Research Article

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Hydroponic phytoremediation of antimony by *Tamarix smyrnensis* and *Nerium oleander*

Petroula Seridou,[a] Konstantinos Fyntrilakis,[a] Evdokia Syranidou[a] and Nicolas Kalogerakis[a,b*]

Abstract

Background: Antimony (Sb) is considered a priority pollutant and its removal from the environment is of great importance. Among the various treatments, phytoremediation is considered an efficient method when the accumulation of this metalloid in plant tissues is sufficiently high. This work focused on removal of Sb(III) from aqueous solution at two Sb concentrations (5 and 10 ppm) and using salt as stressor (100 mmol L^{-1}) only at the lowest Sb concentration (5 ppm). The response of two salt-tolerant plant species – *Tamarix smyrnensis* and *Nerium oleander* – was monitored when subjected to Sb-contaminated hydroponic solutions.

Results: Plant growth and water content remained unaltered within the 15-day experimental period, while the chlorophyll content declined significantly at the higher Sb level (10 ppm) only for *N. oleander*. The antioxidant enzymatic activity in *T. smyrnensis* with respect to guaiacol peroxidase (GPOD) and catalase (CAT) was significantly increased in the treatment under Sb and salt stress. In *N. oleander* there was a strong increase in GPOD activity by the exposure to Sb, whereas the CAT activity was increased considerably with the presence of salinity. Sb concentration was detected on the surface of the leaves, indicating that excretion occurred in both plants. Increased accumulation was observed in *N. oleander* in the absence of salinity whereas, under salt stress, Sb accumulation was considerably higher (60%) in *T. smyrnensis*. Notably, both plants could accumulate a high amount of Sb, preferentially in the roots, with the concentrations in the leaves being significantly lower.

Conclusion: The experimental results from this study demonstrate that both plants are suitable for phytoremediation due to substantial Sb accumulation without visible Sb toxicity.

Keywords: antimony; hydroponic; phytoremediation; *T. smyrnensis*; *N. oleander*

INTRODUCTION

Heavy metal and metalloid pollution is of great concern due to the detrimental effects on the environment as well as human health. One of the most toxic elements is antimony (Sb). This metalloid is typically encountered as the sulfide mineral stibnite (Sb_2S_3) and rarely in its native form due to its strong affinity for sulfur and other metals.[1] Furthermore, it exists in the environment mainly in two oxidation states: antimonite, Sb(III); and antimonate, Sb(V). The trivalent oxidation state is more toxic than the pentavalent.[2,3] The maximum concentration of Sb determined by the World Health Organization (WHO) that is permitted in drinking water is 20 μg L^{-1}, whereas the maximum permissible concentration in soil is 36 mg kg^{-1}.[4] The release of Sb into the environment is derived from geogenic processes and anthropogenic activities such as mining, coal combustion and Sb products (flame retardants, plastics, textiles, etc.).[5-7]

Even though Sb is a trace element and not essential for plants, it can be accumulated in their edible parts according to numerous studies. These findings might indeed pose a risk to human health since Sb can enter the food chain and subsequently the human body.[8,9] Particularly in mining areas with high Sb content in soil, a high accumulation in plants was reported.[10-12] For instance, *Achillea ageratum* accumulates Sb in basal leaves (1367 mg kg^{-1}) and inflorescences (1105 mg kg^{-1}), *Plantago lanceolata* in roots (1150 mg kg^{-1}) and *Silene vulgaris* in shoots (1164 mg kg^{-1}).[13] A study by Murciego *et al.* investigated the Sb accumulation patterns for three plant species: *Cytisus striatus, Cistus ladanifer and Dittrichia viscosa*, which exhibited low, moderate to high, and elevated Sb level in leaf samples, respectively.[14] Conversely, *D. viscosa* extracted Sb from the soil to the root but did not translocate it in large quantities to the aerial parts.[15] In another study, adult pine (*Pinus sylvestris*), birch (*Betula pendula*) and bulrush (*Juncus effusus*) found in old mine areas were examined and it

* *Correspondence to: Nicolas Kalogerakis, School of Chemical and Environmental Engineering, Technical University of Crete, Chania, Greece, E-mail: nicolas.kalogerakis@enveng.tuc.gr*

a *School of Chemical and Environmental Engineering, Technical University of Crete, Chania, Greece*

b *Institute of Geoenergy, Foundation for Research and Technology-Hellas (FORTH), Chania, Greece*

was reported that they were mostly root accumulators with low translocation from roots to shoots.[16] It is clear that the uptake mechanism varied significantly between individual plant species, as Sb can accumulate in roots or be translocated to the above-ground part of the plant. The different behavior around the Sb accumulation can be explored in depth using hydroponic media. This accurate study can investigate the Sb uptake mechanism under controlled conditions.[17]

Among the plant species that have been widely used for phytoremediation of heavy metals, halophytes are suggested as ideal candidates since they can tolerate harsh conditions and develop a tolerance mechanism not specific to salt ions and hence other toxic elements secreted by their salt glands or trichomes.[18] Precisely, halophytes can adopt different strategies upon metal stress in order to moderate the toxicity induced by heavy metals. The main mechanism is that the organic matter exuded from the roots forms a complex with the metals and then adsorbs the metals into the carbohydrates of the cell wall. Subsequently, the metals transported into cells are intracellularly chelated by protein molecules or localized to vacuoles for storage. Finally, some metals are excreted by specific salt glands on leaf surface.[19] In addition, this tolerance mechanism of halophytes to salt stress is correlated with an oxidant defense system considerably more efficient, thus exhibiting a greater capability to cope with heavy metals in relation to common plants.[20]

