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RESEARCH ARTICLE

Growth, photosynthesis, and defense mechanism of antimony (Sb)-contaminated *Boehmeria nivea* L.

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Abstract Ramie (*Boehmeria nivea* L.) is the oldest cash fiber crop in China and is widely grown in antimony (Sb) mining areas. To evaluate the extent of Sb resistance and tolerance, the growth, tolerance index (TI), Sb content in plant parts and in Hoagland solution, bioaccumulation factor (BF), photosynthesis, and physiological changes in Sb-contaminated *B. nivea* (20, 40, 80, and 200 mg L^{-1} Sb) grown hydroponically were investigated. The Sb tolerance and resistance of ramie were clearly revealed by growth inhibition, a TI between 13 and 99 %, non-significant changes in the maximum quantum efficiency of photosystem (F_v/F_m), energy-harvesting efficiency (photosystem II (PSII)) and single-photon avalanche diode (SPAD) value, a significant increase in Sb in plant parts, BF >1, and an increase in catalase (CAT) and malondialdehyde (MDA) at 200 mg L^{-1} Sb. Under increasing Sb stress, nearly the same non-significant decline in the maximum quantum efficiency of photosystem (F_v/F_m), energy-harvesting efficiency (PSII), relative quantum yield of photosystem II (φPSII), and photochemical quenching (qP), except for F_v/F_m at 20 mg L^{-1} Sb, were recorded. SPAD values for chlorophyll under Sb stress showed an increasing trend, except for a slight decrease, i.e., <2 %, than the control SPAD value at 200 mg L^{-1} Sb. With a continuous increase in MDA, superoxide dismutase (SOD), peroxidase (POD), and CAT activities were suppressed under Sb addition up to 40 mg L^{-1} Sb and the addition of Sb enhanced enzyme production at 80 and 200 mg L^{-1} Sb. A continuous decrease in SOD, POD, and CAT up to 40 mg L^{-1} Sb and enhancements at ≥80 mg L^{-1}, along with the continuous enhancement of MDA activity and inhibited biomass production, clearly reveal the roles of these enzymes in detoxifying Sb stress and the defense mechanism of ramie at 80 mg L^{-1} Sb. Thus, *B. nivea* constitutes a promising candidate for Sb phytoremediation at mining sites.

Keywords Ramie · Antimony (Sb) · Chlorophyll fluorescence · Phytoremediation · Antioxidant enzymes

Introduction

The rapid increase of antimony (Sb) in industry (e.g., in medicine, alloy, pigment, ammunition, flame retardant) is accompanied by a growing environmental concern (Okkenhaug et al. 2011, 2012; Pan et al. 2011). The complex chemical speciation, toxicity, fate, and behavior of Sb in soil have recently been addressed. In the environment, Sb exists as antimonite (Sb^{+3}) and antimonate (Sb^{+5}) and Sb^{+3} is 10 times more toxic than Sb^{+5}. Antimony is the ninth most mined element, resulting in drastically increasing pollution, which has to be addressed (Wang et al. 2015; Roper et al. 2012; Wei et al. 2011; Filella and Williams 2012). The US Environment Protection Agency has listed Sb as a priority pollutant (US EPA 1979). China has the most active Sb mining regions, i.e., Guangxi, Hunan (Xikuangshan, Lengshuijiang—the largest antimony-producing site), Yunnan, Guizhou, Gansu, and Jiangxi, and leads in worldwide Sb production, at more than 90 %, followed by Australia, Russia, South Africa, Tajikistan,

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Canada, and the USA (Miao et al. 2014; Okkenhaug et al. 2012; Liu et al. 2010). Intensive accumulation of Sb in mining and adjacent areas has been reported, for instance, 5045 mg kg^{-1} Sb in the soil of Hunan Province, China (Cidu et al. 2014; Wang et al. 2015; He 2007; Liu et al. 2010). Many studies have reported that mining sulfide ore and the downstream leaching have resulted in an alarmingly higher concentration of Sb at and around mining sites (Cidu et al. 2014; Pierart et al. 2015; Corrales et al. 2014; Wei et al. 2015; Zhang et al. 2009; He 2007; Feng et al. 2015; Anawar et al. 2011). The increasing pollution of toxic and carcinogenic Sb is a great threat not only for the health and safety/survival of humans and animals but also for the existence of life on Earth (Cidu et al. 2014; Corrales et al. 2014; Yang et al. 2010). Despite the toxicological effects of Sb on microbes and plant growth, various plants [i.e., *Miscanthus floridulus* (L.) Warb., *Conyza canadensis* (L.) Cronq, *Boehmeria nivea* (L.) Gaudich., *Arachis hypogaea* (L,), and *Pteris vittata*] have been reported as able to grow and flourish in Sb smelting and mining areas, i.e., Xikuangshan and Lengshuijiang (Okkenhaug et al. 2011; Feng et al. 2015; She et al. 2010, 2011a; Wei et al. 2011). Although these species are reported as capable of accumulating higher concentrations of Sb ($\leq$1600 mg kg^{-1}) (Okkenhaug et al. 2011), the potential ranges of Sb accumulation and its effects on plant physiology are not well known.

B. nivea L., ramie or "China grass," is a perennial (harvestable 3–6 times per year) herbaceous fiber plant of India and China that is tolerant, vigorous, high yielding, inedible, safe, profitable, and the most popular textile fiber plant (She et al. 2010, 2011a; Liu et al. 2011). Due to its fiber, it has been widely cultivated in China, with >90 % of the worldwide production (Yi et al. 2013; Zhou et al. 2010; She et al. 2011a). *B. nivea* has been reported to be a tolerant, fast-growing, and large biomass-producing plant under heavy metal stress (e.g., Zn, cadmium (Cd), Pb, Sb, As) (Zhou et al. 2010). Recently, it has been identified as being able to grow and phytoextract 4029 mg kg^{-1} Sb from sulfide mining areas of China (Okkenhaug et al. 2011), though there is no such literature on the growth of *B. nivea* in Sb smelting/mining areas to date. *B. nivea* is known to have a high tolerance to As contamination, with no such report on Sb uptake by *B. nivea* (Huang et al. 2008). Although the existence and growth of *B. nivea* at various abandoned and active sulfide mining sites is documented, the potential range of Sb accumulation by *B. nivea* and the effects of Sb contamination on its physiology remain unaddressed. According to one report, *B. nivea*, though inedible, is economically profitable and can remove elevated concentrations of Sb (Okkenhaug et al. 2011). Thus, using ramie appears to be feasible for Sb phytoextraction from polluted sites, without the risk of its entry into the food chain (She et al. 2011b; Zhou et al. 2010; Yang et al. 2010). Indeed, its metal tolerance, rapid growth, and high biomass production make *B. nivea* economically profitable and a promising candidate for the phytoextraction of Sb from mining and smelting areas. In the present study, Sb accumulation and the effects of Sb pollution on the growth, photosynthesis, physiology, and defense mechanism of *B. nivea* L. were studied.

