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Antimony uptake by different plant species from nutrient solution, agar and soil

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Environmental context. Because of its many industrial and other uses, antimony (Sb) is increasingly emitted into the environment through human activities. We studied the uptake of Sb by crop plants from three different substrates: hydroponic nutrient solutions, agar medium, and potting soil. The uptake of Sb increased linearly with Sb in solution or soluble Sb in soil over a wide range of concentrations until it was limited by toxicity. Antimony was much less toxic than its sister element arsenic compared on a molar basis. The results suggest that Sb may be accumulated by some crop plants on heavily contaminated soils at concentrations that may pose a health risk to humans and animals.

Abstract. We investigated the uptake of antimonate from nutrient solutions, agar and soil by various cultivated plants, including Indian mustard (*Brassica juncea* (L.) Czern), sunflower (*Helianthus annuus* L.), perennial ryegrass (*Lolium perenne* L.), clover (*Trifolium pratense* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). Antimony uptake did not differ between the three growth media. In all tested plants, the shoot Sb concentration was proportional to Sb in solution or soluble Sb in soil, until toxicity eventually limited growth. At a given Sb concentration in the growth medium, Sb accumulation differed between plant species by up to an order of magnitude. Clover grown in agar containing 160 mg L^{-1} Sb in solution accumulated 2151 mg kg^{-1} Sb (dry weight) in the shoots. Maize had the lowest accumulation. In maize and sunflower, most Sb accumulated in the leaves. The results indicate that antimony may accumulate in the edible parts of crop plants grown on heavily contaminated soils at concentrations that may pose a health risk to humans and animals.

Additional keywords: allocation, arsenic, hydroponics, plant uptake, toxicity.

Introduction

With an average concentration between 0.2 and 0.3 mg kg^{-1}, antimony (Sb) is a rare element in the earth's crust.[1] Natural Sb background concentrations in soil were found to vary between 0.3 and 8.6 mg kg^{-1}.[2,3] However, as a component of many industrial products, e.g. in fire retardants, brakes, semiconductors, and metal alloys,[4,5] it has become increasingly emitted through human activities into the environment. One major pathway of Sb entry into soils by human activities is shooting; bullets and pellets contain between 1 and 7% Sb.[6] Switzerland, for example, has more than 2000 shooting ranges. Depending on the duration and intensity of shooting activities, not only soil lead (Pb) but also soil Sb concentrations are generally highly elevated on these sites.[7] As they are often used for grazing sheep and cattle when they are not used for shooting, there is a potential risk that Sb enter the food chain through uptake into plants growing on such Sb-contaminated sites.

Previous studies have shown that plants can accumulate Sb at high concentrations on Sb-contaminated soil. Foliar Sb concentrations of up to 1100 mg kg^{-1} were measured in vegetation growing in a soil polluted with up to 400 mg Sb kg^{-1} dry weight (DW) in the vicinity of an Sb smelter in northeast England.[8] Another study reported foliar Sb concentrations greater than 100 mg kg^{-1} in plants growing on a mine tailing soil with 9000 mg Sb kg^{-1} DW. In the basal leaves of *Achillea ageratum*, more than 1000 mg Sb kg^{-1} DW was found on that site.[9] There are also studies that reported only small Sb concentrations in plants grown on heavily Sb-contaminated soils. Pratas et al. reported maximum stem concentrations of less than 5 mg Sb kg^{-1} DW in various tree and herb species growing on a Portuguese mine spoil with an average total Sb concentration of 663 mg kg^{-1}.[10]

Deposition of Sb-containing dust particles on leaf surfaces may be one reason for the high plant Sb concentrations on contaminated field sites,[8] and thus to some extent may explain the diversity of results reported in the literature, given that most studies did not discriminate between Sb coming from dust deposition or through root uptake. However, very different accumulation rates have also been reported from two pot experiments performed under greenhouse conditions. In one of these studies, barley (*Hordeum vulgare* L.) grown in sand contaminated with 100 mg kg^{-1} soluble Sb was not found to take up more than 2 mg Sb kg^{-1} DW, which was the detection limit in that study, although yields were reduced.[11] In the other study, 19 species of garden and crop plants were grown on potted soil that had been spiked with Sb to give a dissolved Sb concentration of

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45 mg L^{-1}. The plants accumulated up to 399 mg Sb kg^{-1} DW in the shoots without showing toxicity symptoms.[12]

Given the large variation observed in plant Sb concentrations and the uncertain role of roots in Sb uptake by plants, the goal of the present study was to study plant Sb uptake through roots under controlled conditions and to relate the uptake to the Sb concentration in the soil or solution to which the roots were exposed. For this purpose, we performed experiments with six plant species that are cultivated in practice for various purposes, using three systems: nutrient solution (hydroponics), agar medium and potted soil. We also compared the toxicity of As^V and Sb^V in hydroponic solutions and agar cultures.