Tamarix smyrnensis is a salt-excreting halophyte native to the Mediterranean region and is considered a suitable plant since it has great strength at low pH values and high metal concentration in contaminated sites.[21] It was reported in the literature that it is not a metal hyperaccumulator since Pb and Cd were accumulated in roots.[22-24] However, salinity had a significant effect on Cd translocation from the roots to the above-ground tissues of *T. smyrnensis* as exclusively under 3% NaCl the ratio leaf/root Cd concentration was estimated above unity.[24] Another endemic plant of the Mediterranean region is *Nerium oleander*, an ornamental plant of high aesthetic value. This plant exhibits a tolerance mechanism against heavy metals since it is salt tolerant and resistant to drought.[25] It has been applied to phytoremediation of Cd, Zn and Pb, with the latter accumulated in the root, whereas the other metals were found in the aerial part of the plant species.[26] Another study confirmed that Pb was accumulated in the plant roots with a low translocation to the aerial parts. No visible toxicity symptoms were observed or no chlorophyll content reduction was reported when exposed to a high Pb concentration of 2400 mg kg^{-1}, rendering *N. oleander* suitable for phytostabilization.[27]

Under various environmental stress factors, including heavy metal contamination, plants have developed antioxidant systems, consisting of several non-enzymatic and enzymatic mechanisms that are crucial in order to activate their tolerance mechanism.[28] It has been suggested that heavy metal toxicity is due to excessive concentration of reactive oxygen species (ROS) accumulated in plant tissues, like H_2O_2, increasing lipid peroxidation and provoking an alteration in membrane lipid composition that may lead to cellular oxidative damage.[29] This oxidative stress can be alleviated by antioxidant enzymes such as catalase (CAT), peroxidase (POD), guaiacol peroxidase (GPOD), glutathione peroxidase (GPOX) and so forth, which play a key role in the antioxidative response of plants cells since they are mobilized to scavenge and quench ROS.[30] Therefore, it is important to monitor the antioxidant activity in order to investigate the response of the plant species exposed to heavy metal concentration. To our knowledge, no study to date has examined the Sb uptake from *T. smyrnensis* or *N. oleander* under hydroponic conditions. The main objective of this study is to investigate the Sb accumulation and translocation by these plants after exposure to antimonite at two concentrations (5 and 10 ppm) and to Sb(III) at 5 ppm together with salt as stressor. To better understand the detoxification by plants and their tolerance, biomass, water content, chlorophyll content, antioxidant enzyme activity and metalloid excretion were further investigated. This information will shed light on the phytoremediation potential of these plant species, revealing whether they are suitable plants for Sb accumulation.

MATERIALS AND METHODS

Plant material and experimental design

Tamarix smyrnensis cuttings (10–12 cm) were collected from the coastal cliffs in Agioi Apostoloi (Chania, Crete, Greece). The plant propagation method as described by Manousaki *et al.*[23] was followed and was carried out in the greenhouse of Technical University of Crete. The cultivation period was approximately 2 months to achieve sufficient biomass of *T. smyrnensis* for the hydroponic experiment. Regarding *N. oleander*, 6-month-old plants were chosen from a nursery garden in Chania, in the Kounoupidiana district.

The experimental period lasted 15 days. At the beginning of the experiment, all the plants were removed from the soil and washed carefully with tap and distilled water in order to remove any particulate matter, avoiding any potential damage to the root system. In addition, plants were weighed before placing in the vessels. Clean vessels with a volume of approximately 1.5 L were filled with 950 g gravel and covered with aluminum foil. Initially, plants were irrigated with 450 mL of 10% Hoagland's solution, and once a week the required amount of water was added to each vessel to maintain the volume constant for all replicates. The adaptation period was 2 weeks. Plants were exposed to Sb in two concentrations (5 and 10 ppm), and the effect of salinity on metal uptake was investigated. A total of five treatments in four replicates were performed for each plant species; hence the total number of treatments was ten, as shown in Table 1. The plants were divided into samples of similar biomass (height and weight) to ensure homogeneity among the treatments.

At the end of the experiment, the plants were removed from the vessels. Leaves were separated from the roots, rinsed thoroughly with tap water and twice with deionized water, and dried gently. The fresh weight (FW) of roots and shoots was estimated. Samples were collected to be ground for determination of physiological state of plants, expressed in terms of chlorophyll content, protein content, catalase activity and specific guaiacol peroxidase activity. The rest of the plant was oven dried at 70 °C for 48 h and the dry weight (DW) was measured.

Water content (WC, %) was estimated from the FW of the plants and the DW, based on the following formula:

$$WC(\%) = \frac{FW - DW}{FW} \times 100.$$

Chlorophyll measurements

Chlorophyll content was assessed following the method described by Harborne.[31] More specifically, an accurately weighed amount (0.2 g) of fresh leaf samples was collected and homogenized with 10 mL of 80% acetone. Subsequently, the extracts were centrifuged twice at 16 000 × *g* for 1 min and the absorbance was measured at 646 and 663 nm

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Table 1. Experimental design

Treatment No.	Experimental treatment	Plant	Sb concentration (mg L^{-1})	Salt concentration (mmol L^{-1})
1	T/0/0	*T. smyrnensis*	0	0
2	T/0/100	*T. smyrnensis*	0	100
3	T/5/0	*T. smyrnensis*	5	0
4	T/5/100	*T. smyrnensis*	5	100
5	T/10/0	*T. smyrnensis*	10	0
6	N/0/0	*N. oleander*	0	0
7	N/0/100	*N. oleander*	0	100
8	N/5/0	*N. oleander*	5	0
9	N/5/100	*N. oleander*	5	100
10	N/10/0	*N. oleander*	10	0

Note: Treatment notation refers to "Plant species/Sb concentration/Salt concentration".