Materials and methods

Plant materials and growth conditions

Cuttings of young *B. nivea* plants, 14-cm-long leaves containing shoot sections, were collected from an antimony mine deposit in Xikuangshan, Hunan Province, China (29° N, 120° E), and planted in sand for rooting. The cuttings were grown in a greenhouse with illumination maintained using three fluorescent lamps providing a 14:10-h light/dark cycle, day/night temperatures of 25/18 °C was maintained, and the humidity was 60–80 %. All potted plants were well watered and grown for approximately 30 days until they reach the approximate height of 15 cm. The roots of the sand-grown plants were thoroughly rinsed with tap water, followed by deionized water. The plants were then transplanted into the half-strength hydroponic Hoagland solution (2 L) for 20 days until reaching the approximate height of 30 cm.

Hydroponic experiment

After sand culturing and 3 weeks of acclimation, the plants were transferred into 2 L of 1/2 Hoagland nutrient solution spiked with 0, 20, 40, 80, and 200 mg L^{-1} of Sb. All the chemicals used were of analytical reagent (AR) grade. Sb was applied as $KSbC_4H_4O_7·½H_2O$ (Sb(III), >99 % purity). The nutrient content of the Hoagland solution was as follows (mg L^{-1}): macronutrients [94.5 $Ca(NO_3)_2·4H_2O$, 60.7 KNO_3, 49.3 $MgSO_4·7H_2O$, and 11.5 $NH_3H_2PO_4$] and micronutrients [2.86 H_3BO_4, 1.81 $MnCl_2·4H_2O$, 0.22 $ZnSO_4·7H_2O$, 0.08 $CuSO_4·5H_2O$, 0.02 $(NH_4)_4Mo_7O_{24}·4H_2O$, and 2.8 Fe (Fe-EDTA)]. Each treatment was replicated 3 times, and each plant was placed, fixed, and protected in the hole of a tray using a styrofoam plug. The pH of the solution was adjusted to 6.5 with diluted HCl or NaOH. Aeration per pot was ensured using air pumps. The culture conditions of the treated pots were the same during the 3 weeks of acclimation.

After 21 days of Sb exposure, the roots were immersed in 20 mM Na_2-EDTA for 30 min to remove the Sb adsorbed to the roots and the entire plant was rinsed 3 times with deionized water. The plant parts of roots, stems, and leaves were separated. For further analysis, some fresh top leaves of the plant were frozen at −80 °C and some were dried at 70 °C. The remaining Hoagland solution was filtered and refrigerated for later Sb analysis. The chlorophyll content of *B. nivea* in every sixth leaf from the top was assessed for chlorophyll florescence and with a single-photon avalanche diode (SPAD; 502 Plus) meter. The phytotoxicity effect of Sb on ramie or the stress tolerance index of Sb-contaminated *B. nivea* was calculated according to Ismail et al. (2013), Asmare (2013), and Yang et al. (2010).

Measurement of photosynthesis (chlorophyll fluorescence and SPAD measurement)

After 21 days of Sb exposure, the chlorophyll fluorescence of the fully expanded, sixth leaf from the top was measured according to the procedure of Huang et al. (2013, 2014) using the MINI-PAM photosynthesis yield analyzer (Walz Company, Wurzburg, Germany), with some modification. The leaves were kept in the dark for 120 min for minimum fluorescence (F_0) and maximum fluorescence (F_m) measurements. The maximum quantum efficiency of photosystem II $(\mathrm{PSII}) = F_v/F_m = (F_m - F_0)/F_m$, the energy-harvesting efficiency, $(\mathrm{PSII}) = F_v'/F_m' = (F_m' - F_0')/F_m'$, the relative quantum yield of photosystem II (φPSII), photochemical quenching (qP), non-photochemical quenching (NPQ), and the electron transport rate (ETR) were calculated according to Huang et al. (2014) at leaf absorption of 0.85. SPAD (502 Plus; Konica Minolta Sensing Inc., Osaka, Japan) chlorophyll measurements were taken at five sites using a fully expanded, seventh leaf from the top, according to the procedures of Naus et al. (2010) and Uchino et al. (2013).

Antimony analysis

The dried plant samples were ground and sieved through a 1-mm sieve and digested with HNO_3:$HClO_4$ (4:1, *v*/*v*); the antimony (Sb) concentration in different plant parts was analyzed using atomic fluorescence spectrometry (AFS) with a Titan AFS-810. To reveal Sb bioaccumulation and translocation, the bioaccumulation factor (BF) and translocation factor (TF) of Sb in *B. nivea* were calculated as the ratio of Sb concentration in the shoots to the Sb in solution and the Sb in the shoot to the Sb concentration in the roots, respectively, in ramie.

Extractions and antioxidant assays

Physiological measurements were performed on the fifth leaf from the top of plants growing in well-watered Sb conditions. All analyses were done according to amended methods of Giannopolitis and Ries (1977), Lagrimini (1991), Brennan and Frenkel (1977), and Gonzalez et al. (1996). Approximately 0.2 g of fresh tissue was homogenized in a precooled mortar with 5 mL of 50 mmol L^{-1} precooled phosphate buffer (pH 7.8). The homogenate was centrifuged at 11,000*g* for 20 min at 4 °C (Fu and Huang 2001). The supernatant (i.e., the enzyme extract) was used for determinations of enzyme activities [superoxide dismutase (SOD), peroxidase (POD), catalase (CAT)] and the content of malondialdehyde (MDA) (Feng and Wei 2012; Feng et al. 2009, 2011).

A SOD assay briefly was performed, using a method of Giannopolitis and Ries (1977), with 3 mL reaction mixtures containing 0.3 mL of each of 750 μmol L^{-1} nitro blue tetrazolium, 20 μmol L^{-1} riboflavin, 130 mmol L^{-1} methionine, and 100 μmol L^{-1} Na_2-EDTA; 1.5 mL of 50 mmol L^{-1} phosphate buffer (pH 7.8); 0.25 mL of deionized water; and 0.05 mL of the enzyme extract. The test tubes were placed under light with an average photon flux density of 78 μmol photons s^{-1} m^{-2} for 20 min, and the absorbance of the reaction mixture was recorded at 560 nm. Reaction solution placed in the dark was used as the control. One unit of enzyme activity was defined as the amount of the enzyme that resulted in 50 % inhibition of the rate of nitro blue tetrazolium (NBT) reduction.

For POD, 3 mL of the reaction solution containing 1 mL of 0.3 % H_2O_2, 0.95 mL of 0.2 % guaiacol, 1 mL of 50 mmol L^{-1} phosphate buffer (pH 7.0), and 0.05 mL enzyme extract was used. The reaction started on the addition of the enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme that resulted in 1 % absorbance increase in 60 s at 470 nm (Lagrimini 1991).

