Phytoavailability and plant uptake of antimony

Author(s):
Tschan, Martin

Publication Date:
2009

Permanent Link:

https://doi.org/10.3929/ethz-a-006035954 →

Diss. ETH No. 18656

Phytoavailability and Plant Uptake of Antimony

A dissertation submitted to the

ETH ZURICH

for the degree of

Doctor of Sciences

presented by

Martin Tschan

Dipl Biol. Université de Neuchâtel

born 3 May 1978

citizen of Zuzwil BE

accepted on the recommendation of

Prof. Dr. Rainer Schulin, examiner
Dr. Annette Johnson, co-examiner
Dr. Satish Gupta, co-examiner
Dr. Brett Robinson, co-examiner

2009

Acknowledgements

I would like to thank Prof. Dr. Rainer Schulin and Dr. Brett Robinson for giving me the opportunity to carry out my PhD thesis and for their advice, help and guidance during this time.

I would also like to thank my friends, family and colleagues for their help and support which enabled me to carry out and finish this work. In particular I want to thank Erika, Claudia, Kajsa, Irena, Nazanin and Beat whom I had the pleasure to share office with. I would also like to thank Anna Grünwald, Annina Bürgi, Viktor Stadelmann, Björn Studer, Hermann Moench and René Saladin for their technical support in the laboratory.

Lastly I would like to thank the Swiss National Science Foundation for the funding of this work (Grants No. 200021-103768).

Contents

Summary

Antimony (Sb) is a toxic metalloid that is increasingly released into the environment by human activities. Besides mining areas and roadside soil, contamination by Sb is found in particular in stop butts of shooting ranges. The latter is a particularly widespread problem in Switzerland. After lead, antimony is the second component of the alloy making up bullets (2-5 weight %). For this reason lead and antimony are enriched in the soils of stop butts in shooting ranges. Of particular concern is that antimony is much more soluble than lead at neutral pH.

We reviewed the available literature on Sb uptake by plants and on toxicity risks arising from soil contamination by Sb. Reanalyzing the reported data, we found that Sb is generally taken up by terrestrial plants in proportion to the concentration of soluble Sb in soil over a concentration range covering five or more orders of magnitude. However, very little is known about the mechanisms of Sb uptake by plants. Also the deposition of re-suspended soil particles on the surfaces of aerial plant surfaces can result in high plant Sb concentration in the vicinity of Sb-contaminated sites.

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 was similar for the three growth media. In all tested plants, shoot Sb concentrations were approximately 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 more than 2.1 g kg^{-1} Sb (dry weight) in the shoots. Maize showed the lowest accumulation. In maize and sunflower, most Sb accumulated in the leaves.

Using pot experiments, we investigated the uptake of antimony (Sb) by sunflower and maize comparing soil contamination by Sb^{V} with contamination by Sb^{III}. In the first cases Sb was added to the experimental soils as $KSb(OH)_6$ ("Sb^{V}-treatment") and in the other as Sb_2O_3 ("Sb^{III}-treatment"). Two different soils were used: a potting mix and an agricultural soil. Soluble soil Sb concentrations were linearly related to the applied Sb rates, ranging from 0.02 (controls) to 175 mg L^{-1} soil solution. Accumulation of Sb by sunflower was slightly higher in the Sb^{V} than in the Sb^{III} treatment. In maize a similar tendency was only found at low Sb

concentrations. The concentration values at which growth was reduced by half, as determined by means of dose-response curves were higher for the Sb^{V} than for the Sb^{III} treatment, when they were related to soluble soil Sb concentrations, but not when related to shoot Sb concentrations. The fact that the Sb^{III} treatment was not more or even less toxic than the Sb^{V} treatment was unexpected, as Sb^{III} is generally reported to be much more toxic than Sb^{V}.

Additionally, we investigated the effect of phosphate on Sb uptake by maize and sunflowers from nutrient solutions containing concentrations from 3 mg L^{-1} to 24 mg L^{-1} potassium antimonate, with the aim of determining the potential of Sb to enter the food chain. There was no significant difference in Sb uptake between the two investigated plant species. The average bioaccumulation coefficients (plant Sb divided by solution Sb concentration) were 1.02 and 1.93 for *Z. mays* and *H. annuus*, respectively. Phosphate addition did not affect plant growth or Sb uptake.

Although soil pollution by Sb may be rarely severe enough to cause toxicity problems to humans or animals consuming plants or food derived from plants grown on Sb-contaminated sites, such risks may arise under worst-case conditions. This way antimony can enter the food chain in the case of an antimony contaminated area with food or feed plants. The lack of phytotoxicity may facilitate the entry of antimony into the food chain, since healthy biomass can contain up to 0.4 g kg^{-1} dry weight antimony. The results of this work show that the risk of Sb transfer into food chains on contaminated sites requires more attention.

Zusammenfassung

Antimon (Sb) ist ein toxisches Halbmetall das durch menschliche Tätigkeiten zunehmend in die Umwelt freigesetzt wird. Nebst Böden in Bergbaugebieten und Strassenrändern findet sich Sb vor allem in den Kugelfängen von Schiessanlagen. Das Letztere ist vor allem in der Schweiz ein weit verbreitetes Problem. Nach Blei ist Antimon das zweite Element in der Legierung, die für Projektile verwendet wird (2-5 Gewichtsprozent). Aus diesem Grund sind Böden von Kugelfängen in Schiessanlagen mit Blei und Antimon angereichert. Besorgniserregend ist vor allem die Tatsache, dass Antimon viel löslicher ist als Blei bei neutralem pH.

Eine Literaturstudie mit den vorhandenen Publikationen über Antimonaufnahme durch Pflanzen und Toxizitätsrisiken ergab, dass Sb von terrestrischen Pflanzen üblicherweise proportional zur löslichen Antimonkonzentration im Boden aufgenommen wird, über eine Spanne von fünf Grössenordnungen. Es ist jedoch sehr wenig bekannt über die Mechanismen der Aufnahme von Sb durch Pflanzen. In der Nähe von Sb-verschmutzten Gebieten, kann auch die Ablagerung von Bodenstaub auf den Flächen der oberirdischen Teile der Pflanzen kann zu einer höheren Antimonkonzentration führen.

Wir untersuchten die Aufnahme von Antimonat aus Nährlösungen, Agar und Boden durch folgende Kulturpflanzen: Brauner Senf (*Brassica juncea* (L.) Czern), Sonnenblume (*Helianthus annuus* L.), Deutsches Weidelgras (*Lolium perenne* L.), Wiesenklee (*Trifolium pratense* L.) Weichweizen (*Triticum aestivum* L.) and Mais (*Zea mays* L.). Die Antimonaufnahme war ähnlich in allen drei Nährmedien. Die Antimonkonzentration im Spross aller getesteten Pflanzen war ungefähr proportional zur Konzentration von Sb in der Lösung oder löslichem Sb im Boden, bis schliesslich die Giftigkeit das Wachstum der Pflanzen begrenzte. Bei gleicher Konzentration im Nährmedium unterschied sich die Akkumulierung von Sb zwischen den Pflanzen bis zu einer Grössenordnung. Wiesenklee in Agar mit einer löslichen Konzentration von 160 mg L^{-1} Sb nahm mehr als 2.1 g kg^{-1} Sb (Trockengewicht) in den Spross auf. Mais zeigte die niedrigste Akkumulation. In Mais und Sonnenblumen wurde das meiste Sb in den Blättern akkumuliert.

Mit Topfexperimenten verglichen wir die Aufnahme von Antimon (Sb) durch Sonnenblumen und Mais von Böden die mit Sb^{V} oder Sb^{III} verschmutzt waren. Bei Ersteren wurde $KSb(OH)_6$ ("Sb^{V}-Behandlung") und bei den zweiten Sb_2O_3 ("Sb^{III}-Behandlung") zu den Experimentböden hinzugefügt. Es wurden zwei unterschiedliche Böden verwendet: eine Blumenerde und ein landwirtschaftlicher Boden. Lösliche Sb-Konzentrationen im Boden

waren sich in einer linearen Beziehung zu den verwendeten Sb-Dosierungen, welche von 0.02 (Kontrollen) bis zu 175 mg L^{-1} in der Bodenlösung reichten. Die Antimonaufnahme von Sonnenblumen war leicht höher in der Sb^{V}- als in der Sb^{III}-Behandlung. Bei Mais wurde eine ähnliche Tendenz nur bei niedrigen Sb-Konzentrationen festgestellt. Die Konzentrationswerte, bei welchen das Wachstum um die Hälfte reduziert war und die mit Dosis-Wirkungskurven bestimmt wurden, waren höher für die Sb^{V}- als die Sb^{III}-Behandlung, wenn sie in Beziehung zur löslichen Sb-Konzentration im Boden standen. Dies war nicht der Fall, wenn sie in Beziehung zur Sb-Konzentration im Spross betrachtet wurden. Die Tatsache, dass die Sb^{III}-Behandlung nicht mehr oder sogar weniger giftig war als die Sb^{V}-Behandlung, war unerwartet, da Sb^{III} generell als viel giftiger als Sb^{V} betrachtet wird.

Zusätzlich untersuchten wir den Einfluss von Phosphat auf die Aufnahme von Sb durch Mais und Sonnenblumen aus Nährlösungen mit Konzentrationen zwischen 3 mg L^{-1} und 24 mg L^{-1} Kaliumantimonat. Ein Ziel war auch herauszufinden, ob Sb in die Nahrungskette eindringen kann. Es gab keine signifikanten Unterschiede in der Sb-Aufnahme zwischen den beiden Pflanzenarten. Die durchschnittlichen Bioakkumulationskoeffizienten (Konzentration Sb Pflanze geteilt durch Konzentration Sb Lösung) waren 1.02 für *Z. mays* und 1.93 für *H. annuus*. Phosphatzugabe hatte keinen Einfluss auf das Pflanzenwachstum und die Sb-Aufnahme.

Obwohl Bodenverschmutzung durch Sb selten so hoch ist, dass es zu Problemen mit der Giftigkeit von Pflanzen, die auf mit Sb verschmutztem Boden wachsen, oder mit Nahrungsmitteln aus solchen für Menschen und Tiere gibt, können sich solche Risiken doch im ungünstigsten Fall ergeben. So kann Antimon im Fall von einem verschmutzten Gebiet durch Nahrungs- und Futterpflanzen in die Nahrungskette eindringen. Die mangelnde Phytotoxizität kann den Eintritt in die Nahrungskette zusätzlich erleichtern, da gesunde Pflanzenbiomasse bis zu 0.4 g kg^{-1} Antimon Trockengewicht enthalten kann. Die Resultate dieser Arbeit zeigen, dass das Risiko eines Eindringens von Sb in die Nahrungskette auf verschmutzten Böden weiterer Studien bedarf.

1 Introduction

Owing to its many industrial uses, e.g. in fire retardants, semiconductors, and as an agent for metal hardening, antimony (Sb) has become widespread in the environment (Filella et al. 2002). Antimony is a rare element in the earth's crust (0.2 – 0.3 mg kg^{-1}) (Rish 2004) and soil background concentrations vary between 0.3 to 2.3 mg kg^{-1} (Kabata-Pendias and Pendias 1984), but due to emissions of Sb from waste incineration, smelters, traffic, shooting and other human activities, much higher concentrations are found in contaminated soils and sediments.

Very often it occurs as a co-contaminant with more toxic elements, such as arsenic (As) and lead (Pb). As the latter elements usually are the main focus, Sb was usually neglected. For example in shooting ranges, Sb mostly occurs with lead, in orchards with As, in mine tailings with the primary resource element being produced and in incineration dust with other elements that are released after incineration (Nriagu and Pacyna 1988).

Only recently has Sb attracted more attention as a pollutant. The United States Environmental Protection Agency (USEPA) considers Sb as a priority pollutant since the early nineties (1999). Antimony is a non-essential element for both plants and animals. It is toxic to humans at chronic uptake rates exceeding 100 mg day^{-1} per person (Bowen 1979). Rats succumb when fed 11-75 mg day^{-1}. There are no reports of toxicity in plants. Despite the use of sodium stibogluconate as a treatment for Leishmaniasis for the last 75 years (Mishra et al. 2007), not much is known about the chronic toxicity of elemental and methylated antimony. The toxicity of Sb depends on its molecular form; inorganic Sb compounds are more toxic than organic ones. In mammal cells, Sb^{III} compounds are ten times more toxic than Sb^{V} compounds (Krachler et al. 2001).

In Switzerland, there are over 2000 shooting ranges scattered throughout the country (Gresch and Wettstein 2002). These are heavily polluted with Pb and Sb from the bullets. On average, new bullets and pellets consist of > 90% Pb, 1-7% Sb, < 2% As and <0.5% nickel (Ni) (Rooney et al. 1999). The stop butts and the areas around are the most polluted sites in shooting ranges. Concentrations up to 515'800 mg kg^{-1} lead and 17'500 mg kg^{-1} Sb have been reported by Gresch and Wettstein (2002).

A particular problem is that Sb is much more soluble in the soil solution than Pb under neutral to alkaline conditions (Johnson et al. 2005). This explains why antimony was detected in surface waters downstream from a shooting range area by (Mergenthaler and Richner 2002), but the levels remained below WHO drinking water guideline of 20 µg L^{-1} (WHO 2003).

Besides that antimony may reduce plant productivity on contaminated soil, Sb may enter the human food chain through uptake by plants. Antimony in crop plants may present a health risk to humans and animals. Previous studies have shown a large variation in the uptake of Sb concentrations in plant shoot tissue for the different plant species that occur in Sb-contaminated soil.

Some studies reported very high uptake of Sb by plants under field conditions. Ainsworth et al. (1990) measured Sb concentrations of 300 mg kg^{-1} dry weight in the leaves of several grasses nearby an Sb smelter in North East England, where the soil Sb concentrations reached 400 mg kg^{-1}. Baroni et al. (2000) reported over 1000 mg kg^{-1} Sb in the basal leaves of *Achillea ageratum* growing at a tailing pond with a soil containing 9000 mg kg^{-1} Sb. In contrast, Pratas et al. (2005) reported maximum stem concentrations of less than 5 mg kg^{-1} Sb for different tree and herb species growing in a mine spoil with a soil Sb concentration of 663 mg kg^{-1}.

Antimony found in under field-sampled plants must not necessarily have been taken up from the soil. Ainsworth et al. (1990) found that grasses grown near the smelter in pots that contained non-contaminated soil had similar Sb concentrations as plants growing in contaminated soil. This indicated that leaf Sb burden rather originated from surface deposition than from root uptake. Robinson et al. (2008) obtained similar results at a highly polluted shooting range in Switzerland, using iron as indicator element to account for dust deposition.

In study we investigated Sb uptake by different plant species under controlled conditions. Experiments were performed with hydroponics, agar culture and polluted soils. Each system has distinct advantages and disadvantages. The agar system was used as an alternative to the hydroponics system to reduce root damage arising from seedling manipulation. 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. While no transplantation is necessary in agar and soil systems, hydroponics have the important advantage that it is easy to control and also change, if desired, the composition of the solution to which the roots are exposed. Soil provides a more natural environment for plant growth than hydroponics and agar, which means that such experiments are more representative of natural growing conditions. To some extent, the agar system provides conditions in between the hydroponics and the soil system.

The main objectives of this study were to investigate

(i) the uptake of antimony by selected plants from standardized nutrient solutions and soils and its dependence on Sb concentration
(ii) the dependance of Sb uptake by plants on the oxidation state of Sb added to soil
(iii) toxicity of Sb to plants
(iv) the influence of phosphate on the uptake of Sb^V

The following studies were conducted and the results are presented in the four main chapters of this dissertation:

Antimony in the soil-plant system – a review (Chapter 3)

This chapter reviews information about Sb uptake mechanisms and contamination in plants and the soils they are growing in. We elucidate uptake pathways for Sb from different anthropogenic sources. We analysed the Sb data provided in the literature and assessed the toxicity risks associated with soil pollution by Sb.

Antimony uptake by different plant species from nutrient solution, agar and soil (Chapter 4)

We spiked nutrient solutions, agar and potting soil with Sb to assess toxicity of Sb in different growing media. Spiking clean soils allowed us to eliminate the effects of co-contamination occurring in contaminated soils. We used the dicotelydon species Indian mustard (*Brassica juncea* L.), sunflower (*Helianthus annuus* L.) and clover (*Trifolium pratense* L.) and the monocotyledon species perennial ryegrass (*Lolium perenne* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.).

Antimony uptake by sunflower and maize from soil comparing Sb^{III} and Sb^V contamination (Chapter 5)

We spiked a potting soil and an agricultural soil with $Sb^{III}_2O_3$ and $KSb^V(OH)_6$ to study how plant uptake is affected by the form of Sb added to two different soil types. Spiking clean soils allowed us to eliminate the effects of co-contamination occurring in contaminated soils. The experiment was performed with sunflower (*Helianthus annuus*) and maize (*Zea mays*).

Antimony uptake by Zea mays (L.) and Helianthus annuus (L.) from nutrient solution (Chapter 6)

Here we investigated in particular the influence of phosphate on Sb uptake by sunflower (*Helianthus annuus*) and maize (*Zea mays*) from nutrient solution.

References

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .1. Vegetation and Soils, *Environmental Pollution* **1990a**, *65*, 65-77.

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .2. Small Mammals and Invertebrates, *Environmental Pollution* **1990b**, *65*, 79-87.

Baroni, F., Boscagli, A., Protano, G., Riccobono, F., Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area, *Environmental Pollution* **2000**, *109*, 347-352.

Bowen, H. J. M., *Environmental Chemistry of the Elements* **1979**, Academic Press: London.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters I. Occurence, *Earth-Science Reviews* **2002**, *57*, 125-176.

Gresch, M., Wettstein, B., *Antimon- und Bleibelastung bei Schiessanlagen, Fallbeispiel Eschenbach (SG)* Semester Thesis, **2002**, ETH Zurich.

Johnson, C. A., Moench, H., Wersin, P., Kugler, P., Wenger, C., Solubility of antimony and other elements in samples taken from shooting ranges, *J. Environ. Qual.* **2005**, *34*, 248-254.

Kabata-Pendias, A., Pendias, H., *Trace Elements in Soils and Plants* **1984**, CRC Press: Boca Raton, FL.

Krachler, M., Emons, H., Zheng, J., Speciation of antimony for the 21st century: promises and pitfalls, *Trac-Trends in Analytical Chemistry* **2001**, *20*, 79-90.

Mergenthaler, B., Richner, T., *Mobilität und geochemisches Verhalten von Antimon im Boden von Schiessanlagen* Diploma Thesis, **2002,** ETH Zurich.

Mishra, J., Saxena, A., Singh, S., Chemotherapy of leishmaniasis: Past, present and future, *Curr. Med. Chem.* **2007**, *14*, 1153-1169.

Nriagu, J. O., Pacyna, J. M., Quantitative Assessment Of Worldwide Contamination Of Air, Water And Soils By Trace-Metals, *Nature* **1988**, *333*, 134-139.

Pratas, J., Prasad, M. N. V., Freitas, H., Conde, L., Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation, *J. Geochem. Explor.* **2005**, *85*, 99-107.

Rish, M. A., Antimony. In: *Elements and their Compounds in the Environment* (Eds Merian, E., Anke, M., Ihnat, M., Stoeppler, M.) **2004**, pp. 659-670 (Wiley-VHC: Weinheim).

Robinson, B. H., Bischofberger, S., Stoll, A., Schroer, D., Furrer, G., Roulier, S., Gruenwald, A., Attinger, W., Schulin, R., Plant uptake of trace elements on a Swiss

military shooting range: Uptake pathways and land management implications, *Environmental Pollution* **2008**, *153*, 668-676.

Rooney, C. P., McLaren, R. G., Cresswell, R. J., Distribution and phytoavailability of lead in a soil contaminated with lead shot, *Water Air Soil Pollut.* **1999**, *116*, 535-548.

USEPA, *Toxic Release Inventory* **1999**.

WHO, *Guidelines for drinking water - Chemical fact sheet* **2003**.

2 Biogeochemstry of Antimony

2.1 Chemistry

The biogeochemical behaviour of Sb is similar to the other group 15 elements arsenic (As) and phosphorous (P) and bismuth (Bi) (Rish 2004). Because of their identical s^2p^3 outer orbital electron configuration, Sb and As display the same range of oxidation states in environmental systems (-3 to +5). Both most commonly occur as oxides, hydroxides or oxyanions either in the +5 state in relatively oxic environments (antimonates and arsenates) or in the +3 state in anoxic environments (antimonites and arsenites).

While there are many similarities in the chemistry of P, As and Sb, there are many differences. The structures of their pentavalent oxyanions; phosphate, arsenate and antimonate, are different. Arsenate (AsO_4^{3-}) and phosphate (PO_4^{3-}) are both tetrahedral and compete for binding sites on transporter proteins in plants and adsorb to the same sites in soil while $Sb(OH)_6^-$ is octahedral (Baes and Mesmer 1986). Antimonite structure however is similar to arsenite ($As(OH)_3$) and boric acid ($B(OH)_3$).

2.2 Geochemistry of antimony

Antimony (Sb) is a rare element in the earth's crust (0.2 – 0.3 mg kg^{-1}) (Rish 2004). World reserves of Sb are in excess of 2 million tons and are located primarily in Bolivia, China, Russia, South Africa, and Mexico (Filella et al. 2002a). Common minerals are sulphides (in particular stibnite Sb_2S_3) and oxides (valentinite Sb_2O_3 and cervantite Sb_2O_4). Igneous rocks contain 0.1 to 1 mg kg^{-1} Sb and Sb concentrations in sedimentary rocks are reported to be very small, but no exact data is available (Butterman and Carlin 2004).
Mineralisation of stibnite (Sb_2S_3) to senarmonite (Sb_2O_3) through oxidation was observed at a former smelter site in New Zealand (Wilson et al. 2004). The authors proposed the following reaction: $Sb_2S_3 + 3\ H_2O + 6\ O_2 \rightarrow 3\ SO_4^{2-} + Sb_2O_3 + 6H^+$. This process leads to a strong acidification of the soil, which reduces the mobility of Sb. No Sb could be detected in a stream less than 50 m away. However, it might be released once the pH rises.

Soil background concentrations vary between 0.3 to 2.3 mg kg^{-1} (Kabata-Pendias and Pendias 1984). Antimony concentrations in seawater, resulting from rock weathering, soil runoff and anthropogenic activities, are around 0.2 µg L^{-1}. Under oxic conditions in water Sb mostly occurs as Sb^{V}, but also some Sb^{III} can be found. In anoxic conditions, Sb is mostly present as Sb^{III}, but also Sb^{V} is present. The presence of both species in oxic and anoxic conditions is explained with a slow reduction rate (Filella et al. 2002a). In water at pH 4 to 10 and in absence of sulphur, Sb^{V} forms $Sb(OH)_6^-$ and Sb^{III} forms $Sb(OH)_3$ (Filella et al. 2002b).

2.3 Mobility of Sb in soils

In natural soils, there are a lot of SH and OH groups Sb can bind to. Complexes with Cl might occur in highly saline soils. Due to the formation of a negatively charged oxyanion in its oxidized state, Sb is more mobile under neutral than under acidic conditions. Antimony solubility and subsequent mobility depends on the clay mineralogy and organic matter content of the soil. Compared to other common heavy metal contaminants, Sb is relatively soluble. Gresch and Wettstein (2002) found more mobile Sb (0.12 % of total concentration) than Pb (0.000012 % of total concentration) in shooting range soil. The total Pb concentration (130'000 mg kg^{-1}) was more than 56 times higher than the total Sb concentration (2300 mg kg^{-1}). Johnson et al. (2005) reported that the concentration of Sb in soil solution (average 1% through soil strata) was the highest of all trace elements (mobile Pb: 0.01%, Ni 0.02%, Cu 0.04%, Bi 0.05%) in Swiss shooting ranges under unsaturated conditions.

Sb sorbs on various solid metal oxide phases in soil. Similar to As and Bi, Sb sorbs to ferric oxides (Manaka 2006). Sb^{III} sorbs to pure metal oxides: MnOOH > $Al(OH)_3$ > FeOOH, the amount of Sb sorbed generally decreases with pH > 6 (Filella et al. 2002b). In addition, Sb^{V} sorbs to hematite (Fe_2O_3) as do As and Bi. Desorption of ^{119}Sb from the α-Fe_2O_3 surface at pH 4-10 was slow, with a maximum rate at pH 4 (Ambe 1987). Antimonate is more weakly bound than phosphate by the soil matrix. Phosphate displaces both Sb and As from sorption sites (Asher and Reay 1979, Nakamaru and Sekine 2008).

Nakamaru et al. (2006) performed radiotracer experiments with ^{125}Sb to assess the mobility of Sb in soil. With NH_4NO_3 extraction they found values ranging from 0.1 to 0.3 % extractable Sb. Mobility decreased with both decreasing pH and increased with increasing phosphate

concentrations. Flynn et al. (2003) used bacteria with bioluminescence to assess As, Cu and Sb mobility in mining soils and concluded that Sb was far less mobile than As. On average 2.5 % of total Sb were mobile with a range of 0 to 42 %. In the majority of the samples less than 1% of total Sb was soluble.
Hammel et al. found that antimony added Sb to a soil from a mining area (left for aging 6 months) had a significantly higher solubility than in soils with aged Sb concentrations. The concentrations were up to 1000 mg kg^{-1} in spiked soil with 8.9% soluble Sb, whereas 1300 mg kg^{-1} was the maximum in the non-spiked soils with only 0.2% soluble fraction (Hammel et al. 2000).

$Antimony^{III}$ and Sb^{V} form complexes with humic acids, as do As^{III} and As^{V}. Buschmann and Sigg (2004) showed that humic acids increase solubility of antimonite at environmentally relevant conditions, and that low pH favours anion absorption and lowers the mobility of Sb. Steely et al. (2007) demonstrated that humic acids removed Sb^{III} and Sb^{V} from solution.

2.4 Uses of antimony

In Ancient Greece and Rome, women used antimony sulphide, called *stibium* by the Romans, as an eyebrow paint. The standard chemical symbol for antimony derives from this Latin word, *stibium*. In his 1556 book *De re metallica*, Georgius Agricola describes Sb minerals and smelting techniques (Hoover and Hoover 1912).