spectrophotometrically for the determination total chlorophyll (Chl total), chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) concentrations using the following equations:

$$\text{Chl total} = 17.3\,A_{646} + 7.18\,A_{663} \quad (1)$$

$$\text{Chl}\,a = 12.21\,A_{663} - 2.81\,A_{646} \quad (2)$$

$$\text{Chl b} = 20.13\,A_{646} - 5.03\,A_{663} \quad (3)$$

Extraction for protein and antioxidant enzyme assays

Crude extract was prepared by homogenization of the plant sample with a pestle and mortar in buffer medium. The roots from the plant were washed thoroughly with distilled water. 1 g of the root was cut into thin pieces and homogenized in potassium phosphate buffer 100 mmol L^{-1} (pH 7) containing 0.1 mmol L^{-1} ethylenediaminetetraacetic acid and 1% polyvinylpyrrolidone. The homogenate was filtered through three layers of cheesecloth, the filtrate was centrifuged at 16 000 × *g* and the supernatant was collected. Protein concentration was determined using the Bradford assay.[32]

Measurement of antioxidant enzyme activity

Catalase

Catalase (CAT) activity was measured at room temperature by monitoring the decrease in absorbance at 240 nm due to the decomposition of H_2O_2 according to the method of Aebi.[33] The reaction mixture of a total volume of 3.0 mL contained 50 mmol L^{-1} phosphate buffer (pH 7), 36 mmol L^{-1} H_2O_2, and a suitable aliquot of crude extract.

Guaiacol peroxidase

GPOD was determined spectrophotometrically at room temperature by measuring the increase in absorbance at 470 nm. The assay was performed using phosphate buffer (50 mmol L^{-1}, pH 5.8), guaiacol (15 mg mL^{-1}), a suitable amount of plant extract and H_2O_2 (1% v/v).[34]

Enzyme activity was expressed as the change in absorbance per minute in terms of units per milligram of extracted proteins.

Metal excretion determination of the leaf surface

The above-ground parts of plants were washed with 100 mL weak acid (0.1% HNO_3) for 2 min, in order to dissolve all compounds on the leaf surface and inside the crypts, and maintain the conditions of the plant tissues intact. The water collected after the rinsing procedure was filtered and analyzed by inductively coupled plasma–mass spectrometry (ICP-MS).

Heavy metal analysis in the plant tissue and nutrient solution

After drying all the plant tissues at 70 °C for 24 h, they were digested for metal determination. Specifically, the leaves and shoots were separately ground with a blender, followed by ashing in a muffle furnace for 16 h at 480 °C. The fine powder that was obtained was dissolved in concentrated HNO_3 (>69%) and nitric acid digestion was performed.[35] In addition, a 10 mL sample of the nutrient was collected at the beginning of the experiment and every 5 days until the end of the experimental phase. All the samples were filtered (0.45 μm Whatman) prior to analysis for the determination of Sb content by ICP-MS.

Estimation of Sb accumulation

Evaluation of the metal accumulation efficiency in *T. smyrnensis* and *N. oleander* was assessed by estimating two main parameters: the bioconcentration factor and translocation factor, defined by the following equations:[36]

$$\text{BCF} = \frac{\text{Sb concentration in plant tissue}\,(\mu\text{g/g dry weight})}{\text{Initial concentration of Sb in the medium}\,(\text{mg/L})},$$

$$\text{TF} = \frac{\text{Sb concentration in stem and leaves}\,(\mu\text{g/g dry weight})}{\text{Sb concentration in root}\,(\mu\text{g/g dry weight})}.$$

These parameters, the bioconcentration factor (BCF) and translocation factor (TF), can be used to evaluate plant phytoremediation potential. In detail, a BCF value higher than one is indicative of an accumulation from solution to plant tissue and the metal translocation from the root to the shoot is considered efficient when the TF value is higher than one.

Data analysis

All statistical analyses were performed using GraphPad Prism 9 software. Triplicate measurements in the extracts, measurement of calibration blanks, laboratory reagent blanks, as well as analysis of standard reference material, were employed in order to address data quality control. All the results in this study are presented as the mean with standard errors ($n = 4$). Significance of differences was determined using one-way analysis of variance (ANOVA). Significance level was considered at $P < 0.05$. Asterisks indicate the level of significance: $^*P < 0.05$; $^{**}P < 0.001$.

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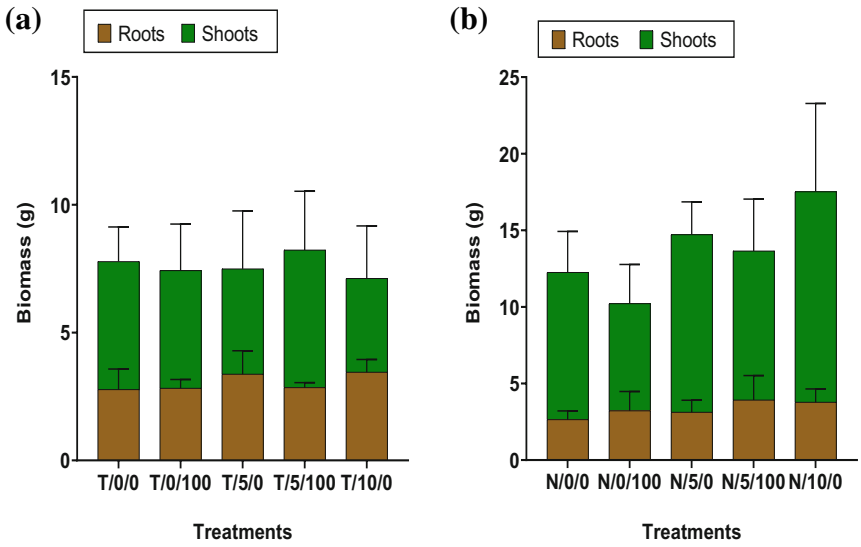

Figure 1. Root and shoot fresh weight after 15 days in (a) *T. smyrnensis* and (b) *N. oleander* (Refer to Table 1 for explanation of the experimental treatments).