Each of the three different substrates we used has distinct advantages and disadvantages. The agar system was used in addition to the hydroponics system to control for root damage that may arise from seedling manipulation in hydroponics experiments; as seeds cannot be germinated in solution, the hydroponics system requires that seeds are germinated first and then the seedlings are transferred to the nutrient solution. This may cause damage to the roots by which pathways are created bypassing the endodermis barrier and thus allowing excessive uptake of solutes that would otherwise be prevented by this barrier. Although no transplantation is necessary in agar and soil systems, as seeds can be directly germinated in these media, hydroponics have the important advantage that it is much easier to control – and also change if desired – the composition of the solution to which the roots are exposed. Heterogeneity of the rhizosphere is on one hand a problem in experiments with soil; however, this reflects natural conditions, and providing a more natural environment for plant growth than hydroponics means that such experiments are more representative of growing conditions in field situations. To some extent, the agar system provides conditions in between the hydroponics and the soil system. It provides much less natural growth conditions than soil, but allows better control of rhizosphere conditions. Nodari et al. found that Sb is 98% available in agar.[13] Care has to be taken that the slow diffusion of gases does not lead to stresses such as shortage in oxygen and accumulation of carbonic acid, as encountered by roots growing in waterlogged soils.[14,15]

In the current study, we focussed on the uptake of Sb^V provided in the form of antimonate, as this is the dominant Sb species in the solution of aerated soils. Moreover, as antimonate is very soluble, it was also possible to apply very high concentrations and, thus, to test for toxicity limits of growth.

Material and methods

Plant cultivation and application of Sb treatments

The plants used in the present study were Indian mustard (*Brassica juncea* (L.) Czern, obtained from TRS, Middlesex, UK), sunflower (*Helianthus annuus* L. cv. Iregi), ryegrass (*Lolium perenne* L. cv. Arvicola), clover (*Trifolium pratense* L. cv. Milvus), wheat (*Triticum aestivum* L. cv. Galaxie) and maize (*Zea mays* L. cv. Magister). All experiments were performed in a climate chamber with a daily photoperiod of 16 h at a light intensity of 11 000 lx, and a day–night temperature rhythm of 22–14°C.

For the hydroponic experiments, plants were germinated in quartz sand. After 2 weeks, the seedlings were transferred to 30-L plastic boxes containing a modified Hoagland nutrient solution.[16] The nutrient solution consisted of 0.4 mmol L^{-1} $Ca(NO_3)_2$, 0.2 mmol L^{-1} $MgSO_4$, 0.1 mmol L^{-1} KH_2PO_4, 0.5 mmol L^{-1} KNO_3, 0.01 mmol L^{-1} $NaFe^{III}EDTA$, 0.01 mmol L^{-1} H_3BO_3, 2 μmol L^{-1} $MnSO_4$, 0.2 μmol L^{-1} $ZnSO_4$, 0.2 μmol L^{-1} $CuSO_4$, 0.1 μmol L^{-1} Na_2MoO_4, 0.02 mmol L^{-1} NaCl and 2 mmol L^{-1} MES (2-(*N*-morpholino)ethanesulfonic acid as a buffer).[17] For the treatments, the seedlings were transferred to 1-L bottles containing the same nutrient solution to which Sb or As were added according to the desired treatment level. The treatment solutions were adjusted to pH 6 by addition of NaOH, continually aerated, and replaced weekly. Antimony was added as $KSb(OH)_6$ and arsenic as KH_2AsO_4.

For the agar cultures, we used either Petri dishes or 300-mL polyethylene boxes filled with 80 mL of a 10% w/v agar medium. The same modified Hoagland solution was used to prepare the agar medium as in the hydroponics system, except that no MES buffer was added. Antimony and arsenic were added as $KSb(OH)_6$ and Na_2HAsO_4, respectively, according to the desired treatment level to the agar before it solidified. After autoclaving the agar for 21 min at 121°C and 961 hPa, seedlings were grown singly per Petri dish or box in three replicates per treatment.

For the pot experiments, we used a standard potting mix consisting of a garden soil enriched with compost. The organic carbon content was 22.9 ± 2.7% and the pH (in 0.1 mmol L^{-1} $CaCl_2$) 7.0 ± 0.1. Antimony was added according to the desired treatment by mixing granular $KSb(OH)_6$ with the dry potting mix. The mixtures were left to equilibrate for 2 weeks, with regular watering, before they were used for planting.

Experiments

The following experiments were performed using the three experimental systems:

(i) In a first experiment, we compared the uptake of Sb and As from nutrient solution and their phytotoxicity to maize, sunflower, ryegrass and wheat. After 4 weeks of growth in uncontaminated nutrient solution, treated plants were exposed for 1 week to either 25 μmol L^{-1} Sb or 25 μmol L^{-1} As added to the nutrient solutions, while neither of the two elements was added to the controls. Four replicates were set up for each treatment.

(ii) Second, we performed a set of experiments in which we compared the dependence of Sb uptake and aboveground growth of Indian mustard, sunflower, clover, wheat and maize grown in hydroponics, agar and soil on the Sb concentration in solution of the hydroponics or agar system and on the KNO_3-extractable Sb concentration in the potted soil system, respectively. Treatment concentrations were 0, 3.0, 6.1, 12.2, 18.3, 24.4 mg L^{-1} Sb in the hydroponics and 0, 10, 20, 40, 80 and 160 mg L^{-1} Sb in the agar system, whereas 0, 12.9, 61.3, 294, 563 and 1240 mg Sb kg^{-1} DW were applied in the experiment with potted soil. The ratio between KNO_3-extractable Sb per litre of soil solution and total Sb per kg of dry soil was 0.031 ± 0.001 kg L^{-1} for all treatments. In the hydroponics system, 4-week old plants were exposed for 1 week to the treatment solutions as in the previous experiment. In the agar experiments, plants were grown for 4 weeks in 300-mL boxes, prepared as described above. In the potted soil system, plants were grown for 5 weeks in 250-mL pots.

