Antimony toxicity in the lichen *Xanthoria parietina* (L.) Th. Fr.

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Abstract

In this paper we tested if treating the lichen *Xanthoria parietina* with Sb-containing solutions causes Sb bioaccumulation as well as physiological and ultrastructural changes. Total and intracellular antimony content in Sb-treated samples increased progressively with increasing concentration in the treatment solutions. Incubation of *X. parietina* thalli with Sb at concentrations as low as 0.1 mM caused a decrease in sample viability, measured as intensity of respiratory activity, and damage to cell membranes, expressed in terms of membrane lipid peroxidation, as well as ultrastructural changes such as plasmolysis, impairment of the thylakoid system of the alga and cytoplasmic lipid droplets. The photosynthetic system hardly responded, at least under the tested experimental conditions.

Key Words: Bioaccumulation, Ecophysiology, Membrane lipid peroxidation, Photosynthesis, Ultrastructure.

1. Introduction

Antimony (Sb) is a toxic element with adverse effects to humans and the environment and without any known physiological function (Bowen, 1979). It can exist in a variety of oxidation states (+5, 0, +3, -3), but it is mainly found in the environment as trivalent and pentavalent. Water-soluble Sb is comparable in its toxicological behaviour to arsenic (As), hence trivalent species are more toxic than pentavalent ones (Gebel, 1997). Since the ‘70s, Sb and its compounds are classified as priority pollutants by the EU and the USEPA and are currently listed among banned hazardous compounds specified in the Basel Convention (Filella et al., 2002).

Antimony is one of the elements that show the highest enrichment in aerosols in relation to its abundance in Earth's crust, and several studies have shown that this phenomenon is relevant in volcanic and geothermal areas and urban environments (Bogen, 1973; Maenhaut et al., 1989).

The most important natural release of Sb to the atmosphere is caused by volcanic activity, while the groundwater Sb content is a consequence of Sb release from the bedrock (Adriano, 1986). Anthropogenic sources of atmospheric Sb include tire, brake, engine and vehicle components deterioration (Fujiwara et al., 2011), and to a lesser extent fuel combustion (Sternbek et al., 2002). In the form of Sb_2O_3, a form considered as possibly carcinogenic to humans by IARC (1998), Sb is used as flame retardant in the process of vulcanization of rubber (Dietl et al., 1997). In recent years Sb has been linked to traffic emissions (Dietl et al., 1997) and recognized as a traffic related element (TRE) associated with particulate matter (Da Silva et al., 2008), and as a consequence Sb has become an element of increasing environmental concern. Antimony is also present in geothermal fluids (Stauffer and Thompson, 1984) and the emissions of geothermal power plants can discharge this element into the atmosphere via steam from the chimney, or in water or soil via disposal of spent fluids (Ármannsson and Kristmannsdóttir, 2002).

Lichens have been widely used in biomonitoring of air pollution since they are highly dependent on the atmosphere for nutrients and are lacking a waxy cuticle and stomata allowing many contaminants to be absorbed over the whole thallus surface (Ferry et al., 1973). Lichens are good

bioindicators of geothermal air pollution (Loppi, 1996) as well as good bioaccumulators of elements of geothermal concern (Loppi et al., 1999).

Despite its widespread use, Sb has so far received little attention from the toxicological point of view, and the hazard associated with the environmental contamination from Sb, as well as possible harmful effects on plants, animals, and humans, still remain poorly understood (Shtangeeva et al., 2011).

Antimony content in plant species is generally between 0.1 and 200 μg/kg (Pais and Jones, 1997), but it is easily absorbed when present in the environment in water-soluble form (Kabata-Pendias and Pendias, 2001). Traffic emissions were found to increase Sb content in the lichen *Xanthoria parietina* (Yenisoy-Karakaş and Tuncel, 2004) and it was suggested that the presence of Sb in the environment may negatively influence lichen diversity, including the distribution of *X. parietina* (van Dobben et al., 2001).

Following projects aimed at investigating the possible toxic effects of elements of geothermal origin such as boron (Pisani et al., 2009), mercury (Pisani et al., 2011a) and arsenic (Pisani et al., 2011b) on lichens, as well as toxicity of new tracers of traffic pollution (Paoli et al., 2013), the aim of this study was to test if treating the lichen *X. parietina* with Sb-containing solutions causes Sb bioaccumulation as well as physiological and ultrastructural changes.

2. Materials and methods

2.1. Lichen material

Thalli of the foliose lichen *Xanthoria parietina* (L.) Th. Fr. were collected in a remote area of Tuscany (43°14'07" N, 11°20'26" E, Ville di Corsano, Siena, Italy). This species was chosen having been previously successfully used to test physiological effects of As and B under controlled conditions (Pisani et al., 2009; 2011b). After collection, samples were transferred to the laboratory.

Peripheral lobes of the thalli, detached using tweezers, were got rid of impurity, immersed for a few sec in deionized water, shaken to wash out deposited particles and left overnight in a climatic-chamber at 15±2 °C, RH 55±5% and photoperiod of 12 h at 40 μmol m^{-2} s^{-1} photons PAR.