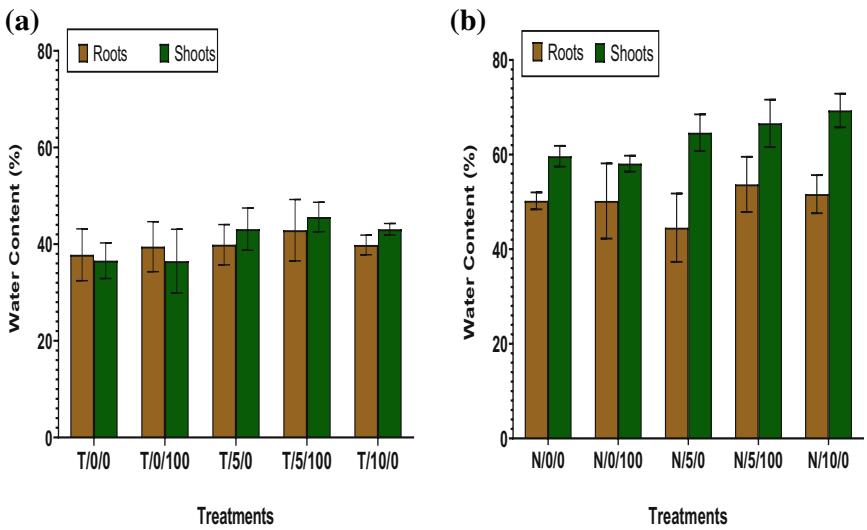

Figure 2. Shoot and root water content after 15 days in (a) *T. smyrnensis* and (b) *N. oleander* (Refer to Table 1 for explanation of the experimental treatments).

RESULTS AND DISCUSSION

Plant growth, chlorophyll content and stress enzymes

The exposure of *T. smyrnensis* plants to salt and Sb did not have any significant effect on biomass in relation to the non-stressed control. The weight of roots and shoots remained unaltered in all plants, with the biomass of shoots being slightly higher than the roots. Regarding *N. oleander*, even though Fig. 1(b) demonstrates a fluctuation of biomass among the treatments, no significant difference was observed, indicating that the metalloid and salt stress did not change the levels of biomass after the end of experiment. In this instance, the biomass of shoots was considerably greater than roots.

The graph in Fig. 2 depicts the water content in different treatments for each plant species. For *T. smyrnensis*, the water content was not affected significantly by the metalloid or salt concentration and remained constant, in the region of 40%, in both roots and shoots. The same trend was followed by *N. oleander* as the water content remained stable during the experiment. However, the water content in this plant species was higher than the former

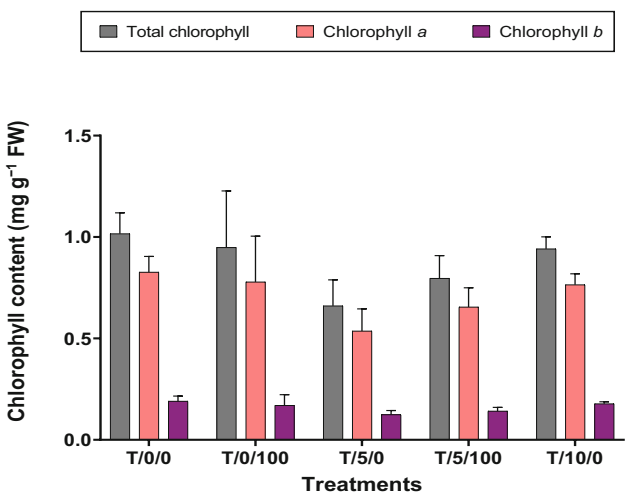

Figure 3. Chlorophyll content (per gram of fresh weight of leaves) of *T. smyrnensis* (Refer to Table 1 for explanation of the experimental treatments).

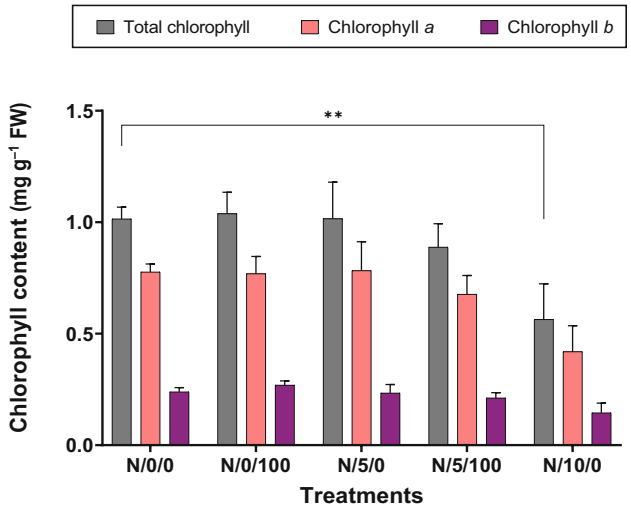

Figure 4. Chlorophyll content (per gram of fresh weight of leaves) of *N. oleander* (asterisks indicate level of significance: $^{**}P < 0.01$) (Refer to Table 1 for explanation of the experimental treatments).

and differed slightly between the roots and shoots, with a percentage of approximately 50% and 63%, respectively.

Figure 3 demonstrates the chlorophyll content (Chl total, Chl *a* and Chl *b*) of *T. smyrnensis*. The Chl total, Chl *a* and Chl *b* in control treatment (T/0/0) were equal to 1.02, 0.83 and 0.190 mg g^{-1} FW, respectively. The chlorophyll concentration was found to display similar values when 100 mmol L^{-1} salt was added (T/0/100). When the plant was exposed to 5 ppm Sb, the chlorophyll content was reduced, although not in a significant manner. The number of photosynthetic pigments of the plant was found not to be influenced in the presence of either Sb or salinity since total chlorophyll, Chl *a* and Chl *b* were not significantly affected exhibiting a slight decrease. Finally, the elevated concentration of Sb did not affect the chlorophyll content.

As shown in Fig. 4, the Chl total, Chl *a* and Chl *b* concentrations of *N. oleander* were estimated at 1.02, 0.78 and 0.24 mg g^{-1} FW, respectively. These values were close to the chlorophyll content of *T. smyrnensis*. Chlorophyll content was not affected by the presence of Sb with and without a salinity content of 5 ppm concentration, while in treatment N/10/0, corresponding to the highest concentration of Sb, a significant decrease to half of initial values was observed.

