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RESPONSE OF TOMATO PLANTS (*SOLANUM LYCOPERSICUM*) TO STRESS INDUCED BY SB(III)

Matúš Peško, Marianna Molnárová & Agáta Fargašová

Department of Environmental Ecology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 842 15 Bratislava, Slovak Republic

Corresponding author: Agáta Fargašová (e-mail: fargasova@fns.uniba.sk)

Abstract

Presented study evaluates effects of various Sb(III) concentrations on tomato plants (*Solanum lycopersicum*) cultivated hydroponically. Visual symptoms of antimony toxicity were observed only at two highest applied concentrations (50 and 100 mg/L). Dry weight of aboveground parts decreased significantly in variants treated with 25, 50 and 100 mg/L Sb(III), by ~12, 35 and 65 %, respectively, in comparison to the control. Statistically significant decrease of chlorophyll a and b was observed only after application of two highest studied concentrations 50 and 100 mg/L Sb(III). On the other hand concentration of total carotenoids in leaves rose with increasing external Sb(III) concentration. High concentrations (50 and 100 mg/L) of Sb(III) in nutrient solution caused that protein content in leaves dropped by ~20 and 39% relative to control. Accumulation of antimony in roots was about 5- (10 mg/L) to 27-times (25 mg/L) greater than that in shoots. The highest BAF factor value determined for shoots was ~55 at 10 mg/L Sb(III) and for roots it was ~821 at 50 mg/L Sb(III). Translocation factor values were in whole studied concentration range 5 – 100 mg/L Sb(III) < 1.The most effective translocation of antimony from roots to shoots was observes for variants treated with 10 mg/L of Sb(III).

Key words: accumulation, antimony, chlorophyll, proteins, *Solanum lycopersicum*, TBARS

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INTRODUCTION

Antimony, a metalloid, is widely spread in environment and considered to be highly toxic for various organisms including humans. Even low environmental concentrations can be threatening. The main sources of Sb are mining sites where the metalloid is released to the environment mainly via oxidative weathering of sulfide minerals (predominantly stibinite (Sb_2S_3)) included in waste products. In Slovakia, such sites are located in vicinity of villages Dúbrava, Medzibrod, Poproč and Čučma (district of Rožňava town). All mines had been closed in 90´s but the remained waste could be marked as potentially health risky (HILLER et al. 2012).

The mechanisms of uptake, assimilation, toxicity and detoxification of Sb in plants are not quite clear as those in animals and humans. The ability of plants to take up Sb depends on these following

factors: (1) phytoavailability of antimony in soils; (2) speciation of Sb; (3) differences in the concentration of other ions in soils, such as P and Ca. Uptake of Sb also varies with plant species (GEBEL 1997; SHTANGEEVA et al. 2011; WAN et al. 2013).

Large amounts of Sb accumulated in plants can be responsible for various negative effects such as excessive production of reactive oxygen species (ROS), micronutrient uptake retardation, decrease in photosynthesis and synthesis of soluble proteins (DING et al. 2015).

Presented study evaluates effects of Sb(III) on tomato plants (*Solanum lycopersicum*). Effect on growth (dry mass and length of plants organs) and biochemical (content of photosynthetic pigments, soluble proteins, thiobarbituric acid reactive substances in leaves) parameters of plants were studied. Concentration of Sb in individual plant parts and corresponding bioaccumulation and translocation factor values were estimated as well.

MATERIAL AND METHODS

Seeds of *Solanum lycopersicum* were germinated at 25 °C for three days in dark on filter paper in Petri dishes filled with 15 mL of demineralized water. After 72 h seedlings were transferred to 1 L beakers filled with Hoagland nutrient solution of pH = 5.5 and cultivated in growing chamber at constant conditions for 17 days. After that plants were transferred to nutrient solution with addition of antimony potassium tartrate ($K_2Sb_2(C_4H_2O_6)$; 5, 10, 25, 50, 100 mg/L) were they last for another 10 days. All beakers were well aerated by air pump. Conditions in growing chamber were as follows: relative humidity 80 ± 5%, mean temperature 25 ± 1 °C, photoperiod: 16h light / 8h dark, light energy 80 μmol.m^{-2}.s^{-1} PAR. After 10 days roots and shoots of control plants as well as Sb(III) treated variants were well dried at 50 °C in order to determine the dry weight and the content of antimony.