A CAT assay was conducted with 0.1 mL enzyme extract, added to the mixture solution of 1 mL of 0.3 % H_2O_2 and 1.9 mL of 50 mmol L^{-1} phosphate buffer (pH 7.0) to initiate the reaction. The activity of CAT was measured by Brennan and Frenkel (1977) as the rate of change of H_2O_2 absorbance in 60 s at 240 nm. One unit of enzyme activity was defined as the amount of the enzyme that resulted in 1 % absorbance reduction in 60 s.

The MDA content was analyzed using the amended method of Gonzalez et al. (1996), such as 2.5 mL of 20 % (*w*/*v*) trichloroacetic acid, including 0.5 % (*w*/*v*) thiobarbituric acid and 1.5 mL enzyme extract. The solution was kept in boiling water bath for 20 min and then quickly cooled. Then, the homogenate was centrifuged at 5000*g* for 10 min at 25 °C. The absorbance of the supernatant was recorded at 532 and 600 nm, respectively. The absorbance at 600 nm was subtracted from the value at 532 nm, and the concentration of MDA was calculated using the MDA's extinction coefficient of 155 mM^{-1} cm^{-1}.

Data analysis

Analysis of variance (ANOVA) at a significance level of $P < 0.05$ was performed using the general linear model (GLM) in the SAS package. The least significant difference (LSD) test and *t* test were employed to compare significant differences between the means for the treatments at $P < 0.05$.

All the results are expressed as the means ± standard deviation (SD). Graphical analyses were carried out using Origin Pro 8.5.

Results

Effect of antimony on *B. nivea* biomass

Based on a review and our survey of Xikuangshan, healthy growing *B. nivea* plants were found in this area. During antimony (Sb)-contaminated hydroponic cultivation, the growth of *B. nivea* was significantly ($P < 0.05$) inhibited with an increase in contamination when compared to the control, as shown in Fig. 1, as indicated by the significant ($P < 0.05$) difference in the means of the fresh weights of the plants. The novelty of this research is that although field surveys to report the presence of *B. nivea* in Sb-contaminated areas (Okkenhaug et al. 2011, 2012; She et al. 2010) are available, no study has yet reported any pot, pilot, or field experiment to confirm the behavior of *B. nivea* under specific Sb ranges, i.e., between 10 and >1000 mg kg^{-1} soil (10 ≥ 1000 mg kg^{-1} soil). The increase in fresh weight of the plants under 0 mg L^{-1} Sb was 80 ± 6.21 g, whereas Sb treatment at 20, 40, 80, and 200 mg L^{-1} reduced the fresh weight to 79.76 ± 2.85, 57.07 ± 13.81, 35.1 ± 18.73, and 10.57 ± 7.70 g (1–5 times < control), respectively. At the time of planting, the leaves were healthy, fully expanded, and green. However, after 2 weeks of Sb application, the plants showed a weight decline and lynching. Later, at and above Sb, 200 mg L^{-1}, leaf wilting at least rate was observed.

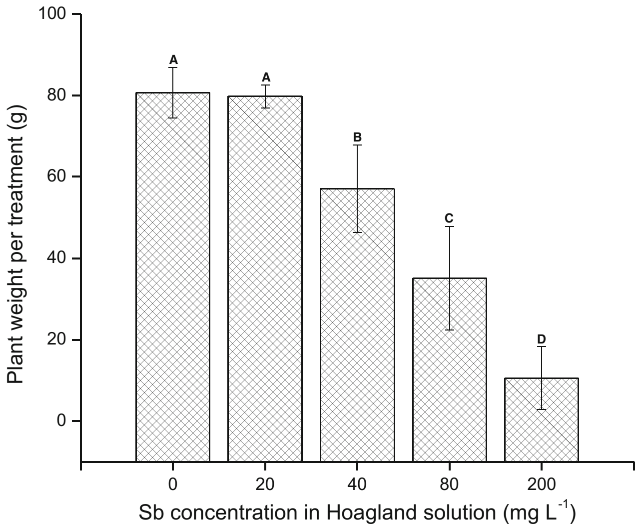

Fig. 1 Effect of Sb application on the weight of *Boehmeria nivea* L. *Different uppercase letters* are significantly different at $\rho < 0.05$ for treatments. *Values in the graph* are mean ($n = 3$), and *error bars* are standard deviation (SD)

The phytotoxicity (% phytotoxicity) of a metal and the tolerance index of a plant help in determining the suitability of that plant for use in the phytoremediation of that metal. The phytotoxicity of a metal (Sb) is inversely proportional to the tolerance index of a specific plant (*B. nivea*) for that metal (Fig. 2). The higher accumulation of Sb by *B. nivea* compared to the other vegetations at the same locality (She et al. 2010), its tolerance for cadmium contamination (She et al. 2011a), and the extraction potential of *B. nivea* for heavy metals (Pb, Cd, and Zn) has been estimated (Zhou et al. 2010) and documented. However, as the potential and suitability of *B. nivea* for Sb is still unverified, a hydroponic experiment was attempted to assess the phytoremediation potential of *B. nivea* for Sb. The tolerance index of *B. nivea* ranged 13.10–98.93 % at the highest to lowest Sb concentrations (200–20 mg L^{-1}), which proves the efficiency of the plant to decontaminate Sb in areas such as Xikuangshan, Hunan, China. Thus, *B. nivea* shows resistance to elevated concentrations of Sb and is a plant species highly tolerant to Sb contamination.

Metal toxicity can cause structural and functional damage to plants, which can adversely affect root/stem elongation and, ultimately, plant growth (Ismail et al. 2013; Shafiq et al. 2011). Sb phytotoxicity was between 1 and 87 % at 20–200 mg L^{-1} Sb (Fig. 2), and critical stage biomass production was observed at 80 mg L^{-1} Sb. At 200 mg L^{-1} Sb, *B. nivea* exhibited reduced capability for new biomass production in large amounts. The inhibited growth, inhibited root/stem elongation, and decreased tolerance index of *B. nivea* clearly reveal the phytotoxic effects of Sb on the plant. Plants respond differently to different metal stresses, but resistant and/or tolerant species activate molecular mechanism, such as metal sequestration, metal ligand binding, and vacuole deposition. Plants