The plant's defense system to combat the oxidative stress induced by Sb concentration was investigated in terms of GPOD and CAT measurements. GPOD activity in the roots of *T. smyrnensis* was found to be increased in all treatments in comparison to control (Fig. 5). However, the enzyme activity was significantly affected only in treatment with Sb and salt concentration (T/5/100). GPOD was increased from 10.35 mU mg^{-1} protein to 35.80 mU mg^{-1} protein, displaying the antioxidant defense under Sb and salt stress. Concomitantly, the CAT activity in roots of *T. smyrnensis* was found to be similarly affected in treatment with Sb and salt concentration. CAT activity was significantly increased from 14.25 to 39.96 mU mg^{-1} protein.

In Fig. 6, GPOD activity in roots of *N. oleander* in all relevant treatments as well as CAT activity was observed. There was a strong increase in GPOD activity in the roots in response to exposure to Sb. For the treatment with 5 ppm and no salinity, the antioxidant enzyme activity was approximately 5773.7 mU mg^{-1} protein – higher in comparison to the roots of the control plants (2829.7 mU mg^{-1} protein). When the salinity was increased to 100 mmol L^{-1}, GPOD activity was increased up to 5040.8 mU mg^{-1} protein. At the highest Sb concentration, the increase in GPOD activity was 2.3 times greater compared to the control. Regarding CAT activity, the behavior was different. There was a significant increase in treatment B without Sb addition and with the presence of salinity. In addition, the results show that there was a significant elevation of this activity in treatment N/5/100. With regard to *N. oleander*, the only treatment that exhibited a significant upward trend in both activities was that with Sb level at 5 ppm and salt concentration at 100 mmol L^{-1} (Fig. 6).

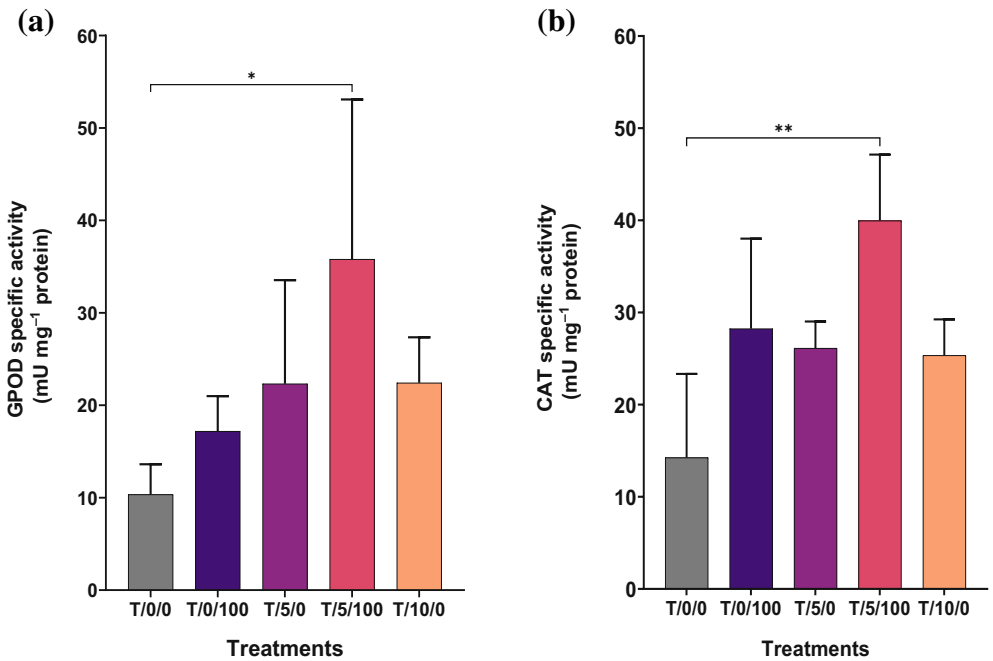

Figure 5. (a) Guaiacol peroxidase (GPOD) and (b) catalase (CAT) activity in roots of *T. smyrnensis* (asterisks indicate level of significance: $^{*}P < 0.05$; $^{**}P < 0.01$) (Refer to Table 1 for explanation of the experimental treatments).

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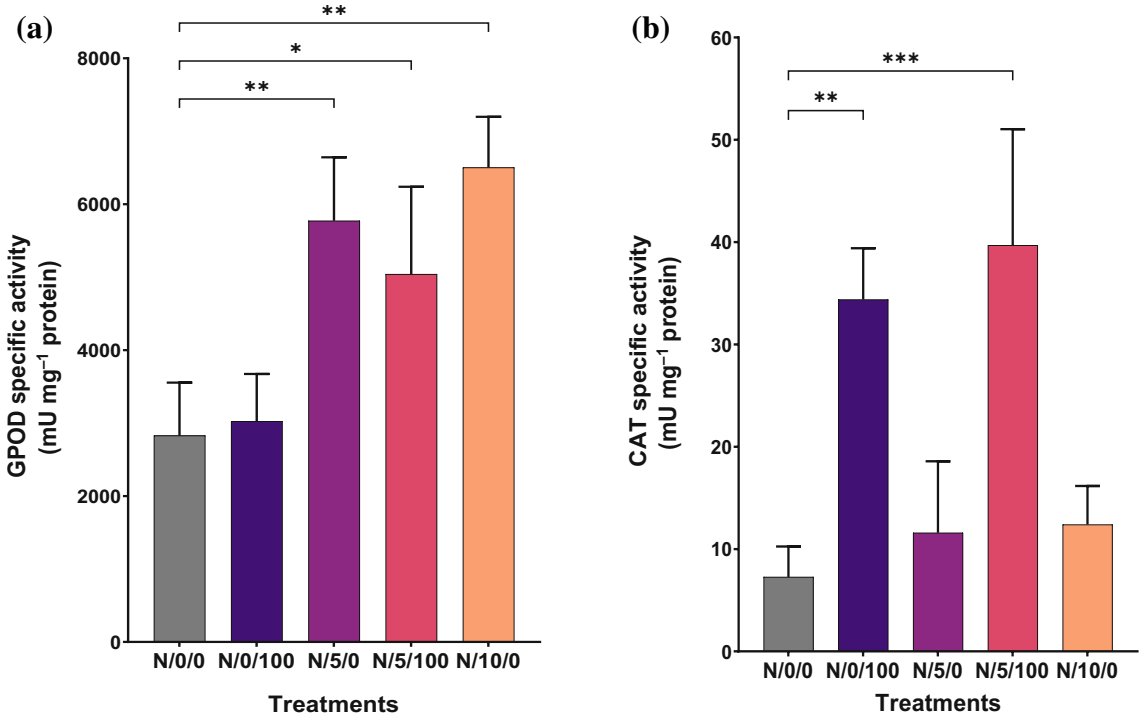

