated in the roots with increasing exposure duration, as indicated in Figure 10A,B. The amount of arsenic accumulated in stems in the 10-day group was 50% of that in the 3-day group. In the leaves, only a small amount of arsenic was detected in the leaf tip in the 10-day group. The remaining arsenic was transported to the lateral veins of the leaves. Additionally, a comparison of Figure 10B,C showed that the higher the arsenic stress, the greater the accumulation of arsenic in the central vascular cylinder of the root. The stem did not effectively intercept arsenic under high concentrations of DMA stress, leading to its accumulation in the veins. As a result, arsenic highly accumulated in the veins, with a small amount of it in the mesophyll. It is believed to be a self-protection mechanism of honeysuckle.

4. Conclusions

The study on the physiological and biochemical effects of organic and inorganic arsenic stress on honeysuckle plants showed that low concentrations (10–20 mg/L) of inorganic arsenic and 10–50 mg/L of organic arsenic stimulated the growth of honeysuckle plants. Also, the activities of antioxidant enzymes, such as POD and CAT, increased, indicating strong self-regulation ability and resistance to arsenic stress. At these concentration ranges, the antioxidant enzyme system was not suppressed by either form of arsenic stress. However, under the stress of high concentrations (20–40 mg/L) of inorganic arsenic and 50–70 mg/L of organic arsenic, the antioxidant system in honeysuckle plants was destroyed and the antioxidant capacity decreased. Especially, the CAT activity in honeysuckle leaves was only 86.3 U/gFW under 70 mg/L DMA stress. Moreover, POD, CAT, and MDA levels increased with the increase in exposure duration to 3–10 days under 30 mg/L $NaAsO_2$ and 50 mg/L DMA stress. It indicated that long-term heavy metal stress caused damage to plant growth, and inorganic arsenic was generally more toxic to plants compared with organic arsenic.

The μ-XRF study showed that under inorganic arsenic stress, the arsenic content in the roots increased with increasing concentrations and exposure duration. Additionally, the accumulated arsenic was transported from the central vascular cylinder to the outer part of the root. In contrast, organic arsenic stress did not result in significant variations in the distribution of arsenic with increasing concentration and was primarily located in the middle woody part of the root. Regardless of the type of arsenic stress, the distribution of arsenic in the stems and leaves was similar, with accumulation primarily occurring in the cortex of the stem and veins of the leaf. After exposure to different forms of arsenic stress, the trend of arsenic accumulation in the honeysuckle plant is mainly observed in the roots. In the case of inorganic arsenic stress, the accumulation of arsenic in the roots shows a trend of diffusion from the central vascular bundle to the outer periphery, with a small amount migrating to the stem cortex. In the case of organic arsenic stress, arsenic accumulates in the middle woody part of the root, with a small amount directly migrating to the leaf veins.

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