(iii) In a third experiment, we investigated the toxicity effect of Sb on root growth of the five plant species using agar cultures in Petri dishes. The treatment concentrations were 0, 10, 30, 100 and 300 mg L^{-1} Sb as in the previous agar

experiment. Root length was measured after 14 days of growth.

(iv) Finally, we also studied the allocation of Sb in maize and sunflower grown in Sb-spiked potted soil. For this experiment, we used 5-L pots and applied a KNO_3-extractable Sb concentration of 1.03 mg L^{-1} (mass per volume unit of soil solution). Plants were harvested after 4 months of growth by cutting off the shoots ~1 cm above the soil surface, and separated into stems, leaves and seeds. Leaves were further grouped by position along the stem into three age categories: 'old', 'medium' and 'young'. These groups were analysed separately, as were the stem and seeds.

Sample analysis

Soil samples were taken from each treatment batch after preparation and before the soil was put into the pots. The soil samples were oven-dried at 65°C for 1 week, weighed and stored at 4°C until they were analysed. Soluble Sb in the soil samples was extracted using potassium nitrate as described by Massard et al.[18] For this purpose, 5-g soil samples were mixed with 12.5-mL aliquots of a 0.1 mol L^{-1} potassium nitrate solution in polypropylene bottles. The bottles were tightly closed and longitudinally shaken for 2 h at a frequency of 120 min^{-1} and with an amplitude of 55 mm. The resulting slurries were left for 10 min to settle; then the supernatants were collected using 60-mL single-use syringes and filtered through 45-μm membrane filters. The filtrates were collected in 20-mL volumetric flasks, which contained 0.8 mL of 65% nitric acid.

The plant samples were oven-dried at 65°C for 48 h and weighed, the dried samples were digested for chemical analysis using aqua regia in closed Teflon vessels, at first for 2 h at room temperature and then for 30 min in a microwave oven (MLS) at 100°C.

Soil solution samples and plant extracts from the agar experiments were analysed by inductively coupled plasma mass spectrometry (ICP-MS) (Varian). The extracts and solution samples from the other experiments were analysed for Sb by means of hydride generation atomic fluorescence spectroscopy (HG-AFS, PSAnalytical) and for As by means of inductively coupled plasma optical emission spectrometry (ICP-OES, Varian). For quality assurance, we used Virginia Tobacco leaves (CTA-VTL-2) as reference material obtained from LGC Standards for all plant sample analyses using AFS. The mean ± standard error of our measurements was 0.306 ± 0.023 mg kg^{-1}, which agreed well with the certified values (0.312 ± 0.025 mg kg^{-1}) of these standards. For the ICP-MS measurements, we employed standard addition, using a series of CTA-VTL-2 standards; the measured values exceeded the expected values on average by 14.4%. All sample measurements were corrected for this deviation.

Statistical analysis

Statistical analyses (ANOVA and regression) were performed using *SPSS 13.0* (SPSS Inc., Chicago, MA). Differences between estimated parameters were tested using the *t*-test proposed by Sachs.[19]

Results

Plant uptake and phytotoxicity of Sb in comparison with As

Exposed to the same molar concentration of 25 μmol L^{-1} antimonate or arsenate in hydroponic solution, sunflower, wheat and ryegrass seedlings accumulated between ~1.5 (sunflower) and 3 (ryegrass) times higher concentrations (by mass) of As than Sb in their shoots (Fig. 1). Plant Sb concentrations are given on the basis of mass in Fig. 1 for reasons of easier comparability with literature data. Expressed on a molar basis, the differences would increase by a factor of 1.63 owing to the correspondingly higher atomic weight of Sb. Maize accumulated an order of magnitude less of both elements than the other three plant species. On the basis of mass, there was no difference, but on the basis of molar concentrations, more As than Sb was also taken up by this crop.

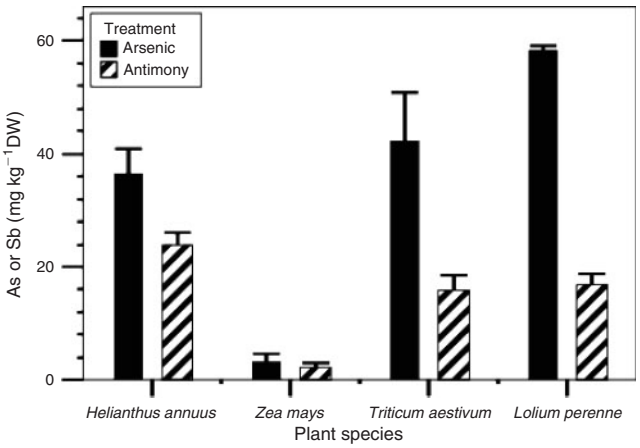

Fig. 1. Concentrations of As and Sb (mg kg^{-1} DW, dry weight) in the shoots of plant seedlings exposed for 1 week to either 25 μmol L^{-1} As or 25 μmol L^{-1} Sb, respectively, in nutrient solution after 4 weeks' growth in uncontaminated solution. Error bars represent standard errors.