Antimony has many industrial uses. In particular it is used as a component in fire retardants and in semiconductors, and as an agent for metal hardening (Filella et al. 2002a). As antimony consumption has increased with global economic growth, so have emissions into the environment through dust emissions and waste disposal. This has resulted in elevated Sb concentrations in soils and natural waters, where it may affect plants, animals, and humans.
Antimony trioxide (Sb_2O_3) is widely used as a flame retardant, e.g. in textiles, papers, plastics and adhesives. Therefore textiles and plastics are major sources of Sb in municipal wastes (Paoletti et al. 2001). Furthermore, Sb trioxide is used as a paint pigment, ceramic opacifier, catalyst, mordant and glass decolouriser.
Estimated worldwide consumption for 2001 is 110’900 tons, of those 60 % is used as fire retardants, 20 % for metal products and 20 % for non-metal products (Mathys et al. 2007).

2.5 Environmental Contamination

Antimony is also used in brake pads to increase effectiveness and released to the atmosphere through abrasion. Measurements along roadsides show a positive correlation of Sb with traffic volume and a negative correlation with the distance from the road. Cal-Prieto et al. (2001) measured Sb in the topsoil at two distances from a highway in Spain. At 1 m distance the average Sb concentration was 3.81 mg kg^{-1} and at 100 m it was 0.53 mg kg^{-1}. In Austria, Amereih et al. (2005) measured 8.68 mg kg^{-1} at 0.2 m distance from a highway and 1.16 mg kg^{-1} at 10 m. Lehndorff and Schwark (2008) compared enrichment factors (EF = $(c_x/c_{reference\ element})_{biomass}/(c_x/c_{reference\ element})_{geogenic\ background}$) of Sb to those of Cd, Mo, Pb, Ba, Fe, Na, V, Ti, Zr in pine needles (*P. nigra*) in a heavy traffic area in Cologne. As reference elements the rare earth elements: La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu and Hf were used. Sb concentration in needles was 502 µg kg^{-1} DW, resulting in an EF of 2149.

Mining activity can produce very high Sb concentrations that are over an order of magnitude higher than geogenic concentrations. Gemici and Tarcan (2007) for example found up to 418 mg L^{-1} Sb in waters and 52 mg kg^{-1} Sb in soils near a mercury mine in Turkey.

Hirner et al. (2000) investigated volatilization from contaminated soils at industrial sites and waste deposits of gaseous stibine and methylated Sb compounds. The total concentration of methylated Sb species in the soil was 1.0 µg kg^{-1} and the concentrations in the air were more than 10 µg m^{-3}. This is two orders of magnitude higher than background and may endanger people living and working nearby.

Also farming practices, recreation, and military activities can increase Sb contamination in the environment. In Switzerland, elevated soil Sb concentrations in particular occur in the soils of the more than 2000 shooting ranges scattered throughout the country (Gresch and Wettstein 2002). Johnson et al. (2005) found up to 13'800 mg kg^{-1} Sb in the topsoils of shooting ranges. This comes from bullets, which contain on average over 90% Pb, 1-7% Sb, <2% arsenic (As) and <0.5% nickel (Ni) (Rooney et al. 1999).

Wagner et al. (2003) traced elevated Sb concentrations in orchard soils to a Pb-As pesticide that was commonly used in the first half of the 20^{th} century. Antimony: As ratios were the same in pesticides and soil with a 0.01 Sb:As ratio and soil concentration of 0.4 to 1.5 mg kg^{-1}

Sb. This points to impurities in the pesticide as the source for Sb, even if the concentrations stayed within the worldwide observed range of 0.3 to 2.3 mg kg^{-1}, and stayed below health protection standards.

Usually, Sb is a co-contaminant in soil: In shooting ranges Sb is associated with Pb, in orchards with As, in mine tailings with the primary resource element, in incineration dust with other elements (As, Cd, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Se, V, Zn (Nriagu and Pacyna 1988)) that are released after incineration.

2.6 Toxicity of Sb

Antimony is a non-essential element for both plants and animals. Antimony is toxic to humans at chronic uptake rates exceeding 100 mg day^{-1} per person (Bowen 1979). Rats succumb when fed 11-75 mg day^{-1}. Leonard and Gerber (1996) report toxic effects in dogs and cats after ingestion of 4 mg, respectively 10 mg per kg body weight potassium antimony tartrate. A dose of 11-75 mg day^{-1} is lethal to rats (Bowen 1979) and a dose of over 100 mg kg^{-1} to rabbits (Leonard and Gerber 1996). The WHO set the tolerable daily (TDI) intake at 6 µg kg^{-1} body weight (WHO 2003). Eckhoff et al. (1973) found that 45 mg kg^{-1} Sb in liver and 33 mg kg^{-1} Sb in kidney were lethal for livestock.

2.7 Uptake and membrane transporters

Filella et al. (2007) reviewed microbiota relevant interactions with Sb in natural waters and concluded that aquaglyceroporins mediate the transport of the neutral $Sb(OH)_3$ molecule. Porquet at al. (2007) found structural evidence for the similarity in the conformation of the molecules and the charge distribution between $Sb(OH)_3$, $As(OH)_3$ and glycerol. Antimonite and arsenite are slightly smaller than glycerol. Three families of transporters are involved in Sb^{III} efflux from cells: the ArsB protein, which belongs to the ion transporter super family and is found in various prokaryotes (Cai et al. 1998); the Acr3p family, identified in both bacteria and yeast (Wysocki et al. 2003); and the ABC (ATP-binding cassette) transporter super family (Gayet et al. 2006).

ArsB proteins are ATP dependant efflux transporters for arsenite. Cai et al. (1998) found that the same genes coding for the ArsB protein in *Pseudomonas aeruginosa* also contributed to Sb resistance when they were cloned and inserted into *E. coli*. This example illustrates that in the case of antimonite and arsenite there is analoguous behaviour of the ArsB protein, due to the chemical and structural similarity.

Gayet et al. (2006) showed that insertion of the genes for the human ATP-binding cassette (ABC) transporters into the genome of *Arabidopsis thaliana* did not increase the tolerance of *Arabidopsis* to Sb^{III} salts. ABC transporter are associated with resistance mechanisms and also responsible for detoxification of As and Sb. However, the expression of the transporter in the transgenic plants might have been insufficient to confer a strong resistance to antimony salts.

2.8 Metabolism

Despite the use of sodium stibogluconate as a treatment for Leishmaniasis for the last 75 years, a disease caused by the protozoan parasite *Leishmania* sp., with a global prevalence of 12 million cases, occurring in 88 countries (Mishra et al. 2007), not much is known about the effect of elemental and methylated Sb on the metabolism of different organisms.

It is known that the Sb^{V} contained in drugs used against Leishmaniasis depend on the reduction of Sb^{V} to Sb^{III} in the liver to reach their full effectiveness against the parasite (Mishra et al. 2007). In the absence of adequate experimental data for Sb, it has been common practice to assume that Sb behaves similar to arsenic (uptake, tolerance mechanisms, and methylation) because of the chemical similarities of the two elements. However, whether such may exist with some phenomena, this may not be the case at all in others.

Sb^{III} and As^{III} are known to form strong bonds with functional groups such as the thiolates of cysteine residues. Like Sb^{III}, also Sb^{V} prefers SH to OH groups, but binds to both.
When they react with these groups in proteins, they frequently inhibit catalytic or biological activity (Bhattacharjee and Rosen 1996, Rosen 2002). This may lead then to oxidative stress when enzymes in the oxygen metabolism are affected. This effect is known for various heavy metals that are not directly involved in the oxygen metabolism. One may theorize that also high concentrations of Sb may damage tissues in this way (Schutzendubel and Polle 2002).

Only a few toxicity tests have been performed with animals, plants or other organisms exposed to Sb contaminated soil. Aqueous extracts of a spiked Luvisol and Chernozem that had been spiked with Sb_2S_3 and Sb_2S_5 (aged for 6 months) were toxic to the soil alga *Chlorococcum infusionum* (Hammel et al. 1998). Kuperman et al. (2006) determined EC_{50} values for the reproduction of worms and collembola in $Sb_2(SO_4)_3$ spiked soils. They obtained 70 mg kg^{-1} for cocoon production of the earthworm *Eisenia fetida*, 316 mg kg^{-1} and 169 mg kg^{-1} for juvenile production of the enchytraeid *Enchytraeus crypticu*s and the collembolan *Folsomia. candida*, respectively. Oorts et al. (2008) determined the EC_{50} values of total and soluble soil Sb concentrations for plant growth. They spiked soil with up to 10'000 mg kg^{-1} Sb_2O_3 and found mainly Sb^V, around 70%, in soil solutions, extracted by centrifugation. They found a 50% reduction of root elongation for barley at 39 mg L^{-1} Sb in soil solution and a 50% shoot yield reduction for lettuce at 41 mg L^{-1} Sb in solution.

The toxic effects of trace elements are usually most severe when they are present as free ions. Therefore for example in plants, most trace elements found in the tissues are complexed with organic ligands (Hall 2002), such as low molecular weight organic acids, plants produce two classes of metal-binding polypeptides: phytochelatins and metallothioneins (Cobbett and Goldsbrough 2002). Metallothioneins are low-molecular-weight, cystine-rich, metal binding polypeptides (Kagi 1991). Maeda and Ohki (1998) detected metallothionein-like polypeptides in algae of the species *Chlorella vulgaris* exposed to Sb^{III}. Phytochelatins have the general structure (γ-Glu-Cys)n-Gly, where n = 2 to 11. They are rapidly produced in plants under heavy metal stress (Rauser 1995, Zenk 1996). Phytochelatins were shown to contribute to Sb tolerance in yeast and wheat (Wysocki et al. 2003).

2.9 Regulations and health risks

McCallum (2005) investigated health risk of process workers in the Sb industry who were exposed to dust in the 1970s. The men suffered mostly from skin irritations (Sb spots) and upper respiratory tract irritation. Workers in the Sb industry had a 15% higher incidence of pneumoconiosis than workers of an adjacent zircon plant, they had 15 % more. The average concentration measured in the processing plant was 0.5 mg m^{-3}, which was frequently exceeded with maximum concentrations up to 36.7 mg m^{-3}. Today's limiting concentration is 0.5 mg m^{-3} (Rish 2004). Working conditions have vastly improved since the 1970s and the

number of exposed people has been significantly reduced. Cavallo et al. (2002) studied two groups of workers, one directly exposed to Sb_2O_3 applied as a flame retardant and one not directly exposed, using a micronucleus assay and a sister chromatide exchange test they found no significant differences between the two groups.

In drinking water, the EU limit for Sb is 5 μg L^{-1} (Filella et al. 2002a). The WHO guideline value for drinking water is 20 μg L^{-1} (WHO 2003). The European Commission has set the specific migration limit to 0.04 mg kg^{-1} for plastic materials and articles intended to come in contact with food (EU 2005). The Dutch intervention values for soil and groundwater are 15 mg kg^{-1}, and 20 μg L^{-1}, respectively (Swartjes 1999).

2.10 References

Ambe, S., Adsorption-Kinetics Of Antimony(V) Ions Onto Alpha-Fe2o3 Surfaces From An Aqueous-Solution, *Langmuir* **1987**, *3*, 489-493.

Amereih, S., Meisel, T., Scholger, R., Wegscheider, W., Antimony speciation in soil samples along two Austrian motorways by HPLC-ID-ICP-MS, *J. Environ. Monit.* **2005**, *7*, 1200-1206.

Asher, C. J., Reay, P. F., Arsenic Uptake by Barley Seedlings, *Australian Journal of Plant Physiology* **1979**, *6*, 459-466.

Baes, C. F., Mesmer, R. E., Arsenic, Antimony, Bismuth. In: *The Hydrolysis of Cations* (Eds **1986**, pp. 366-375 (Krieger: Malabar, FL).

Bhattacharjee, H., Rosen, B. P., Spatial proximity of Cys(113), Cys(172), and Cys(422) in the metalloactivation domain of the ArsA ATPase, *J. Biol. Chem.* **1996**, *271*, 24465-24470.

Bowen, H. J. M., *Environmental Chemistry of the Elements* **1979**, Academic Press: London.

Buschmann, J., Sigg, L., Antimony(III) Binding to Humic Substances: Influence of pH and Type of Humic Acid, *Environ. Sci. & Technol.* **2004**, *38*, 4535.

Butterman, W. C., Carlin, J. F. J., *Antimony, Mineral Commodity Profiles, Open file Report 03-019* **2004**, U.S. Department of the Interior.

Cai, J., Salmon, K., DuBow, M. S., A chromosomal ars operon homologue of Pseudomonas aeruginosa confers increased resistance to arsenic and antimony in Escherichia coli, *Microbiology-(UK)* **1998**, *144*, 2705-2713.

Cal-Prieto, M. J., Carlosena, A., Andrade, J. M., Martinez, M. L., Muniategui, S., Lopez-Mahia, P., Prada, D., Antimony as a tracer of the anthropogenic influence on soils and estuarine sediments, *Water Air Soil Pollut.* **2001**, *129*, 333-348.

Cavallo, D., Iavicoli, I., Setini, A., Marinaccio, A., Perniconi, B., Carelli, G., Iavicoli, S., Genotoxic risk and oxidative DNA damage in workers exposed to antimony trioxide, *Environ. Mol. Mutagen.* **2002**, *40*, 184-189.

Cobbett, C., Goldsbrough, P., Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis, *Annu. Rev. Plant Biol.* **2002**, *53*, 159-182.

Eckhoff, N. D., Clack, R. W., Anthony, H. D., Gray, A. P., Measurement Of Toxic Heavy-Metals By Neutron-Activation Analysis, *Toxicol. Appl. Pharmacol.* **1973**, *24*, 178-181.

EU, *Commission directive 2005/79/EC* **2005**.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters I. Occurence, *Earth-Science Reviews* **2002a**, *57*, 125-176.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters II. Relevant solution chemistry, *Earth-Sci. Rev.* **2002b**, *59*, 265-285.

Filella, M., Belzile, N., Lett, M. C., Antimony in the environment: A review focused on natural waters. III. Microbiota relevant interactions, *Earth-Sci. Rev.* **2007**, *80*, 195-217.

Flynn, H. C., Meharg, A. A., Bowyer, P. K., Paton, G. I., Antimony bioavailability in mine soils, *Environmental Pollution* **2003**, *124*, 93-100.

Gayet, L., Picault, N., Cazale, A. C., Beyly, A., Lucas, P., Jacquet, H., Suso, H. P., Vavasseur, A., Peltier, G., Forestier, C., Transport of antimony salts by *Arabidopsis thaliana* protoplasts over-expressing the human multidrug resistance-associated protein 1 (MRP1/ABCC1), *FEBS Lett.* **2006**, *580*, 6891-6897.

Gemici, U., Tarcan, G., Assessment of the pollutants in farming soils and waters around untreated abandoned Turkonu Mercury Mine (Turkey), *Bull. Environ. Contam. Toxicol.* **2007**, *79*, 20-24.

Gresch, M., Wettstein, B., *Antimon- und Bleibelastung bei Schiessanlagen, Fallbeispiel Eschenbach (SG)* Semester Thesis, **2002**, ETH Zurich.

Hall, J. L., Cellular mechanisms for heavy metal detoxification and tolerance, *J. Exp. Bot.* **2002**, *53*, 1-11.

Hammel, W., Debus, R., Steubing, L., Mobility of antimony in soil and its availability to plants, *Chemosphere* **2000**, *41*, 1791-1798.

Hammel, W., Steubing, L., Debus, R., Assessment of the ecotoxic potential of soil contaminants by using a soil-algae test, *Ecotox. Environ. Safe.* **1998**, *40*, 173-176.

Hirner, A. V., Gruter, U. M., Kresimon, J., Metal(loid)organic compounds in contaminated soil, *Fresenius J. Anal. Chem.* **2000**, *368*, 263-267.

Hoover, H. C., Hoover, L. H., *Georgius Agricola - De re metallica* **1912**, The Mining Magazine, Salisbury House, London.

Johnson, C. A., Moench, H., Wersin, P., Kugler, P., Wenger, C., Solubility of antimony and other elements in samples taken from shooting ranges, *J. Environ. Qual.* **2005**, *34*, 248-254.

Kabata-Pendias, A., Pendias, H., *Trace Elements in Soils and Plants* **1984**, CRC Press: Boca Raton, FL.

Kagi, J. H. R., Overview Of Metallothionein, *Method Enzymol.* **1991**, *205*, 613-626.

Kuperman, R. G., Checkai, R. T., Simini, M., Phillips, C. I., Speicher, J. A., Barclift, D. J., Toxicity benchmarks for antimony, barium, and beryllium determined using reproduction

endpoints for Folsomia candida, Eisenia fetida, and Enchytraeus crypticus, *Environ. Toxicol. Chem.* **2006**, *25*, 754-762.

Lehndorff, E., Schwark, L., Accumulation histories of major and trace elements on pine needles in the Cologne Conurbation as function of air quality, *Atmos. Environ.* **2008**, *42*, 833-845.

Leonard, A., Gerber, G. B., Mutagenicity, carcinogenicity and teratogenicity of antimony compounds, *Mutation Research-Reviews in Genetic Toxicology* **1996**, *366*, 1-8.

Maeda, S., Ohki, A., Bioaccumulation and Biotransformation of Arsenic, Antimony, and Bismuth Compounds by Freshwater Algae. In: *Wastewater Treatment with Algae* (Eds Wong, Y. S., Tam, N. F. Y.) **1998**, pp. 73-92 (Springer - Verlag and Landes Biosciences).

Manaka, M., Amount of amorphous materials in relationship to arsenic, antimony, and bismuth concentrations in a brown forest soil, *Geoderma* **2006**, *136*, 75-86.

Mathys, R., Dittmar, J., Johnson, C. A., *Antimony in Switzerland - A substance flow analysis* **2007**, Swiss Federal Office for the Environment.

McCallum, R. I., Occupational exposure to antimony compounds, *J. Environ. Monit.* **2005**, *7*, 1245-1250.

Mishra, J., Saxena, A., Singh, S., Chemotherapy of leishmaniasis: Past, present and future, *Curr. Med. Chem.* **2007**, *14*, 1153-1169.

Nakamaru, Y., Tagami, K., Uchida, S., Antimony mobility in Japanese agricultural soils and the factors affecting antimony sorption behavior, *Environ. Pollut.* **2006**, *141*, 321-326.

Nakamaru, Y. M., Sekine, K., Sorption behavior of selenium and antimony in soils as a function of phosphate ion concentration, *Soil Science and Plant Nutrition* **2008**, *54*, 332-341.

Nriagu, J. O., Pacyna, J. M., Quantitative Assessment Of Worldwide Contamination Of Air, Water And Soils By Trace-Metals, *Nature* **1988**, *333*, 134-139.

Oorts, K., Smolders, E., Degryse, F., Buekers, J., Gasco, G., Cornelis, G., Mertens, J., Solubility and toxicity of antimony trioxide (Sb_2O_3) in soil, *Environ. Sci. Technol.* **2008**, *42*, 4378-4383.

Paoletti, F., Sirini, P., Seifert, H., Vehlow, J., Fate of antimony in municipal solid waste incineration, *Chemosphere* **2001**, *42*, 533-543.

Porquet, A., Filella, M., Structural evidence of the similarity of Sb(OH)(3) and As(OH)(3) with glycerol: Implications for their uptake, *Chem. Res. Toxicol.* **2007**, *20*, 1269-1276.

Rauser, W. E., Phytochelatins And Related Peptides - Structure, Biosynthesis, And Function, *Plant Physiol.* **1995**, *109*, 1141-1149.

Rish, M. A., Antimony. In: *Elements and their Compounds in the Environment* (Eds Merian, E., Anke, M., Ihnat, M., Stoeppler, M.) **2004**, pp. 659-670 (Wiley-VHC: Weinheim).

Rooney, C. P., McLaren, R. G., Cresswell, R. J., Distribution and phytoavailability of lead in a soil contaminated with lead shot, *Water Air Soil Pollut.* **1999**, *116*, 535-548.

Rosen, B. P., Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes, *Comp. Biochem. Physiol. A-Mol. Integr. Physiol.* **2002**, *133*, 689-693.

Schutzendubel, A., Polle, A., Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization, *J. Exp. Bot.* **2002**, *53*, 1351-1365.

Steely, S., Amarasiriwardena, D., Xing, B., An investigation of inorganic antimony species and antimony associated with soil humic acid molar mass fractons in contaminated soils, *Environmental Pollution* **2007**, *148*, 590-598.

Swartjes, F. A., Risk-based assessment of soil and groundwater quality in the Netherlands: Standards and remediation urgency, *Risk Analysis* **1999**, *19*, 1235-1249.

Wagner, S. E., Peryea, F. J., Filby, R. A., Antimony Impurity in Lead Arsenate Insecticide Enhances the Antimony Content of Old Orchard Soils, *Journal of Environmental Quality* **2003**, 736-738.

WHO, *Antimony in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality* **2003**.

Wilson, N. J., Craw, D., Hunter, K., Antimony distribution and environmental mobility at an historic antimony smelter site, New Zealand, *Environ. Pollut.* **2004**, *129*, 257-266.

Wysocki, R., Clemens, S., Augustyniak, D., Golik, P., Maciaszczyk, E., Tamas, M. J., Dziadkowiec, D., Metalloid tolerance based on phytochelatins is not functionally equivalent to the arsenite transporter Acr3p, *Biochem. Biophys. Res. Commun.* **2003**, *304*, 293-300.

Zenk, M. H., Heavy metal detoxification in higher plants - A review, *Gene* **1996**, *179*, 21-30.

3 Antimony in the soil-plant system – a review

Martin Tschan, Brett Robinson and Rainer Schulin

Environmental Chemistry **2009**, *6*, 106-115

Environmental Context

Soil contamination by antimony (Sb) has become an environmental problem of much concern in recent years, because increasing mining and industrial use has led to widespread soil contamination by this biologically unessential, but potentially carcinogenic element. We reviewed the available literature and found that Sb is generally taken up by terrestrial plants in proportion to the concentration of soluble Sb in soil over a concentration range covering five or more orders of magnitude, a finding that is relevant in particular for the assessment of environmental and health risks arising from Sb contaminated soils. But very little is known about the mechanisms of Sb uptake by plants.

Abstract

Soil contamination by antimony (Sb) due to human activities has considerably increased in the recent past. We reviewed the available literature on Sb uptake by plants and toxicity risks arising from soil contamination by Sb and found that Sb is generally taken up by terrestrial plants in proportion to the concentration of soluble Sb in soil over a concentration range covering five or more orders of magnitude. However, very little is known about the mechanisms of Sb uptake by plants. Also the deposition of resuspended soil particles on the surfaces of aerial plant surfaces can result in high plant Sb concentration in the vicinity of Sb-contaminated sites. Although soil pollution by Sb may be rarely so severe as to cause toxicity problems to humans or animals consuming plants or food derived from plants grown on Sb-contaminated sites, such risks may arise under worst-case conditions.

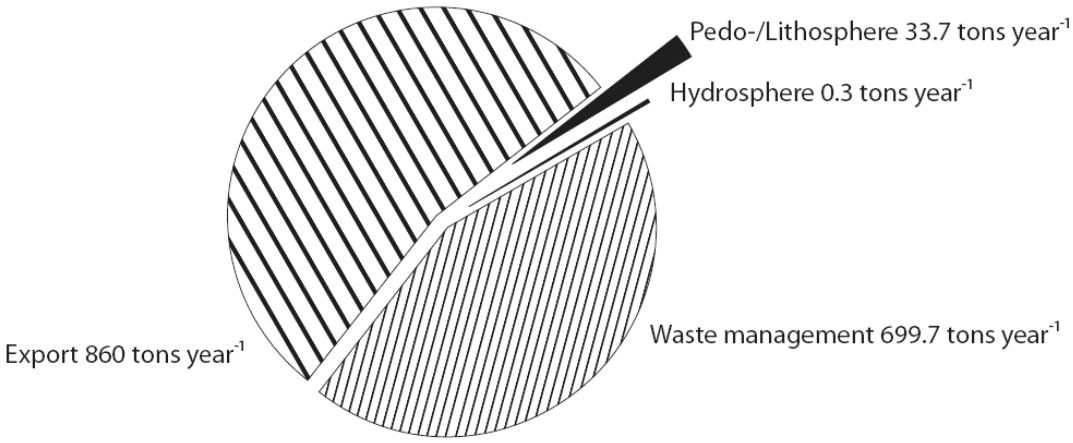

Fig. 1. Fate of Sb consumed in Switzerland. Adapted from Mathys et al.(2007)

3.1 Introduction

Natural concentrations of antimony (Sb) in the environment are low. Its abundance in the earth's crust is in the order of 0.2 – 0.3 mg kg^{-1} (Fowler and Goering 1997). In topsoils, Sb tends to be slightly enriched. Background concentrations of antimony in soils were reported to range between 0.3 and 8.6 mg kg^{-1} (Table 1) (Johnson et al. 2005, Kabata-Pendias and Pendias 1984). In general they are below 1 mg kg^{-1} (Lintschinger et al. 1998). Higher concentrations are usually related to anthropogenic sources. Antimony has many uses, and these are still increasing (Filella et al. 2002, Mathys et al. 2007). In its metallic form its major use is as a hardener for lead, e.g. in lead-acid batteries, cable sheathings and ammunition, and it is also an important component in semiconductors. Antimony trioxide (Sb_2O_3) is widely used as a flame retardant, e.g. in textiles, papers, plastics and adhesives. Therefore textiles and plastics are major sources of Sb in municipal wastes (Paoletti et al. 2001). Furthermore, antimony trioxide is used as a paint pigment, ceramic opacifier, catalyst, mordant and glass decolouriser. Antimony tetroxide (Sb_4O_8) is used as an oxidation catalyst, particularly for the oxidative dehydrogenation of olefins. The wide use of Sb has led to considerable inputs of this element into the environment (Figure 1). Important anthropogenic sources of antimony in the environment are emissions from vehicles (where it is used as fire retardant in brake linings), waste disposal and incineration, fuel combustion, metal smelters, and shooting activities (Amereih et al. 2005, Cal-Prieto et al. 2001).

Particularly high Sb concentrations in soils have resulted from mining and shooting activities. For example, Sb concentrations up to 15 g kg^{-1} were found in an area in southern Tuscany where Sb ores have been mined and smelted until recently (Baroni et al. 2000). Reuse of

Table 1. Published values of Sb concentrations in the environment.