2.2. Antimony treatments

Treatment solutions containing trivalent antimony (Sb^{3+}) at concentrations 0.1, 1, 10 and 100 mM were prepared using potassium antimony tartrate (PAT: $C_8H_4K_2O_{12}Sb_2$ $3H_2O$). These quite high concentrations have been chosen to obtain a clear and rapid response in order to identify the most sensitive and responsive parameters, as already done in the case of As (Pisani et al., 2011b), B (Pisani et al., 2009) and N (Munzi et al., 2009). Lichen thalli were incubated by shaking for 1 h in 50 mL of solutions, let air-dry on absorbing paper and then conserved at light and room temperature for 24 h. Control samples were treated in the same way, but incubated in deionized water. Treatments were repeated three times; all treatments and analyses were run on five replicates.

2.3. Total and intracellular Sb content

To distinguish between total amounts and intracellular fractions of Sb, the sequential elution technique (Brown and Brown, 1991) was followed. Treated samples were divided into two batches: one batch was analysed directly to measure the total Sb content; to remove Sb bound to the cell wall (Branquinho and Brown, 1994), the other batch was soaked by shaking for 20 min in 10 mL of 20 mM Na_2EDTA solution and then rinsed in deionised water. The Sb content of the samples after this washing cycle corresponds to the intracellular fraction. Lichen thalli were air-dried to constant weight then pulverized and homogenized in liquid nitrogen with a ceramic mortar and pestle. About 200 mg of lichen powder were mineralized with a mixture of 6 mL of 70% HNO_3, 0.2 mL of 60% HF and 1 mL of 30% H_2O_2 (ultra-pure reagents). Digestion of samples was carried out in a microwave digestion system (Milestone Ethos 900). Antimony concentrations, expressed on a dry

101 weight basis, were determined by ICP-MS (Perkin-Elmer Sciex 6100). Analytical quality was
102 checked by analysing the Standard Reference Material IAEA-336 'lichen'. Precision of analysis
103 was estimated by the coefficient of variation of 4 replicates and was found to be within 10%.
104
105 ***2.4. Sample viability***
106 Triphenyltetrazolium chloride (TTC) reduction to triphenylformazan (TPF) is a good indicator of
107 dehydrogenase activity and was used to assess sample viability (Bačkor and Fahselt, 2005). Ca. 15
108 mg of lichen material was incubated in the dark for 20 hours in 2 mL of 0.6% TTC and 0.005%
109 Triton X 100 solution in 50 mM phosphate buffer (pH 6.8). Solutions were then removed and
110 samples rinsed in distilled water until bubbles of Triton X were produced. Water-insoluble formazan
111 was extracted with 6 mL of ethanol at 65 °C for 1 h. Tubes were then centrifuged at 4,000 g for 10
112 min and absorbance red at 492 nm. Results were expressed as absorbance units/g (dw).
113
114 ***2.5. Membrane lipid peroxidation***
115 Membrane lipid peroxidation was estimated using the thiobarbituric acid reactive substances
116 (TBARS) assay, as suggested by Huang et al. (2004), and was used as indicator of stress conditions
117 (Pisani et al., 2011b). Fragments of lichen thalli were rinsed in deionized water and then
118 homogenized in a mortar using 0.1% (w/v) trichloracetic acid (TCA) with the addition of sand. 1.5
119 mL of the homogenate was put in eppendorf tubes and centrifuged at 12,000 g for 20 min. 0.5 mL
120 of the supernatant were collected and added to 1.5 mL of 0.6% thiobarbituric acid in 10% TCA and
121 put in glass tubes. Tubes were put in the oven at 95 °C for 30 min, cooled in an ice bath and then
122 solutions were centrifuged again at 12,000 g for 10 min. The absorbance of the supernatant was
123 measured at 532 nm and corrected for non-specific absorption at 600 nm. Concentration of TBARS
124 was calculated using the extinction coefficient for the TBA-MDA complex (155 mM^{-1} cm^{-1}) and the
125 results expressed as μmol/g (dw).

126

127 ***2.6. Water-soluble proteins***

128 The content of water-soluble proteins was estimated by the dye binding technique (Bradford, 1976).
129 Samples were rinsed in deionized water and homogenized in a mortar using 2 mL of 50 mM
130 phosphate buffer (pH 6.8) and sand, and the homogenate was centrifuged at 12,000 g at 4 °C for 20
131 min. Then 100 µL of the supernatant were added to 1.5 mL of Bradford solution, the mixture shaken
132 and allowed to react at least for 10 min. Absorbance was red at 595 nm and the concentration (mg/g
133 dw) was determined using bovine serum albumin as a protein standard.

134

135 ***2.7. Chlorophyll* a *fluorescence***

136 Chlorophyll *a* fluorescence emission was analysed by the standard physiological indicator of
137 photosynthetic efficiency F_V/F_M, representing the potential quantum yield of primary
138 photochemistry (Maxwell and Johnson, 2000). In addition, the performance index (PI_{ABS}), a global
139 indicator of the photosynthetic performance was calculated to express the overall vitality of the
140 samples (Strasser et al., 2000). Prior to measurements, each lichen sample was hydrated and then
141 dark-adapted with a clip for 10 min to allow full dark adaptation of the photosynthetic pigments.
142 Lichens rested on a foam pad whilst in the clip to minimize damage to the thalli. Samples were then
143 lightened 1 sec with a saturating 3,000 µmol m^{-2} s^{-1} light pulse. Fluorescence emission was recorded
144 for 1 sec.