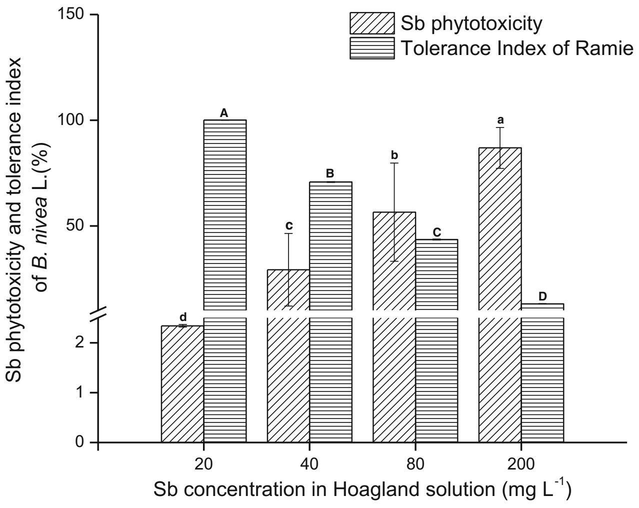

Fig. 2 Phytotoxicity of Sb application and tolerance index of *Boehmeria nivea* L. *Different uppercase and lowercase letters* are significantly different at $\rho < 0.05$ for treatments. *Values in the graph* are mean ($n = 3$), and *error bars* are standard deviation (SD)

can have multiple mechanisms for metal tolerance, employing one or any combination of resistance mechanisms (Ismail et al. 2013), and the tolerance process can occur rapidly through the synthesis of enzymatic and non-enzymatic antioxidants.

Effect of Sb on *B. nivea* L. photosynthesis

Chlorophyll florescence is a measure of the ability of a plant to convert photosynthetic energy into biomass or is the measurement of the efficiency of a plant to produce biomass. Thus, the measurement of photosynthesis (chlorophyll florescence and/or SPAD value of chlorophyll) is an indication of plant stress, as metabolism is affected under stress. Some researchers consider PSII to be a measure of photosynthesis (Dias et al. 2013; Pan et al. 2011; Hichem et al. 2009), whereas others favor the electron transport rate and carbon dioxide (CO_2) fixation, which essentially are correlated (Amari et al. 2014; Zhang et al. 2014; Lichtenthaler et al. 2007; Sarijeva et al. 2007). Indeed, qP can be used as a proxy of PSII. Chlorophyll florescence measurements and SPAD readings of chlorophyll are presented in Tables 1 and 2, respectively.

No significant changes ($P<0.05$) in chlorophyll florescence, i.e., F_v/F_m, PSII, φPSII, qP, NPQ, and ETR, in *B. nivea* were observed at 20–200 mg L^{-1} Sb, except for φPSII, qP, and ETR at 200 mg L^{-1} Sb. The obtained values of chlorophyll florescence parameters (mean ± SD) were of the order ETR > qP > F_v/F_m > PSII > φPSII > NPQ. Almost the same non-significant decline in F_v/F_m and PSII in *B. nivea* up to Sb stress at 80 mg L^{-1} was observed, except for F_v/F_m at 20 mg L^{-1} Sb (Table 1). F_v/F_m remained stable at 40 and 80 mg L^{-1} Sb, as it was in the control, but decreased from 0.04 ± 0.05 to 0.05 ± 0.02 units (5.68–7 % < control) at 20 and 200 mg L^{-1} Sb, respectively. PSII remained stable at 20 mg L^{-1} Sb and then decreased gradually, with the greatest decrease of 0.08 ± 0.02 units (13.6 % < control) at the highest applied Sb, i.e., 200 mg L^{-1}. φPSII and qP displayed the same decreasing trends. φPSII decreased from 0.51 ± 0.04 to 0.32 ± 0.10 (~38 % < control), with the highest decrease at 200 mg L^{-1} Sb, except for an increase (9.75 % > control) at 80 mg L^{-1}. The decrease in qP fluctuated (5–29 % < control) (Table 1). The smallest range of values, i.e., mean ± SD (0.13 ± 0.05–0.26 ± 0.12), was for NPQ, and the highest range (22.25 ± 6.81–35.95 ± 2.82) was for ETR. The highest decrease in NPQ and ETR was at 20 and 200 mg L^{-1} (~38 % < control), respectively.

Table 2 Effect of Sb application on SPAD of *Boehmeria nivea* L.

Sb concentration (mg L^{-1})	SPAD
0	31.57 ± 13.33 A
20	33.03 ± 9.13 A
40	36.40 ± 2.08 A
80	33.87 ± 5.41 A
200	30.95 ± 1.20 A

Values are mean ± SD ($n=5$). Values followed by uppercase different letters are significantly different at $\rho<0.05$

Non-significant changes ($P<0.05$) in the SPAD readings of the chlorophyll content of *B. nivea* were observed at 40–80 and 200 mg L^{-1} Sb (high and the highest). The SPAD values of chlorophyll showed an increasing trend from 5 to 15.3 % greater than the control at 20–80 mg L^{-1} Sb. However, the values dropped to 30.95 ± 1.20 (≤2 % < control) at 200 mg L^{-1} Sb (Table 2).

In this study, non-significant changes in F_v/F_m, PSII, φPSII, qP, NPQ, ETR, and SPAD values occurred at increasing (20, 40, and 80 mg L^{-1}) and the highest (200 mg L^{-1}) Sb concentration compared to the control, revealing the resistance and phytoremediation potential of *B. nivea* for Sb. However, our results are contrary to the results of Huang et al. (2013, 2014), who showed significant decreases in F_v/F_m, PSII, φPSII, and ETR and a significant increase in NPQ for *B. nivea* under increased salinity and drought stresses. As in Huang et al. (2013, 2014), our results for the photosynthesis of *B. nivea* under increased Sb stress are contrary to the significant decrease in F_v/F_m and chlorophyll of Dias et al. (2013) and Shi and Cai (2009) in *Lactuca sativa* L. and peanut

Table 1 Effect of Sb application on the chlorophyll florescence of *Boehmeria nivea* L.

Sb concentration (mg L^{-1})	F_v/F_m	PSII or F_v'/F_m'	φPSII	qP	NPQ	ETR
0	0.73 ± 0.02 A	0.60 ± 0.06 A	0.51 ± 0.04 A	0.84 ± 0.03 A	0.20 ± 0.08 A	35.95 ± 2.82 A
20	0.69 ± 0.07 A	0.60 ± 0.05 A	0.44 ± 0.14 AB	0.73 ± 0.20 AB	0.13 ± 0.05 A	31.31 ± 10.25 AB
40	0.73 ± 0.01 A	0.59 ± 0.04 A	0.45 ± 0.01 AB	0.77 ± 0.05 AB	0.23 ± 0.06 A	31.80 ± 0.81 AB
80	0.73 ± 0.02 A	0.57 ± 0.07 A	0.46 ± 0.08 AB	0.80 ± 0.10 AB	0.26 ± 0.12 A	32.45 ± 6.03 AB
200	0.68 ± 0.04 A	0.52 ± 0.04 A	0.32 ± 0.10 B	0.60 ± 0.15 B	0.23 ± 0.07 A	22.25 ± 6.81 B

Values are mean ± SD ($n=5$). Values followed by different uppercase letters in the same column are significantly different at $\rho<0.05$, where F_v/F_m, PSII, φPSII, qP, NPQ, and ETR are the maximum quantum efficiency of photosystem, energy-harvesting efficiency, relative quantum yield of photosystem II, photochemical quenching, non-photochemical quenching, and electron transport rate, respectively

(*A. hypogaea* L.) under medium and high Cd (1, 10, and 50 μM Cd $(NO_3)_2$) and heavy metal stress (Cd, Cu, and Zn), respectively. Our results are in agreement with the non-significant results of Amari et al. (2014) for *Mesembryanthemum crystallinum* and *Brassica juncea* under Ni stress (25, 50, and 100 μM $NiCl_2$).