Medium	Source of pollution	Concentration (mg kg^{-1}, unless given otherwise)	Reference
Igneous rocks	-	0.1-1	(Butterman and Carlin 2004)
Sedimentary rocks	-	0.05-1.5	(Bowen 1979)
Soil background	-	0.3-2.3	(Kabata-Pendias and Pendias 1984)
Seawater	-	0.0002	(Filella et al. 2002)
Water near mine	Mercury mine	418	(Gemici and Tarcan 2007)
Soils near mine	Mercury mine	0.5-52	(Gemici and Tarcan 2007)
Emissions waste deposit	Waste deposit	10 μg m^{-3}	(Hirner et al. 2000)
Roadside highway (100m distance)	Traffic pollution	0.53	(Cal-Prieto et al. 2001)
Needles of trees	Residential area	2.4	(Pohl et al. 2003)
Orchard soil	Pesticide	0.4-1.5	(Wagner et al. 2003)
Grass	Antimony smelter	400	(Ainsworth et al. 1990a)
Garden soil near disused mine	Past mining activities	500	(Hammel et al. 2000)
Detritivore invertebrates	Smelter	290	(Ainsworth et al. 1990b)
Ectomycorrhizal fungi	Lead smelter	100-1400	(Borovicka et al. 2006)
Aquatic plants	Mine tailing pond	19	(Hozhina et al. 2001)
Garden and crop plants	Spiked pots	399	(Hammel et al. 2000)
Shooting range	Bullets	13800	(Johnson et al. 2005)

material from mining dumps has also been identified as a cause of severe Sb contamination of agricultural land and residential areas (Flynn et al. 2003, Hammel et al. 2000). Recently, also shooting ranges were found not only to be hot spots of soil pollution by lead (Pb), but also by Sb (Johnson et al. 2005). Antimony is used in bullets to harden them. Next to Pb, Sb generally

is their second-most important component with a content ranging between 1 and 7% by mass (Rooney et al. 1999). Johnson et al. found up to 13.8 g kg^{-1} Sb in the topsoils of Swiss shooting ranges (2005). Although such pollution is usually restricted to very small areas, it constitutes a major environmental problem because of the abundance of shooting ranges. Switzerland alone has more than 2000 shooting ranges that are still in use.

Antimony has no known essential biological function. Similar to other trace elements, it can be toxic at elevated concentrations, and some Sb compounds are even considered potentially carcinogenic (1996). A potentially important Sb exposure pathway of humans and animals to antimony in areas with contaminated soils is through food and feed plants (Ainsworth et al. 1990, Hammel et al. 2000). However, owing to the fact that Sb mostly occurs as a co-contaminant of more toxic elements such as Pb or As, research into its biogeochemistry and ecotoxicity has been neglected in the past. Thus, little is still known also about the factors determining the phytoavailability of Sb in soils and its uptake by crop plants. The objective of this study was to review the available literature on the transfer of Sb from soil into plants and on the toxicity risks associated with soil pollution by this element.

3.2 Toxicity of antimony to plants and soil organisms

In general, inorganic Sb compounds have been found to be more toxic than organic ones, and Sb^{III} more than Sb^{V} species (Filella et al. 2002). In its trivalent form, Sb may have a similar level of genotoxicity to trivalent As (Gebel 1997). Flynn et al. found that the bioluminescence of As and Sb-specific biosensors (*Escherichia coli* strain CM1166 pC200) was suppressed at Sb^{III} concentrations in solution exceeding 1 mg L^{-1} (Flynn et al. 2003). Although sodium stibogluconate has been used for more than 75 years to treat leishmaniasis, a disease caused by the protozoan parasite *Leishmania* sp. and affecting 12 million people in 88 countries (in 2007) (Mishra et al. 2007), not much is known about the toxicity of elemental and methylated Sb.

Only a few toxicity tests have been performed with plants or other organisms exposed to Sb-contaminated soil. Table 2 summarises half maximal effective concentration (EC_{50}) values for various soil organisms and terrestrial plants. According to the data given in Table 2, plants were generally found to be more tolerant to soil Sb than soil fauna.

Hammel et al. found that aqueous extracts of a Luvisol and a Chernozem that had been spiked with 1000 mg kg^{-1} dry weight (DW) Sb_2S_3 and Sb_2S_5 and aged for 6 months were more toxic to the soil alga *Chlorococcum infusionum* than similar extracts from a Cambisol (1998).

Table 2. Published half maximal effective concentration (EC_{50}) values for Sb toxicity to plants and soil organisms.

Organism		Soil type	Test variable	Sb compound	EC_{50} mg kg^{-1}	EC_{50} mg L^{-1}	References
common name	Latin name				total Sb concentration in soil	soil solution concentration	
Earthworm	*Eisenia fetida*	Typic Haplodult	Reproduction	$Sb_2(SO_4)_3$	70		(Kuperman et al. 2006)
Potworm	*Enchytraeus crypticus*				316		
Collembola	*Folsomia candida*				169		
Soil algae	*Chlorococcum infusionum*	Cambisol	Chlorophyll density	$K(SbO)C_4H_4O_6$	>1000		(Hammel et al. 1998)
		Luvisol			>1000		
		Chernozem			>1000		
		Cambisol		Sb_2S_3	>1000		
		Luvisol			257		
		Chernozem			125		
		Cambisol		Sb_2S_5	>1000		
		Luvisol			172		
		Chernozem			314		
Barley	*Hordeum vulgare* cv. Mauritia	Haplic Luvisol	Root elongation	Sb_2O_3	6819	39	(Oorts et al. 2008)
Lettuce	*Lactuca sativa* cv. Pontiac		Shoot yield		7549	41.4	

Table 2 (continued)

Organism		Substrate	Test variable	Sb compound	EC_{50} mg kg $^{-1}$	EC_{50} mg L^{-1}	References
common name	Latin name				total Sb concentration in soil	soil solution concentration	
Clover	*Trifolium pratense* cv. Milvus	agar	Root elongation	$KSb(OH)_6$	1247		(Tschan et al. 2009)
		potting soil	Shoot yield	$KSb(OH)_6$	1111	10	
Maize	*Zea mays* cv. Magister	agar	Root elongation	$KSb(OH)_6$	829		
		potting soil	Shoot yield	$KSb(OH)_6$	1178	10.6	
Indian Mustard	*Brassica juncea*	agar	Root elongation	$KSb(OH)_6$	> 2463		
Sunflower	*Helianthus annuus* cv. Iregi	agar	Root elongation	$KSb(OH)_6$	829		
		potting soil	Shoot yield	$KSb(OH)_6$	1122	10.1	
Wheat	*Triticum aestivum* cv. Galaxie	agar	Root elongation	$KSb(OH)_6$	631		

Oorts et al. found 50% reduction of root elongation in barley and 50% reduction of shoot growth in lettuce at ~40 mg L^{-1} Sb in centrifuged soil solution after spiking the soil used in their study with ~7 g kg^{-1} Sb in form of Sb_2O_3 (2008). At the time of sampling, ~70% of the Sb in solution was present as Sb^V. He and Yang found no significant difference in the toxicity of Sb^{III} and Sb^V on root and shoot growth of rice grown in pots (1999).

Davis et al. showed that phytotoxicity of Sb does not necessarily require Sb accumulation in the shoots. Growth of barley (*Hordeum vulgare*) was depressed in sand cultures at concentrations of 50-100 mg L^{-1} Sb in solution, although Sb was below the detection limit in the shoots (< 2 mg kg^{-1}) (1978).

3.3 Uptake of antimony by plants

3.3.1 Relationship between plant and soil Sb concentrations

Antimony uptake by plants has been found to vary widely among plant species and study sites (Table 3). Particularly high plant Sb concentrations occur in Sb mining areas. Baroni et al. (2000) found up to 1367 mg kg^{-1} Sb in the basal leaves of *Achillea ageratum* growing in southern Tuscany at a tailing pond in an abandoned Sb mining area where the soil contained ~9000 mg kg^{-1} Sb with an extractable concentration of 793 mg kg^{-1}. Foliar Sb concentrations exceeded 100 mg kg^{-1} also in other plant species on this site.

In contrast to these high concentrations, there are also reports of low Sb uptake from plants growing on heavily contaminated soils. Pratas et al. (2005) reported maximum Sb concentrations of less than 5 mg kg^{-1} Sb in tree stems and herbaceous plants growing on mine spoils of abandoned mines in Portugal with an average soil Sb concentration of 663 mg kg^{-1}. In comparison to the study of Baroni et al., Sb uptake was not only much less in absolute terms, but also relative to the soil Sb concentration in the Portuguese study. Relatively low soil-to-plant transfer or bioaccumulation coefficients were also reported by Dominguez et al. (2008) from the Guadiamar valley of southern Spain, where soils had been covered by mine tailing sludges after the dam break at Aznalcóllar. Antimony concentrations of woody plant leaves ranged between 0.03 and 0.07 mg kg^{-1} on soil that contained between 4.5 and 37.7 mg kg^{-1}.

Sb, which corresponds to bioaccumulation coefficients of less than 0.03. Similar bioaccumuation rates were also obtained by Leduc and Gardou, who analysed plants growing on Sb-rich ore deposits near Brouzils in the Vendée department, France (1992). The Sb

Table 3 Summary of studies investigating Sb uptake by plants.

Author	Origin of Sb	Plants species	Wild pl. or Lab pl.	Conc of Sb in soil. in mg kg–1	Method for extr Sb
Affolter and Enggist 1995	Shooting ranges	Pooled samples, grasses or herbs, tested unwashed (feed quality)	wild plants	6.1–208	–
Ainsworth et al. 1990	Sb smelter	*Dactylis glomerata* and *Festuca rubra*	wild plants	at 100 m from source: 360 ± 20; at 250 m: 210 ± 10	–
Baghour et al. 2001	Field irrigated polluted water	*Solanum tuberosum*	Field exp.	Extractable Sb 0.021	unknown
Baroni et al. 2000	Abandoned Sb mining site, Tuscany, Italy	*Achillea ageratum*, *Plantago lanceolata*, *Silene vulgaris*	wild plants	Total conc.: old field: 27.7 ± 4.3, mine dump: 192.4–15112.9, tailing pond: 6529.7–7591.7; Extractable fraction in soil: old field: 0.18 ± 0.05, mine dump: 1.8–14.9, tailing pond: 105.7–792.6	1/40 soil/liquid 0.43 mol acetic acid
Borovicka et al. 2006	Mining area	different species of Macrofungi	wild plants	Median total conc.: unpolluted 4.9 and polluted 62.3	–
Craig et al. 1999	Mining Area (Louisa Sb mine, Scotland)	Liverwort and moss, no exact Latin names given	wild plants	n.d.	–
Davis et al. 1978	Sand culture	Spring barley (*Hordeum vulgare*)	greenhouse	Sand cultures with solutions added every second day: Sb2(SO4)3 at levels: 10, 20, 50, 100	–
De Gregori et al. 2001	Mining activities especially emissions	alfalfa (*Medicago sativa*)	wild plants	La Greda: 6.8, Campiche: 13	–
De Gregori et al. 2004	Mining sites in Chile	alfalfa (*Medicago sativa*)	field experiment	0.42–11.0 mg kg–1	2g in 20 mL 0.05 M EDTA, HG-AFS
Dodd et al. 1996	Gold mine area, collection areas in lakes affected by effluent	Pondweed (*Potamogetan pectinatus*)	wild plants	Sediment Keg Lake: 18.0, Kam Lake: 257, data from Bright et al. 1994	–
Dominguez et al. 2008	Area affected by mine spill	*Olea europaea*, *Phyllirea angustifolia*, *Pistacia lentiscus*, *Populus alba*, *Quercus ilex*, *Retama sphaerocarpa*, *Rosmarinus officinalis*, *Tamarix africana*	wild plants	Soil: 4.5–37.7	–

Sb conc. in plants mg kg–1 DW	Sb detection method	Key findings
0.6–201	HCl extraction for plants, according to Swiss law, detection method unknown	Limit acces to stop butts, because of the high lead concentrations. Lead, rather than Sb, is considered the primary contaminant. There are no regulatory values for Sb in soil. Soil Sb concentrations are correlated with lead, reflecting the bullets' composition.
at 100 m from source: 48–336; at 250 m: 8–30; in glasshouse with soil from 100 m site: 1.72–2.23; from 250 m site: 0.26–0.33	F-AAS, HG-AAS	Antimony concentrations in surface soils were decreased with increasing distance from an Sb smelter. Field exposure of grass in pots of uncontaminated soil and a laboratory experiment using soils from near the smelter indicated that the Sb in vegetation was largely due to continued aerial deposition and not to uptake from soil.
2.2–2.7 in leaves varying with root temperature 16–30°C	GFAAS	Root temperatures significantly influenced the phytoaccumulation of Sb in different organs of potato plants, with root-zone temperature of 20°C resulting in the highest uptake and accumulation of Sb in the leaves of S. tuberosum.
Sb conc. in shoots: *A. ageratum*: old field: 6.09 ± 1.4, mind dump: 8.1–228.0, tailing pond: 96.4–215.8; *P. lanceolata*: mine dump: 28.5–53.6, tailing pond: 274.6–569.3; *S. vulgaris*: mine dump: 17.6–35.5, tailing pond: 473.6–1163.8	HG-GFAAS	In the three plant species considered, Sb uptake was more efficient when the concentration of the element in the soil solution was low or intermediate (< 3 mg/L). There was a saturation of uptake pathways at higher concentrations in soil solution.
10–100 mg kg–1, up to 1423 in *Chalciporus piperatus*	INAA	Antimony content of soils was much higher than that of macrofungal fruit bodies. The Sb concentration of macrofungi was somewhat higher than that of vascular plants.
Liverwort: 8.9–57.3 Sb Dw total, 0.18–0.19 DW Dimethyl-Sb; moss: 19.9 Sb DW total, 0.10 DW Dimethyl-Sb	HG-QF-AAS for Methyl-Sb, ICP-MS (for totals)	These results indicate that previously reported problems with methylantimony analysis may be due to traces of oxygen, which in conjunction with the excess of hydride reagent used, may cause the formation of rearranged products.
Sb was not measurable due to low concentrations in plants. It may have depressed barley yields by reducing the availability or translocation of other nutrients, perhaps phosphate.		Barley yields were depressed by high concentrations of Sb (50 and 100 mg L–1) in the nutrient solutions but these elements could not be detected in the plant tops (< 2 mg kg–1).
La Greda: 0.39, Campiche: 0.17	HGAFS, ETAAS	All Sb concentration values were higher than those reported as average concentrations for soils (1 mg kg–1) and herbaceous vegetables (0.2 mg kg–1). These data possibly reflect the degree of contamination of the sampling sites.
0.02–1.7 mg kg–1 DW	HGAFS	There were significant correlations were obtained between the total and extractable Sb concentration of soils and the Sb concentration in alfalfa. Plant concentrations were better correlated with extractable antimony conecntrations whant the total concentrations in soil.
48–68 mg kg–1 DW in pondweed at affected lakes	Total Sb: NAA	These are the first results that indicate the presence of methylantimony compounds in biological systems. Corresponding methylated arsenic compounds are widespread in the environment and in samples of biological origin.
O. europaea: 0.031, *P. angustifolia*: 0.046, *P. lentiscus*: 0.028, *P. alba*: 0.037, *Q. ilex*: 0.070, *R. sphaerocarpa*: 0.067, *R. officinalis*: 0.046, *T. africana*: 0.070	ICP-MS	Despite high soil concentrations of Sb, there was a little transfer to the aboveground parts of woody plants.

Table 3 (continued)

Author	Origin of Sb	Plants species	Wild pl. or Lab pl.	Conc of Sb in soil. in mg kg–1	Method for extr Sb
Foster et al. 2005	Kerosene Creek, NZ	Moss and algae	wild plants	nd	–
Gordillo et al. 2001	San Antonio Mine, Spain	*Cistus ladanifer*	wild plants	nd	–
Hammel et al. 2000	Sb Mining area, Italy	19 vegetable and crop species	wild plants, cultivated	Up to 500, however only 0.06 to 0.59% extractable (0.3 –2.95)	2g in 50 mL 1 M NH4NO3, HG-GFAAS
Hozhina et al. 2001	Mining area collection ponds	*Typha latifolia*, *Scirpus sylvaticus*, *Phragmites australis*	wild plants	Bulk water of collection pond 3.6	–
Jung et al. 2002	Cu-W mine, South Korea	*Allium cepa*, *Capsicum annuum*, *Glycine max*, *Perilla frutescens*, *Zea mays*, *Zizyphus jujuba*	wild plants	Korea: acidic, sandy soils, Mine dump soils: 6–165, alluvial and high land: 2.0–3.7, household garden soils: 1.4–12.6, control site soils: 0.9–1.5 mg kg–1	–
Kanias et al. 1992	Saronikos Gulf of Greece	8 brown algae species	wild plants	nd	–
Kawamoto and Morisawa 2003	River, with treated waste waters from dye factories	Aquatic plants and algae	wild plants	River water: 0.3–4.0, Sediment 2.7–3.4, shellfish 0.1–2.2, fish 0.06–3.85	–
Koch et al. 1997	Yellowknife Bay, Canada, mine tailings	pondweed (*Potomogetan pectinatus*)	wild plants	nd	–
Krachler et al. 1999	Residential area	Leaves from elder bushes (*Sambucus nigra*)	wild plants	nd	–
Leduc and Gardou 1992	Sb, As ore body	*Twigs of Quercus pedunculata*, *Carpinus betulus*, *Crataegus laevigata.*	wild plants	Average 38, max: 105	–
Li and Thornton 1993	Old lead mining sites	*Holcus lanatus*, *Lolium perenne*, *Festuca rubra*	wild plants	range: 0.65–130 in top soil, depending on site	–
Massard et al. 2006	Shooting range soil	*Trifolium pratense*, *Lolium perenne*	greenhouse	Spiked soils from a shooting range, solube Sb conc.: 0.8–22	1:2,5 w/v 0.1 mol/L KNO3
Miravet et al. 2005	Abandoned Sb mine, Spain	*Hypnum cupressiforme*, *Dryopterix filix-max*, *Stellaria halostea*, *Chaenorhinum asarina*	wild plants	nd	–

Sb conc. in plants mg kg–1 DW	Sb detection method	Key findings
Moss: 49 mg kg–1, Algae: 1.5 mg kg–1	HPLC-ICP-MS	Analogous to arsenic and other elements such as cadmium, Sb in algae and plants is likely to be present in the inorganic form and complexed with phytochelatins or may be present as Sb nano-crystallites
Mean: 27.3 mg kg–1, range = 1.7–70 mg kg–1	PSA	A potentiometric stripping analysis method ihad sufficient precision and accuracy to determine antimony concentrations in selected vegetation samples.
Storage organs: up to 0.09, shoots and leavers: up to 0.34 and 2.2. However, for the generative parts (grain, fruit) a transport barrier exists.	HGAAS	Consumption of the analyzed crops and vegetables should present no health risk. However, a pot experiment with soils spiked with similar antimony contents had a high mobile fraction that may leach or be taken up by plants.
T. latifolia: 15, *S. sylvaticus*: 19, *P. australis*: 15	FAAS/GFAAS	The plants studied could be used to decrease Sb discharges into the environment: hornwort as hyperaccumulator for decontaminating drainage water and cattail and bulrush for decontaminating soil around collection ponds.
Conc. in all plants below 0.01, except *G. max* leaves 0.01–0.04 at mining site and *A. cepa* at mining site 0.01–0.04	HG-ICP-AES	Concentrations of Sb in crop plants are species dependant. Higher concentrations occur in leaves than in grains and fruit. There was no relationship between Sb concentrations in plants and those in the soils.
Average Sb conc.: 0.16, range: 0.06–0.29	INAA, X-ray spectroscopy	There was no correlation between antimony concentration and the fatty acids in algae.
Sb conc. in fresh weight, green algae 6.8–8.2, leaves of plants 2.8–6.7, roots of plants 7.1–7.2	ICP-MS	There was no clear relationship between the concentrations in river water and those in biota.
Conc. in pondweed: extract average 0.27 (0.21 control), digestion average: 39.5 (37.2 control)	Extraction (water methanol, sonication) and acid digestion, ETAAS, (ICPMS: control)	Both ETAAS and ICPMS gave good results. They were no significant differences between the two.
153 ± 5 ng g–1	HGAAS	The developed procedure for the determination of Sb in plant materials, based on flow injection hydride generation atomic absorption spectrometry, proved to be a reliable method for concentrations in the digests down to the low pg g–1 level.
Max conc. on highest soil conc.: *Q. pedunculata*: 0.23, *C. laevigata*: 0.19, *C. betulus*: 0.12	INAA	The results of the biogeochemical tests carried out on the lodging with Sb of Brouzils showed that the ends of branches of pedunculate oak and, to a lesser degree, common hawthorn were adequate materials for the biogeochemical prospecting of Sb ore bodies in the Vendée.
0.06–0.27 in herbage	HG-ICP-AES	Concentrations of Sb in pasture herbage were generally low even on the highly onctaminated soils, however, unwashed herbage samples showed markedly higher concentrations. Sb concentrations in herbage reflected the degree of soil contamination.
T. pratense: 4–44, *L. perenne*: 2–5, depending on soluble Sb concentration.	HG-AFS	The Sb concentrations in plants had no observable toxic effects and did not reduce yield.
H. cupressiforme: 27.1 ± 4.0, *D. filix-max*: 17.1–28.2, *S. halostea*: 17.9 ± 1.0, *C. asarina*: 26.2 ± 5.5	HG-AFS, ICP-MS	Both inorganic, Sb(V) and Sb(III), and methylated species were measured in several terrestrial plants grown in this polluted area.

Table 3 (continued)

Author	Origin of Sb	Plants species	Wild pl. or Lab pl.	Conc of Sb in soil. in mg kg–1	Method for extr Sb
Miravet et al. 2006	Abandoned Sb mine, Spain	*Hydnum cupressiforme*, *Dryopteris filix-max*, *Stellaria halostea*, *Chaenorhinum asarina*	wild plants	nd	–
Murciego Murciego et al. 2007	Sb-mining area	*Cytisus striatus*, *Cistus ladanifer*, *Dittrichia viscosa*	wild plants	Means for total conc. range from 225 to 2243.8 for polluted areas, mobile fraction ranges from 1.37 to 2.10%	100 g in 1000 mL water
Oorts 2008	Pot experiment with uncontaminated soil	*Lactuca sativa*, *Hordeum vulgare*	lab	1996.9 mg kg–1	centrifugation
Picard and Bosco 2003	Abandoned Sb mining site, Tuscany, Italy	*Achillea ageratum*	lab	Soil total conc.: 27.7–16388.8, extractable: 0.2–798.4	1/40 soil/liquid 0.43 mol acetic acid
Pohl et al. 2003	Urban areas, villages in Poland and Norway	Pine (*Pinus sylvestris*), Spruce (*Picea abies*), Yew (*Taxus baccata*), Thuya (*Thuja occidentalis*)	wild plants	nd	–
Pratas et al. 2005	Abandoned mine area, Portugal	*Pinus pinaster*, *Chamaespartinum tridentatum*, *Genista triacanthos*, *Calluna vulgaris*, *Erica umbellata*, *Quercus ilex*, *Q. suber*, *Helichrysum stoechas*, *Andryala integrifolia*, *Digitalis purpurea*, *Agrostis curtisii*, *Cistus ladanifer*, *Halimium ocymoides*, *Eucalyptus globulus*, *Lavandula stoechas*, *Rubus ulmifolius*	wild plants	mean: 663.1, median: 87.8, range: 30.5–5986.4	–
Rached-Mosbah et al. 1992	Abondoned mine, Algeria	*Limonium ramosissimum*, *Hedyrasum pallidum*, *Plantago coroponus*, *Thymus algeriensis*, *Artemis herba-alba*, *Carduncellus pinnatus*, *Centaurea incana ssp. Pubescens*, *Asperula hirsute*, *Convolvulus lineatus*, *Ampelodesma mauritanica*, *Lygeum spartum*, *Stipa parviflora*, *Dactylis glomerata*, *Stipa tenacissima*, *Atractylis cancellata*	wild plants	Extractable Sb in soil: <1.25–167.5	2mol L–1 HNO3 extraction
Tighe et al. 2005	Mining site and floodplain downstream	*Cynodon dactylon*, *Paspalum distichum*	wild plants	Soil mean: 9.9, range: 1.8–18.1	–

Sb conc. in plants mg kg–1 DW	Sb detection method	Key findings
Average total Sb conc.: *H. cupressiforme*: 7.8; *D. filix-max* (sample a): 4.9; *S.halostea*: 2.2; *D. filix-max* (sample b): 8.0; *C. asarina*: 7.5	IC-UV-HG-AFS	On-line UV photooxidaton by peroxodisulfate coupled to ion chromatography hydride generation atomic fluorescene spectroscopy was a suitable method for the seperation and determination of Sb species in plant samples.
C. striatus: 1.0, *C. ladanifer*: 3.9–59.3, *D. viscosa*: 266.6–1136.0	Total Sb in soils and plants: INAA, soil extracts: HG-AAS	Antimony concentrations in the leaves of *Cistus ladanifer* were greater in autumn than in spring in the two mining areas examined.
0.24–24.4 mg kg–1 DW	ICP-MS ICP-OES	The long term equilibration test (31 weeks) was still insufficient to bring about equilibrium soil solution concentrations (i.e. complete transformation of Sb2O3). Toxic effect could be detected in plants grown in soil containing with 2000 mg kg–1 Sb in soil.
Auxin production in bacteria from rizhosphere is the highest in most polluted soil and at the time of flowering.	–	The results of this research have practical importance in the context of using plant growth-promoting agents in heavy metal phytoextraction experiments. Only the ARDRA-1 auxþ genotype was present in the *Achillea ageratum* rhizosphere throughout plant development, regardless of the Sb concentration present in the soil. It was hypothesised that if it were included in an inoculum, it would efficiently colonise the rhizosphere.
P. sylvestris: 0.02–1.09; *P. abies*: 0.5–0.95; *T. baccata*: 0.58 ± 0.21; *T. occidentalis*: 2.37 ± 0.53	HG-ICP-AES	Inductively coupled plasma atomic emission spectrometry with on-line hydride generation in the system and the introduction of aerosolised sample into the plasma was suitable for the determination of Sb of conifer needles.
Sb conc. in plants: mean: 7.16, range: 0.04–139.9	HG-AAS	*Digitalis purpurea*, *Cistus ladanifer* and *Calluna vulgaris* were good indicators of Sb in soil.
Sb conc. range: <1.25–98	AAS	Antimony does not appear to be toxic for plants, which can result in high concentrations in the aerial tissues. Plants adapt either an exclusion or accumulation strategy. Plants are grazed by the cattle or collected as food or aromatic plants could result in the entry of Sb into the food chain.
C. dactylon: 0.4, 0.1–2.2; *P. distichum*: 0.3, 0.06–0.86	ICP-MS	Analysis of pastures shows variable enrichment of Sb in the plant species tested, and a strong relationships between total soil Sb levels and pasture Sb concentrations.