145

146 ***2.8. Transmission Electron Microscopy (TEM)***

147 Lichen samples were kept for 24 h on filter paper moistened with deionized water to ensure
148 complete hydration of thalli. TEM preparations (three replicates) and observations were performed
149 according to the method reported by Basile et al. (1994). Specimens were fixed with glutaraldehyde
150 3%, post-fixed with osmium tetroxide 1%, dehydrated with ethanol to propylene oxide and

151 embedded in Spurr's epoxy medium. Ultrathin sections (70 nm thick) were collected on copper
152 grids and stained with uranyl acetate and lead citrate. A FEI EM 208S TEM, with an accelerating
153 voltage of 80 kV, was used for the observations. The image analysis of the cellular ultrastructural
154 characters within a median section of algal and fungal cells was performed on electron micrographs
155 by the software program analySIS (FEI); cytoplasmic and chloroplast droplets, altered organelles
156 and other ultrastructures were examined.
157
158 ***2.9. Statistics***
159 Significance of differences ($P<0.05$) was checked by one-way analysis of variance (ANOVA), using
160 the HSD Tukey test for post-hoc comparisons. Data not matching a normal distribution
161 (Kolmogorov-Smirnov test at the 95% confidence interval) were log-transformed prior to analysis.
162
163 **3. Results**
164
165 ***3.1. Bioaccumulation***
166 The results of analytical determinations are summarized in Table 1. Antimony content in control
167 samples was consistent with Sb values reported for samples from background areas (Bergamaschi et
168 al., 2004). Total concentrations of Sb in treated samples increased progressively with increasing
169 concentration in the treatment solutions, and the same held true also for intracellular concentrations,
170 which ranged from 60% in control samples to 17-40% in treated samples. All differences among
171 samples were statistically ($P<0.05$) significant.
172
173 ***3.2. Physiological effects***
174 Physiological effects of treatments with Sb solutions are shown in Figures 1-5. Treatments with Sb
175 solutions induced a significant ($P<0.05$) reduction of sample viability and increase in membrane

lipid peroxidation already at the concentration 0.1 mM. The content of water-soluble proteins was affected ($P<0.05$) from the concentrations of 1 mM Sb. Photosynthetic parameters were the least sensitive to Sb treatments, with the Performance Index being decreased ($P<0.05$) only from the concentration of 10 mM Sb and the F_V/F_M responding ($P<0.05$) only at the highest Sb concentration.

3.3. Ultrastructural effects

The ultrastructural organization of control samples is shown in Figures 6a-b. Algal cells showed lobate chloroplasts with very undulated thylakoids, arranged as sheaves spreading from the chloroplast membrane towards the pyrenoid (Fig. 6a). Thylakoids are in a clear stroma. A pyrenoid with numerous pyrenosomes is well delimited in the centre of the chloroplast. Pyrenosomes are distributed along membranes crossing the pyrenoid (Fig. 6a). Ribosomes, vacuoles, mitochondria, nucleus and a few cytoplasmic lipid droplets were regularly shaped (Fig. 6b). Fungal cells had thick cell walls (Fig. 6a); cytoplasm appeared rather electron-dense and contained nucleus, mitochondria and a few multivesicular bodies.

Treatment with Sb affected cell ultrastructural features in a dose-dependent way (Fig. 6c-i). At the lowest Sb concentration (0.1 mM), ultrastructural changes were already visible. In the algae the cytoplasm showed lipid droplets and vacuolization, and the thylakoid system appeared depleted (Fig. 6c, d). Nonetheless, membrane organelles, i.e. mitochondria, and the whole algal symbiont ultrastructure appeared well preserved (Fig. 6d). Fungal cells were also altered at the lowest Sb concentration. The cytoplasm appeared very clear and contained vesicles, membrane residues and lipid droplets. Treatment with 1 mM Sb caused notable alterations (Fig. 6e-f). In the algae, the thylakoid system became poor and less regularly arranged; stromal electron-dense droplets were visible and plasmolysis was heavy (Fig. 6e). Pyrenoid appeared less electron dense, inhomogeneous and with a reduced volume, but pyrenogloblues were still present. Lipid droplets and multivesicular bodies occurred in the cytoplasm (Fig. 6f). Fungal cells showed a damage similar to that at 0.1 mM

Sb (Fig. 6e). Treatments with 10 and 100 mM Sb solutions determined the loss of the ultrastructure in the algal cells (Fig. 6g-i). Nevertheless, even at the highest Sb concentration, a 10% of algal cells showed an ultrastructure identifiable as alga, where thylakoid membranes and a pyrenoid were barely recognizable (Fig. 6i). Furthermore, those algal cells were heavily plasmolysed too. Only a few fungal cells had an ultrastructure comparable to that at lower Sb concentrations.

4. Discussion

Antimony accumulation clearly showed to have detrimental effects on the lichen *X. parietina*. In fact, at increasing Sb concentration of treatment solutions, Sb was increasingly accumulated and taken up also intracellularly, affecting, with different sensitivities, all the physiological parameters investigated. The ability of lichens to accumulate Sb is documented also by the study by Uluozlu et al. (2010), which showed that the lichen *Physcia tribacia* has high biosorption capacity for Sb(III) and has potential for being used for the treatment of Sb(III) containing waste-waters. Our results are consistent with those of a field study on the impact of vehicle emissions, which showed that a progressive accumulation of Sb and other TREs in thalli of the lichen *E. prunastri* was a function of the intensity of road traffic and that a high damage to cell membranes occurred in association with high concentrations of bioaccumulated Sb (Pisani, 2008).