Antimony concentration in *B. nivea* L.

The antimony (Sb) content in the dried roots, leaves, and stem of *B. nivea* with increasing applied Sb is presented in Fig. 3. Upon review of the limited available data on Sb uptake by plants, it is clear that Sb accumulation within and among plant species varies greatly (Pan et al. 2011). Compared to the control, Sb significantly decreased plant weight after Sb at 20 mg L^{-1} but it significantly ($P < 0.05$) increased Sb in the root, stem, and leaves in the order of root > stem > leaf. Sb in the leaf, root, and stem of *B. nivea* gradually significantly increased within a certain range, i.e., 18.50–24.50 mg kg^{-1} compared to the control (36–82 % > control). Sb is mostly accumulated in the underground parts of tolerant plants. In accordance with our results, Hammel et al. (2000), Pratas et al. (2005), and Hozhina et al. (2001) reported higher underground (root) Sb accumulation in 10 vegetable species, 16 plant species growing in abandoned Portuguese mines, and 3 aquatic plant species, respectively. Thus, the performance of *B. nivea* for Sb accumulation improves with increasing Sb addition. The mobilization of Sb from roots to leaves is the greatest threat for the food chain and the survival of life on Earth, but this would not occur in the case of *B. nivea* because it annually sheds the older leaves (which can be collected removed/recycled), and it is a commercial fiber crop; hence, food chain contamination is automatically avoided.

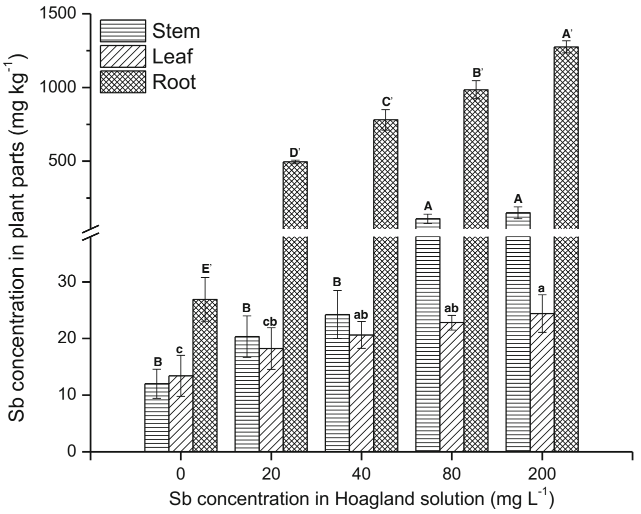

Fig. 3 Effect of Sb application on the Sb concentration in plant parts of *B. nivea* L. *Different uppercase and lowercase letters* are significantly different at $\rho < 0.05$ for treatments. *Values in the graph* are mean ($n = 3$), and *error bars* are standard deviation (SD)

Measurement of bioaccumulation and translocation factors can assess the level of phytoextraction by a plant. If the plant accumulates >3 % of its biomass of the metal, then it is considered to be suitable for phytoremediation of that specific metal; the reference value for BF and TF is >1.0 (Bahri et al. 2015; Mganga 2014; Liu et al. 2014). In our experiment, BF of Sb in *B. nivea* was higher than the reference value whereas TF was low, i.e., 0.94 Sb at 0 mg L^{-1}. Compared to the control, *B. nivea* accumulated Sb (BF_{Sb}) in the range of 98.62–264.74 % (8–66 % < control), whereby the smallest BF_{Sb} (66 % < control) was for Sb at 80 mg L^{-1} (Fig. 4). Translocation of Sb through *B. nivea* compared to the control was slow i.e., between 0.06 and 0.14 (86–94 % < control), whereby the smallest (94 % < control) was for Sb at 40 mg L^{-1}. Our results of low TF and high BF are in accordance with the results of Tisarum et al. (2014), Pierart et al. (2015), Pan et al. (2011), and Feng et al. (2009), who found the bioaccumulation factor of Sb (BF_{Sb}) in the arsenic hyperaccumulator *P. vittata*, agricultural plants (edible plants and herbs), aquatic plants (*Typha latifolia*, *Scirpus sylvaticus*, and *Phragmites australis*), and four ferns (*Pteris cretica*, *Cyrtomium fortunei*, *Cyclosorus dentatus*, and *Microlepia hancei*), respectively, to be higher than the translocation factor of Sb, i.e., $BF_{Sb} \gg TF_{Sb}$.

Effect of antimony on *B. nivea* antioxidant enzymes

Stress on plants and/or living organisms initiates the generation of reactive oxygen species (ROS), which may disturb metabolism through oxidative damage to proteins and

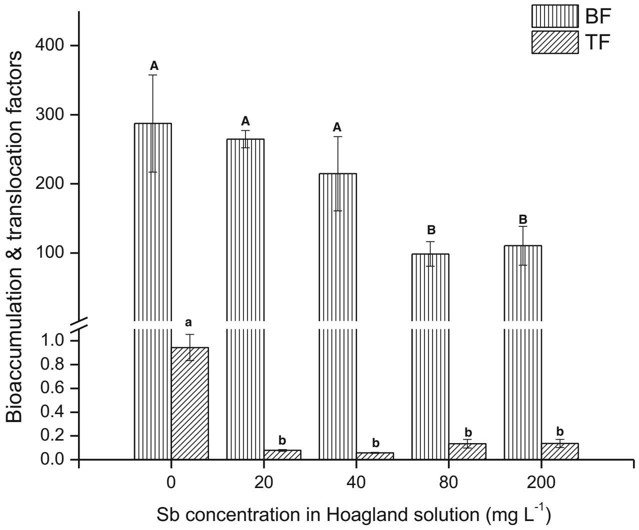

Fig. 4 Bioaccumulation and translocation of Sb in *Boehmeria nivea* L. *Different uppercase and lowercase letters* are significantly different at $\rho < 0.05$ for treatments. *Values in the graph* are mean ($n = 3$), and *error bars* are standard deviation (SD)

enzymes (Huang et al. 2013; Shehab et al. 2010). Increasing ROS increases MDA, the last product of membrane liposome peroxidation, which demonstrates the instability of lipid membranes as a result of excess ROS (Liu et al. 2005; Feng et al. 2013a, b). To reduce the effects of oxidative damage, plants initiate enzymatic and non-enzymatic antioxidant defense mechanisms, of which the synthesis of SOD, POD, and CAT are the most important. SOD eliminates oxygen radical (O^{2-}), which forms H_2O_2, and POD and CAT, being intrinsic antioxidative enzymes, control the level of produced H_2O_2 by degrading H_2O_2 to form H_2O and O_2 (Feng et al. 2009). Thus, SOD, POD, and CAT protect plant cells from ROS by eliminating excessive active oxygen in metal-affected areas. Hence, maintaining a high level of antioxidants may protect a plant from the toxicity of metal (Huang et al. 2013; Feng et al. 2013a, b). Increased antimony (Sb) had a non-significant ($P < 0.05$) effect on POD, CAT, and MDA concentrations in *B. nivea* (Fig. 5a–d).