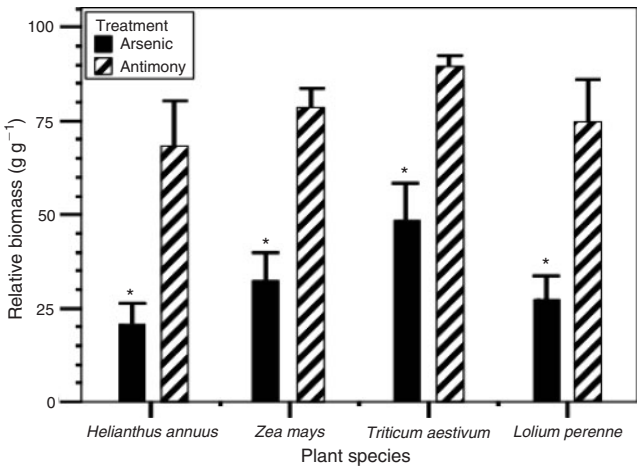

Fig. 2. Relative shoot biomass (control = 100%) of plant seedlings exposed for 1 week to either 25 μmol L^{-1} As or 25 μmol L^{-1} Sb in nutrient solution after 4 weeks' growth in uncontaminated solution. Error bars represent standard errors. An asterisk denotes that the decrease was significant in comparison with the control treatment. Error bars represent standard errors.

Fig. 2 shows that at the applied concentration of 25 μmol L^{-1}, Sb was also less toxic than As to the shoot growth of all four tested plant species. In the As treatment, the plants produced on average only between 20% (sunflower) and 50% (wheat) of the biomass in the control treatment. Growth was also slightly reduced in the Sb treatment, but this effect was not significant at the $P < 0.05$ level. In the As treatments, we observed chlorosis and wilting

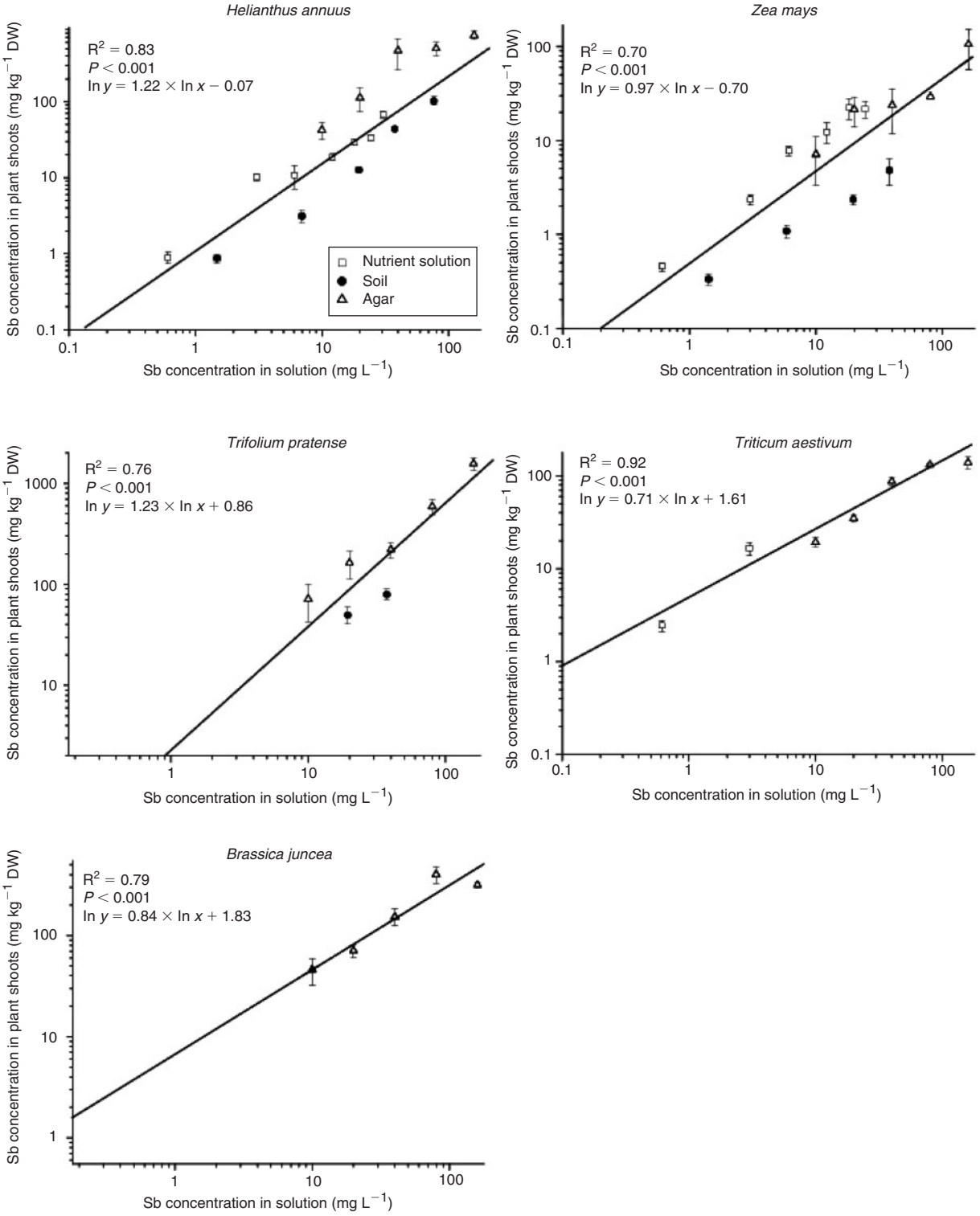

Fig. 3. Dependence of Sb accumulation in the shoots of sunflower, maize, wheat, Indian mustard and clover seedlings on the available Sb concentration (mg kg^{-1} DW, dry weight) in the growth medium: hydroponic solution (squares), agar (triangles), and potted soil (filled circles). In the case of the soil-grown plants, the Sb concentration given refers to the KNO_3-extractable (= available) Sb concentration in the soil. The linear equations give the regression of the logarithms of the Sb concentrations in the shoots (y) on the available (i.e. dissolved or soluble) Sb concentration (x) in the respective growth medium, taking the measurements from all three systems into account.