The physiological parameters investigated showed a differential sensitivity to Sb accumulation, with sample viability and increase in membrane lipid peroxidation being the most sensitive, significantly responding already at the lowest Sb concentration tested of 0.1 mM. These results agrees perfectly with those of Pisani et al. (2011b) for As toxicity in *X. parietina*: also for As, sample viability and membrane damage were the most sensitive parameters. This is consistent with the fact that being chemically and physically similar to arsenic, toxicity of Sb is similar to that of As (Gebel, 1997).

225 Reduction of sample viability is caused by decreased activity of dehydrogenase enzymes, which are
226 involved in respiratory processes. Reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to
227 triphenyl formazan (TPF) is directly linked to the activity of the mitochondrial respiratory chain
228 (Ruf and Brunner, 2003) and is commonly used for the assessment of vitality of lichens exposed to
229 environmental stress (Bačkor and Fahselt, 2005). Antimony interacts with sulphydryl groups of
230 enzymes and other proteins, leading to inhibition of cellular function and also to cell death (Gebel,
231 1997). Antimony is known to inhibit succinic oxidase, pyruvate oxidase, and phosphofructokinase,
232 suggesting interference with cellular respiration (Gebel, 1997). Fitting a model of dose-response
233 curve and taking as EC_{50} the concentration of Sb at which sample viability is reduced by 50%
234 compared with the control incubated in the absence of Sb, we obtained a value of ca. 55 mg/L Sb,
235 which agrees well with the EC_{50} values of 40-50 mg/L obtained for growth reduction in sunflower
236 (Tschan et al., 2010), 40 mg/L obtained for lettuce shoot yield (Oorts et al., 2008), 59 and 43 mg/L
237 reported for the green algae *Scenedesmus subspicatus* and *Chlorococcum infusionum* (Hammel et
238 al., 1998).
239 Like sample viability, a significant increase in membrane lipid peroxidation products (TBARS) in
240 treated samples compared with control samples emerged already after treatment with the solution
241 0.1 mM Sb. Increased production of TBARS was found in samples of *X. parietina* incubated with
242 solutions containing arsenic at concentrations as low as 0.1 ppm (Pisani et al., 2011b). The addition
243 of Sb to the hydroponic culture of some fern species significantly increased their TBARS content
244 (Feng et al., 2009). TBARS are a decomposition product of polyunsaturated fatty acids which are
245 produced during peroxidation of membrane lipids (Mittler, 2002) and are known to occur when
246 lichens are exposed to metals, as a consequence of oxidative stress (Bačkor and Loppi, 2009).
247 Probably oxidative stress induces the activation of defence mechanisms composed by antioxidant
248 enzymes such as peroxidase, catalase, and ascorbate peroxidase (Feng et al., 2009; Pan et al., 2011),
249 which may be effective in contrasting deleterious effects of Sb until concentrations of 0.1 mM (12

mg/L); above this threshold, antioxidant mechanisms may be exceeded and peroxidation processes may take place.

Pan et al. (2011) reported the ability of these antioxidant enzymes to respond to the stress induced by Sb in maize: the activity of peroxidase increased in response to Sb levels in the soil up to 50 mg/kg and decreased at 500 mg/kg, whereas high levels of Sb in the soil were found to enhance catalase and reduce the action of superoxide dismutases. The lichen *X. parietina* also showed to activate detoxification mechanisms mediated by antioxidant enzymes, glutathione, ascorbic acid and phytochelatins against the action of some toxic metals (Sanità di Toppi et al., 2005a, 2005b). These processes might contribute to maintain the activity of dehydrogenase enzymes and membrane lipid peroxidation at normal values. However, increasing the Sb concentration in the growing medium causes progressive intracellular Sb uptake and likely, these protection mechanisms gradually fail until their almost complete ineffectiveness with the solution 100 mM, as suggested by the 82.4% reduction in sample viability and 423% increase in TBARS.

Soluble proteins decreased significantly from ca. 10 mg/g dw in control samples and samples treated with 0.1 mM solutions, to ca. 6 mg/g dw in samples treated with 1 mM Sb, down to <5 mg/g in samples incubated with 10 and 100 mM Sb. A significant decrease in the content of soluble proteins is generally observed in lichen thalli and isolated photobionts exposed to metal stress (Bačkor and Fahselt, 2005; Bačkor et al., 2009; Monnet et al., 2006). Incubation of *X. parietina* samples with 0.01 ppm As for 24 h caused a reduction in the content of water-soluble proteins (Pisani et al., 2011b).

Antimony is known to induce oxidative stress in lichens, ferns and higher plants (Öztetik and Çiçek, 2010; Feng et al., 2009; Pan et al., 2011) throughout the production of reactive oxygen species (ROS) that, besides membrane lipid peroxidation, may also cause protein inactivation, as in our case with water-soluble proteins. Our results are thus consistent with a status of generalized cellular stress, paralleled by oxidative processes and cell membrane impairment.

Exposure to Sb significantly decreased the F_V/F_M value only in the case of samples incubated with the more concentrated solution (100 mM). It has been suggested that in lichens PI_{ABS} is a more sensitive than F_V/F_M to stress conditions (Paoli et al., 2010). Also in the case of Sb stress, PI_{ABS} was more sensitive than F_V/F_M, being significantly decreased from the concentration 10 mM. Ecophysiological parameters related with photosynthetic efficiency and functioning were the least sensitive to Sb treatment. Consistently with our results, Pan et al. (2011) investigated the chlorophyll content and the photosynthetic efficiency of plants of maize treated for 2 weeks with 10, 50, 100, 500 and 1000 mg/kg Sb in the soil and found that both parameters were negatively influenced only at the highest concentrations. Pisani et al. (2011b) found that culturing *X. parietina* thalli with As caused a significant reduction of the photosynthetic efficiency, expressed by the F_V/F_M ratio, only at the highest concentration tested (10 ppm).