According to the LSD test, Sb treatment had a non-significant effect ($P < 0.05$) on the POD, CAT, and MDA contents of *B. nivea*, except for SOD and CAT at the highest Sb, 200 mg L^{-1}. Compared to the control, with increasing Sb addition, SOD, CAT, and POD concentrations in *B. nivea* non-significantly ($P < 0.05$) decreased whereas MDA showed a non-significant ($P < 0.05$) increasing trend. Overall, at 80 mg L^{-1} Sb, a fluctuating performance of *B. nivea* with regard to SOD, POD, CAT, and MDA was observed (Fig. 5a–d). In *B. nivea*, the highest decrease in CAT and MDA (<control) was at 80 mg L^{-1} Sb (Fig. 5a–c), demonstrating that POD and CAT are H_2O_2 scavengers in ramie. SOD, POD, and CAT activities followed almost the same suppressive effects and enhanced trends with Sb addition (Fig. 5a–c), except for POD at 80 mg L^{-1} Sb.

Concentrations of SOD and POD were suppressed at lower applied Sb, i.e., 20 and 40 mg L^{-1}, but Sb addition at 80 and 200 mg L^{-1} Sb enhanced SOD and POD activities, except for POD decline at 200 mg L^{-1} Sb (Fig. 5a, b). Critical-stage SOD and POD performance in *B. nivea* occurred at 80 mg L^{-1} Sb. The SOD contents of *B. nivea* were suppressed approximately by 1.7, 2.8, 2.0, and 1.5 times (less than the control) at 20, 40, 80, and 200 mg L^{-1} Sb, respectively (Fig. 5a). Based on the suppressions and fluctuations, it can be concluded that SOD had little contribution to the tolerance mechanism of *B. nivea* under Sb contamination. At 20, 40, 80, and 200 mg L^{-1} Sb, POD declined by approximately 1.30, 1.70, 1.07, and 2.0 times (less than the control), respectively (Fig. 5b). Although SOD is considered to be a non-sensitive indicator for Sb tolerance (Feng et al. 2009), decreases in SOD and POD with applied metal stress have been reported, including in wheat (*Triticum durum*) and ryegrass (*Lolium perenne*) exposed to cadmium and selenium, respectively. In a study by Saidi et al. (2013), suppressed activities of SOD and POD were also reported in cadmium (Cd)-contaminated bean plants. In hybrid ramie, Huang et al. (2014) reported increased SOD and POD contents under increased salinity.

Compared to the control, a gradual decrease in the CAT content at 20, 40, and 80 mg L^{-1} Sb in *B. nivea*, i.e., 1.13, 1.61, and 2.07 times (less than control), respectively, was observed (Fig. 5c). In contrast, a rapid increase, i.e., 1.40 times (greater than control), was observed at 200 mg L^{-1} Sb. In accordance to our results (compared to control), a decreasing CAT content in rye (Silva et al. 2013) and ramie (Huang et al. 2014) under long-term exposure to aluminum (Al) and salinity was reported. Decreased CAT activity with increasing Sb contamination confirms the role of CAT in quenching H_2O_2 and preventing oxidative damage in *B. nivea*.

With the addition of Sb, the concentration of MDA in *B. nivea* continuously increased, i.e., 1.26, 1.36, and 1.22, with the highest increase of 1.72 times (greater than control) at 200 mg L^{-1} Sb (Fig. 5d). The constant increase in MDA with increased Sb addition reveals its role in lipid peroxidation to overcome and eliminate the after effects of ROS at higher applied Sb and maintain the homeostasis of *B. nivea*. An increase in MDA activity with inhibited biomass production (at and above 80 mg L^{-1} Sb) in *B. nivea* is a clear indication of tolerance to Sb. From the results, it can be predicted that Sb exposure at and above 80 mg L^{-1} Sb resulted in an increase in the production of ROS and excessive ROS were regulated by increased activities of SOD, POD, and CAT at and below 80 mg L^{-1} Sb. In comparison, a higher concentration of SOD, POD, and CAT below 80 mg L^{-1} Sb suggests their limited role in the tolerance mechanism of ramie below 80 mg L^{-1} Sb addition. Increases in the activity of SOD, POD, and CAT in *B. nivea* under higher, i.e., 80 and 200 mg L^{-1}, Sb exposure confirm their role in the resistance of *B. nivea* to high Sb addition. Our results of enhanced MDA and CAT with the addition of Sb stress are in agreement with the increases in MDA and CAT in plants (ferns, rice, and maize), drought-resistant ramie cultivars (Huangketong, Qingkezi, Ningduramie, Xiyeqing, Zhuzima, and Yushanma), and hybrid ramie (*B. nivea*) reported by Feng et al. (2013b), Liu et al. (2005), and Huang et al. (2013), respectively, under drought stress. Corrales et al. (2014) reported non-significant decreases in MDA content in the root and shoot of Sb-contaminated clover species (*Trifolium pratense* L. and *Trifolium repens* L.). Huang et al. (2014) reported the same tolerance behavior with an increase in the concentration of enzymatic and non-enzymatic antioxidants in *B. nivea* under increased salinity stress. The present results suggest that *B. nivea* is capable of alleviating oxidative stress and preventing lipid peroxidation under the specified range of Sb contamination.