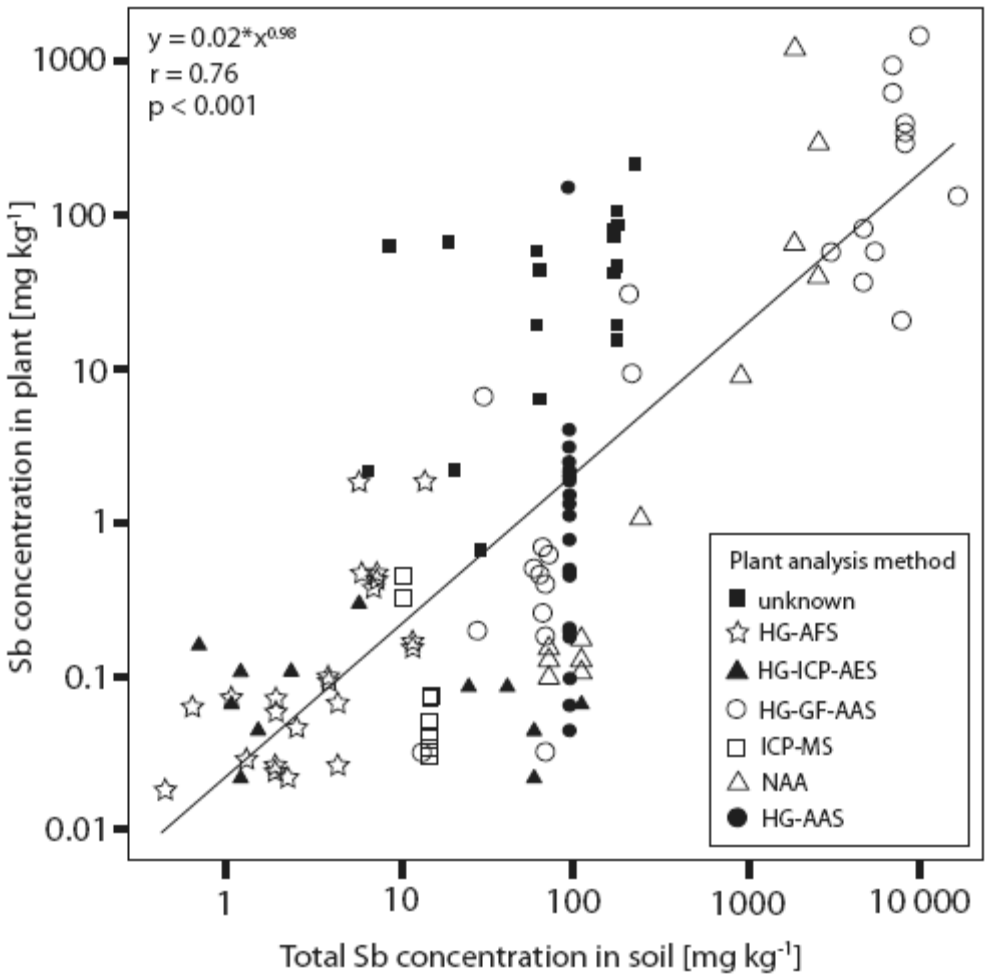

Figure 2. Relationship between total Sb concentrations of field-contaminated soils and Sb in plants grown on these soils, based on published data.(Affolter and Enggist 1995, Baroni et al. 2000, De Gregori et al. 2004, De Gregori et al. 2003, De Gregori et al. 2001, Dominguez et al. 2008, Hammel et al. 2000, Jung et al. 2002, Leduc and Gardou 1992, Li and Thornton 1993, Murciego Murciego et al. 2007, Pratas et al. 2005, Rached-Mosbah et al. 1992, Tighe et al. 2005) The points represent averages of replicate samples or pot trials. Regression was performed on the log-transformed data. The slope of the log-log regression line is not significantly different from 1. Methods used for Sb analysis: HG-AFS, hydride generation atomic fluorescence spectrometry; HG-ICP-AES, hydride generation inductively coupled plasma atomic emission spectrometry; HG-GF-AAS, hydride generation graphite furnace atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; NAA, neutron activation analysis; and HG-AAS, hydride generation atomic absorption spectrometry.

concentration of the sampled soil averaged 38 mg kg^{-1}. On soil with the maximum Sb concentration of 105 mg kg^{-1}, oak branches accumulated 0.23 mg kg^{-1} Sb in their tips and hawthorn branches 0.19 mg kg^{-1} Sb.

Rather low plant Sb concentrations were also reported in studies of roadside soils and residential areas where soil Sb concentrations were elevated to some, but not high degree, presumably owing to atmospheric deposition. Lehndorff and Schwark measured Sb concentrations of around 0.5 mg kg^{-1} dry weight in the needles of pines (*Pinus nigra*) growing in a heavy traffic area in Cologne, Germany (2008). Krachler at al. found concentrations up to 0.15 mg kg^{-1} Sb dry weight in leaves of elder bushes in a residential area (1999). Also in a

residential area, Pohl et al. measured up to 2.4 mg kg^{-1} Sb in the dry mass of thuja needles (2003).

These results indicate that plant Sb accumulation increases with the Sb concentration of soil over a very wide range of concentrations. In fact, combining available published data from field studies in which Sb concentrations have been analyzed in plants as well as in the soils on which the sampled plants were growing produced a relationship with a significant correlation between plant and total soil Sb (Figure 2). Despite considerable scatter, this relationship is described well by a linear log-log regression model. The fact that the slope of the regression line approximately equals 1 means that the two variables are on average almost proportional to each other. A large part of the scatter is due to the variation in Sb solubility among the soils included in the studies. Performing the same analysis with soluble instead of total soil Sb concentrations, a similar but much closer relationship was obtained, although a variety different analytical methods were used and very different plants examined in the studies on which this analysis is based (Figure 3). Again the slope of the log-log line was almost exactly equal to 1, indicating proportionality. The average proportionality factor (i.e. ratio between plant and soil concentration or bioaccumulation coefficient) was 0.02 (kg soil per kg plant dry matter) for total soil Sb and 2.57 for soluble soil Sb. Similar linear relationships were also found within individual studies (Hammel et al. 2000, He and Yang 1999, Leduc and Gardou 1992, Tschan et al. 2008), indicating that proportionality between plant and soluble soil Sb indeed represents a common pattern. That fact that this proportionality is found to extend over at least five orders of magnitude of Sb concentrations suggests a very general and rather simple mechanism. A mechanism easily explaining it would be passive transport by convection with the stream of transpirational water into and through the plants. However, this hypothesis has to be reconciled with the existence of selective barriers in plant roots controlling the root-to-shoot transfer of water and solutes.

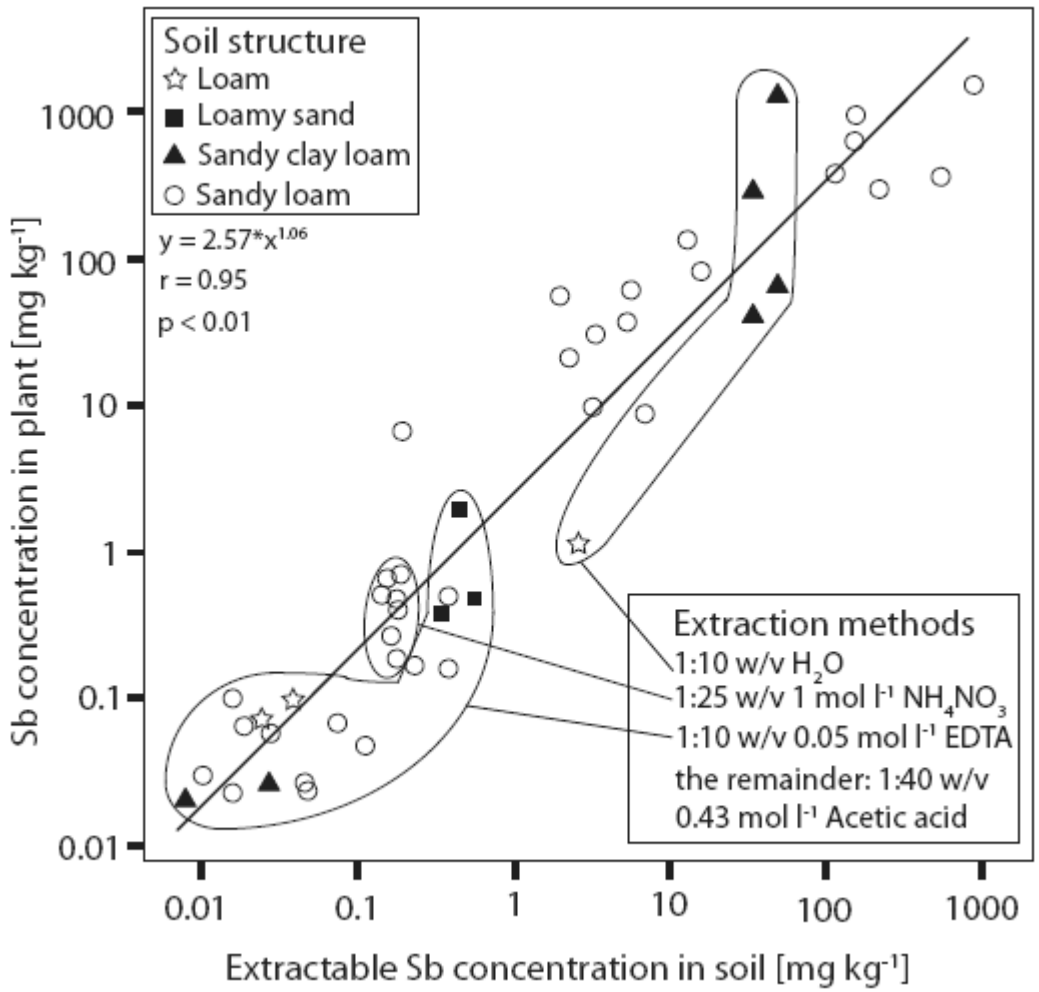

Figure 3. Relationship between soluble Sb concentrations of field-contaminated soils and Sb in plants grown on these soils, based on published data.(Baroni et al. 2000, De Gregori et al. 2004, De Gregori et al. 2003, De Gregori et al. 2001, Dominguez et al. 2008, Hammel et al. 2000, Murciego Murciego et al. 2007, Pratas et al. 2005, Rached-Mosbah et al. 1992) The points represent averages of replicate samples or pot trials. Regression was performed on the log-transformed data. The slope of the log-log regression line is not significantly different from 1.

3.3.2 Mechanisms of Sb uptake and root-to-shoot transfer

Little is known about the mechanisms of Sb uptake by plants. In microorganisms, $Sb(OH)_3$ was found to be taken up through aquaglyceroporins like $As(OH)_3$, which can be attributed to the small size of these two neutral molecules and their similarity in conformation and charge distribution with glycerol (Filella et al. 2007, Porquet and Filella 2007). Also active membrane transport of arsenite and antimonite is known in microorganisms. This transport is mediated by ArsB proteins, which are ATP-dependant efflux transporters for arsenite. Cai et

al. (1998) found that the same genes coding for the ArsB protein in *Pseudomonas aeruginosa* also contributed to Sb resistance when they were cloned and inserted into *E. coli*.

No such analogy has been found between Sb and its sister element As with respect to their uptake in oxidation state V. Whereas arsenate is transferred through cell membranes by the same transporters as phosphate, resulting in mutual inhibition of uptake due to competition between these two anions for transporter binding sites (Asher and Reay 1979), plant uptake of antimonate was not found to be affected by phosphate, suggesting that it does not occur along the same pathway (Tschan et al. 2008). This difference may be due to structural factors. The structure of antimonate is octahedral, whereas that of phosphate and arsenate is tetrahedral, and together with the larger size and lower charge density set antimonate clearly apart from other oxyanions (Table 4) (Tschan et al. 2008).

Also antimonite and arsenite do not always show similar effects. The deletion of genes responsible for the production of phytochelatins in *Schizosaccharomyces pombe* reduced the tolerance to arsenite and arsenate, but not to antimonite (Wysocki et al. 2003).

The presented evidence suggests that also the mechanisms of cellular uptake and transport in plants are also probably not the same for antimonate and antimonite. Antimonite may pass cell membranes passively with water through aquaporins. Such transport would be in line with the observed proportionality between plant and soluble soil Sb concentrations. Aquaporins, however, are not open for anions like antimonate, and cellular uptake of antimonate would require mediation by transporters. A linear rate law extending over a concentration range of several orders of magnitudes may be produced by a cascade of transporters with different kinetics, but is highly unlikely in the case of Sb, given that this element is not essential for organisms. However, as antimonate usually is the dominating Sb species in soil solution, a mechanism with a linear rate-dependence on concentration is also needed for this species in order to explain the observed linearity in the Sb uptake characteristics of plants. To understand how this may work, we have to consider the pathways along which solutes are transported from the soil solution into plant roots and shoots. There are two parallel transport pathways for water and solutes through plant tissues: the apoplastic pathway through intercellular spaces including pores in the cell walls and the symplastic pathway from cell to cell (selective transport across membranes). The symplastic pathway is only accessible by crossing a cell membrane. The apoplast of the root cortex is directly accessible to solutes from the external solution, whereas the apoplast of the root stele is separated from the cortex by the Casparian bands, thickenings impregnated with

Table 4. Structure and other molecular properties of antimonate in comparison to other common oxyanions in. Volumes and charge densities are calculated on the basis of bond lengths and van der Waals radii.

Molecule formula	Radius (Å)	Structure	Predominant species at pH 7	Volume ($Å^3$)	Charge density soils (e $Å^{-3}$)	Reference for bond length
$Sb(OH)_6^-$	3.68	Octahedral	$Sb(OH)_6^-$	209.2	-0.0048	(Palenik et al. 2005)
$B(OH)_4^-$	3.13	Tetrahedral	$B(OH)_4^-$	129.0	-0.0078	(Liu et al. 2007)
PO_4^{3-}	3.12	Tetrahedral	$H_2PO_4^-$	127.5	-0.0078	(Hoppe et al. 1998)
AsO_4^{3-}	3.36	Tetrahedral	$HAsO_4^{2-}$	158.3	-0.0126	(Hilmer and Dornberger-Schiff 1956)
MoO_4^{2-}	3.29	Tetrahedral	MoO_4^{2-}	149.3	-0.0134	(Kolitsch and Tillmanns 2003)
SeO_4^{2-}	3.16	Tetrahedral	SeO_4^{2-}	132.0	-0.0151	(Stalhandske 1981)
CrO_4^{2-}	3.14	Tetrahedral	CrO_4^{2-}	129.7	-0.0154	(Montgomery 1979)
SO_4^{2-}	2.99	Tetrahedral	SO_4^{2-}	112.0	-0.0179	(Zahrobsky and Baur 1965)

hydrophobic materials, in particular suberin, in the radial and transverse walls of the endodermis (Waisel et al. 1996). The function of the Casparian bands, is to force water and solutes to enter the symplastic pathway and pass through the endodermis cells in order to reach the inner cylinder of the root, which allows the plant to exert control on the uptake of solutes. The Casparian bands, however, are not a perfect barrier for apoplastic transport. Apart from 'leaks' at branching points where lateral roots emerge it is not fully developed at the root tips (Huang and Van Steveninck 1989), and the endodermis may also be damaged by chemical or biological agents, such as herbicides, toxic metals or root pathogens (Wenger et al. 2005). Using this bypass, even big molecules such as ethylenediamine-*N,N'*-disuccinic acid (EDDS) can be taken up along the apoplastic pathway and translocated from the roots into the shoots without any membrane passage (Tandy et al. 2006). Transport along this pathway would plausibly explain why Sb accumulation by plants was found to be proportional to the concentration of Sb in the soil solution.

Passive apoplastic transport also explains the pattern of Sb allocation within plants. Jung et al. (2002), who investigated Sb accumulation and allocation in crop plants on soil polluted with Sb, As and Bi around a mining area in Korea, found highest Sb concentrations in plant leaves and lowest in grains and fruits. Baroni et al. (2000) found that Sb was not deposited in the roots, but in the epigeal parts of *Achillea ageratum* and *Silene vulgaris* that died at the end of the growing season.

Although most of the available evidence suggests that Sb is translocated within plants primarily along the apoplastic pathway through the xylem, this does not exclude that some symplastic transport may occur. Studying the environmental distribution of the radioisotope ^{125}Sb emitted from a nuclear fuel processing plant, Ghuman et al. (1993) found that Sb deposited onto the leaves of *Agropyron dasystachyum, Artemisia tridentata* and *Chrysothamnus viscidiflorus* was transferred into the roots of these plants, indicating Sb transport through the phloem.

3.3.3 Influence of other factors than soil Sb concentration on plant Sb uptake

If Sb uptake by plant roots were primarily a passive process of convection with root water uptake, a close relationship with the rate of transpiration would be expected. No studies on such a relationship have been published so far, however. Also, it has not been investigated to what extent water consumption may explain differences in Sb uptake or accumulation observed among different plants. Such differences can be large as shown by studies of plants growing under the same conditions. Rached-Mosbah et al. (1992) found that some plant species in the area of the Djebel Hamimat, Algeria, seemed to exclude Sb, while others apparently accumulated it. An example for exclusion was *Lygeum spartum* L. Plants of this species growing on soil with 168 mg kg^{-1} Sb only had 17.5 mg kg^{-1} Sb in the leaves. Conversely, *Carduncellus pinnatus* (Desf.) DC. accumulated up to 61 mg kg^{-1} Sb on a soil with only 17.5 mg kg^{-1} Sb.

Temperature is another factor that has been found to influence Sb accumulation in plants. Baghour et al. (2001) investigated the effect of root-zone temperature on the accumulation of As, Ag, Cr and Sb in different organs of potato plants under field conditions by applying mulches. The soil was an alkaline loam with 21 µg kg^{-1} extractable Sb, associated with As, Ag, and Cr. The temperature range tested was 16°C to 30°C. The highest accumulation of Sb was 0.85 mg kg^{-1} in roots, 0.14 mg kg^{-1} in tubers and 1.64 mg kg^{-1} in stems at 30°C, and 2.70 mg kg^{-1} in leaves at 20°C. Although the growth was maximal, the Sb concentration of tubers and leaves was minimal at 23°C, indicating that Sb was more 'diluted' in the larger biomass produced at this temperatures. Also, at higher temperatures increased diffusion and desorption rates of soil Sb may have contributed to enhanced Sb uptake.

The study performed by Hammel et al. (2000) indicates that rates of short-range transport and phase transfer processes may be an important factor in limiting the transfer of Sb from soil

into plants. These authors conducted a pot experiment with spinach using contaminated garden and agricultural soils, soils from a mining area and artificially Sb-contaminated arable soils (aged for 6 months after spiking) and compared the uptake of Sb from the potted soils with the Sb concentrations of plant samples collected at the field sites. Approximately linear relationships between soil and plant Sb concentrations were found in the pot experiment, with soil-to-plant transfer factors ranging between 4.5 and 12.9 for NH_4NO_3-extractable soil Sb and 0.17 and 0.54 for total soil Sb (depending primarily on the soil type), whereas the uptake under field conditions was two to three orders of magnitude lower (ranging from <0.02 to 2.2 mg Sb per kg dry weight) and did not show a significant dependence on the NH_4NO_3-extractable soil Sb concentration (ranging from <0.02 to 0.29 mg Sb per kg soil) owing to the relatively large scatter in the measurements in this concentration range (Hammel et al. 2000). These results suggest that the availability of Sb for plant uptake was highly increased by the structural disruption and physical homogenization of the soils for the pot experiment.

In the context of bioaccessibility and bioavailability of Sb to uptake by plant roots, mycorrhyzal fungi may also play an important role. Borovicka et al. (2006) analyzed more than hundred species of macrofungi, ectomycorrhizal fungi and terrestrial saprobes in the vicinity of a lead smelter. Several of the tested ectomycorrhizal species were found to have accumualated more than 100 mg kg^{-1} Sb, and specimens of *Chalciperus piperatus* were found with up to 1400 mg kg^{-1} Sb. However, whether Sb is transferred from mycorrhizal fungi to host plants is not known.

3.3.4 Antimony uptake by plants via surface deposition

Plants may take up contaminants into their aboveground parts not only through the roots, but also through deposition of aerosols from the atmosphere onto the surfaces or aerial plant parts, where part of the contamination then can become so tightly bound or incorporated that they cannot be removed even by vigorous washing (Hinton et al. 1995). Like lead and mercury, antimony can also be transported over long distances through the atmosphere, before it is deposited on plant, soil or water surfaces. Analysing samples of the two moss species *Hylocomium splendens* and *Pleurozium schreberi*, Berg and Steinnes (1997) showed that Sb is among the elements that are transported over long distances even to the remotest parts of northern Norway. Cloy et al. (2005) and Shotyk et al. (2004) found that pattern of Sb and Pb concentrations in peat cores from remote areas of Switzerland and Scotland reflected the

history of anthropogenic air pollution over the past two millennia. Sb was found to be immobile in peat like Pb and also similarly distributed. Current anthropogenic Sb fluxes in the atmosphere are ~10 times greater than natural fluxes. Short-range atmospheric transport may be even more important, at least locally, than long-distance transport. In particular on highly contaminated sites with reduced ground vegetation cover, resuspension of soil particles by wind erosion or rain splash can be an important pathway of contaminant transfer from soil to plants.

As the following evidence demonstrates, atmospheric deposition of Sb onto plant surfaces may in fact be a dominating pathway in the soil-to-plant transfer of Sb under field conditions. It may explain part of the variability in plant Sb concentrations shown in Figs 2 and 3 in addition to the factors discussed before. Ainsworth et al. (1990a) measured ~300 mg kg^{-1} Sb in leaf samples of various grasses near an Sb smelter in north-east England, where soil Sb concentrations reached 400 mg kg^{-1}. A control experiments in which plants were grown under open-air conditions in pots with uncontaminated soil revealed that almost all this Sb uptake could be attributed to dust deposition onto the plant leaves. Robinson et al. (2008) obtained similar results at a highly polluted shooting range in Switzerland. Using iron as a reference element to determine the rate of resuspended soil deposition, they concluded that almost all Sb found in leaf and shoot samples from the study site was attributable to this pathway.

Short-range transfer of resuspended soil particles from contaminated soil to aerial plant parts would not invalidate the general relationship between total concentrations of Sb in soil and Sb in the aboveground parts of plants shown in Fig. 2. However, it cannot be expected to produce a close relationship between plant Sb and soluble Sb in soil. In fact, it may be one of the major reasons for the scatter in this relationship shown in Fig. 3.

Direct transfer of contaminants to the aerial parts of plants by dust deposition and rain splash can be avoided under greenhouse conditions. Unfortunately, only few experiments have been performed on Sb uptake by plants under such controlled conditions so far. These experiments, however, show the same type of linear relationships between plant and soil Sb concentrations as those shown in Figs 2 and 3 with comparable bioaccumulation coefficients, showing that high uptake of Sb is possible via the root-shoot pathway and not necessarily implies atmospheric deposition of resuspended contaminated soil particles (Hammel et al. 2000, Tschan et al. 2008).

In aquatic environments, Sb may also be directly transferred to submerged shoot and leaf surfaces through the water phase. This phenomenon has been shown for arsenic in aquatic vegetation and may also be involved in the uptake of Sb by aquatic plants reported in some

studies (Robinson et al. 2006). Hozhina et al. (2001) found that *Typha latifolia* and *Scirpus sylvaticus* growing around a mine tailing pond that contained up to 3.6 mg L^{-1} Sb, accumulated up to 19 mg kg^{-1} Sb. Similarly, Kawamoto and Morisawa (2003) reported Sb concentrations of up to 7 mg kg^{-1} not only in green algae, but also in the roots and leaves of higher plants growing along a river in which the water contained 3.4 to 4.0 mg L^{-1} Sb due to discharges from into from a dye factory.

3.4 Toxicity risks posed by consumption of plants grown on Sb-contaminated soil

If Sb is taken up by crop plants it may enter the food chain and present a health risk for animals and humans, even if plant themselves remain unaffected.

Li and Thornton (1993) investigated soil and pasture herbage contaminated by of As, Sb and Bi. They came to the conclusion that very little of these elements is usually absorbed by grazing animals and that health problems in grazing livestock therefore are uncommon. The chemical forms of these elements and other related metals in soil and herbage, and possible subclinical effects of long-term, low-level exposure require further study.

A possible danger is the low Sb toxicity to plants. As has been mentioned before, plants can take up high amounts of Sb while still being and looking healthy. Humans as well as animals consuming such plants over longer periods of time may thus become poisoned.

Gebel et al. (1998) studied Sb exposure by taking urine, blood and scalp hair sample from a population living in contaminated area in northern Palatinate, Germany. No significant differences in Sb concentrations compared with the control group were found. Also, risk factors like consuming seafood or home-grown produce apparently did not affect Sb concentrations in urine, blood or scalp hair.

Owing to the limited knowledge about Sb toxicity, it is difficult to assess the health risks of exposure to elevated concentrations of Sb. Acute Sb poisoning of humans or animals via ingestion of Sb-contaminated soil or consumption of plants grown on Sb-contaminated soil is extremely unlikely. Also, chronic effects are to be expected only under rare circumstances. Assuming an average Sb concentration in vegetables grown in a contaminated garden of 100 mg kg^{-1} dry weight (a value that has rarely been found exceeded under field conditions), then a person hypothetically would have to consume in average 1 kg dry matter of these plants per day in order to reach a dose of 100 mg day^{-1} Sb, i.e. the threshold of Sb intake considered to be critical (Bowen 1979).

3.5 Treatment of Sb-contaminated soil

In principle at least, phytoextraction would be the method of choice to remove Sb from a polluted soil without destroying it. Murciego Murciego et al. (2007) proposed to use *Dittrichia viscosa* for phytoextraction of Sb from mine waste. The practical feasibility of this approach has not been demonstrated yet, however, and simple spreadsheet calculations reveal that this will not be easy. With a constant bioaccumulation coefficient of 0.022 (the average ratio between plant and total soil concentrations derived from the data presented in Fig. 2), an annual yield of 10 t plant dry mass per ha, a soil bulk density of 1.3 kg L^{-1}, and a contamination depth of 0.1 m, the time required to halve the Sb concentration of the soil by phytoextraction would be more than 4000 years. This value is independent of the initial Sb concentration because the assumption of a constant bioaccumulation coefficient means that the rate of Sb extraction decreases in proportion to the concentration of Sb in the soil. For other scenarios, estimates of the time required to reduce a given initial Sb contamination to a specified target concentration by phytoextraction are presented in Table 5. These example calculations demonstrate that phytoextraction is generally not a realistic option, unless high-biomass Sb hyperaccumulator plants are found that are much more efficient for this purpose.