The ultrastructural changes observed in the algal cells (plasmolysis, impairment of the thylakoid system, appearance of lipid droplets) after Sb treatment are comparable to the alterations found by Sorbo et al. (2011) in the photobiont of *Pseudervernia furfuracea* exposed to heavy metals. These authors found that in *P. furfuracea* the effects of heavy metals on ultrastructure were similar, irrespective if samples were exposed for six months to a heavily polluted urban and industrial environment, sprayed weekly for six months with solutions containing Cd, Cu, Pb and Zn in an unpolluted area, or cultured in vitro with the same metals for one month, and argued that the reason could be that heavy metals affect many different target sites, impairing the whole cell physiology (Sanità di Toppi and Gabbrielli, 1999) and induce oxidative stress (Gallego et al., 1996; Dixit et al., 2001; Shah et al., 2001; Sharma and Dietz, 2009), and concluded that heavy metals may cause different kind of injuries and affect the whole cellular ultrastructure. Moreover, the similar response of *X. parietina* and *P. furfuracea* to trace element stress could be explained also by the fact that the algal symbiont of both lichen species usually belongs to the same genus *Trebouxia* (Purvis et al., 1992).

Our findings of injury to thylakoid arrangement in Sb-treated *X. parietina* samples is consistent with the ultrastructural damage observed in samples of the lichen *Bryoria fuscescens* trated with heavy metals (Tarhanen, 1988). Moreover, our results of injury to chloroplast associated with impairment of the photosynthetic function agree with similar results reported for Pb-treated lichens (Branquinho et al., 1997) and for Cd-treated *Elodea canadensis* and *Triticum aestivum* (Dalla Vecchia et al., 2005; Ouzounidou et al., 1997).

The increase of cytoplasmic lipid droplets observed in algal and fungal cells of Sb-treated samples agrees with the findings of Tarhanen (1998) for heavy metal treated *B. fuscescens* and Sorbo et al. (2011) for heavy metal treated *P. furfuracea*. Lipid droplets can be regarded as accumulated lipids derived from membrane damage (Dalla Vecchia et al., 2005).

The appearance of plasmolysis in treated samples may be related to the loss of membrane selective permeability, resulting from direct damage to the cell membrane. In fact, Branquinho et al. (1997) observed that cellular components can swell or shrink when ions move across the membrane.

The observed reduction in the volume of the pyrenoid is consistent with the study of Aguilera and Amils (2005) on the Cd-stressed green alga *Chlamydomonas*.

Our finding of photosynthetic impairment and the Sb inducted damage of enzymes involved in starch synthesis could be related to pyrenoid alteration, as already suggested by Demirevska-Kepova (2004).

It is noteworthy that ultrastructural changes such as plasmolysis, impairment of the thylakoid system and cytoplasmic lipid droplets already occurred even at the lowest Sb concentration tested (0.1 mM).

In spite of the impairment to the thylakoid system, the limited negative effects on photosynthesis emerged from our results could be determined by the peculiar structure of the lichen thallus, where the algae are surrounded and protected by fungus hyphae and are located in the inner part of the thallus, in such a way that the mycobiont is the first to interact with the environment and hence with

potentially toxic substances, which once taken up by lichens are firstly transferred through the mycobiont-derived outermost hyphal cortex into a fungal apoplastic (Basile et al., 1994). In short they are translocated within the apoplastic continuum that encloses the photobiont cells, and later on absorbed (Honegger, 1993). Our results evidenced that the ecophysiological parameters related with chlorophyll fluorescence, such as F_V/F_M and PI_{ABS}, may still give an unaltered signal even if algal cells seem partially damaged, indicating that these parameters are not very sensitive to Sb toxicity.

5. Conclusions

Total and intracellular antimony content in Sb-treated samples increased progressively with increasing concentration in the treatment solutions. Incubation of *X. parietina* thalli with Sb at concentrations as low as 0.1 mM caused a decrease in sample viability, measured as intensity of respiratory activity, and damage to cell membranes, expressed in terms of membrane lipid peroxidation, as well as ultrastructural changes such as plasmolysis, impairment of the thylakoid system of the alga and cytoplasmic lipid droplets. From these results we can suggest that the above parameters can be used as indicators of the biological effects of acute Sb pollution. The photosynthetic system hardly responded, at least under the tested experimental conditions.

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	Control	0.1 mM	1 mM	10 mM	100 mM
Total Sb	0.48 ± 0.03a*	508 ± 24b*	946 ± 5c*	2490 ± 22d*	3452 ± 14e*
Intracellular Sb	0.29 ± 0.02a*	200 ± 10b*	273 ± 3c*	391 ± 4d*	1373 ± 10e*

Table 1. Total and intracellular content of Sb in control and treated samples (μg/dw). * = significant difference ($P<0.05$) between total and intracellular Sb; different letters indicate significant differences ($P<0.05$) between treatments.