Concentrations of chlorophyll *a*, *b* and total carotenoids (chl *a*, chl *b*, carot) in plant leaves were determined spectrophotometrically (chl *a* at 663.2 nm, chl *b* at 646.8 nm, and carot at 470.0 nm) after extraction into 80% (v/v) acetone (Genesys 6, Thermo Scientific, U.S.A) according to LICHTENTHALER (1987). Concentration of thiobarbituric acid reactive substances (TBARS) in leaves of tomato plants was determined according to the method described in detail in PEŠKO et al. (2012). Briefly: 2 mL of supernatant was incubated at ~95 °C for 30 min with 1 mL of mixture containing 0.5% (w/v) thiobarbituric acid, 20% (v/v) trichloroacetic acid, and 100 μL of 4% butylated hydroxytoluene, followed by cooling in an ice bath for 10 min and centrifuged 2 min at 2900g. The absorbance of the solution was determined at λ = 532 nm spectro-photometrically (Genesis 6, Thermo Scientific) and concentration of TBARS was calculated using extinction coefficient ε = 155 L/mmol.cm.

Soluble protein concentration in leaves was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA) according to BRADFORD (1976) using Bradford reagent prepared by dissolving of 20 mg Coomassie Brilliant Blue G-250 in 10 mL of 95% ethanol, thereafter 20 mL H_3PO_4 and 70 mL deionized water was added, respectively. The reaction mixture (200 L of supernatant and 2 mL of Bradford reagent) was incubated at room temperature for 5 min. Absorbance of incubated mixture was measured at λ = 412 nm and protein concentration evaluated by using calibration curve. Serum bovine albumin was used as a standard.

Dried plant samples from control and Sb(III) treatments were digested in solution containing HNO_3 and H_2O_2 (4:1), than heated in the oven at 160 °C for 1 h and diluted with deionized water. Antimony concentration in shoots and roots was determined by means of galvanostatic dissolving chronopotentiometry on EcaFlow 150 GLP (Istran, Slovakia).

The results were evaluated by the multifactorial ANOVA algorithm ($p \leq 0.05$) after verification of normality and homogeneity of the variance.

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RESULTS AND DISCUSSION

Thirty days old tomato plants exposed to different Sb(III) concentrations for 10 days exhibited visual symptoms of antimony toxicity only at two highest studied concentrations (50 and 100 mg/L). Leaves of these plants were chlorotic, wilted and some of them even desiccated. Roots were slightly brownish. Growth of plants treated with 100 mg/L Sb(III) was stunt. Slight stimulation of biomass growth (Fig. 1) of plants was observed for variants were Sb(III) was applied in low concentrations (5 and 10 mg/L).

Application of concentrations 25, 50 and 100 mg/L (Sb(III)) caused that the dry weight of aboveground parts (leaves + stems) decreased by ~12, 35 and 65%, respectively, in comparison to the control. On the other hand, adverse effect of Sb(III) on dry weight of roots was observed only in variants treated with 100 mg/L (Fig. 1). Decrease in root weight of these plants was about ~30%, comparing to the untreated variants. Inhibition of plant growth as a result of application of Sb(III) was observed also in experiments with other species, e.g. paddy rice (DING et al. 2015), fern plants (FENG et al. 2009), maize (PAN et al. 2011), and lichen (PAOLI et al. 2013).