Table 5. Estimates of the time necessary to reduce Sb concentrations in polluted topsoil (0–10 cm depth) to certain target levels by phytoextraction, based on model calculations (see text for further explanation). Soil concentrations in the examples were taken from Table 1. For the calculations an annual biomass yield of 10 t dry matter and a soil-to-plant accumulation coefficient of 0.022 was used, based on the data given in Fig. 2. Soil bulk density was assumed to be 1.3 kg L^{-1}.

Site	Initial soil Sb concentration (mg kg^{-1})	Time necessary to halve concentration in soil (years)	Time required to reach a certain Sb concentration in the topsoil	
			Target concentration (mg kg^{-1})	Years
Mine spoil	26.3	4096	1	19307
Shooting range	13800	4096	100	29113
Roadside along highway	0.53	4096	0.5	344
Garden soil near mine	500	4096	100	9510
Orchard	0.95	4096	0.5	3792

3.6 Conclusions

The main findings of this literature review are that Sb is generally taken up in proportion to the concentration of soluble Sb in the soil and that this proportionality holds over a concentration range of five or more orders of magnitude. Almost nothing is known about the mechanisms of Sb uptake by plants, but the general occurrence of the proportionality suggests passive uptake by convection along the apoplastic pathway with the stream of transpiration water. Crossing of the endodermis could occur through leaks in the Casparian bands or, in the case of antimonite, also through aquaporins. Although soil pollution by Sb may be rarely so severe as to cause toxicity problems to humans or animals consuming plants or food derived from plants grown on Sb-contaminated sites, such risks also cannot be excluded in all cases. There is a general need for more controlled laboratory and green house experiments to elucidate the mechanisms and processes that govern the fate of Sb in soil-plant systems, including the ecotoxicity of Sb and its transfer through the food chain.

3.7 References

Affolter, R., Enggist, A., *Schadstoffbelastung des Bodens und der Vegetation im Bereich von Schiessanlagen, Folgeuntersuchungen an drei Schiessanlagen im Kanton Solothurn* **1995**.

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .1. Vegetation and Soils, *Environmental Pollution* **1990a**, *65*, 65-77.

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .2. Small Mammals and Invertebrates, *Environmental Pollution* **1990b**, *65*, 79-87.

Asher, C. J., Reay, P. F., Arsenic Uptake by Barley Seedlings, *Australian Journal of Plant Physiology* **1979**, *6*, 459-466.

Baghour, M., Moreno, D. A., Hernandez, J., Castilla, N., Romero, L., Influence of root temperature on phytoaccumulation of As, Ag, Cr, and Sb in potato plants (*Solanum tuberosum* L. Var. Spunta), *Journal of Environmental Science and Health Part A* **2001**, *36*, 1389-1401.

Baroni, F., Boscagli, A., Protano, G., Riccobono, F., Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area, *Environmental Pollution* **2000**, *109*, 347-352.

Berg, T., Steinnes, E., Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: From relative to absolute deposition values, *Environ. Pollut.* **1997**, *98*, 61-71.

Borovicka, J., Randa, Z., Jelinek, E., Antimony content of macrofungi from clean and polluted areas, *Chemosphere* **2006**, *64*, 1837-1844.

Bowen, H. J. M., *Environmental Chemistry of the Elements* **1979**, Academic Press: London.

Butterman, W. C., Carlin, J. F. J., *Antimony, Mineral Commodity Profiles, Open file Report 03-019* **2004**, U.S. Department of the Interior.

Cai, J., Salmon, K., DuBow, M. S., A chromosomal ars operon homologue of Pseudomonas aeruginosa confers increased resistance to arsenic and antimony in Escherichia coli, *Microbiology-(UK)* **1998**, *144*, 2705-2713.

Cal-Prieto, M. J., Carlosena, A., Andrade, J. M., Martinez, M. L., Muniategui, S., Lopez-Mahia, P., Prada, D., Antimony as a tracer of the anthropogenic influence on soils and estuarine sediments, *Water Air Soil Pollut.* **2001**, *129*, 333-348.

Cloy, J. M., Farmer, J. G., Graham, M. C., MacKenzie, A. B., Cook, G. T., A comparison of antimony and lead profiles over the past 2500 years in Flanders Moss ombrotrophic peat bog, Scotland, *J. Environ. Monit.* **2005**, *7*, 1137-1147.

Craig, P. J., Forster, S. N., Jenkins, R. O., Miller, D., An analytical method for the detection of methylantimony species in environmental matrices: methylantimony levels in some UK plant material, *Analyst* **1999**, *124*, 1243-1248.

Davis, R. D., Beckett, P. H. T., Wollan, E., Critical Levels Of 20 Potentially Toxic Elements In Young Spring Barley, *Plant and Soil* **1978**, *49*, 395-408.

De Gregori, I., Fuentes, E., Olivares, D., Pinochet, H., Extractable copper, arsenic and antimony by EDTA solution from agricultural Chilean soils and its transfer to alfalfa plants (*Medicago sativa* L.), *J. Environ. Monit.* **2004**, *6*, 38-47.

De Gregori, I., Fuentes, E., Rojas, M., Pinochet, H., Potin-Gautier, M., Monitoring of copper, arsenic and antimony levels in agricultural soils impacted and non-impacted by mining activities, from three regions in Chile, *J. Environ. Monit.* **2003**, *5*, 287-295.

De Gregori, I., Pinochet, H., Fuentes, E., Potin-Gautier, M., Determination of antimony in soils and vegetables by hydride generation atomic fluorescence spectrometry and electrothermal atomic absorption spectrometry. Optimization and comparison of both analytical techniques, *J. Anal. At. Spectrom.* **2001**, *16*, 172-178.

Dodd, M., Pergantis, S. A., Cullen, W. R., Li, H., Eigendorf, G. K., Reimer, K. J., Antimony speciation in freshwater plant extracts by using hydride generation gas chromatography mass spectrometry, *Analyst* **1996**, *121*, 223-228.

Dominguez, M. T., Maranon, T., Murillo, J. M., Schulin, R., Robinson, B. H., Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain: A large-scale phytomanagement case study, *Environ. Pollut.* **2008**, *152*, 50-59.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters I. Occurence, *Earth-Science Reviews* **2002**, *57*, 125-176.

Filella, M., Belzile, N., Lett, M. C., Antimony in the environment: A review focused on natural waters. III. Microbiota relevant interactions, *Earth-Sci. Rev.* **2007**, *80*, 195-217.

Flynn, H. C., Meharg, A. A., Bowyer, P. K., Paton, G. I., Antimony bioavailability in mine soils, *Environmental Pollution* **2003**, *124*, 93-100.

Foster, S., Maher, W., Krikowa, F., Telford, K., Ellwood, M., Observations on the measurement of total antimony and antimony species in algae, plant and animal tissues, *J. Environ. Monit.* **2005**, *7*, 1214-1219.

Fowler, B. A., Goering, P. L., Antimony. In: *Metals and Their Compounds in the Environment, Occurence Analysis and Biological Relevance* (Eds Ernest, M.) **1997**, pp. 743-750 (VCH: Weinheim).

Gebel, T., Arsenic and antimony: comparative approach on mechanistic toxicology, *Chemico-Biological Interactions* **1997**, *107*, 131-144.

Gebel, T., Claussen, K., Dunkelberg, H., Human biomonitoring of antimony, *Int. Arch. Occup. Environ. Health* **1998**, *71*, 221-224.

Gemici, U., Tarcan, G., Assessment of the pollutants in farming soils and waters around untreated abandoned Turkonu Mercury Mine (Turkey), *Bull. Environ. Contam. Toxicol.* **2007**, *79*, 20-24.

Ghuman, G. S., Motes, B. G., Fernandez, S. J., Guardipee, K. W., McManus, G. W., Wilcox, C. M., Weesner, F. J., Distribution Of Antimony-125, Cesium-137, And I-129 In The Soil-Plant System Around A Nuclear-Fuel Reprocessing Plant, *J. Environ. Radioact.* **1993**, *21*, 161-176.

Gordillo, M. C. T., Gil, E. P., Gonzalez, M. A. R., Murciego, A. M., Ostapczuk, P., Potentiometric stripping analysis (PSA) for monitoring of antimony in samples of vegetation from a mining area, *Fresenius J. Anal. Chem.* **2001**, *370*, 434-437.

Hammel, W., Debus, R., Steubing, L., Mobility of antimony in soil and its availability to plants, *Chemosphere* **2000**, *41*, 1791-1798.

Hammel, W., Steubing, L., Debus, R., Assessment of the ecotoxic potential of soil contaminants by using a soil-algae test, *Ecotox. Environ. Safe.* **1998**, *40*, 173-176.

He, M. C., Yang, J. R., Effects of different forms of antimony on rice during the period of germination and growth and antimony concentration in rice tissue, *Sci. Total Environ.* **1999**, *244*, 149-155.

Hilmer, W., Dornberger-Schiff, K., Die Kristallstruktur Von Lithiumpolyarsenat (LiAsO3)X, *Acta Crystallographica* **1956**, *9*, 87-88.

Hinton, T. G., Kopp, P., Ibrahim, S., Bubryak, I., Syomov, A., Tobler, L., Bell, C., A Comparison Of Techniques Used To Estimate The Amount Of Resuspended Soil On Plant-Surfaces, *Health Physics* **1995**, *68*, 523-531.

Hirner, A. V., Gruter, U. M., Kresimon, J., Metal(loid)organic compounds in contaminated soil, *Fresenius J. Anal. Chem.* **2000**, *368*, 263-267.

Hoppe, U., Walter, G., Barz, A., Stachel, D., Hannon, A. C., The P-O bond lengths in vitreous P2O5 probed by neutron diffraction with high real-space resolution, *J. Phys.-Condes. Matter* **1998**, *10*, 261-270.

Hozhina, E. I., Khramov, A. A., Gerasimov, P. A., Kumarkov, A. A., Uptake of heavy metals, arsenic, and antimony by aquatic plants in the vicinity of ore mining and processing industries, *J. Geochem. Explor.* **2001**, *74*, 153-162.

Huang, C. X., Van Steveninck, R. F. M., The role of particular pericycle cells in the apoplastic transport in root meristems of barley, *J. Plant Physiol.* **1989**, *135*, 554-558.

Johnson, C. A., Moench, H., Wersin, P., Kugler, P., Wenger, C., Solubility of antimony and other elements in samples taken from shooting ranges, *J. Environ. Qual.* **2005**, *34*, 248-254.

Jung, M. C., Thornton, I., Chon, H. T., Arsenic, Sb and Bi contamination of soils, plants, waters and sediments in the vicinity of the Dalsung Cu-W mine in Korea, *Sci. Total Environ.* **2002**, *295*, 81-89.

Kabata-Pendias, A., Pendias, H., *Trace Elements in Soils and Plants* **1984**, CRC Press: Boca Raton, FL.

Kanias, G. D., Skaltsa, H., Tsitsa, E., Loukis, A., Bitis, J., Study Of The Correlation Between Trace-Elements, Sterols And Fatty-Acids In Brown-Algae From The Saronikos Gulf Of Greece, *Fresenius J. Anal. Chem.* **1992**, *344*, 334-339.

Kawamoto, Y., Morisawa, S., The distribution and speciation of antimony in river water, sediment and biota in Yodo River, Japan, *Environ. Technol.* **2003**, *24*, 1349-1356.

Koch, I., Harrington, C. F., Reimer, K. J., Cullen, W. R., Simplex optimisation of conditions for the determination of antimony in environmental samples by using electrothermal atomic absorption spectrometry, *Talanta* **1997**, *44*, 1241-1251.

Kolitsch, U., Tillmanns, E., $Li_3Sc(MoO_4)_3$: substitutional disorder on three (Li,Sc) sites, *Acta Crystallographica Section E* **2003**, *59*, i55-i58.

Krachler, M., Burow, M., Emons, H., Development and evaluation of an analytical procedure for the determination of antimony in plant materials by hydride generation atomic absorption spectrometry, *Analyst* **1999**, *124*, 777-782.

Kuperman, R. G., Checkai, R. T., Simini, M., Phillips, C. I., Speicher, J. A., Barclift, D. J., Toxicity benchmarks for antimony, barium, and beryllium determined using reproduction endpoints for *Folsomia candida*, *Eisenia fetida*, and *Enchytraeus crypticus*, *Environ. Toxicol. Chem.* **2006**, *25*, 754-762.

Leduc, C., Gardou, C., Biochemical prospecting for antimony - Results of an orientation study on the Brouzils deposit (Vendee, France), *Bulletin de la société botanique de France - Actualités botaniques* **1992**, *139*, 123-131.

Lehndorff, E., Schwark, L., Accumulation histories of major and trace elements on pine needles in the Cologne Conurbation as function of air quality, *Atmos. Environ.* **2008**, *42*, 833-845.

Leonard, A., Gerber, G. B., Mutagenicity, carcinogenicity and teratogenicity of antimony compounds, *Mutation Research-Reviews in Genetic Toxicology* **1996**, *366*, 1-8.

Li, X. D., Thornton, I., Arsenic, Antimony And Bismuth In Soil And Pasture Herbage In Some Old Metalliferous Mining Areas In England, *Environ. Geochem. Health* **1993**, *15*, 135-144.

Lintschinger, J., Michalke, B., Schulte-Hostede, S., Schramel, P., Studies on speciation of Antimony in soil contaminated by industrial activity, *Intern. J. Environ. Anal. Chem.* **1998**, *72 (1)*, 11-25.

Liu, G. Z., Zheng, S. T., Yang, G. Y., B3O4(OH) · 0.5(C4H10N2): First organic-inorganic hybrid borate with a neutral layered framework, *Inorg. Chem. Commun.* **2007**, *10*, 84-87.

Massard, G., Reiser, R., Stadelmann, V., Gupta, S. K., *Antimony-Contaminated Soils on Shooting-Ranges* **2006**, Swiss Federal Departement of Economic Affairs.

Mathys, R., Dittmar, J., Johnson, C. A., *Antimony in Switzerland - A substance flow analysis* **2007**, Swiss Federal Office for the Environment.

Miravet, R., Bonilla, E., Lopez-Sanchez, J. F., Rubio, R., Antimony speciation in terrestrial plants. Comparative studies on extraction methods, *J. Environ. Monit.* **2005**, *7*, 1207-1213.

Miravet, R., Bonilla, E., Lopez-Sanchez, J. F., Rubio, R., On-line photodecomposition for the determination of antimony species, *Appl. Organomet. Chem.* **2006**, *20*, 12-19.

Mishra, J., Saxena, A., Singh, S., Chemotherapy of leishmaniasis: Past, present and future, *Curr. Med. Chem.* **2007**, *14*, 1153-1169.

Montgomery, H., Tuttons Salts .9. Nickel Ammonium Chromate Hexahydrate, *Acta Crystallogr. Sect. B-Struct. Commun.* **1979**, *35*, 155-156.

Murciego Murciego, A., Garcia Sanchez, A., Rodriguez Gonzalez, M. A., Pinilla Gil, E., Toro Gordillo, C., Cabezas Fernandez, J., Buyolo Triguero, T., Antimony distribution and mobility in topsoils and plants (*Cytisus striatus*, *Cistus ladanifer* and *Dittrichia viscosa*) from polluted Sb-mining areas in Extremadura (Spain), *Environmental Pollution* **2007**, *145*, 15-21.

Oorts, K., Smolders, E., Degryse, F., Buekers, J., Gasco, G., Cornelis, G., Mertens, J., Solubility and toxicity of antimony trioxide (Sb_2O_3) in soil, *Environ. Sci. Technol.* **2008**, *42*, 4378-4383.

Palenik, R. C., Abboud, K. A., Palenik, G. J., Bond valence sums and structural studies of antimony complexes containing Sb bonded only to O ligands, *Inorg. Chim. Acta* **2005**, *358*, 1034-1040.

Paoletti, F., Sirini, P., Seifert, H., Vehlow, J., Fate of Sb in minicipal solid wast incineration, *Chemosphere* **2001**, *42*, 533-534.

Picard, C., Bosco, M., Soil antimony pollution and plant growth stage affect the biodiversity of auxin-producing bacteria isolated from the rhizosphere of Achillea ageratum L, *FEMS Microbiol. Ecol.* **2003**, *46*, 73-80.

Pohl, P., Lesniewicz, A., Zyrnicki, W., Determination of As, Bi, Sb and Sn in conifer needles from various locations in Poland and Norway by hydride generation inductively coupled plasma atomic emission spectrometry, *Int. J. Environ. Anal. Chem.* **2003**, *83*, 963-970.

Porquet, A., Filella, M., Structural evidence of the similarity of Sb(OH)(3) and As(OH)(3) with glycerol: Implications for their uptake, *Chem. Res. Toxicol.* **2007**, *20*, 1269-1276.

Pratas, J., Prasad, M. N. V., Freitas, H., Conde, L., Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation, *J. Geochem. Explor.* **2005**, *85*, 99-107.

Rached-Mosbah, O., Gardou, C., Pauwels, J. M., Accumulator plants in a steppe upon an antimonious contaminated soil, *Bulletin de la société botanique de France - Actualités botaniques* **1992**, *139*, 133-139.

Robinson, B. H., Bischofberger, S., Stoll, A., Schroer, D., Furrer, G., Roulier, S., Gruenwald, A., Attinger, W., Schulin, R., Plant uptake of trace elements on a Swiss military shooting range: Uptake pathways and land management implications, *Environmental Pollution* **2008**, *153*, 668-676.

Robinson, B. H., Greven, M., Green, S., Sivakumaran, S., Davidson, P., Clothier, B., Leaching of copper, chromium and arsenic from treated vineyard posts in Marlborough, New Zealand, *Sci. Total Environ.* **2006**, *364*, 113-123.

Rooney, C. P., McLaren, R. G., Cresswell, R. J., Distribution and phytoavailability of lead in a soil contaminated with lead shot, *Water Air Soil Pollut.* **1999**, *116*, 535-548.

Shotyk, W., Krachler, M., Chen, B., Antimony in recent, ombrotrophic peat from Switzerland and Scotland: Comparison with natural background values (5,320 to 8,020 C-14 yr BP) and implications for the global atmospheric Sb cycle, *Glob. Biogeochem. Cycle* **2004**, *18*.

Stalhandske, C., Structure of cadmium selenate monohydrate, *Acta Crystallographica Section B* **1981**, *37*, 2055-2057.

Tandy, S., Schulin, R., Nowack, B., The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers, *Chemosphere* **2006**, *62*, 1454-1463.

Tighe, M., Ashley, P., Lockwood, P., Wilson, S., Soil, water, and pasture enrichment of antimony and arsenic within a coastal floodplain system, *Sci. Total Environ.* **2005**, *347*, 175-186.

Tschan, M., Robinson, B., Schulin, R., Antimony uptake by *Zea mays* (L.) and *Helianthus annuus* (L.) from nutrient solution, *Environmental Geochemistry and Health* **2008**, *30*, 187-191.

Tschan, M., Robinson, B. H., Nodari, M., Schulin, R., Antimony uptake by different plant species from nutrient solution, agar and soil, *Environmental Chemistry* **2009**, *6*, 144-152.

Wagner, S. E., Peryea, F. J., Filby, R. A., Antimony Impurity in Lead Arsenate Insecticide Enhances the Antimony Content of Old Orchard Soils, *Journal of Environmental Quality* **2003**, 736-738.

Waisel, Y., Eshel, A., Kafkafi, U., editors. Plant Roots - The Hidden Half. 2nd ed. New York, Basel, Hong Kong: Marcel Dekker, Inc.; **1996**.

Wenger, K., Tandy, S., Nowack, B., Effect of chelating agents on trace metal speciation and bioavailability. In: *Biogeochemistry of Chelating Agents* (Eds Vanbriesen, J., Nowack, B.) **2005**, pp. 204-224 (American Chemical Society).

Wysocki, R., Clemens, S., Augustyniak, D., Golik, P., Maciaszczyk, E., Tamas, M. J., Dziadkowiec, D., Metalloid tolerance based on phytochelatins is not functionally equivalent to the arsenite transporter Acr3p, *Biochem. Biophys. Res. Commun.* **2003**, *304*, 293-300.

Zahrobsky, R., Baur, W. H., Crystal Structure Of Copper (2) Sulfate Trihydrate, *Naturwissenschaften* **1965**, *52*, 389-&.

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

Martin Tschan, Brett Robinson, Matteo Nodari and Rainer Schulin

Environmental Chemistry **2009**, *6*, 144-152

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 results show that plant uptake of Sb increases 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. Antimony may thus 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.

4.1 Introduction

With an average concentration between 0.2-0.3 mg kg^{-1}, antimony (Sb) is a rare element in the earth's crust (Rish 2004). Natural Sb background concentrations in soil were found to vary between 0.3 - 8.6 mg kg^{-1} (Johnson et al. 2005, Kabata-Pendias and Pendias 1984). However, as a component of many industrial products, e.g. in fire retardants, brakes, semiconductors, and metal alloys (Filella et al. 2002, Mathys et al. 2007), 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 (Rooney et al. 1999). Switzerland for example has more than 2000 shooting ranges. Depending on the duration and intensity of shooting activities, not only soil Pb but also soil Sb concentrations are generally highly elevated on these sites (Gresch and Wettstein 2002). As they are often used for grazing sheep and cattle in the time when they are not used for shooting, there is a potential risk that Sb enters 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 on a soil polluted with up to 400 mg kg^{-1} Sb dry weight (DW) in the vicinity of an Sb smelter in north-east England (Ainsworth et al. 1990). Another study reported foliar Sb concentrations greater than 100 mg kg^{-1} in plants growing on a mine tailing soil with 9000 mg kg^{-1} Sb DW. In the basal leaves of *Achillea ageratum*, more than 1000 mg kg^{-1} Sb were found on that site (Baroni et al. 2000). There are also studies that reported only small Sb concentrations in plants grown on heavily Sb-contaminated soils. Pratas et al. (2005) reported maximum stem concentrations of less than 5 mg kg^{-1} Sb DW in various tree and herb species growing on a Portuguese mine spoil with an average total Sb concentration of 663 mg kg^{-1}.

Deposition of Sb-containing dust particles on leaf surfaces may be one reason for the high plant Sb concentrations on contaminated field sites (Ainsworth et al. 1990), 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 kg^{-1} Sb DW, which was the detection limit in that study, although yields were reduced (Davis et al. 1978). 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 45 mg L^{-1}. The plants accumulated up to 399 mg kg^{-1} Sb DW in the shoots without showing toxicity symptoms (Hammel et al. 2000).

Given the large variation observed in plant Sb concentrations and the uncertain role of roots in Sb uptake by plants, the goal to 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 are 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 damages 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 for 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. (2007) found that Sb is 98 % available in agar. 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 (Drew 1983, Jackson and Drew 1984).

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 also very high concentrations and, thus, to test for toxicity limits of growth.

4.2 Material and Methods

4.2.1 Plant cultivation and application of Sb treatments

The plants used in this study were Indian mustard (*Brassica juncea* (L.) Czern, obtained from TSR, 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 (Hoagland and Arnon 1938). 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) (Krämer et al. 1996). 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 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.

4.2.2 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) Secondly, 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, while 0, 12.9, 61.3, 294, 563 and 1240 mg Sb kg^{-1} DW were applied in the experiment with potted soil. The latter applications resulted in proportional KNO_3-extractable Sb concentrations. 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 one week to the treatment solutions as in the previous experiment. In the agar experiments, plants were grown for four 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-liter 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 analyzed separately, as were the stems and seeds.

4.2.3 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 in at 4^{o}C until they were analysed. Soluble Sb in the soil samples was extracted using potassium nitrate as described by Massard et al. (2006). 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 ^{o}C for 48 hours 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 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.

4.2.4 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 (2004).

4.3 Results

4.3.1 Plant uptake and phytotoxicity of Sb in comparison to 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 (Figure 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 by 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.

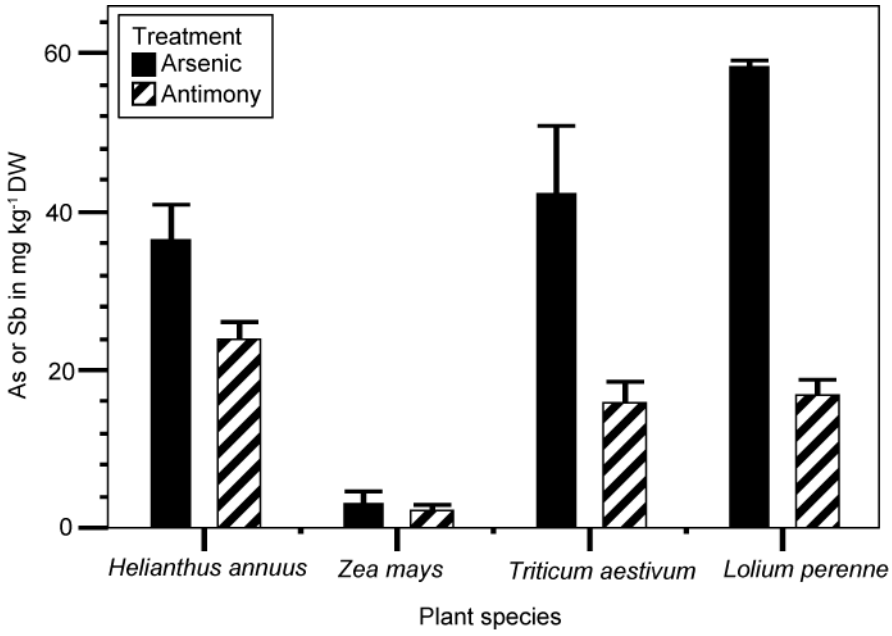

Fig. 1: Concentrations of As and Sb (mg kg^{-1} DW, dry weight) in the shoots of plant seedlings exposed for 1 week to either 25 µmol L^{-1} As or 25 µmol L^{-1} Sb, respectively, in nutrient solution after 4 weeks growth in uncontaminated solution. Error bars represent standard errors.

Figure 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 in 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 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.