Figure 6. (a) Guaiacol peroxidase (GPOD) and (b) catalase (CAT) activity in roots of *N. oleander* (asterisks indicate level of significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) (Refer to Table 1 for explanation of the experimental treatments).

According to reported studies, the response of plants against oxidative damage is influenced by various factors such as plant species, the metal and type of stress conditions.[37] With regard to Sb, the activities of antioxidant enzymes POD and CAT were found to be significantly higher in the fern *Pteris cretica* with no Sb concentration and under different rates of Sb than those of three other fern plants, namely *Microlepia hancei*, *Cyrtomium fortunei* and *Cyclosorus dentatus*. Furthermore, upon Sb addition, the activities of these enzymes in the plants were enhanced, with the only exception for peroxidase in *C. fortunei*.[38] Prior research suggests that the CAT activity of maize (*Zea mays*) showed an increasing trend when Sb concentration in soil increased, while POD activity declined.[39] The same pattern was followed in the antioxidative response of maize roots, where the CAT and POD activity increased and decreased, respectively, with increasing concentration of Sb.[40] More recent evidence shows that CAT activity in the roots of *N. oleander* was not significantly affected by exposure to Sb, while the GPOD in the roots was found to be

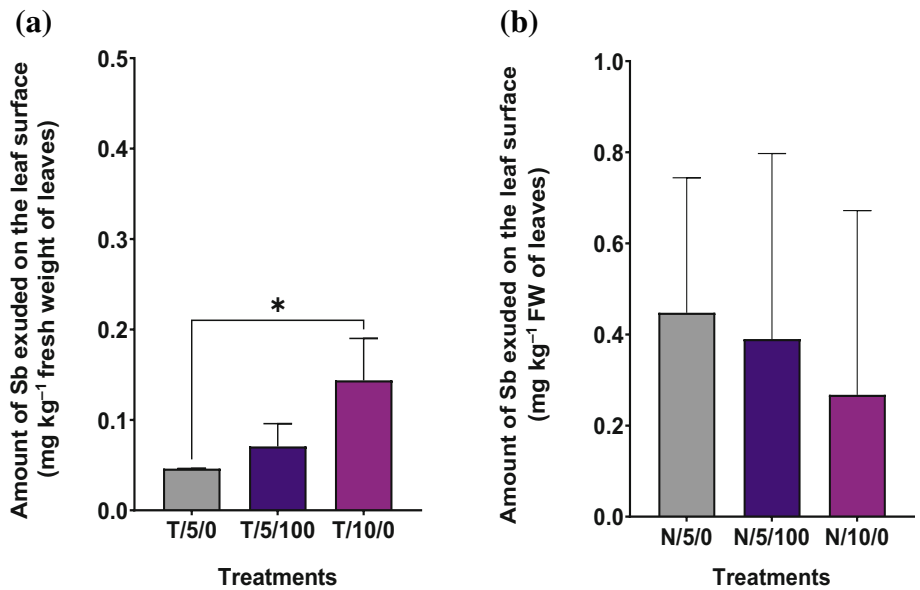

Figure 7. Sb concentration excreted on the leaf surface (per fresh weight of leaves) of (a) *T. smyrnensis* and (b) *N. oleander* (asterisks indicate level of significance: *$P < 0.05$) (Refer to Table 1 for explanation of the experimental treatments).

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significantly higher in the presence of Sb.[41] As the selected plant species accounts for the activity of antioxidant enzymes, it is essential to monitor their response in order to provide precise information about the antioxidant defense under heavy metal stress.

Sb concentration in the solution and within the plants

According to Fig. 7, the concentration of Sb in solution after washing the leaves, which corresponded to excreted Sb, was higher in *N. oleander* than *T. smyrnensis*. In treatments T/5/0, T/5/100 and T/10/0, Sb concentrations were approximately 10, 6 and 2 times lower, respectively, compared to the excreted amount of *N. oleander* exposed to same treatments. With regard to *T. smyrnensis*, the excreted concentration was slightly higher in the presence of salinity at an Sb level of 5 ppm, and a statistical difference in Sb excretion was found between the two Sb concentrations. Conversely, another study demonstrated that more metals are released in treatments without salt condition.[22] No significant difference was observed in excreted concentration in *N. oleander*.

As mentioned above, BCF is described as the ability of plants for elemental accumulation from the soil and the TF as an important factor to assess whether the plant is an hyperaccumulator, given that these factors are estimated to be above 1. Table 2 demonstrates the effect of Sb treatment on BCF and the TF factors. It is obvious that both plants exhibited a great ability to accumulate Sb in the tissues. A statistical difference was observed between the two plant species at the lowest concentration of Sb with and without the presence of salinity. At the highest Sb concentration, there was no significant difference between the two plants. On the other hand, it can be seen that translocation was substantially lower for all treatments in both cases (below 0.1). Slightly higher values were reported in *T. smyrnensis* in relation to *N. oleander* at the lowest concentration of Sb with the highest recorded values without the presence of salinity.