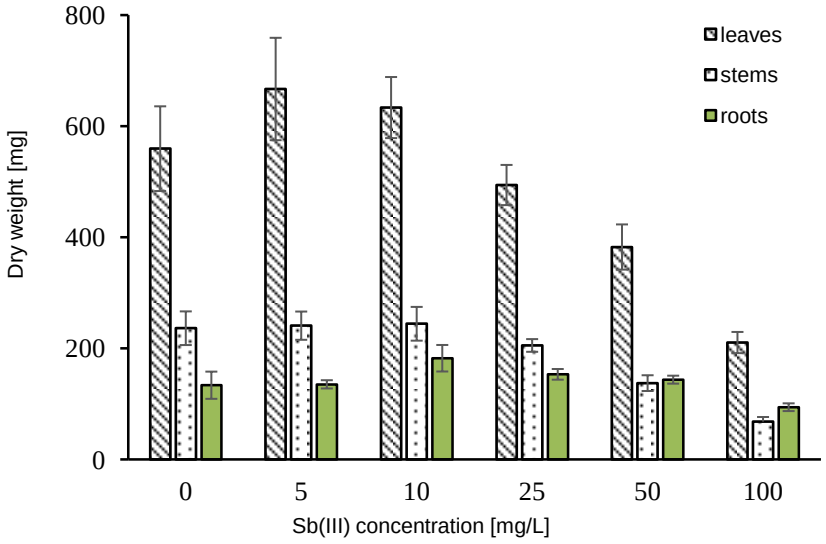

Fig. 1: Dependence of dry weight of leaves, stems and roots of tomato plants on different Sb(III) concentrations. Mean ± S.E.; n = 5; S.E. – standard error

Relationship between external concentration of antimony potassium tartrate and concentration of photosynthetic pigments in leaves of tomato plants is shown in Fig. 2. Statistically significant decrease of chlorophyll *a* and *b* was observed only after application of two highest studied concentrations 50 and 100 mg/L Sb(III). Comparing to the control plants, chlorophyll *a* dropped by ~31 and 55 %, and chlorophyll *b* by ~39 and ~59%, respectively. Similar results were observed also by PAN et al. (2011) in their experiments with *Zea mays* plants. This suggest that Sb is interfering with biomolecules involved in synthesis of chlorophylls. Antimony could also interfere with or damage important molecules and enzymes within chloroplasts, which is supported by results from experiments of DING et al. (2015). They found that more than 10% of Sb accumulated in shoots of paddy rice plants was located in chloroplasts. Concentration of total carotenoids in leaves rose with increasing concentration of Sb(III) in nutrient solution. Application of 100 mg/L Sb(III) caused that concentration of total carotenoids was almost twice the control. Excessive production of carotenoids is probably a part of protection mechanism against oxidative stress.

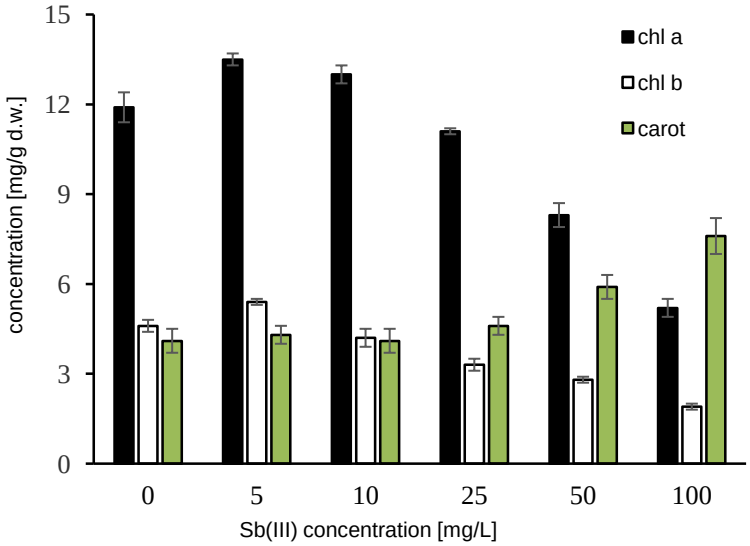

Fig. 2: Dependence of chlorophyll *a* (chl a) and *b* (chl b) as well as carotenoids (carot) concentration in leaves of tomato plants on different Sb(III) concentrations. Mean ± S.E.; n = 5; S.E. – standard error; d.w. – dry weight