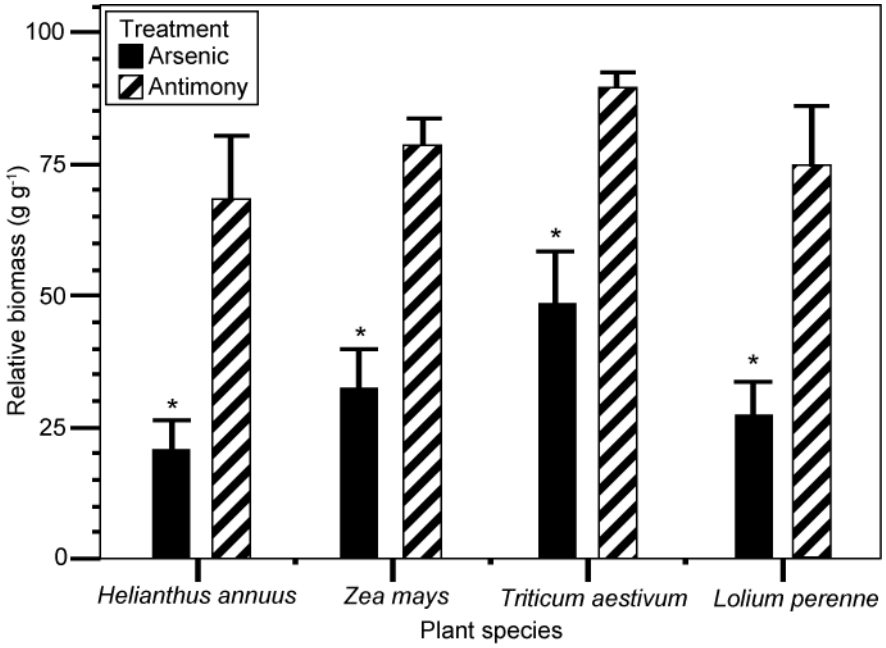

Fig. 2: Relative shoot biomass (control = 100%) of plant seedlings exposed for 1 week to either 25 µmol L^{-1} As or 25 µmol L^{-1} Sb in nutrient solution after 4 weeks' growth in uncontaminated solution. An asterisk denotes that the decrease was significant in comparison to the control treatment. Error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

4.3.2 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

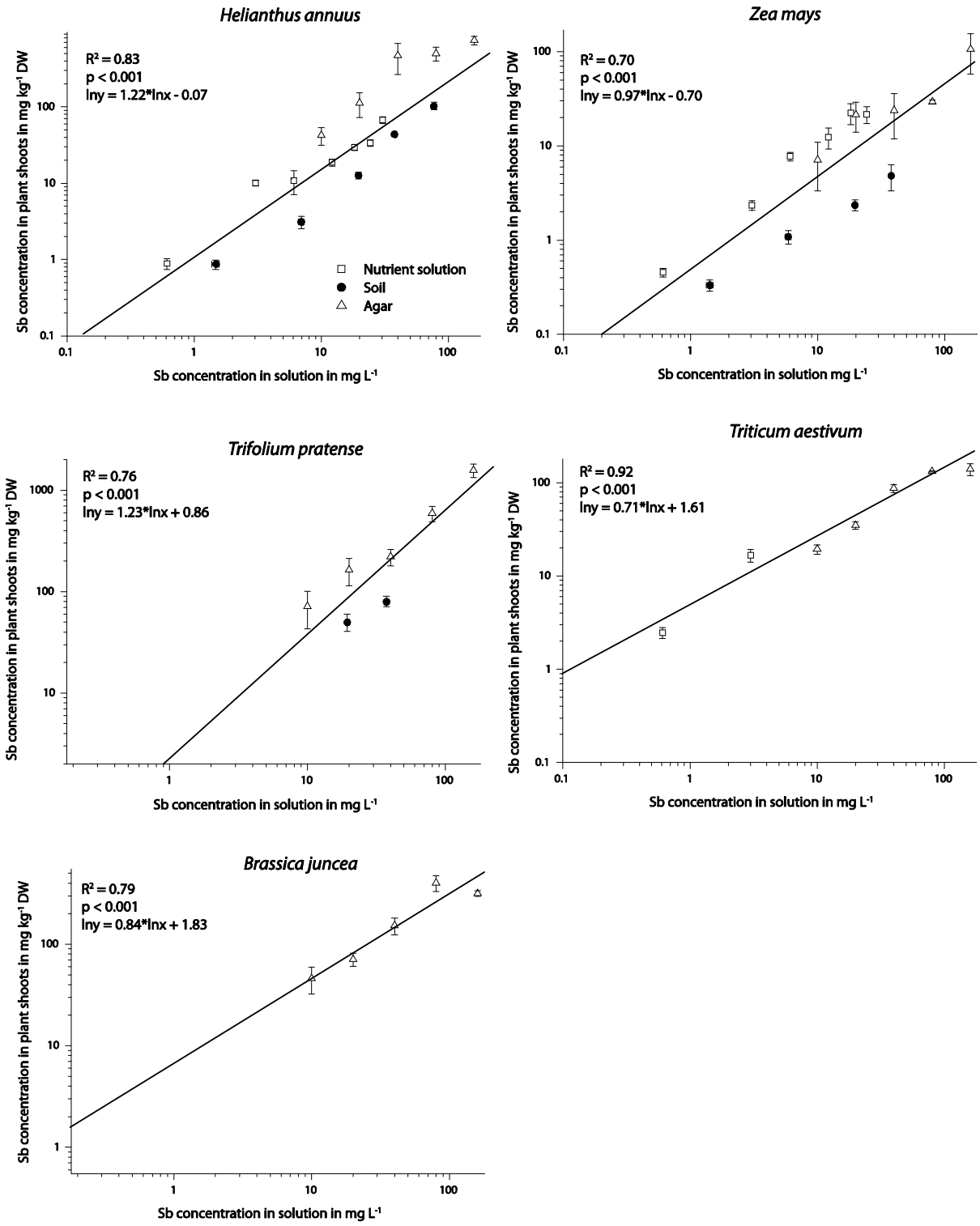

Fig. 3: Dependance of Sb accumulation in the shoots of sunflower, maize, wheat, Indian mustard and clover seedlings on the available Sb concentration (mg kg^{-1} DW, dry weight) in the growth medium: hydroponic solution (squares), agar (triangles), and potted soil (filled circles). In the case of the 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.

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

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

concentrations, was highest in Indian mustard, followed by clover, sunflower and wheat, and lowest in maize. The bioconcentration factor was about 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 to 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, while exposure lasted only one 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.

Figure 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

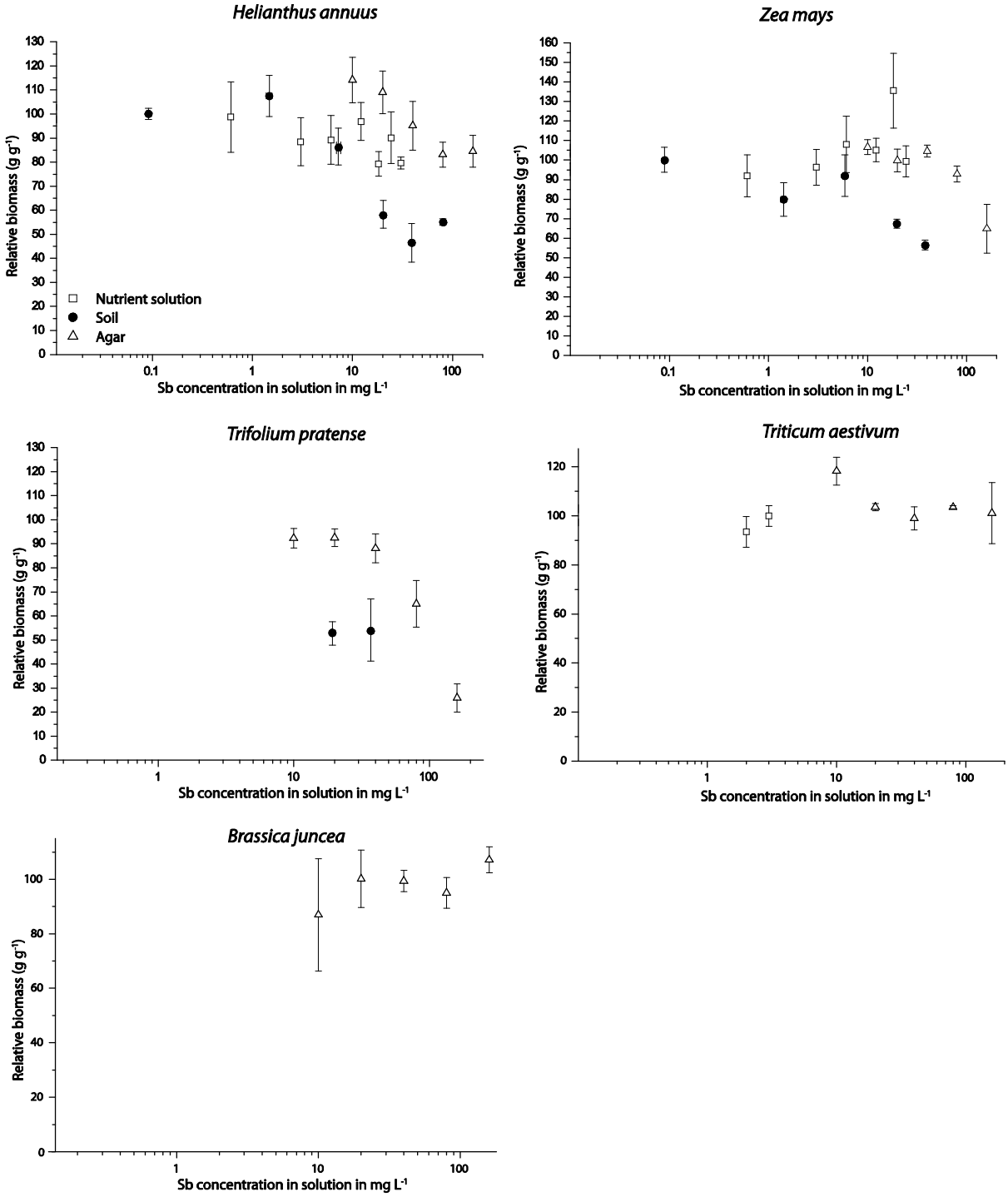

Fig. 4: Dependance of the relative biomass (control = 100%) of sunflower, maize, wheat, Indian mustard and clover seedlings on the available Sb concentration in the growth medium: hydroponic solution (squares), agar (triangles), and potted soil (filled circles). In the case of the soil-grown plants,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

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 has been an additional stress factor in the soil system.

4.3.3 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 (Figure 5). Even an Sb concentrations 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 µmol L^{-1}), but a clear toxicity effect at 300 mg L^{-1} (= 2464 µmol L^{-1}) Sb. Clover was similar, with a clearer reduction in root growth already at 100 mg L^{-1} Sb, while the root growth of the two monocotyledons wheat and maize was inhibited already at Sb concentrations of 30 mg L^{-1} (= 246 µmol 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 compared on the basis of molar concentrations, there is still a clear difference. Already at As concentrations of 10 mg L^{-1} (= 133 µmol 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 µmol 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 µmol L^{-1}), the next highest treatment level, virtually none the seedlings showed significant root growth any more. In contrast, even at the highest Sb treatment level (= 2464 µmol L^{-1}), there was still more than 21% root growth in comparison to the controls, even in wheat and maize.

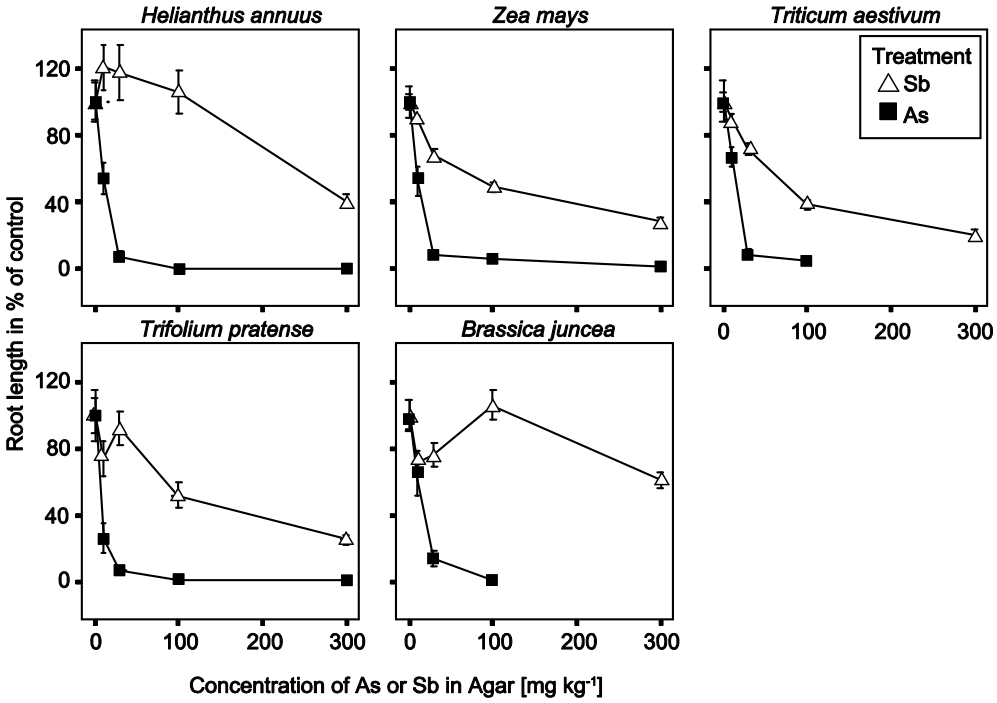

Fig. 5: Relative root length of plant seedlings grown for 2 weeks in Petri dishes with agar at different concentrations of either As or Sb.

4.3.4 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 (Figure 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.

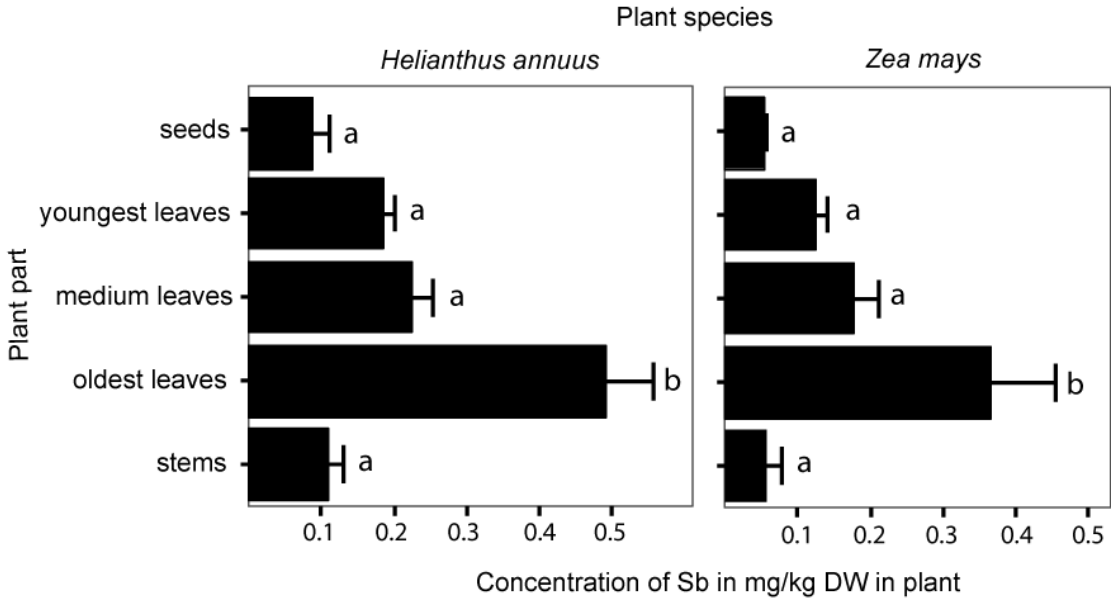

Fig. 6: Allocation of accumulated Sb (concentrations in mg kg^{-1} DW, dry weight) in different aboveground parts of maize and sunflower grown in soil with a KNO_3-extractable Sb concentration of 1.03 mg L^{-1}. Bars with different letters are significantly different from each other.

4.4 Discussion and conclusions

The approximate proportionality between available Sb concentration in the growth medium and accumulation of Sb in the 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 (2000). In the latter study three different soils were spiked with up to 1000 mg kg^{-1} Sb and then left to age for six months before the plants were grown. This treatment resulted in NH_4NO_3-extractable Sb concentration 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 (Reid and Hayes 2003). Thus, at least at low external concentrations, uptake of antimonate into the root symplast would require anion transporters of low selectivity, in which antimonite 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 (Bell et al. 2003, Wenger et al. 2005). 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 form of antimonite, passive uptake of Sb with the transpiration stream could theoretically also occur through aquaporins. Passage of Sb(III) into cells via aquaglyceroporins was found in microorganisms, i.e. in *E. coli*, *Saccharomyces cerevisiae*, *Leishmania major* and *L. tarentolae* (Filella et al. 2007). 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 in form of antimonite only, 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 antimonite 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. (2008), 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.

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. (2005) found concentrations of up to 6 mg L^{-1} Sb in leachates from shooting-range soils where total Sb concentrations reached values up to 10 g kg^{-1}. 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 (Bowen 1979), 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 (Bowen 1979). 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, also a wider variety of plants should be tested for their capability to accumulate Sb.

4.5 Acknowledgements

We gratefully acknowledge Anna Grünwald, René Saladin and Viktor Stadelmann for their generous help and support in the laboratory. This project was financially supported by the Swiss National Science Foundation (Grants No. 200021-103768).

4.6 References

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .1. Vegetation and Soils, *Environmental Pollution* **1990**, *65*, 65-77.

Baroni, F., Boscagli, A., Protano, G., Riccobono, F., Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area, *Environmental Pollution* **2000**, *109*, 347-352.

Bell, P. F., McLaughlin, M. J., Cozens, G., Stevens, D. P., Owens, G., South, H., Plant uptake of ^{14}C-EDTA, ^{14}C-citrate, and ^{14}C-histidine from chelator-buffered and conventional hydroponic solutions, *Plant and Soil* **2003**, *253*, 311-319.

Bowen, H. J. M., *Environmental Chemistry of the Elements* **1979**, Academic Press: London.

Davis, R. D., Beckett, P. H. T., Wollan, E., Critical Levels Of 20 Potentially Toxic Elements In Young Spring Barley, *Plant and Soil* **1978**, *49*, 395-408.

Drew, M. C., Plant Injury And Adaptation To Oxygen Deficiency In The Root Environment - A Review, *Plant Soil* **1983**, *75*, 179-199.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters I. Occurence, *Earth-Science Reviews* **2002**, *57*, 125-176.

Filella, M., Belzile, N., Lett, M. C., Antimony in the environment: A review focused on natural waters. III. Microbiota relevant interactions, *Earth-Sci. Rev.* **2007**, *80*, 195-217.

Gresch, M., Wettstein, B., *Antimon- und Bleibelastung bei Schiessanlagen, Fallbeispiel Eschenbach (SG)* Semester Thesis, **2002**, ETH Zurich.

Hammel, W., Debus, R., Steubing, L., Mobility of antimony in soil and its availability to plants, *Chemosphere* **2000**, *41*, 1791-1798.

Hoagland, D. R., Arnon, D. I., The water-culture method for growing plants without soil, *Circular of the Agricultural experiment station Berkeley, California* **1938**, *347*.

Jackson, M. B., Drew, M. C., Effects of Flooding on Growth and Metabolism of Herbaceous Plants. In: *Flooding and Plant Growth* (Eds Kozlowski, T. T.) **1984**, pp. 47-128 (Academic Press: Orlando).

Johnson, C. A., Moench, H., Wersin, P., Kugler, P., Wenger, C., Solubility of antimony and other elements in samples taken from shooting ranges, *J. Environ. Qual.* **2005**, *34*, 248-254.

Kabata-Pendias, A., Pendias, H., *Trace Elements in Soils and Plants* **1984**, CRC Press: Boca Raton, FL.

Krämer, U., Cotter-Howells, J. D., Charnock, J. M., Baker, A. J. M., Smith, J. A. C., Free histidine as a metal chelator in plants that accumulate nickel, *Nature* **1996**, *379*, 635-638.

Massard, G., Reiser, R., Stadelmann, V., Gupta, S. K., *Antimony-Contaminated Soils on Shooting-Ranges* **2006**, Swiss Federal Departement of Economic Affairs.

Mathys, R., Dittmar, J., Johnson, C. A., *Antimony in Switzerland - A substance flow analysis* **2007**, Swiss Federal Office for the Environment.

Nodari, M., Schulin, R., Reyer, H. U., Robinson, B. H., *Investigating Plant - Antimony Interactions: Method Development, Plant Tolerance, and Plant Uptake* Master Thesis, **2007**, Universität Zürich.

Oorts, K., Smolders, E., Degryse, F., Buekers, J., Gasco, G., Cornelis, G., et al., Solubility and toxicity of antimony trioxide (Sb_2O_3) in soil, *Environ. Sci. Technol.* **2008**, *42*, 4378-4383.

Pratas, J., Prasad, M. N. V., Freitas, H., Conde, L., Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation, *J. Geochem. Explor.* **2005**, *85*, 99-107.

Reid, R., Hayes, J., Mechanisms and control of nutrient uptake in plants. In: *International Review Of Cytology - A Survey Of Cell Biology, Vol 229* (Eds **2003**, pp. 73-114 (Academic Press Inc: San Diego).

Rish, M. A., Antimony. In: *Elements and their Compounds in the Environment* (Eds Merian, E., Anke, M., Ihnat, M., Stoeppler, M.) **2004**, pp. 659-670 (Wiley-VHC: Weinheim).

Rooney, C. P., McLaren, R. G., Cresswell, R. J., Distribution and phytoavailability of lead in a soil contaminated with lead shot, *Water Air Soil Pollut.* **1999**, *116*, 535-548.

Sachs, L., *Angewandte Statistik* **2004**, Springer: Berlin, Heidelberg, New York.

Wenger, K., Tandy, S., Nowack, B., Effect of chelating agents on trace metal speciation and bioavailability. In: *Biogeochemistry of Chelating Agents* (Eds Vanbriesen, J., Nowack, B.) **2005**, pp. 204-224 (American Chemical Society).

5 Antimony uptake and toxicity in sunflower and maize growing in Sb^{III} and Sb^{V} contaminated soil

Martin Tschan, Brett Robinson, C. Annette Johnson, Annina Bürgi and Rainer Schulin

Submitted to Plant and Soil

Abstract

Using pot experiments, we investigated the uptake of antimony (Sb) by sunflower (*Helianthus annuus* L. cv. Iregi), and maize (*Zea mays* L. cv. Magister) in two different soils, a potting mix and an agricultural soil. In one treatment Sb was added to the experimental soils as $KSb(OH)_6$ ("Sb^{V}-treatment") and in the other as Sb_2O_3 ("Sb^{III}-treatment"). Soluble soil Sb concentrations were linearly related to the applied Sb rates, ranging from 0.02 (controls) to 175 mg L^{-1} soil solution. Accumulation of Sb tended to be slightly higher in the Sb^{V} treatment in sunflower, while no difference in Sb uptake between the two Sb treatments was found in maize. The half maximal effective concentration (EC_{50}) values derived from the dose-response curves were higher for the Sb^{V} than for the Sb^{III} treatment when they were related to soluble soil Sb concentrations, but differences became insignificant when they were related to shoot Sb concentrations. Maize was substantially more sensitive to Sb toxicity than sunflower, indicating physiological differences in Sb tolerance between the two plant species. Our results show that on soils with high Sb contamination, as often found in shooting ranges, plants may suffer from Sb toxicity.

5.1 Introduction

The concentration of antimony (Sb) in soils is generally below 10 mg kg^{-1}, with most soils having <1 mg kg^{-1} Sb (Johnson et al. 2005; Kabata-Pendias and Pendias 1984). Higher concentrations of Sb in soil are usually a result of human activities. Due to its many industrial uses, e.g. in fire retardants, semiconductors, and as an agent for metal hardening, Sb is released into the environment in increasing amounts (Filella et al. 2002). High soil Sb concentrations are often found in areas affected by Sb-mining activities (210 to 360 mg kg^{-1} in Ainsworth et al. 1990; 192 to 15'112 mg kg^{-1} in Baroni et al. 2000; 31 to 5'986 mg kg^{-1} in Pratas et al. 2005), along roadsides (1.2 to 8.7 mg kg^{-1} in Amereih et al. 2005) and particularly in shooting ranges (Johnson et al. 2005). The latter is due to the fact that Sb is used to harden lead bullets, making up 1-7% of their weight (Rooney et al. 1999). Soil contamination by Sb in shooting ranges is an important environmental problem in Switzerland, where more than 2000 shooting ranges are scattered over the country (total area of only 41'000 km^2). Concentrations of up to 2400 mg kg^{-1} have been found in stop butt soils (Gresch and Wettstein 2002).

While lead (Pb) is the main contaminant in shooting range soils on a mass basis, soil contamination by Sb may be of greater concern, given that Sb is much more soluble than Pb in the near-neutral pH range characteristic of many Swiss soils (Blaser et al. 2008) and that the toxicity of Sb to humans and animals is considered comparable to its sister element arsenic (Belzile et al. 2001; Bowen 1979). Similar to As, inorganic forms of Sb are generally more toxic than organic Sb compounds (Gebel 1997). Likewise, Sb toxicity also depends on its oxidation state, with Sb^{III} being much more toxic than Sb^{V} (Gurnani et al. 1994). In mammal cells Sb^{III} compounds were found to be ten times more toxic than Sb^{V} compounds (Krachler et al. 2001).