Discussion

Reduced and stunted growth is the most common response of stressed plants, but these effects could be severe under higher

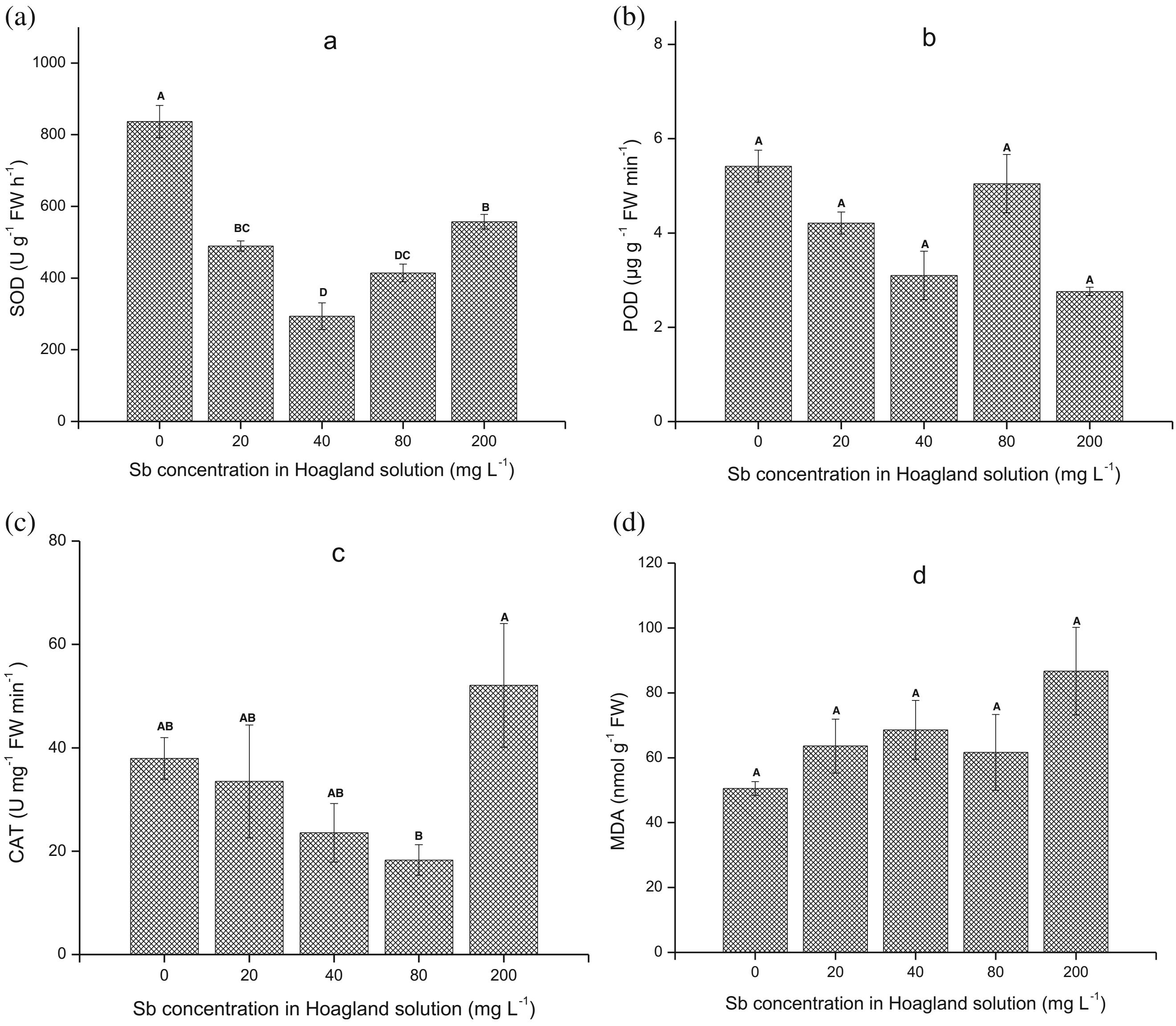

Fig. 5 Effect of Sb application on antioxidant enzymes of *Boehmeria nivea*: **a** SOD, **b** POD, **c** CAT, and **d** MDA. *Different uppercase letters* are significantly different at $\rho < 0.05$ for treatments. *Values in the graph* are mean ± SD ($n = 3$), and *error bars* are SD

stress (Koyro et al. 2013; Huang et al. 2014). In this study, increasing Sb adversely affected ramie in terms of inhibited growth, chlorophyll fluorescence, the SPAD value, antioxidant enzymes, and increased MDA. The lack of a significant difference between the control plants and those grown in increasing Sb contamination suggests that under hydroponic cultivation, *B. nivea* is tolerant to high Sb stress. Sb is toxic and carcinogenic and is a reported threat to life (Pierart et al. 2015; Wilson et al. 2010; Filella et al. 2002, 2009a, b; Smichowski 2008; US EPA 1979). Inhibited growth and a significant decrease in chlorophyll florescence and antioxidant enzymes at the highest Sb concentration are clear indications of the phytotoxic effect of Sb on *B. nivea*.

Plant growth, i.e., increases in weight, plant height, and root length, is an important parameter for the classification of heavy metal tolerance (Amin et al. 2014). Under Sb contamination, the weight of ramie was negatively affected by about 99, 71, 44, and 13 % at 20 to 200 mg kg^{-1} Sb, respectively. This could be due to the inhibitory effect of Sb pollution on the growth of plant. The inhibitory effect of Sb on the biomass production of ramie, as observed in the present study, has already been reported in other metal-stressed crops such as Cd-, heavy metal-, Ni-, and Sb-stressed bean plants (Saidi et al. 2013), *Paulownia tomentosa* (Bahri et al. 2015), *B. juncea* (Amari et al. 2014), and *Trifolium* species (Corrales et al. 2014), respectively. Various mechanisms help tolerant plants maintain growth even in the presence of potentially toxic metal concentrations (Islam et al. 2007, 2008). In this study, the plant tolerance index represented the maximum viability of *B. nivea* against Sb. The results of this research are a useful indicator of the Sb tolerance of *B. nivea* and its use in contaminated mining areas. Indeed, as a tolerant plant, *B. nivea* shows great potential to counteract the deleterious effect of Sb-contaminated soils at higher levels.

The survival and growth of plants depends on photosynthesis; therefore, if environmental stress affects growth, it must be influencing photosynthesis (Dubey 1997). Flagella et al. (1998) reported that φPSII is related to the Calvin cycle in metabolism and decreases only under drastic water deficiency, with only long-term water reduction causing water depletion in the PSII core of pea. According to Efeoğlu et al. (2009), a significant decrease in qP indicates that a large percentage of PSII reaction centers remain closed at any time of severe stress. ETR was significantly affected under serious Sb stress (200 mg L^{-1}), indicating that under high Sb, the electron transport through PSII was inhibited. The reduced state of PSII maintains the balance between excited electrons and ETR, which consequently results in a significant drop in PSII activity. In the present study, the non-significant decrease in chlorophyll florescence, i.e., F_v/F_m, PSII, φPSII, qP, NPQ, ETR, and SPAD reading at high Sb, suggests no inhibition of photosynthesis or only mild photosynthesis damage of PSII in *B. nivea* under the specified range of Sb contamination. At the highest applied Sb, a significant decrease in qP (71 %) and ETR (62 %) resulted in a significant decrease in φPSII (60 %). The changes in the fluorescence parameters (qP, ETR ,and φPSII) of Sb-stressed ramie agreed with those found by Efeoğlu et al. (2009), Inamullah and Isoda (2005), and Huang et al. (2013) in water-stressed maize, soybean, and extreme drought-stressed ramie, respectively. This decrease in photosynthesis to avoid damage under the highest Sb concentration is to protect the photosynthetic apparatus from excess energy (Li et al. 2010; Qiu et al. 2003). Significant drops in qP and ETR at the highest Sb concentration suggest that a large portion of PSII complexes were closed and that photosynthetic electron transport through PSII was inhibited, which may have caused some damage to PSII. qP induced photosynthesis inhibition, leading to the reduction of ETR, which prevented severe damage to the plants (Huang et al. 2014). The same phenomena of a significant drop of qP, ETR, and, eventually, a decrease in φPSII have already been reported in drought (Huang et al. 2013) and salinity-stressed hybrid ramie (Huang et al. 2014).