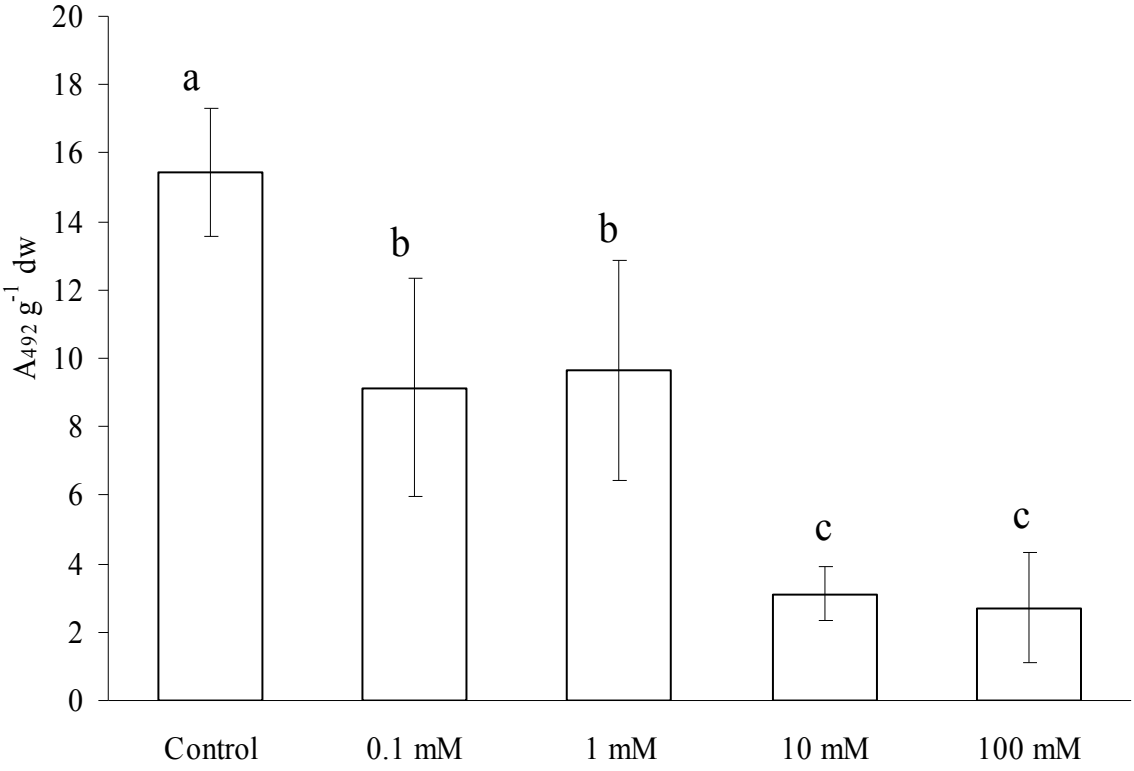

Figure 1. Mean (±SD, N=15,) viability (expressed as absorbance at 492 nm on a dry weight basis) of lichen samples treated with different antimony concentrations. Different letters indicate statistically significant differences ($P<0.05$).

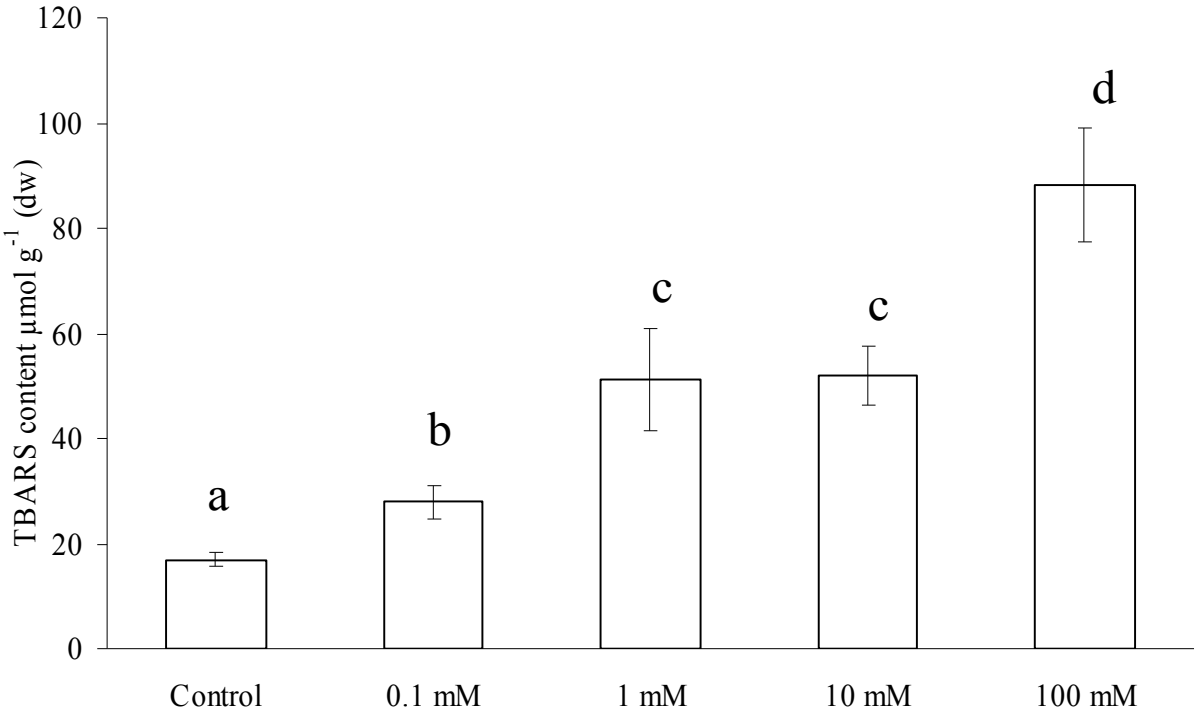

Figure 2. Mean values (±SD, N=15) of membrane lipid peroxidation (expressed as TBARS content, µmol/g, on a dry weight basis) of lichen samples treated with different antimony concentrations. Different letters indicate statistically significant differences ($P<0.05$).