In soils Sb is usually present as Sb^{III} and Sb^{V}. Antimony entering soil as elemental Sb, for example as part of the lead amalgam of bullets in the case of shooting range soils, is rapidly oxidized to Sb^{III} and Sb^{V}, depending on soil pH and redox conditions (Johnson et al. 2005). Trivalent antimony is present in soil solution as antimonite, i.e. as the neutral species $Sb(OH)_3$ between pH 2.5 and pH 10.8, the soil pH range relevant under normal environmental conditions (Baes and Mesmer 1986). Apart from the oxidation of elemental Sb entering soil in the form of metal alloys, Sb^{III} in soil can originate from flame retardants, which contain Sb_2O_3, or by reduction of Sb^{V}. Pentavalent antimony occurs in soil solution as antimonate, i.e. as the oxyanion $Sb(OH)_6^-$ between pH 2.7 and pH 14 (Baes and Mesmer 1986). Iron plays an

important role in the redox chemistry of Sb. In alkaline conditions Fe^{II} can reduce Sb^{V}, while in acidic to neutral conditions the oxidation of Fe^{II} with oxygen can produce intermediate products that oxidise Sb^{III} to Sb^{V} (Leuz 2002; Leuz et al. 2006a). The oxidation of Sb^{III} is also catalyzed when sorbed to amorphous Fe- and Mn-oxyhydroxides, for which Sb^{III} has a strong affinity (Belzile et al. 2001; Blay 2000; Leuz et al. 2006b). Oorts et al. (2008) found that more than 70% of the Sb added in solution as dissolved antimony trioxide to a soil was oxidized to antimonate within two days.

The neutral molecule $Sb(OH)_3$ was found to be taken up by bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*) and protozoa (*Leishmania major* and *Leishmania tarentolae*) through aquaglyceroporins. Thus, it is likely that aquaglyceroporins can also mediate the uptake of $Sb(OH)_3$ by plants. Conversely, although the mechanisms of Sb^{V} uptake by plants are unclear (Filella et al. 2007), it is unlikely that antimonate can pass through aquaporins, because of its negative charge. Furthermore, the observation by Tschan et al. (2008) that phosphate did not affect antimonate uptake by sunflower and maize in hydroponic experiments indicates that antimonate is not taken up by plants via phosphate transporters like arsenate (Asher and Reay 1979; Gulz et al. 2005; Woolson et al. 1973). Tschan et al. (2009a) found that antimonate is accumulated by plants approximately in proportion to soil soluble Sb over a concentrations range of several orders of magnitude, suggesting that antimonate uptake is dominated by a non-selective pathway, possibly an apoplastic bypass of the endodermis barrier separating the outer parts of a plant root from the central root cylinder with the vascular bundles. This latter pathway would also be available for antimonite.

Given the likelihood that Sb is taken up by plants from soil solution both as antimonate and also as antimonite, the influence of oxidation state on uptake rate and phytotoxicity needs further examination. Considering that even under oxidizing conditions substantial fractions of soil Sb may be present as Sb^{III} (Belzile et al. 2001), this question is particularly relevant for Sb-contaminated shooting range soils, where bullet weathering and oxidation of Sb(0) first lead to Sb^{III} before it is further converted to Sb^{V}. Thus, the objective of this study was to investigate how the form in which Sb was added to soil would affect its uptake by plants and its phytotoxicity. For this purpose we performed pot experiments in which two crop plant species, maize and sunflower, were grown on two different soil substrates, an agricultural topsoil and a commercial potting mix, to which Sb was added either as antimonite or as antimonate at various concentrations.

5.2 Material and Methods

Sunflower (*Helianthus annuus* L. cv. Iregi) and maize (*Zea mays* L. cv. Magister) in 250-mL pots were grown in a climate chamber (photoperiod 16 h, day/night temperature 22/14°C, light intensity 11000 lux). Two soils were used: a standard potting mix (obtained from Migros Co-operative, Zürich), consisting of garden soil enriched with compost (organic carbon content: 229 ± 27 g kg^{-1}, pH in $CaCl_2$ extract: 7.0 ± 0.1) and soil from the plough layer of an agricultural field at Birr (Canton Aargau) on the Swiss plateau (organic carbon content: 15 ± 1.4 g kg^{-1}, pH in $CaCl_2$ extract: 6.6 ± 0.1). Either antimonite ("Sb^{III} treatment") or antimonate ("Sb^{V} treatment") was added by mixing granular Sb_2O_3 or $KSb(OH)_6$, respectively, with the sieved and dried soil substrates. Apart from the control (no Sb added), the applied total Sb concentration levels were 156, 313, 625, 1250, 2500, 5000, and 10000 mg kg^{-1} for the potting mix and 20, 39, 78, 156, 313, 625, 1250, 2500, and 5000 mg kg^{-1} for the agricultural soil. Regularly watered, the mixtures were left to equilibrate for 2 weeks and then, after taking samples for chemical analysis (one composite sample per batch), filled into the pots. Three replicates were prepared for each concentration. Three seeds of either maize or sunflower were planted in each pot, of which only one was left to grow after a week. Plants were harvested after four weeks of growth, separated into roots and shoots, carefully washed with de-ionized water, oven-dried for 48 h at 65°C, weighed and then digested for chemical analysis using aqua regia (Sample weight for digestion was 0.2 g which was digested in 2 ml HNO_3, 6 ml HCl, 2 ml H_2O_2 and 8 ml H_2O) in closed Teflon vessels (2 h at room temperature and then for 30 min in a microwave oven (EM-2, Lavis ETHOS, MLS Microwave Laboratory Systems GmbH, Leutkirch i. A., Germany) at 100°C).

Soil samples were oven-dried at 65°C for 1 week, weighed and then stored at 4°C until they were analysed. Soluble soil Sb concentrations relating to soil solution were determined by extraction with potassium nitrate as described by Tschan et al. (2009a): Subsamples of 5 g 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 with a frequency of 120 min^{-1} and an amplitude of 55 mm (KS 250, Janke & Kunkel IKA Labortechnik GmbH, Staufen i. Br., Germany). The resulting slurries were left for 10 min to settle, before 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 containing 0.8 mL of 65% nitric acid.

The plant and soil extracts from the potting mix experiments were analysed for Sb by means of hydride generation atomic fluorescence spectroscopy (HG-AFS) (10.055 Excalibur Millenium System, PSAnalytical, Orpington, Kent, UK) and those from the experiments with agricultural soil by means of stripping voltametry with a mercury anode (797 VA Computrace, Metrohm, Herisau, Switzerland). Total soil Sb concentrations were measured by means of XRF (X-Ray fluorescence spectrometry) (Spectro). As reference material for the analysis of plant samples, we used Virginia tobacco leaves (CTA-VTL-2) obtained from LGC Standards (Wesel, Germany). 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 the standards. Statistical analyses (ANOVA and regression) were performed using SPSS 17.0 (SPSS 2009). Slopes of regression lines were compared by means of t-tests (Sachs 2004).

5.3 Results

5.3.1 Soluble Soil Antimony Concentrations

The soluble Sb concentrations determined after the initial equilibration phase prior to planting were significantly correlated to the total Sb concentrations for both treatments and soil substrates, showing linear relationships on log-log scales (Figs. 1 and 2). The form in which Sb had been added showed no effect on this relationship in the agricultural soil, but there was a clear treatment effect in the case of the potting mix. Sb was more soluble in the Sb^{V} than in the Sb^{III} treatment with potting mix,, suggesting that a substantial fraction of the added Sb^{III} was still present in that form, bound to the soil matrix. Interestingly, the relationships between total and soluble soil Sb was very similar for the two substrates in the Sb^{III} treatment, meaning that Sb solubility was about the same in the two substrates where Sb had been added as Sb^{III}, whereas there was a pronounced difference in Sb solubility between the two substrates where Sb had been added as Sb^{V}. Given that there was no solubility difference between the two Sb treatments in the agricultural soil, the main difference is the elevated Sb solubility in the potting mix in the Sb^{V} treatment as compared to the other treatments.

The slopes of the log-log relationships did not differ significantly from 1 for both soils and both treatments, which means that soluble Sb concentrations were approximately proportional to total soil Sb concentrations in all cases. Thus, the partitioning of Sb between soil matrix and soil solution could be expressed by a constant distribution coefficient K_D (K_D =

concentration sorbed / concentration dissolved). The K_D values found here were 195.0 ± 13.2 L kg^{-1} for the Sb^{III} treatment and 169.8 ± 9.9 L kg^{-1} for the Sb^{V} treatment in the case of the agricultural soil, and 144.5 ± 12.5 L kg^{-1} for the Sb^{III} treatment and 33.9 ± 6.2 L kg^{-1} for the Sb^{V} treatment in the case of the potting mix.

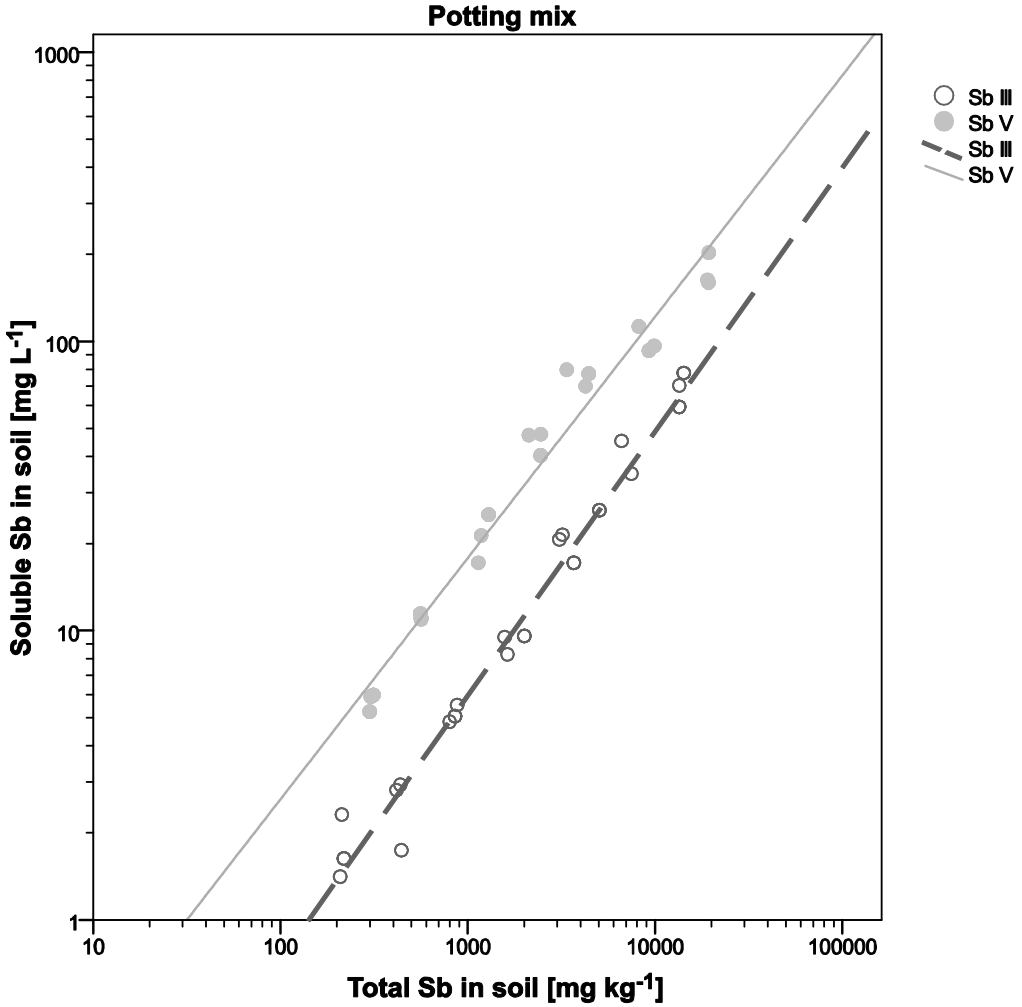

Fig. 1 Concentrations of soluble (mg L^{-1} soil solution) and total Sb (mg kg^{-1} dry soil) in the potting mix after adding various amounts of either antimonite (Sb^{III} treatment) or antimonite (Sb^{V} treatment). Points represent experimental data; lines were determined by linear regression on the log-transformed data.

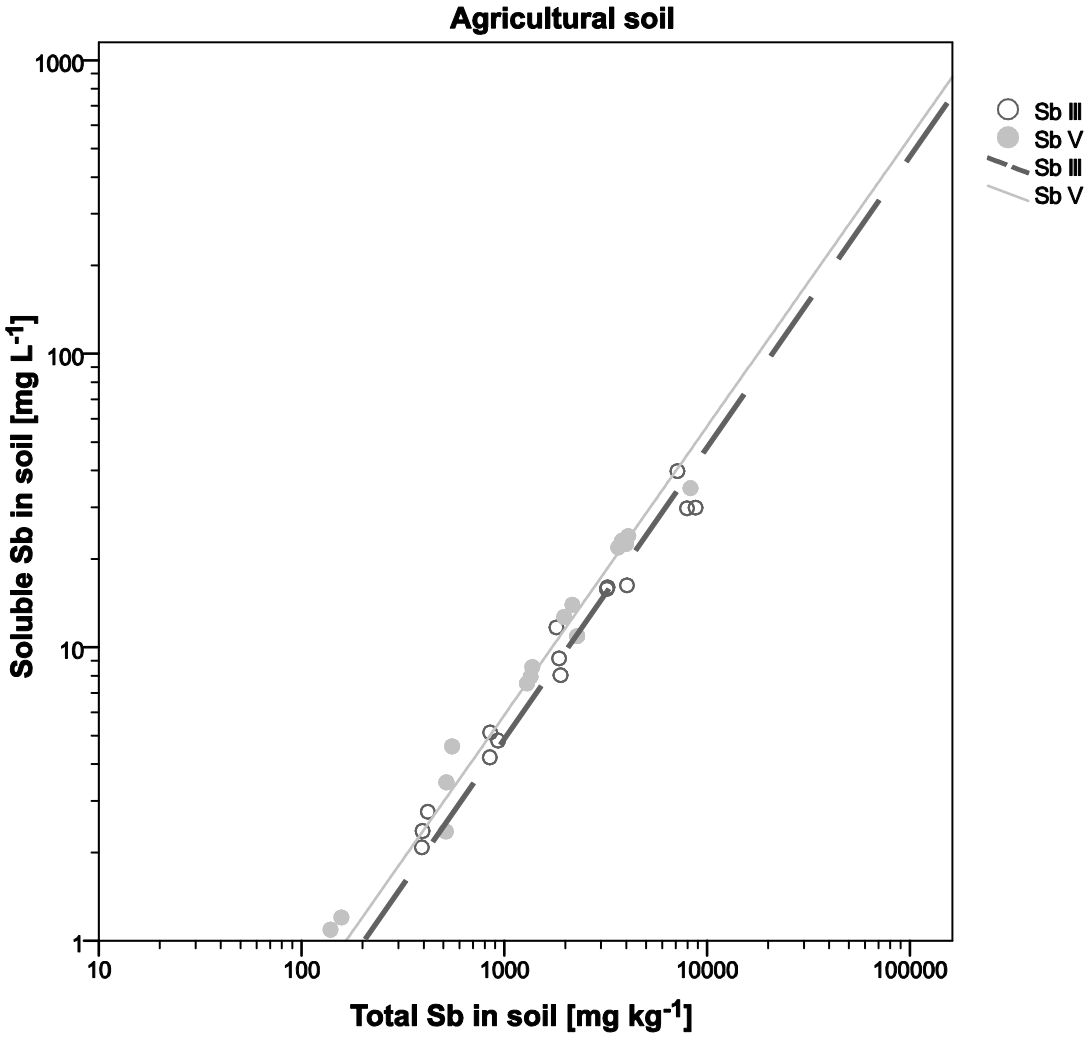

Fig. 2 Concentrations of soluble (mg L^{-1} soil solution) and total Sb (mg kg^{-1} dry soil) in the agricultural soil after adding various amounts of either antimonite (Sb^{III} treatment) or antimonite (Sb^{V} treatment). Points represent experimental data; lines were determined by linear regression on the log-transformed data.

5.3.2 Phytotoxicity and Uptake of Antimony

At the highest Sb concentrations applied in this study, toxicity symptoms such as reduced growth, chlorosis and leaf necrosis were observed in the two plants. Maize also showed anthocyane coloring of the stem base. Based on the shoot biomass data shown in Figures 3 and 4, maize appeared to be more sensitive to Sb in soil than sunflower. To quantify Sb toxicity on growth, we fitted the following 2-parameter empirical model commonly used in toxicology to the recorded dose-response curves:

$DW = exp(b+a*c)$ (Equation 1)

where *DW* is the dry weight of the aboveground biomass, c denotes the soluble Sb concentration of the soil, and a and b are fitting parameters. For comparison, because of its higher flexibility, we also fitted the following 3-parameter logistic model to the data:

$DW = (1/u + v\,(w^{c}))^{-1}$ (Equation 2)

where *u*, *v* and *w* are the fitting parameters. The parameter *u* corresponds to exp(*b*) in the 2-parameter model: both represent the dry mass DW_o produced in absence of Sb, i.e. at c=0. The fitted models were used to calculate EC_{50} values, i.e. the values of the soluble soil Sb concentrations at which growth was reduced to 50% of DW_o. Both models gave very similar results (Tables 1 and 2). The results, which are listed in Table 1, indicate that both plants were more sensitive to soluble soil Sb in the Sb^{V} than in the Sb^{III} treatment, although the difference was only significant for sunflower on the agricultural soil. Furthermore they reveal that soluble soil Sb was approximately 5 times more effective in reducing growth in the agricultural soil than in the potting mix. In the potting mix experiment, no toxicity was observed in sunflower even at the highest rate of Sb^{III} application. Table 2 shows that the Sb treatment effect on EC_{50} became insignificant, when *DW* was analysed as a function of shoot Sb concentrations, indicating that there were little or no substantial differences between the two Sb treatments in the oxidation state of the Sb that had been taken up by the plants and translocated into the shoots. In the case of maize, the disappearance of a significant difference between the two Sb treatments was primarily due to a larger scatter in the latter dose-response curves compared to the curves relating to soluble soil Sb. In contrast to the reduction in the difference between Sb treatments effect, the differences in growth response to Sb between the two plant species became more pronounced when their shoot biomass was related to the concentration of Sb in the shoots (Fig. 4). This finding shows that maize is physiologically more sensitive to Sb than sunflower, and that the difference between the two plant species is not due to different accumulation rates.

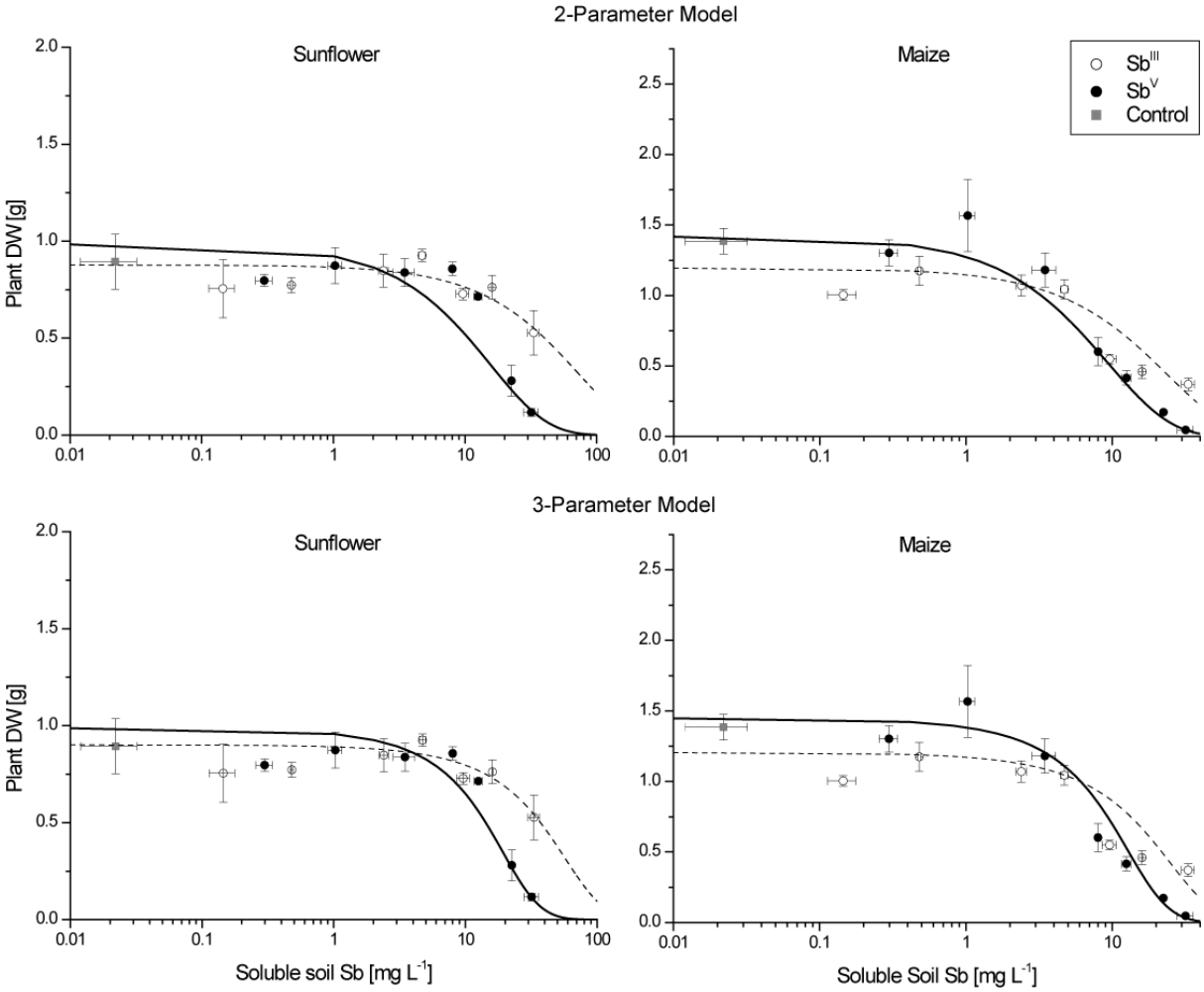

Fig. 3 Shoot biomass (g per plant) of 4-weeks old sunflower and maize plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonite (Sb^{V} treatment) had been added. Points represent averages of 3-4 replicates each. Error bars represent standard errors. Lines are model curves fitted to the experimental data using the 2-parameter model given by Equation 1 and the 3-parameter model given by Equation 2 in the text.

Figures 5, 6 and 7 show that Sb accumulation in the shoots was linearly related to the soluble soil Sb concentration on a log-log scale. The parameter values given in Table 3, which were determined by linear regression on the logarithms of the respective concentration values, excluding plants that showed clear toxicity symptoms, reveal that the slopes of the lines were close to 1 for sunflower on the agricultural soil in both Sb treatments (Table 3). This means that Sb accumulation by sunflower was approximately proportional to the soluble soil Sb concentration. The relationship was similar in both Sb treatments, with a tendency for higher Sb accumulation by sunflower in the Sb^{V} treatment. Also, we found no significant difference in Sb uptake between the two Sb treatments in maize, although here a treatment effect may have been masked by the larger scatter in the data compared to sunflower. The slopes of the

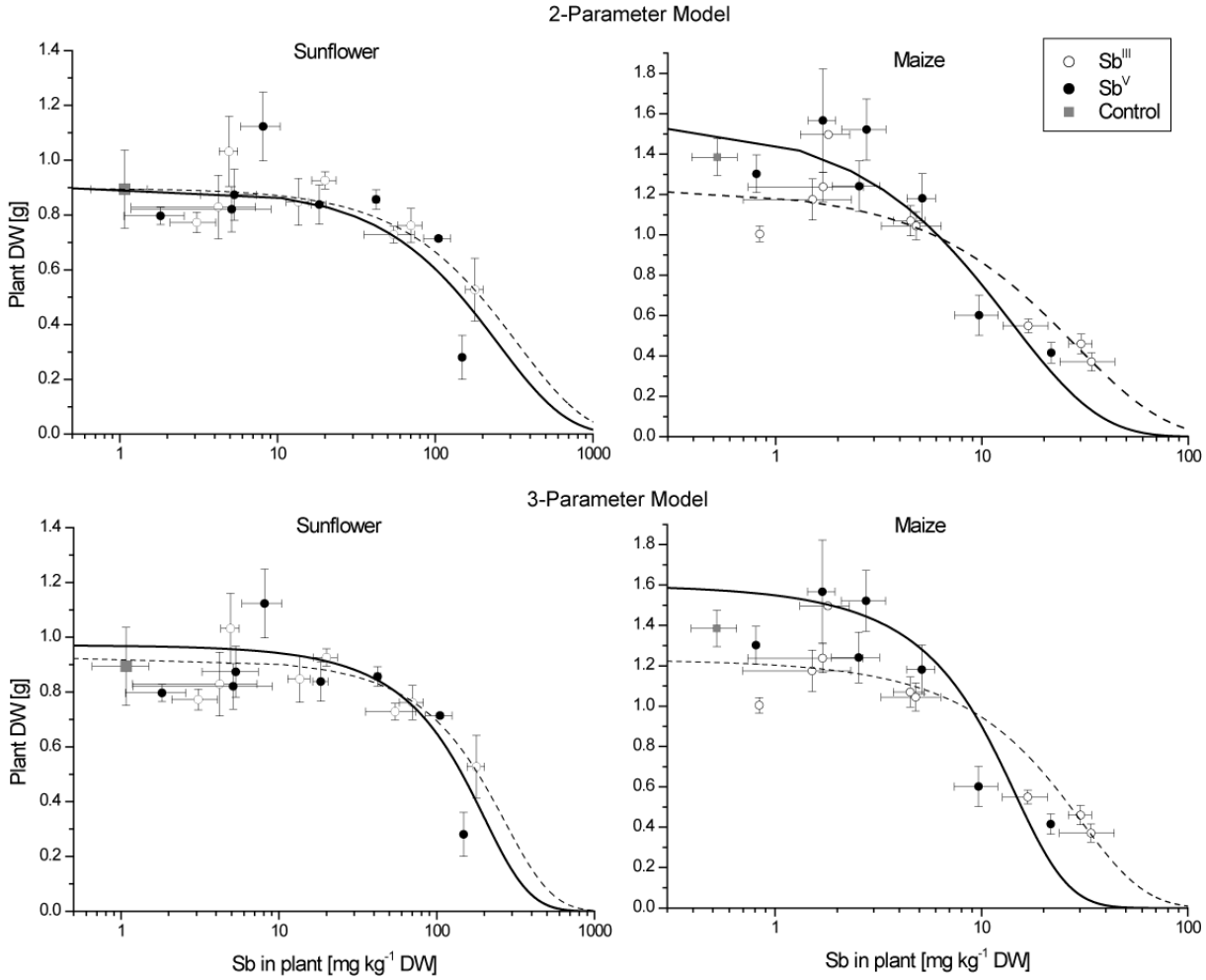

Fig. 4 Shoot biomass (g per plant) of 4-weeks old sunflower and maize plants as a function of the concentration of plant Sb (mg kg^{-1} dry weight) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonite (Sb^{V} treatment) had been added. Points represent averages of 3-4 replicates each. Error bars represent standard errors. Lines are model curves fitted to the experimental data using the 2-parameter model given by Equation 1 and the 3-parameter model given by Equation 2 in the text.

log-log regression line between shoot and soluble soil Sb concentrations, however, were smaller for maize than for sunflower. For both Sb treatments they were clearly less than 1 for maize on the agricultural soil, which means that the accumulation efficiency decreased with increasing concentration of Sb. As a result maize also accumulated less Sb than sunflower at high soil Sb concentrations, while there was no difference at low soil Sb concentrations.