Sb accumulation in plants varies greatly with the species (Wei et al. 2015; Corrales et al. 2014; Pratas et al. 2005). In *B. nivea*, the Sb content in plant parts increased with added Sb, with greater accumulation in roots than aboveground parts. Sb translocation to aboveground parts varies greatly with the species and even varies greatly among individuals of the same species. Previously, it has been reported that Sb has an extremely low translocation factor and is thus not easily translocated to aboveground tissue (Wei et al. 2015; Baroni et al. 2000; Hammel et al. 2000; Hozhina et al. 2001). In accordance with the previous reports for various plants, *B. nivea* showed an almost similar trend of increased bioaccumulation and decreased translocation of Sb. Higher accumulation of Sb in plant roots and reduced accumulation in the shoots of rice (*Oryza sativa* L. cv Jiahua) in paddy soil in Xikuangshan, China, has also been reported by Okkenhaug et al. (2012). *B. nivea* has been reported as accumulating 4029 mg kg^{-1} extractable Sb (Okkenhaug et al. 2011), whereas the present research reports 1450 mg kg^{-1} total Sb accumulation by *B. nivea*. *B. nivea* is reported to accumulate far more Sb than *M. floridulus*, *A. hypogaea* L., and *C. canadensis* (L.) Cronq., in Xikuangshan, China (Okkenhaug et al. 2011). Based on studies by Zhou et al. (2010), using two varieties of *B. nivea* [triploid Tri (two) and diploid Xiangzhu (three)] and heavy metals (Pb, Zn, and Cd), it is clear that *B. nivea* (both varieties—soils from three different areas) can accumulate Pb and Cd in the order of roots > aerial parts (shoot and/or leaf). However, in a study of both varieties in soil from three different areas, the opposite trend of Zn accumulation was found for *B. nivea* (aerial parts > roots) (Zhou et al. 2010), which was confirmed by Pachura et al. (2015) and Zhu et al (2013), who reported Cd accumulation in *B. nivea* as roots > stems > leaves.

In the present study, the TF of Sb in ramie was less than 0.5, which is in agreement with the previous report of Feng et al. (2009) for four ferns. The BF of Sb in ramie was in the range of 99–265, which is far higher than the reported range of BF of Sb (7–25) in ferns (Feng et al. 2009). Metal immobilization in root cells or least translocation to the aboveground plant parts is the exclusion strategy of plant tolerance. According to Saidi et al. (2013), a moderate resistant plant tolerates the contamination by selective exclusion and/or lowered uptake, leading to lower cytoplasmic metal contents. In accordance to the present report, Feng et al. (2009) have reported higher Sb accumulation in the roots than in the fronds of four ferns.

Reactive oxygen species (ROS) are generated in excess in plants under stress. Depending on the concentration of metal stress, ROS play dual roles of lipid peroxidation and protection (Breusegem et al. 2001; Feng et al. 2013a, b). However, mostly, the enhanced generations of ROS pose a threat to plants and enhance the lipid peroxidation. However, the mechanisms of Sb-induced oxidative stress have to be fully elucidated. In the present experiment, SOD appeared to be contributing to scavenging in *B. nivea* up to the higher Sb contamination though, at the highest Sb application, its function was suppressed. In contrast to SOD scavenging at the high Sb contamination level, CAT and POD functioned together, converted H_2O_2 to water, and decreased lipid peroxidation in *B. nivea*, promoting tolerance. The non-significant increase of SOD and the increased activities of POD and CAT under highest Sb revealed their involvement in resisting Sb stress. Previously, Pan et al. (2011), Feng et al. (2013a, b) have reported the same enhanced content of SOD and increased activities of POD and CAT, at high Sb levels. Thus, under higher Sb stress, oxidative injury was prevented but, at extreme Sb contamination, the activities of these enzymes were impaired. MDA is an index for excessive ROS and is the cytotoxic product of lipid peroxidation (Meloni et al. 2003). In our experiment, MDA continuously accumulated with the progression of Sb stress, indicating Sb-induced lipid peroxidation via ROS. However, under low and higher Sb contamination in *B. nivea*, ROS induced a protective mechanism. Indeed, under low and higher Sb, ROS scavenging performed properly (SOD, POD, and CAT were functioning) and damage to membranes and oxidative stress was decreased, which led to the increased tolerance of *B. nivea*. The increased accumulation of MDA at the highest Sb suggests that SOD did not contribute but POD and CAT did, directly hydrolyzing H_2O_2 to H_2O and O_2. Obviously, Sb contamination influences the activity of antioxidative enzymes and its effects on MDA vary with the severity of Sb contamination. Our results are in agreement with the studies of Feng et al. (2009, 2013b), who reported elevated MDA contents in high accumulation of Sb, in the fronds of Sb-stressed *C. dentatus* and *M. hancei* (MH). These results of increased SOD, POD, CAT, and MDA are in agreement with the previous reports of other metal- or metalloid-treated plants, i.e., ferns, duck weed, Araceae, and wheat (Feng et al. 2009, 2011, 2013b, 2015; Pan et al. 2011).

Upon exposure to Sb contamination, *B. nivea* respond through alterations in morphological and physiological processes, which led to inhibited growth. However, changes in morphological and physiological characteristics depend on the severity of Sb stress. *B. nivea* proved to be tolerant to Sb stress. Thus, *B. nivea* can be a promising candidate for Sb phytoremediation in mining sites.

Conclusion

This study revealed the resistance and tolerance of *B. nivea* toward Sb in terms of inhibited growth, high Sb in plant parts, chlorophyll fluorescence, and increased SPAD values, antioxidant enzymes, and MDA in the plants. The concentration of Sb in ramie increased with increases in the applied Sb, with 200 mg L^{-1} Sb contamination inhibiting ramie biomass and growth. In *B. nivea*, the highest amount of Sb was found in the roots, followed by the leaf and stem. The plants accumulated higher amounts of Sb with inhibited growth, a scarcely affected metabolism and mildly altered physiology. The results indicated that ramie tolerates and can overcome Sb contamination by changing its internal physiology. The strength and intensity of these physiological changes are proportional to the increase in applied Sb.

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