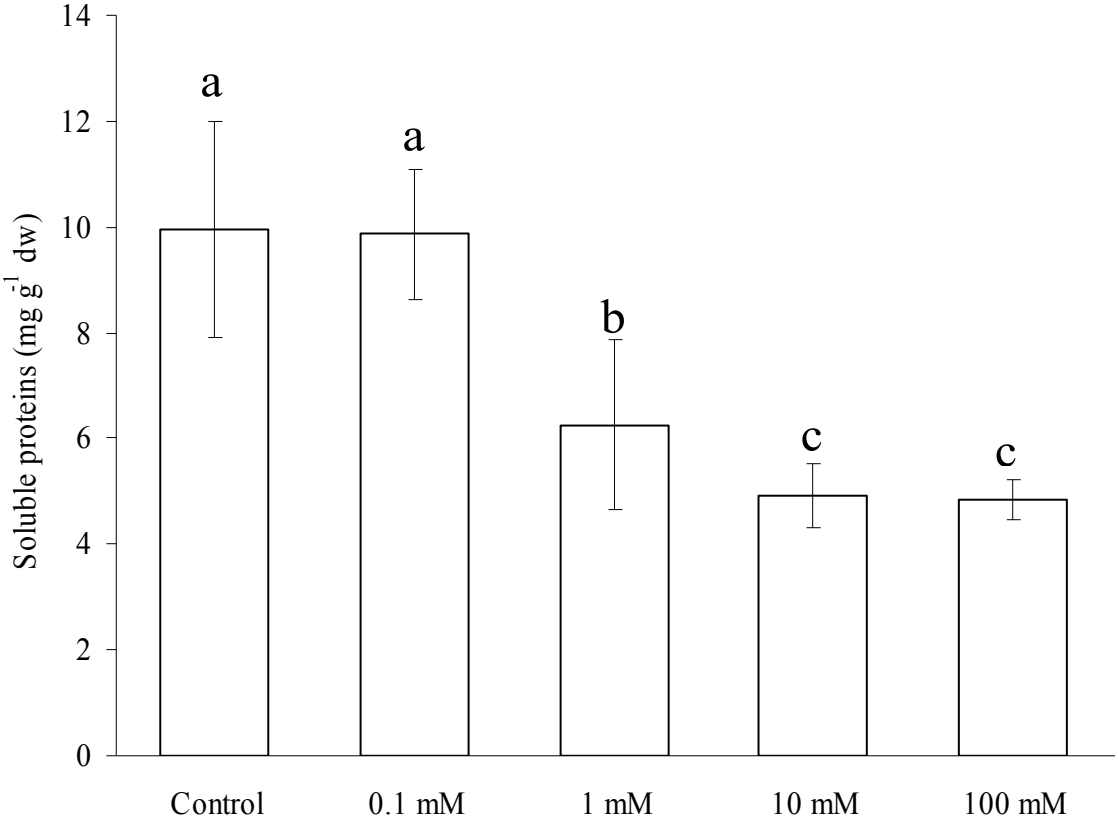

Figure 3. Mean (±SD, N=15) soluble proteins content (expressed as mg g^{-1} on a dry weigh basis) of lichen samples treated with different antimony concentrations. Different letters indicate statistically significant differences ($P<0.05$).