Concentration of TBARS and soluble proteins in leaves of plants treated with different concentrations of Sb(III) are summarized in Tab. 1. In our study concentration of these products (Tab. 1) in leaves of tomato plants rose as the external concentration of Sb(III) increased. Massive accumulation of TBARS in leaves was observed for variants treated with two highest studied concentrations (50 and 100 mg/L Sb(III)). Accumulation of these products reached ~129 and 240% of control. Thiobarbituric acid reactive substances (TBARS), products of peroxidation of membrane lipids, are very good marker of oxidative stress caused by the presence of metals. Our results are in good conformity with experiments of many other authors. For example accumulation of TBARS in shoots of *Hedysarum pallidum* plants grown on highly contaminated Sb-mining sites was 5-times greater than in those grown on uncontaminated control sites (BENHAMDI et al. 2014).

High concentrations (50 and 100 mg/L) of Sb(III) in nutrient solution caused that protein concentration in leaves dropped by ~20 and 39% relative to the control. Reactive oxygen species (ROS) that are produced as a result of oxidative stress, are responsible not only for membrane lipid peroxidation but cause protein inactivation and degradation as well. The bulk of the oxidized proteins is then degraded by proteolysis (SHRINGARPURE et al. 2003).

Tab. 1: Concentration of TBARS and soluble proteins in leaves of tomato plants treated with different concentrations of Sb(III)

c Sb(III) [mg/L]	TBARS [µmol/g d.w.]	Soluble proteins [mg/g d.w.]
0	1.7 ± 0.10	41.4 ± 2.1
5	2.3 ± 0.10	43.3 ± 2.0
10	2.2 ± 0.05	42.2 ± 1.6
25	2.6 ± 0.11	40.4 ± 3.1
50	3.9 ± 0.12	33.3 ± 2.9
100	5.8 ± 0.15	26.1 ± 2.3

Concentration of Sb in roots and shoots increased with increasing external Sb(III) concentration. Accumulation of antimony in roots was about 5- (10 mg/L) to 27-times (25 mg/L) greater than that in shoots. The fact that tomato plants were able to accumulate in their shoots more than 10 μg Sb/g d.w. already at the lowest applied external concentration, 5 mg/L Sb(III), suggests that *Solanum lycopersicum* is Sb-tolerant. But it can't be marked as an accumulator of this metalloid because most of Sb remained accumulated in roots. Higher amount of Sb accumulated in shoots was observed by HAMMEL et al. (2000). Spinach plants grown on contaminated soil (100 μg Sb(III)/g) were able to accumulate 399 μg Sb /g d.w. in shoots.

Tab. 2: Concentration of antimony in shoots and roots as well as corresponding bioaccumulation factor (BAF) and translocation factor (TF) values, and percentage share of Sb accumulated in shoots of tomato plants treated with different Sb(III) concentrations

c Sb(III) mg/L	c Sb [μg/g d.w.]		BAF		TF	% of Sb in shoots
	Shoots	Roots	Shoots	Roots		
5	43.9	688.6	46.2	577.7	0.064	19.0
10	105.1	548.8	55.3	362.4	0.192	26.8
25	132.3	3 612.5	27.8	760.5	0.037	6.8
50	308.1	7 798.6	32.4	820.9	0.040	7.4
100	998.5	13 162.9	54.8	692.8	0.090	9.2

The highest BAF factor value determined for shoots was ~55 at 10 mg/L Sb(III) and for roots it was ~821 at 50 mg/L Sb(III). This suggests that bioaccumulation of antimony within aboveground parts is most effective at lower external concentrations.

Translocation factor values were in whole studied concentration range 5 – 100 mg/L Sb(III) < 1, suggesting restricted translocation of Sb into the aboveground parts. The most effective translocation of antimony from roots to shoots was observed for variants treated with 10 mg/L of Sb(III). TF value was in this case 0.192.