Figure 8 reveals the accumulation of Sb in plant tissues (roots and shoots) for the two tested plants. It can be clearly observed that in treatment with low concentration of Sb and absence of salinity *N. oleander* exhibited significantly higher accumulation in roots compared to *T. smyrnensis*. Upon addition of salt, there was a change in accumulation as it was substantially higher in *T. smyrnensis* and dropped markedly in *N. oleander* compared to treatment without salinity. Sb accumulation was increased by increased Sb concentration in both plant species and no statistical difference was observed between them. With regard to translocation, it was obvious that the accumulation in shoots was dramatically lower in relation to the roots; thus these plants are not considered hyperaccumulators.

These findings are in agreement with previous hydroponic studies as increased accumulation at increased Sb concentrations was also observed, while low translocation from roots to aerial parts of plants was recorded.[38,42,43] Four species of fern plants – *Pteris cretica*, *Microlepia hancei*, *Cyrtomium fortunei* and *Cyclosorus dentatus* – were investigated, and it was found that more Sb accumulated in the roots.[38] However, at the same Sb rate (5 ppm) without the presence of salt concentration, the average Sb level was greater in our study since the highest accumulation in roots was reported in *P. cretica* and was equal to around 100 mg kg^{-1}, whereas we found an Sb content of 321 mg kg^{-1} for *T. smyrnensis* and 648 mg kg^{-1} for *N. oleander*. Also in this study, no translocation was observed, as the ratio of Sb concentration in the shoots to that in the roots was less than 0.5 for all the plants.[38] Antimonite accumulation in rice has been investigated in another hydroponic experiment and the results showed that Sb(III) concentration in roots was 12.5 mg kg^{-1}, while the concentration in the aboveground parts of the plant was approximately 1.5 mg kg^{-1}, indicating that no translocation from roots to shoots was achieved.[4] In our experiment, at the same initial antimonite concentration, the Sb(III) content in roots was significantly higher, with accumulation estimated as approximately 25 times and 50 times greater in *T. smyrnensis* and in *N. oleander*, respectively, but displaying inefficiency in translocation of the metal to the upper parts of the plant. On the other hand, the results from previous studies reported in the literature significantly differed from our experiments. *Pteris vittata*, an arsenic hyperaccumulator, had a limited ability to translocate Sb(III) to the fronds. Overall, it displayed an efficient root uptake as it accumulated 3119 mg kg^{-1} Sb in the roots after growing in media containing 8 ppm Sb(III). In our study, a maximum accumulation of 1078 mg kg^{-1} was reported in plant tissues of *T. smyrnensis* when the initial Sb concentration was 10 ppm – significantly lower than the aforementioned – indicating that *T. smyrnensis* has a lower Sb uptake capacity than *P. vittata*.[44] In another study, an accumulation of 4900 mg kg^{-1} was reported in maize plants in growth media containing 10 ppm. However, Sb

Table 2. Bioconcentration factor (BCF) and translocation factor (TF) for *T. smyrnensis* and *N. oleander* for all treatments with Sb above background level

Treatment	BCF	TF
T/5/0	59.0	0.077
N/5/0	101.4*	0.014
T/5/100	133.4*	0.037
N/5/100	88.24	0.018
T/10/0	116	0.009
N/10/0	96.2	0.011

Note: Asterisks indicate level of significance: *$P < 0.05$ (Refer to Table 1 for explanation of the experimental treatments).

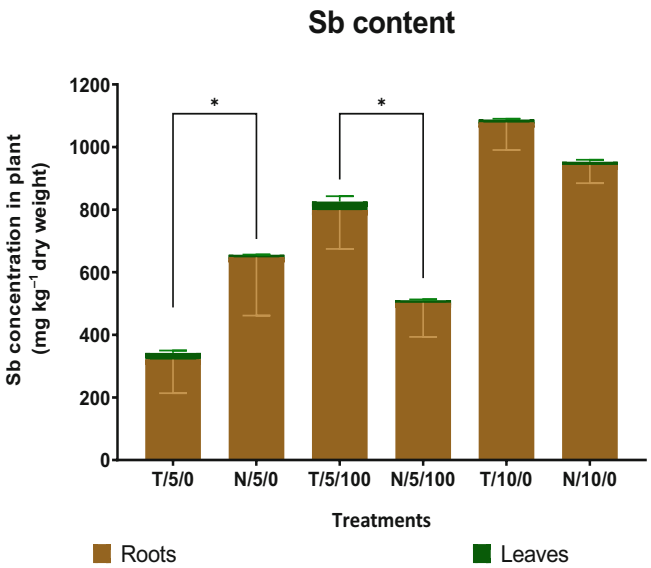

Figure 8. Sb accumulation in roots and leaves of *T. smyrnensis* and *N. oleander* (asterisks indicate level of significance: *$P < 0.05$) (Refer to Table 1 for explanation of the experimental treatments).