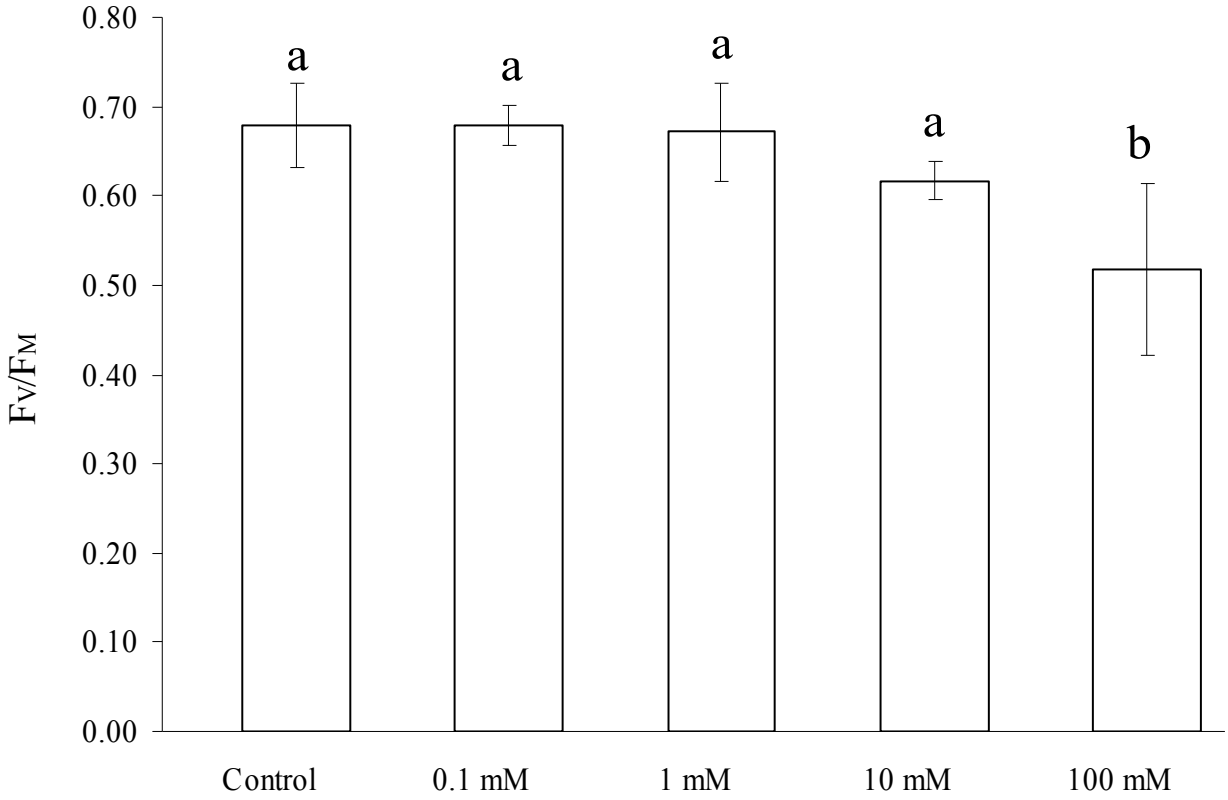

Figure 4. Mean values (±SD, N=15) of the potential quantum yield of primary photochemistry (F_V/F_M) of lichen samples treated with different antimony concentrations. Different letters indicate statistically significant differences ($P<0.05$).

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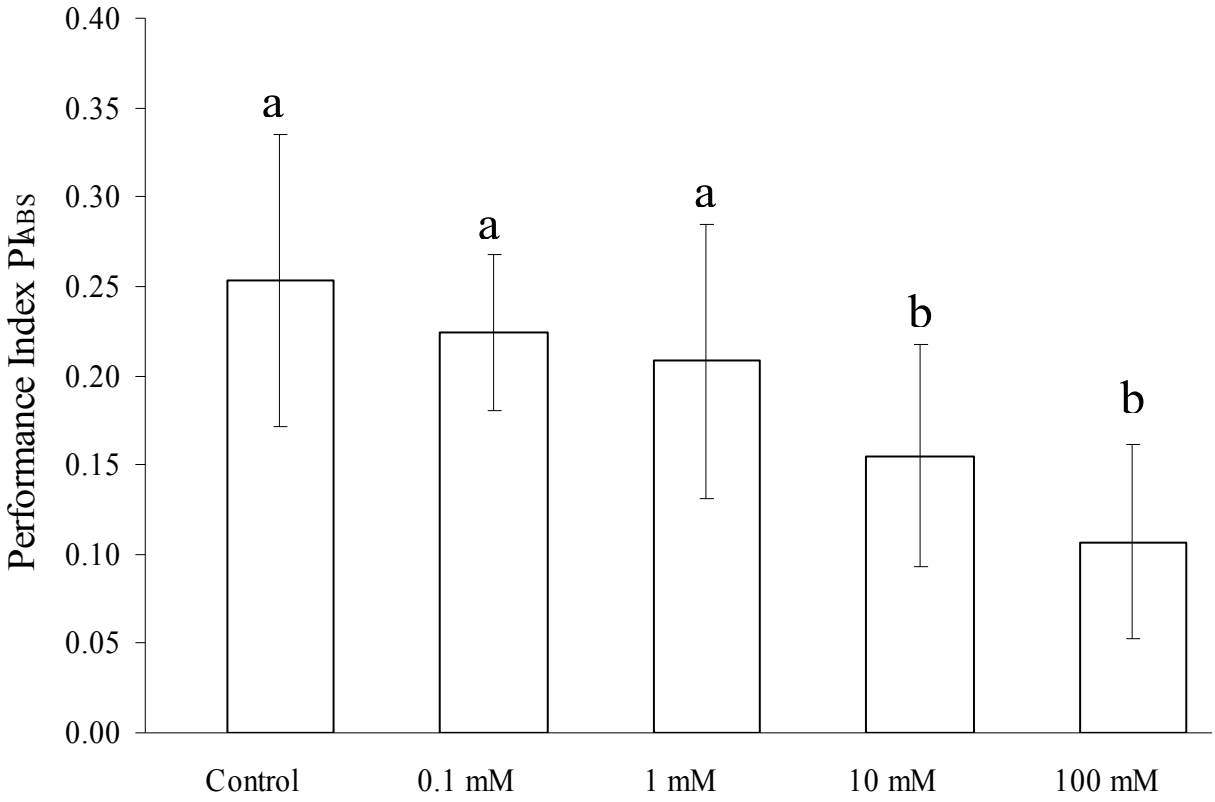

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615 **Figure 5.** Mean values (±SD, N=15) of the performance index (PI_{ABS}, a global indicator of the
616 photosynthetic performance) of lichen samples treated with different antimony concentrations.
617 Different letters indicate statistically significant differences ($P<0.05$).