of leaves, whereas in the Sb treatments, no such symptoms were observed. The results suggest that in all four plants, the toxicity threshold was below 25 μmol L^{-1} for As and approximately that level for Sb. Comparison of Fig. 2 with Fig. 1 suggests that the lower toxicity of Sb in this experiment may be closely related to the lower accumulation of this element, whereas the toxicity of the two elements may actually not be so different if their concentrations in the tissues are considered.

Table 1. Variation of the parameters B (bioconcentration coefficient; the logarithms of the numbers in parentheses give the standard error of log B) and A (slope of the regression line; standard error in parentheses) among plant species and experimental systems

The values were obtained by linear regression on log-transformed concentrations (c) using the model $\ln(c_{plant}) = A\ln(c_{sol}) + \ln(B)$

Substrate	*Brassica juncea*	*Helianthus annuus*	*Trifolium pratense*	*Triticum aestivum*	*Zea mays*
B values					
Solution	6.24 (1.58)	1.29 (1.15)	3.79 (1.73)	3.82 (1.36)	0.71 (1.22)
Agar		3.93 (1.89)			0.76 (2.32)
Soil		0.53 (1.19)			0.26 (1.17)
A values					
Solution	0.84 (0.12)	1.06 (0.07)	1.15 (0.14)	0.76 (0.08)	1.10 (0.10)
Agar		1.08 (0.17)			0.88 (0.22)
Soil		1.13 (0.06)			0.75 (0.35)

Dependence of Sb accumulation in plants on Sb in the growth medium

As Fig. 3 shows, there was an approximately linear relationship in all plant species tested between the concentrations of Sb in the shoot biomass and the available Sb concentration in the growth medium (i.e. dissolved Sb concentration in the case of the hydroponics and agar system and the KNO_3-soluble Sb concentration in the potted soil system) if both were plotted on a log scale. Linear regression on the logarithms of the concentrations did not only show that these relationships were highly significant, with R^2 values between 0.70 and 0.92, but also revealed that the slope of the regression lines, denoted as A, varied closely around 1. (Note that for *Brassica juncea*, *Trifolium pratense* and *Triticum aestivum*, this statement is subject to a rather large uncertainty though, owing to the relatively small number of data points.) This means that in all plants, the accumulation of Sb was approximately proportional to the available Sb in the growth medium. However, the ratio B between Sb concentration in plant and growth medium, which was determined from the offset log(B) of the log-log regression lines, varied by an order of magnitude among the different plant species (Table 1). This ratio, which represents an averaged bioconcentration factor over the investigated range of Sb concentrations, was highest in Indian mustard, followed by clover, sunflower and wheat, and lowest in maize. The bioconcentration factor was approximately three times higher in the hydroponics system than in the soil system in those cases where sufficient data were available for both media, i.e. for sunflower and maize. This reflects a lower mobility of Sb in the soil solution compared with hydroponics. In the case of maize, the same bioconcentration was found in the agar as in the hydroponics system, whereas Sb accumulation by sunflower was three times higher in agar than in hydroponics. The latter difference can be attributed to the fact that seedlings were exposed to the Sb treatments over the entire period of their growth in the agar system, whereas exposure lasted only 1 week in the hydroponics system after 4 weeks of unexposed growth. In maize, where accumulation was very low over the entire period of seedling growth, uptake probably was negligible at the early stages of growth, so that the difference in exposure during this period did not matter. The fact that Sb accumulation by the plants was similar or even higher in agar than in nutrient solution also demonstrates that root damage due to seedling transfer at the beginning of the experiment did not play a role in the hydroponics system.

Fig. 4 shows the dependence of shoot biomass on the available Sb concentration in the growth medium for the different experiment systems. No shoot growth reduction was observed in wheat and Indian mustard even at the highest Sb concentrations applied. No significant decrease in growth was also found in sunflowers grown in agar and nutrient solution. However, sunflower showed reduced shoot growth at soluble Sb concentrations above $6\,mg\,L^{-1}$ in the potted soil system. A similar difference in toxicity between Sb treatments in agar and soil was also found in maize and clover. These two species showed increasing reduction of shoot growth only at the two highest levels applied in the agar system, i.e. at 80 and $160\,mg\,L^{-1}$ Sb, but no sign of toxicity up to the level of $40\,mg\,L^{-1}$ Sb. In contrast, shoot growth of maize and clover was reduced to a similar degree as that of sunflower already at soluble Sb concentrations above $6\,mg\,L^{-1}$ in the potted soil system. Also, many leaves were necrotic and chlorotic in the latter treatments. We have no explanation for this difference, but it appears as if there was an additional stress factor in the soil system.