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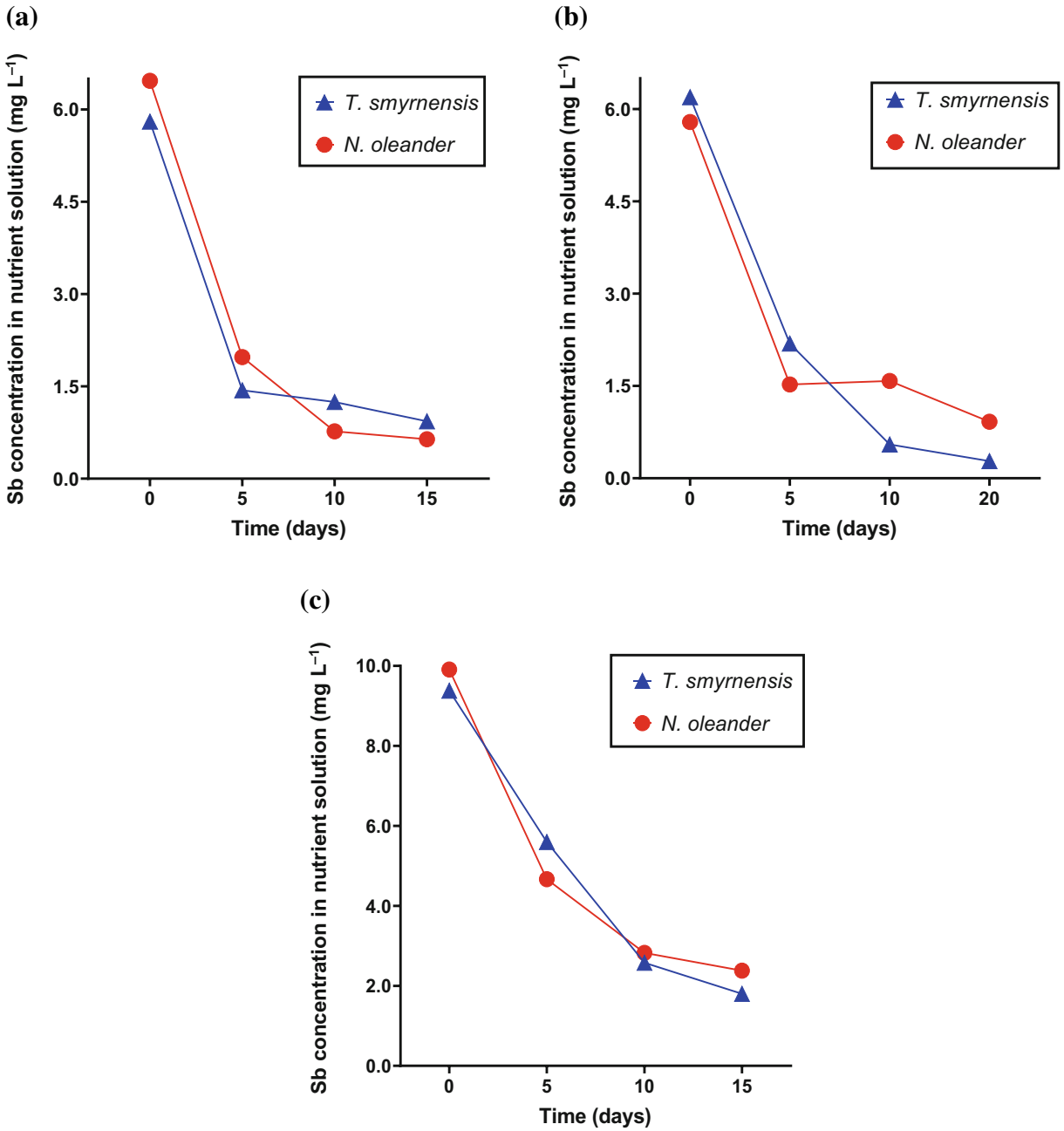

Figure 9. Sb removal in the aqueous phase over the experimental period with various treatments: (a) Sb 5 ppm/salinity 0 mmol L^{-1}; (b) Sb 5 ppm/salinity 100 mmol L^{-1}; (c) Sb 10 ppm/salinity 0 mmol L^{-1}.

induced toxicity symptoms such as decreased root length, and fresh and dry root biomass.[40] Previous work demonstrated that Sb treatment of 5 ppm in sunflower plants (*Helianthus annuus* L.) accumulated the metalloid at approximately 7700 and 50 mg kg^{-1} in roots and shoots, respectively. Also, in this instance there was a decline in photosynthetic pigment content and plant growth.[43] When comparing our results to those that reported higher accumulation, it must be pointed out that *T. smyrnensis* and *N. oleander* were not significantly affected in terms of plant growth and water content – parameters that are also important for phytoremediation.

Nutrient solution was analyzed every 5 days to monitor the fate of Sb in the aqueous phase. Figure 9 shows the removal efficiencies over the experimental period. Overall, the Sb removal efficiencies by *T. smyrnensis* and *N. oleander* were high in all treatments. In treatments, a low concentration of Sb was indicated by the total removal of Sb, which reached approximately 83% in *T. smyrnensis* and 90% *in N. oleander*. When salt was added, the removal was increased to 96% in *T. smyrnensis*, while Sb elimination was decreased to 84% in N. oleander (Fig. 9(b)). Under the highest level of Sb, Fig. 9(c) displays the Sb concentration in the aqueous phase, which was reduced to 81% and to 76%, in *T. smyrnensis* and *N. oleander*, respectively.

CONCLUSIONS

Given the ability of these plants to accumulate high root Sb concentrations without growth reduction and with stable water content, it can be considered that these plant species are suitable

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candidates for Sb phytoremediation. The rise in GPOD and CAT activity was indicative of a high capacity for antioxidant response. Accumulation in the plant tissues was increased by Sb addition and no difference was observed between the plant species in Sb content in the roots at the highest Sb level. *Nerium oleander* accumulated markedly higher Sb concentration in the roots under Sb stress at low concentration whereas, when salt was added as stressor, an opposite trend was observed as *T. smyrnensis* accumulated a substantially higher concentration in the presence of 100 mmol L^{-1} salt. Neither plant was efficient at translocating Sb from roots to the above-ground tissues, since the translocation factor was low (<0.1). Since these plants are not Sb hyperaccumulators and they are not edible by animals, there is no risk of Sb entering the food chain. Certainly, the real uptake mechanism under field conditions will be different as it is influenced by many factors, such as bioavailability, Sb speciation and concentration of coexisting ions in soils like phosphorus and calcium. As a whole, the results from this study recommend the use of *T. smyrnensis* and *N. oleander* for Sb extraction in Sb-contaminated sites.

AUTHOR CONTRIBUTIONS

Conceptualization: NK, PS; methodology: PS, ES; formal analysis: PS, NK; investigation: PS, ES, KF; chemical analysis: PS, ES, KF; data curation: PS; writing – original draft preparation: PS; writing – review and editing: NK, ES, PS; supervision: NK; funding acquisition: NK. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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