The amount of antimony accumulated in shoots depends not only on applied external concentration but also on dry weight of plant organs. The percentage share of Sb accumulated in shoots from total amount accumulated by tomato plants was for low external concentrations relatively high, 19% (5 mg/L) and 26.8% (10 mg/L). In case of variants treated with higher concentrations, the percentage share was below 10%.

CONCLUSION

According to the presented results it can be said that toxic effects of antimony on tomato plants (*Solanum lycopersicum*) were exhibited only in variants treated with higher concentrations (50 and 100 mg/L) of studied metalloid. These variants exhibited moderate to severe signs of Sb toxicity, including chlorosis, stunt plant growth, decreased concentration of chlorophylls, soluble proteins, and increased accumulation of products of lipid peroxidation in leaves, nevertheless *Solanum lycopersicum* could be marked as Sb-tolerant because in presence of lower Sb(III) concentrations (5, 10 and 25 mg/L) was the accumulation of antimony in aboveground parts relatively high, which in turn could be utilized in phytoremediation of lightly to moderate polluted sites, but further field experiments should be done to support our results.

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REFERENCES

BENHAMDI A., BENTELLIS A., RACHED O., DU LAING G. & MECHAKRA A. 2014. Effects of antimony and arsenic on antioxidant enzyme activities of two steppic plant species in an old antimony mining area. Biol. Trace. Elem. Res. 158: 96-104.

BRADFORD M.M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

DING Y., WANG R., GUO J., WU F., XU Y. & FENG R. 2015. The effect of selenium on the subcellular distribution of antimony to regulate the toxicity of antimony in paddy rice. Environ. Sci. Pollut. Res. Int. 22(7): 5111-5123.

FENG R. W., WEI C. Y., TU S. X., WU F. C. & YANG L. S. 2009. Antimony accumulation and antioxidative responses in four fern plants. Plant Soil 317: 93-101.

GEBEL T. 1997. Arsenic and antimony: comparative approach on mechanistic toxi-cology. Chem. Biol. Interact. 107: 131-144.

HAMMEL W., DEBUS R. & STEUBING L. 2000. Mobility of antimony in soil and its avail-ability to plants. Chemosphere 41: 1791-1798.

HILLER E., LALINSKÁ B., CHOVAN M., JURKOVIČ L., KLIMKO T., JANKULAR M., HOVORIČ R., ŠOTTNIK P., FĽAKOVÁ R., ŽENIŠOVÁ Z. & ONDREJKOVÁ I. 2012. Arsenic and antimony contamination of waters, stream sediments and soils in the vicinity of abandoned antimony mines in the Western Carpathians, Slovakia. Appl. Geochem. 27: 598-614.

LICHTENTHALER H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Method. Enzymol. 148: 350-382.

PAN X., ZHANG D., CHEN X., BAO A. & LI L. 2011. Antimony accumulation, growth performance, antioxidant defense system and photosynthesis of *Zea mays* in response to antimony pollution in soil. Water Air Soil Pollut. 215: 517-523.

PAOLI L., FIORINI E., MUNZI S., SORBO S., BASILE A. & LOPPI S. 2013. Antimony toxicity in the lichen *Xanthoria parietina* (L.) Th. Fr. Chemosphere 93: 2269-2275.

PEŠKO M., KRÁĽOVÁ K. & BLAŠKO J. 2012. Phytotoxic effects of trivalent chromium on rapeseed plants. Fresen. Environ. Bull. 21(3A): 761-768.

SHRINGARPURE R., GRUNE T., MEHLHASE J. & DAVIES K. J. A. 2003. Ubiquitin conjugation is not required for the degradation of oxidized proteins by proteasome. J. Biol. Chem. 278: 311-318.

SHTANGEEVA I., BALI R. & HARRIS A., 2011. Bioavailability and toxicity of antimony. J. Geochem. Explor. 110: 40-45.

WAN X. M., TANDY S., HOCKMANN K. & SCHULIN R. 2013. Changes in Sb speciation with waterlogging of shooting range soils and impacts on plant uptake. Environ. Pollut. 172: 53-60.