Toxicity of Sb to root growth

Also, the 2-week root growth tests performed with agar as growth medium revealed a high Sb tolerance of Indian mustard (Fig. 5). Even an Sb concentration of $300\,mg\,L^{-1}$ was tolerated without reduction in root length. Sunflower was second in this test, showing little effect up to $100\,mg\,L^{-1}$ ($= 821\,\mu mol\,L^{-1}$), but a clear toxicity effect at $300\,mg\,L^{-1}$ ($= 2464\,\mu mol\,L^{-1}$) Sb. Clover was similar, with a clearer reduction in root growth already at $100\,mg\,L^{-1}$ Sb, whereas the root growth of the two monocotyledons wheat and maize was inhibited already at Sb concentrations of $30\,mg\,L^{-1}$ ($= 246\,\mu mol\,L^{-1}$) or less.

In all five species investigated here, As was much more toxic to root growth than Sb (Fig. 5). The large difference in rhizotoxicity of the two elements visible in Fig. 5 is partially due the fact that concentrations are given on a mass basis, for reasons of easier comparability with the literature. However, if the effects of the two elements on root growth are also compared on the basis of molar concentrations, there is still a clear difference. Already at As concentrations of $10\,mg\,L^{-1}$ ($= 133\,\mu mol\,L^{-1}$), root growth was clearly reduced, least in Indian mustard and most strongly in clover; at an As concentration of $30\,mg\,L^{-1}$ ($= 400\,\mu mol\,L^{-1}$), it was reduced to less than 15% of the controls in all plants and close to zero in some like clover, maize and wheat; and at $100\,mg\,L^{-1}$ ($= 1335\,\mu mol\,L^{-1}$), the next highest treatment level, virtually none of the seedlings showed significant root growth any more. In contrast, even at the highest Sb treatment level ($= 2464\,\mu mol\,L^{-1}$), there was still more than 21% root growth in comparison with the controls, even in wheat and maize.

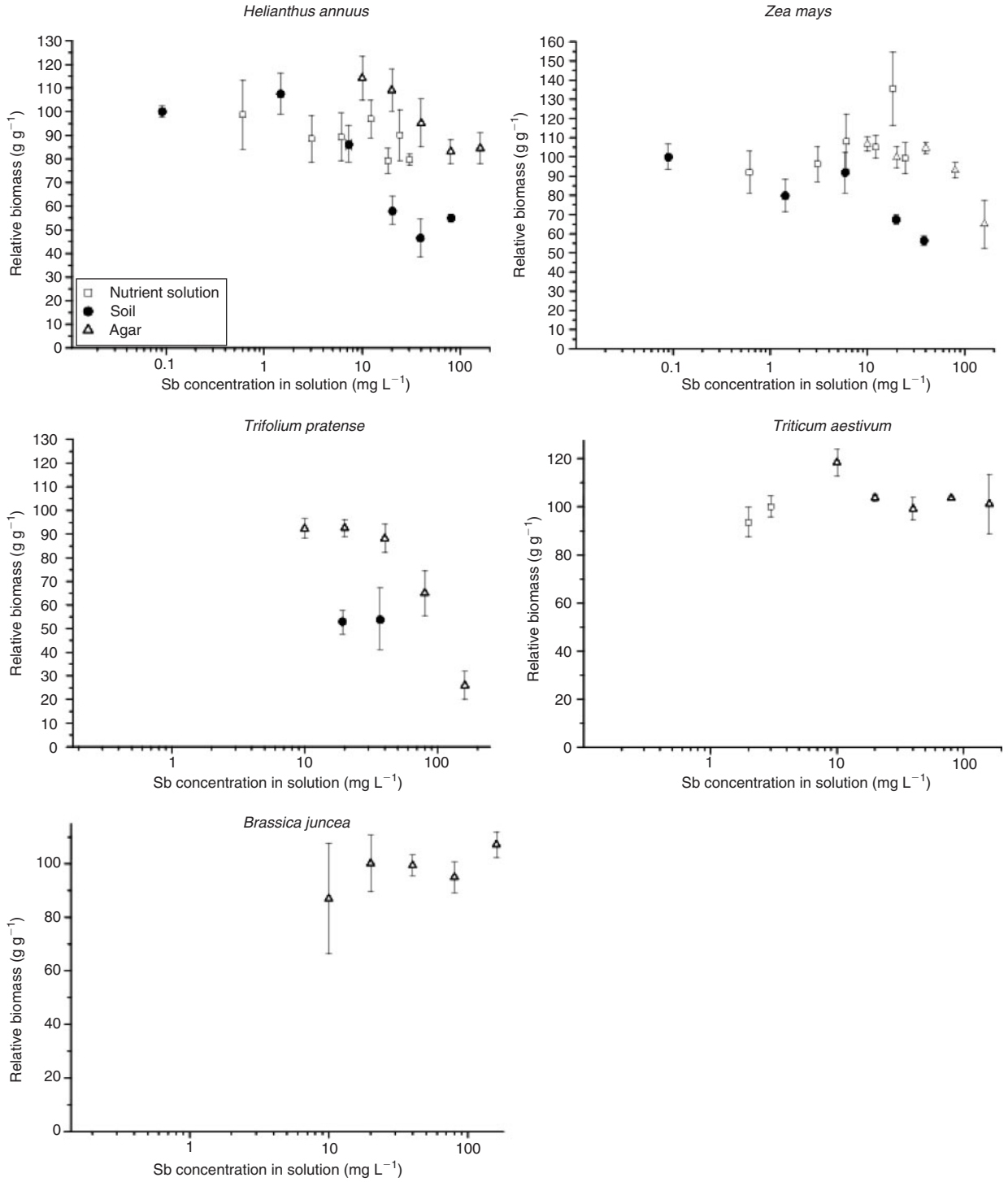

Fig. 4. Dependence of the relative biomass (control = 100%) of sunflower, maize, wheat, Indian mustard and clover seedlings on the available Sb concentration in the growth medium: hydroponic solution (squares), agar (triangles), and potted soil (filled circles). In the case of the soil-grown plants, the Sb concentration given refers to the KNO_3-extractable (= available) Sb concentration in the soil.

Allocation of accumulated Sb in sunflower and maize

Both plant species tested, i.e. maize and sunflower, showed similar patterns of Sb allocation in the aboveground parts at harvest after 4 months of growth. Antimony concentrations were highest in the oldest (i.e. bottom) leaves, decreased with the age of the leaves (i.e. towards the top), and were lowest in the seeds and stems (Fig. 6). Compared with average leaf Sb concentrations, the concentration ratio between Sb in stems and Sb in leaves was 0.34 in sunflower and 0.28 in maize, whereas the concentration ratio between Sb in seeds and Sb in leaves was 0.25 in both plants.

Discussion and conclusions

The approximate proportionality between available Sb concentration in the growth medium and accumulation of Sb in the

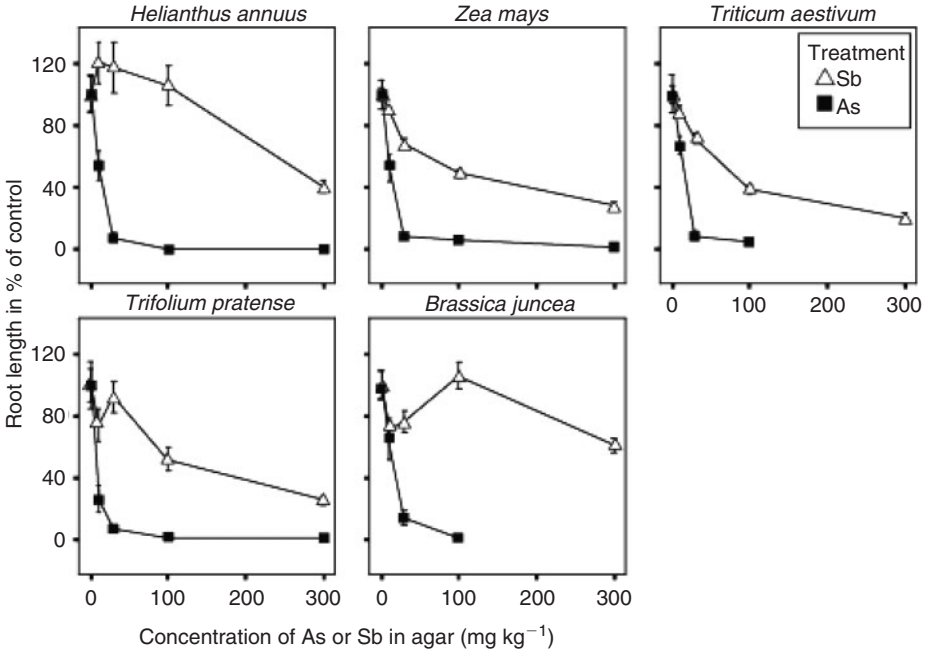

Fig. 5. Relative root length of plant seedlings grown for 2 weeks in Petri dishes with agar at different concentrations of either As or Sb.

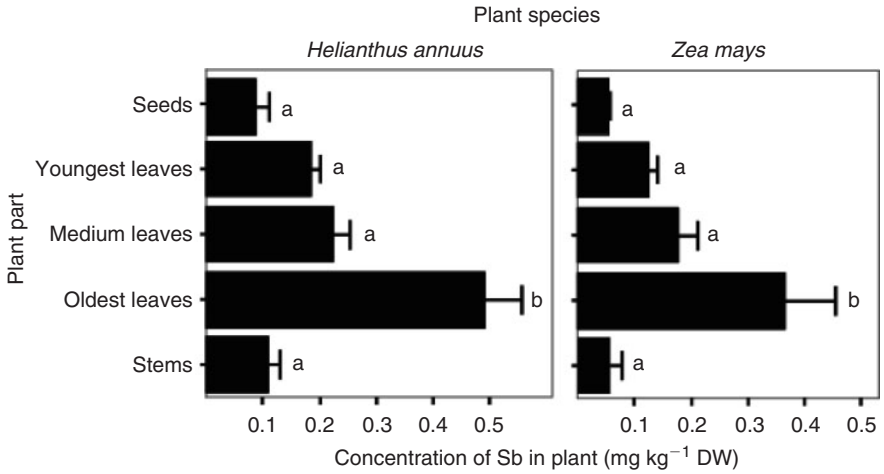

Fig. 6. Allocation of accumulated Sb (concentration in mg kg^{-1} DW, dry weight) in different above-ground parts of maize and sunflower grown in soil with a KNO_3-extractable Sb concentration of 1.03 mg L^{-1}. Bars with different letters are significantly different from each other.

shoots of all plants investigated in the present study is in agreement with a similar proportionality found by Hammel et al. in a pot experiment with spinach.[12] In the latter study, three different soils were spiked with up to 1000 mg Sb kg^{-1} DW and then left to age for 6 months before the plants were grown. This treatment resulted in NH_4NO_3-extractable Sb concentrations of up to 90 mg kg^{-1} dry soil and an Sb accumulation in the spinach leaves of up to 399 mg kg^{-1} dry mass. The observed proportionalities between soil and plant Sb suggest that Sb uptake by these plants is not controlled and mediated by membrane-bound transporters. In the latter case, saturation of the transporter binding sites would be expected at high Sb concentrations, leading to a levelling of the uptake rate. The existence of a mechanism for the specific uptake of Sb also is not likely because it is not an essential element.

Antimony was supplied in our experiments as antimonate. Antimonate speciates as a monovalent anion ($Sb(OH)_6^-$) between pH 2 and pH 10, i.e. over the entire range of pH occurring in soils. As an anion, antimonate entering a cell has to overcome an electrical potential difference across the membrane in the range of −100 to −200 mV, which would require an outer concentration two to three orders of magnitude higher than internally to drive passive uptake.[20] Thus, at least at low external concentrations, uptake of antimonate into the root symplast would require anion transporters of low selectivity, in which antimonate anions could substitute for essential nutrient anions such as Cl^- or NO_3^-.

An alternative uptake route would be via the apoplastic pathway as in the case of negatively charged metal chelates.[21,22] As the Casparian strip does not completely seal the intercellular space of the root cortex from the inner root cylinder, in particular

at the root tips and at branching-points of lateral roots, transport to the xylem can partly bypass the endodermis barrier without transfer through a cell membrane. Purely apoplastic transport would mean that Sb is simply taken up with the transpiration water stream, in proportion to the concentration of Sb in solution, and accumulated where the water evaporates. Also, the very similar ratios between Sb concentrations in various plant parts for maize and sunflower are in line with the hypothesis that Sb is primarily translocated with the transpiration stream and thus accumulated in the leaves, where the water is transpired and evaporated into the atmosphere, leaving behind solutes that are not volatilised.

In the form of non-ionic antimonite ($Sb(OH)_3$), passive uptake of Sb with the transpiration stream could theoretically also occur through aquaporins. Passage of $Sb(OH)_3$ into cells via aquaglyceroporins was found in microorganisms, i.e. in *Escherichia coli*, *Saccharomyces cerevisiae*, *Leishmania major* and *L. tarentolae*.[23] This pathway is not open to ions, however, apart from the thermodynamic problem of overcoming the electrochemical gradient. Antimonite very likely did not play a role in the uptake of Sb in our experiments though. In addition to supplying Sb only in the form of antimonate, we took care that all the experimental systems were well aerated. With the agar system, we performed preliminary tests with a dye tracer showing that there was sufficient air-filled pore space around the roots to allow rapid infiltration. The fact that the growth medium had little or no influence on the uptake rate provides additional evidence that reduction of antimonate to antimonite, which should have differed in degree between the three systems, was negligible, if it occurred at all.

It is not clear why maize and wheat showed a much higher tolerance to external Sb in shoot growth than in root growth. In the case of maize, it could be hypothesised that the aboveground parts were protected by a low degree of Sb translocation from roots to shoots in these two species. But in wheat shoots, Sb accumulation and toxicity was similar to that in the dicotyledons that were tested here, where toxicity effects emerged in shoots and roots at similar levels of external Sb exposure. The magnitude of Sb concentrations at which toxicity effects were manifested here agrees well with findings by Oorts et al., who found a 50% reduction of root elongation in barley and a 50% shoot biomass reduction in lettuce at concentrations of 39 and 41 mg L^{-1} Sb in soil solution (collected by centrifugation), respectively.[24]

Antimony was accumulated less from solution or soil than arsenic in our experiments. Being also less phytotoxic, Sb contamination of soil could still be a problem for human or animal health, because this means that plants can survive much higher soluble Sb than As concentrations. Johnson et al. found concentrations of up to 6 mg L^{-1} Sb in deionised water leachates from shooting-range soils where total Sb concentrations reached values up to 10 g kg^{-1}.[3] Using these data and the average bioaccumulation coefficients found in our study here, the predicted shoot Sb accumulation would be 45.7 mg kg^{-1} in clover, 4.4 mg kg^{-1} in maize and 23.6 mg kg^{-1} in sunflower. We did not find any tolerance or critical values for Sb consumption by animals. But taking as a surrogate a chronic toxicity threshold of 1.4 mg kg^{-1} day^{-1}, which has been proposed for Sb ingestion by humans,[25] cattle consuming more than 15.4 kg of clover, 160 kg of maize or 29.4 kg of sunflower grown on such a site per day over a longer period to exceed the threshold of 1.4 mg kg^{-1} day^{-1} may be at risk of adverse health effects.[25] Although such situations are probably rare, there may be other plants accumulating even more Sb. Leaf vegetables grown on soils with high soluble Sb concentrations might even present some chronic health risk for human self-suppliers. Thus, in addition to the influence of soil factors such as redox conditions on Sb uptake by plants, a wider variety of plants should also be tested for their capability to accumulate Sb.

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