Similar linear relationships between plant and soluble soil Sb as for the agricultural soil were also obtained for the potting mix. Although Sb was more soluble in the Sb^{V} than in the Sb^{III} treatment (Figure 1), this difference did not clearly translate into Sb uptake by the plants.

Table 1: EC_{50} values derived from the best-fit model dose-response curves relating shoot biomass production (dry weight) of sunflower and maize to the concentration of soluble soil Sb (mg L^{-1}). The two models are defined in the text.

EC_{50} relating to soluble soil Sb concentration

			H. annuus		*Z. mays*	
Substrate		Treatment	EC_{50}	SE	EC_{50}	SE
Potting mix	2-parameter model	Sb III	*	*	85.8	57.8
		Sb V	52.6	10.4	35.9	3.2
	3-parameter model	Sb III	*	*	83.0	63.5
		Sb V	62.0	24.7	46.8	17.1
Agricultural soil	2-parameter model	Sb III	48.3	14.2	16.6	1.5
		Sb V	10.8	0.9	6.5	0.2
	3-parameter model	Sb III	42.7	17.9	18.1	2.7
		Sb V	14.4	3.8	9.7	2.2

* no toxicity effect even at the highest treatment level

Table 2: EC_{50} values derived from the best-fit model dose-response curves relating shoot biomass production (dry weight) of sunflower and maize to the concentration of Sb in plant shoots (mg kg^{-1} DW). The two models are defined in the text.

EC_{50} relating to shoot Sb concentration

			H. annuus		*Z. mays*	
Substrate		Treatment	EC_{50}	SE	EC_{50}	SE
Agricultural soil	2-parameter model	Sb III	231.3	61.1	19.9	2.2
		Sb V	193.2	64.1	9.5	1.5
	3-parameter model	Sb III	205.0	77.0	21.9	4.1
		Sb V	150.7	56.2	11.3	7.8

Uptake of Sb by sunflower was less at low soil Sb concentrations from the potting mix than from the agricultural soil. However, as Sb accumulation increased slightly more than proportional with soluble soil Sb concentration, this difference disappeared at high soil Sb concentrations in potting mix while Sb accumulation was proportional to soluble soil Sb on agricultural soil. Figure 7 shows that uptake in leaves and stems was similar to the uptake in the whole shoot. While there was no significant difference between Sb^{III} and Sb^{V} treatments,

Sb accumulation was two- to threefold higher in leaves than in stems. Unfortunately, the data for the maize plants grown in the potting mix were much more scattered than for the maize plants grown in the agricultural soil.

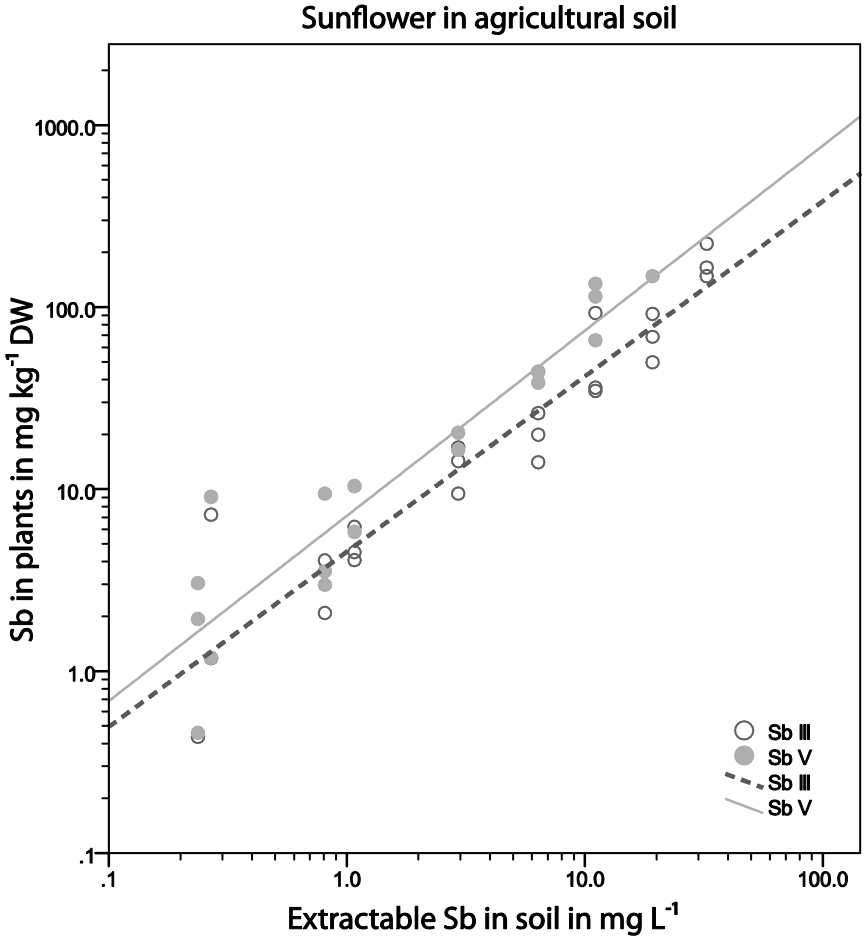

Fig. 5 Accumulation of Sb in 4-weeks old sunflower plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonite (Sb^{V} treatment) had been added. Points represent experimental data; lines were determined by linear regression on the log-transformed data.

5.4 Discussion

As mentioned in the introduction a number of authors have reported rapid oxidation of Sb^{III} to Sb^{V} in soil or soil mineral suspension, in particular Fe and Mn oxyhydroxides (Belzile et al. 2001; Oorts et al. 2008). Belzile et al. (2001) found that the oxidation of dissolved Sb^{III} to Sb^{V} followed a pseudo-first order rate kinetics and was complete within a few days in presence of suspended amorphous Fe and Mn oxyhydroxides at neutral to weakly alkaline pH, while the rate was reduced at lower pH, which they attributed to a decreased stability of the

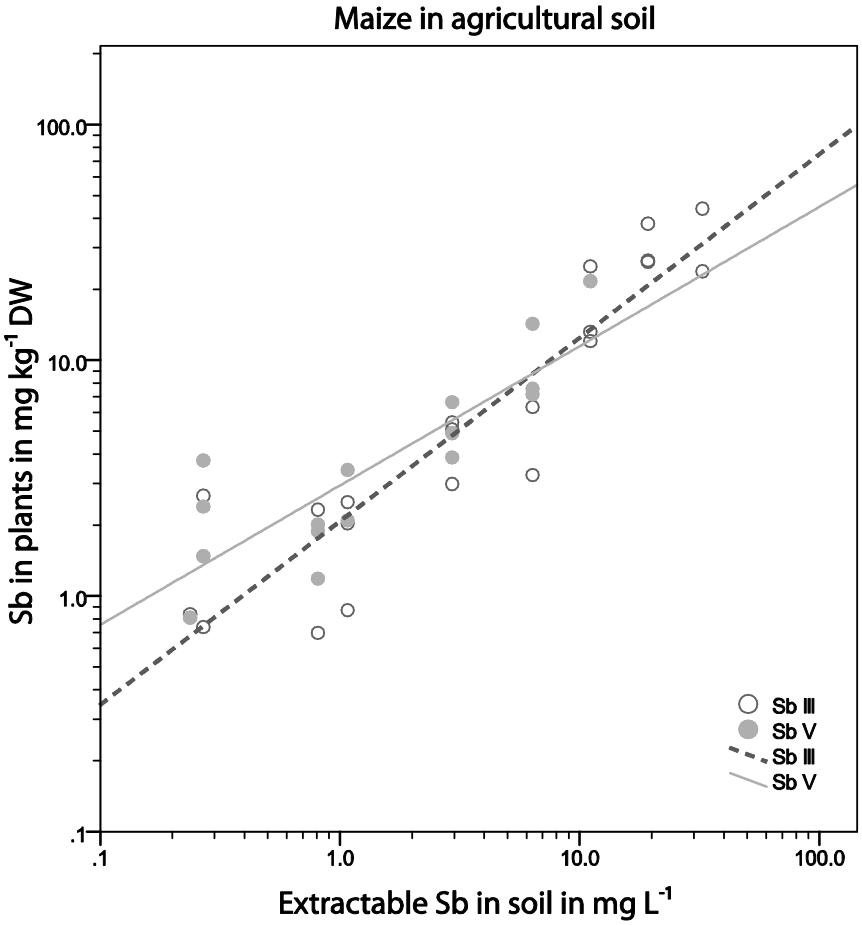

Fig. 6 Accumulation of Sb in 4-weeks old maize plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in agricultural soil to which various amounts of either antimonite (Sb^{III} treatment) or antimonite (Sb^{V} treatment) had been added. Points represent experimental data; lines were determined by linear regression on the log-transformed data.

oxyhydroxides. Oxidation was also slower in presence of natural Fe oxhydroxides than with synthetic Fe oxyhydroxides, which can be explained by the increased crystallinity and lower purity of the natural compounds. Oorts et al. (2008) added Sb_2O_3 in suspension to topsoil collected from an uncontaminated agricultural Haplic Luvisol and found that 70% of the Sb in solution was present as Sb^{V} after two days. Similarly, we found in a preliminary experiment, in which we added crystalline Sb_2O_3 to the agricultural soil used in our experiments here, that up to 80% of the Sb obtained by KNO_3-extraction was Sb^{V} after 6 hours incubation.

Based on the high oxidation rates reported in the literature, almost complete Sb^{III} oxidation was expected in our experiments. However, Sb solubility differed between the two Sb treatments for the potting mix. This difference may be attributed to kinetic limitations in the dissolution of the added Sb_2O_3. In the aforementioned preliminary experiment, we observed

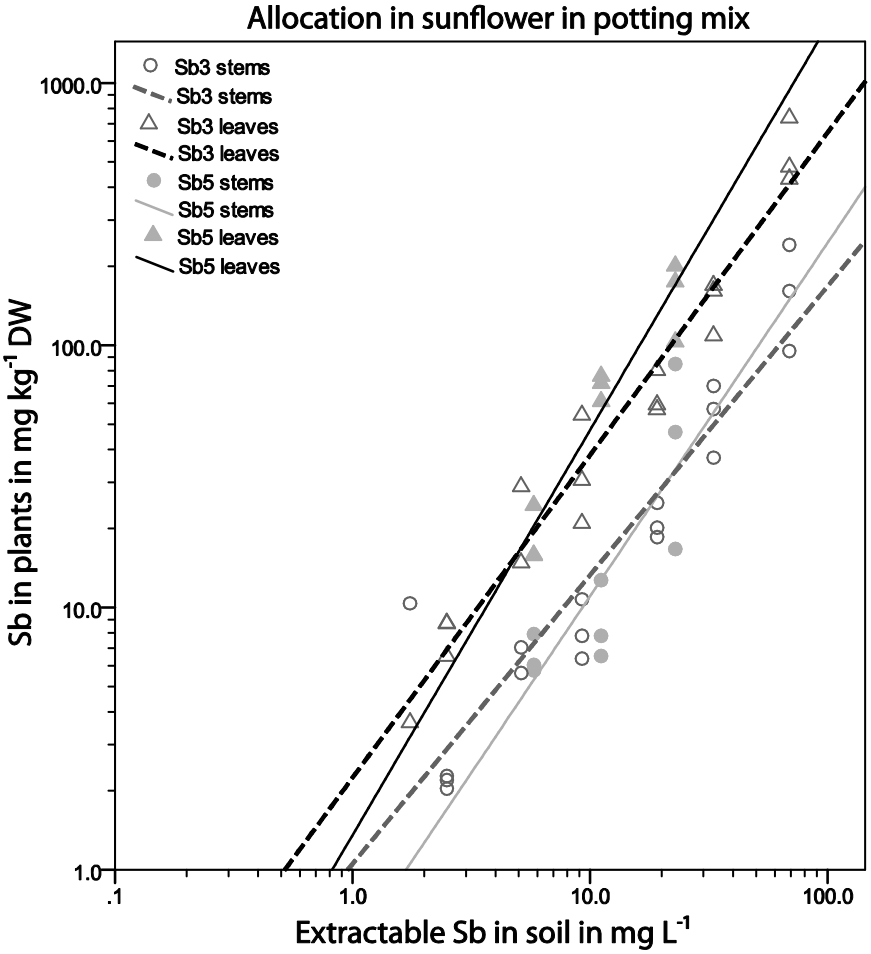

Fig. 7 Accumulation of Sb in leaves and stems of 4-weeks old sunflower plants as a function of the concentration of soluble Sb (mg L^{-1} soil solution) in potting mix to which various amounts of either antimonite (Sb^{III} treatment) or antimonite (Sb^{V} treatment) had been added. Points represent experimental data; lines were determined by linear regression on the log-transformed data.

that dissolution was the limiting process in the conversion of the added Sb_2O_3 to Sb^V in solution. This observation agrees well with the results of Oorts et al. (2008) who found that the concentration of Sb in solution continued to increase linearly with time over 5 weeks after addition of Sb_2O_3, while the final concentration at this time represented only 25% of the added amount of Sb. Thus, it seems likely that also in our experiment dissolution may not have been complete after the two weeks of equilibration, and that calculated K_D values thus only represent apparent partition coefficients, which would continue to decrease over time. Oorts et al. (2008) reported K_D values averaging 38 L kg^{-1} for Sb in soil amended with Sb_2O_3 soil and aged for 5 years. The solubility in the aged soil was similar to that of Sb that had been freshly added to the same soil as $SbCl_3$, indicating that dissolution kinetics was the limiting factor, while sorption occurred rapidly. The (apparent) K_D values calculated in our study are four to six times higher than the average K_D value reported by Oorts et al. (2008) for aged Sb contamination. While these values are still within the range of K_D values reported for

Table 3: Parameter values for the regression equation $\log(c_{plant}) = A*\log(c_{sol}) + \log(B)$ fitted to the experimental relationships between the log-transformed concentrations of soluble soil Sb (c_{sol}, mg L^{-1}) and Sb accumulated in sunflower and maize shoots (c_{plant}, mg kg^{-1} DW) for the two plants, soil substrates and Sb treatments. The numbers in parentheses give the standard errors for A (slope of the regression line) and the anti-logarithms of the standard errors of log(B), respectively.

		H. annuus			*Z. mays*		
Substrate	Treat-ment	B	A	R^2	B	A	R^2
Potting mix	Sb III	1.77 (1.26)	1.17 (0.08)	0.93*	4.60 (1.36)	0.53 (0.14)	0.50*
	Sb V	0.93 (1.40)	1.52 (0.14)	0.95*	0.44 (3.58)	0.93 (0.61)	0.37^{ns}
Agricultural soil	Sb III	4.53 (1.16)	0.96 (0.07)	0.89*	2.07 (1.17)	0.78 (0.08)	0.84*
	Sb V	7.12 (1.16)	0.85 (0.06)	0.88*	2.94 (1.14)	0.59 (0.10)	0.73*

*: significant at $P < 0.01$, ns: not significant

Sb in the literature (Cornelis et al. 2006; Tighe et al. 2005), the discrepancy indicates that thermodynamic equilibrium conditions had not been reached. Furthermore, Oorts et al. (2008) observed that the dissolution rate coefficient increased almost three-fold (from 0.005 to 0.014 day^{-1}) when the applied dose of Sb_2O_3 was increased from 0.41 to 81 mmol Sb kg^{-1} soil. The proportionality between soluble Sb concentration and applied Sb dose, however, indicates that the dissolution rate coefficient did not substantially increase with total (nominal) soil Sb concentration in our case.

The fact that the Sb treatment effect on Sb solubility was particularly pronounced for the potting mix can be entirely attributed to higher solubility of antimonate in this substrate, because the soluble soil Sb concentrations did not differ between the two substrates for a given level of Sb_2O_3 application in the Sb^{III} treatments. Buschmann and Sigg (2004) found that oxidation of Sb^{III} can be prevented by complexation with humic matter. However, such an effect cannot explain why there was a difference in Sb solubility between the two substrates in the Sb^{V}, but not in the Sb^{III} treatment. Higher solubility of antimonate may have been due to the higher organic matter content of the potting mix compared to the agricultural soil, in which the effect of the Sb treatment on Sb solubility was low. Complexation with soluble organic ligands possibly enhanced the solubility of antimonate in the potting mix more than in the agricultural soil. Oorts et al. (2008) found a plateau in Sb solubility at high concentrations of Sb in solution and suggested that this was due to Ca-antimonate

precipitation. Here, we found no such plateau and, thus, conclude that such precipitates did not limit Sb^V solubility in our substrates.

While Sb solubility differed little, especially in the agricultural soil, between the Sb^{III} and Sb^V treatments, a clear tendency for higher Sb uptake was observed in the Sb^V treatment in the case of sunflower suggesting that different Sb species were present in solution with different availability for plant uptake and that the concentrations of these Sb species were different in the two Sb treatments. The approximate proportionality between soluble soil Sb and accumulated plant Sb concentrations, extending over two orders of magnitude for sunflower on the agricultural soil, agrees well with previous findings as reviewed by Tschan et al. (2009b), indicating that uptake was dominated by non-selective transport with the transpiration water stream, passing through leaks in the endodermis or in the case of antimonite also through aquaporins as mentioned already in the introduction. The decrease in Sb accumulation efficiency with increasing Sb concentration that was observed in maize on agricultural soil, but to a slight degree also in sunflower in the Sb^V treatment may reflect to some degree a trend of concentration-dependent Sb speciation; but the differences in these trends between the two plant species show that plant factors also played a role. Unfortunately, the data from the potting mix experiment were too scattered to establish similarly clear trends as for the agricultural soil.

Given that Sb^{III} is generally considered more toxic than Sb^V the lower tolerance of both plants to soluble soil Sb in the Sb^V treatments may be surprising. However, for sunflower, at least, the difference between the two treatments can be explained by the greater Sb accumulation in the Sb^V treatment. For maize, relating toxicity to accumulation did not fully explain the difference in toxicity between the two Sb treatments; it only made it less significant due to increased uncertainty in dose-response curve fitting. A difference in toxicity between the two Sb treatments would mean that Sb speciation in maize could not have been exactly the same or that the toxicity effects on shoot growth were due to interactions between soil Sb and plant roots. In any case, contrary to expectation, the Sb^V treatment was more toxic than or at least as toxic as the Sb^{III} treatment in maize. In sunflower the observed toxicity effects on shoot growth appeared to be related solely to the accumulation of Sb in the shoot tissue. In this case, there either was no difference in toxicity between different Sb species accumulated by the plants or all accumulated Sb was rapidly converted to species of similar toxicity.

The EC_{50} values estimated here from the reponse of shoot growth to soluble Sb in the agricultural soil amended with Sb^{III} ranged between 40 and 50 mg L^{-1} for sunflower and between 16 and 18 mg L^{-1} for maize, depending on the model used to fit the experimental

curves. These values are comparable to the EC_{50} values varying around 40 mg L^{-1} (0.34 mmol L^{-1}) that were obtained by Oorts et al. (2008) for lettuce shoot yield. The good agreement between the EC_{50} values obtained with the two models indicates that the estimates were robust.

The dose-response curves obtained here confirm the findings of Oorts et al. (2008) that background soil Sb concentrations are two orders of magnitude lower than typical phytotoxic levels. However, Sb may well occur at phytotoxic concentrations in Sb contaminated soils, in particular in shooting range soils. Our results suggest that even when the predominate form of Sb is antimonate Sb toxicity may still remain a serious risk in Sb contaminated soil.

5.5 Acknowledgements

We gratefully acknowledge Anna Grünwald, Björn Studer, René Saladin and Viktor Stadelmann for their generous help and support in the laboratory. This project was financially supported by the Swiss National Science Foundation (Grants No. 200021-103768).

5.6 References

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .1. Vegetation and Soils, *Environmental Pollution* **1990**, *65*, 65-77.

Asher, C. J., Reay, P. F., Arsenic Uptake by Barley Seedlings, *Australian Journal of Plant Physiology* **1979**, *6*, 459-466.

Baes, C. F., Mesmer, R. E., Arsenic, Antimony, Bismuth. In: *The Hydrolysis of Cations* (Eds **1986**, pp. 366-375 (Krieger: Malabar, FL).

Baroni, F., Boscagli, A., Protano, G., Riccobono, F., Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area, *Environmental Pollution* **2000**, *109*, 347-352.

Belzile, N., Chen, Y. W., Wang, Z. J., Oxidation of antimony (III) by amorphous iron and manganese oxyhydroxides, *Chem. Geol.* **2001**, *174*, 379-387.

Blaser, P., Pannatier, E. G., Walthert, L., The base saturation in acidified Swiss forest soils on calcareous and noncalcareous parent material. A pH-base saturation anomaly, *Journal of Plant Nutrition and Soil Science* **2008**, *171*, 155-162.

Blay, K., *Sorption wässriger Antimon-Spezies an bodenbildende Festphasen und Remobilisierung durch natürliche Komplexbildner.* PhD Thesis, **2000**, Technische Universität München.

Bowen, H. J. M., *Environmental Chemistry of the Elements* **1979**, Academic Press: London.

Buschmann, J., Sigg, L., Antimony(III) Binding to Humic Substances: Influence of pH and Type of Humic Acid, *Environ. Sci. & Technol.* **2004**, *38*, 4535.

Cornelis, G., Van Gerven, T., Vandecasteele, C., Antimony leaching from uncarbonated and carbonated MSWI bottom ash, *Journal of Hazardous Materials* **2006**, *137*, 1284-1292.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters I. Occurence, *Earth-Science Reviews* **2002**, *57*, 125-176.

Filella, M., Belzile, N., Lett, M. C., Antimony in the environment: A review focused on natural waters. III. Microbiota relevant interactions, *Earth-Sci. Rev.* **2007**, *80*, 195-217.

Gebel, T., Arsenic and antimony: comparative approach on mechanistic toxicology, *Chemico-Biological Interactions* **1997**, *107*, 131-144.

Gresch, M., Wettstein, B., *Antimon- und Bleibelastung bei Schiessanlagen, Fallbeispiel Eschenbach (SG)* Semester Thesis, **2002**, ETH Zurich.

Gulz, P. A., Gupta, S. K., Schulin, R., Arsenic accumulation of common plants from contaminated soils, *Plant Soil* **2005**, *272*, 337-347.

Gurnani, N., Sharma, A., Talukder, G., Effects of antimony on cellular systems in animals - a review, *Nucleus* **1994**, *37*, 71-96.

Johnson, C. A., Moench, H., Wersin, P., Kugler, P., Wenger, C., Solubility of antimony and other elements in samples taken from shooting ranges, *J. Environ. Qual.* **2005**, *34*, 248-254.

Kabata-Pendias, A., Pendias, H., *Trace Elements in Soils and Plants* **1984**, CRC Press: Boca Raton, FL.

Krachler, M., Emons, H., Zheng, J., Speciation of antimony for the 21st century: promises and pitfalls, *Trac-Trends in Analytical Chemistry* **2001**, *20*, 79-90.

Leuz, A. K., *Redox Chemistry of Antimony in Aquatic Systems: e.g. Lakes* Diploma, **2002**, Carl von Ossietzky Oldenburg.

Oorts, K., Smolders, E., Degryse, F., Buekers, J., Gasco, G., Cornelis, G., Mertens, J., Solubility and toxicity of antimony trioxide (Sb_2O_3) in soil, *Environ. Sci. Technol.* **2008**, *42*, 4378-4383.

Pratas, J., Prasad, M. N. V., Freitas, H., Conde, L., Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation, *J. Geochem. Explor.* **2005**, *85*, 99-107.

Rooney, C. P., McLaren, R. G., Cresswell, R. J., Distribution and phytoavailability of lead in a soil contaminated with lead shot, *Water Air Soil Pollut.* **1999**, *116*, 535-548.

Sachs, L., *Angewandte Statistik* **2004**, Springer: Berlin, Heidelberg, New York.

Tighe, M., Lockwood, P., Wilson, S., Adsorption of antimony(v) by floodplain soils, amorphous iron(III) hydroxide and humic acid, *Journal of Environmental Monitoring* **2005**, *7*, 1177-1185.

Tschan, M., Robinson, B., Schulin, R., Antimony uptake by *Zea mays* (L.) and *Helianthus annuus* (L.) from nutrient solution, *Environmental Geochemistry and Health* **2008**, *30*, 187-191.

Tschan, M., Robinson, B. H., Nodari, M., Schulin, R., Antimony uptake by different plant species from nutrient solution, agar and soil, *Environmental Chemistry* **2009a**, *6*, 144-152.

Tschan, M., Robinson, B. H., Schulin, R., Antimony in the soil-plant system - a review, *Environmental Chemistry* **2009b**, *6*, 106-115.

Woolson, E. A., Axley, J. H., Kearney, P. C., Chemistry and Phytotoxicity of Arsenic in Soils .2. Effects of Time and Phosphorus, *Soil Science Society of America Journal* **1973**, *37*, 254-259.

6 Antimony uptake by *Zea mays* (L.) and *Helianthus annuus* (L.) from nutrient solution

Martin Tschan, Brett Robinson & Rainer Schulin

Environmental Geochemistry and Health **2008**, *30*, 187-191

Abstract

We investigated the extent of Sb uptake by maize (*Zea mays*) and sunflower (*Helianthus annuus*) from nutrient solutions containing concentrations from 3 mg L^{-1} to 24 mg L^{-1} potassium antimonate, with the aim of determining the potential of Sb to enter the food chain. The maximum shoot Sb concentrations in *Z. mays* and *H. annuus* were 41 mg kg^{-1} and 77 mg kg^{-1} dry wt, respectively. There was no significant difference in Sb uptake between species. The average bioaccumulation coefficients (the plant / solution concentration quotients) were 1.02 and 1.93 for *Z. mays* and *H. annuus*, respectively. Phosphate addition did not affect plant growth or Sb uptake. Antimony uptake by the *Z. mays* and *H. annuus* is unlikely to pose a health risk to animals and humans.

6.1 Introduction

Antimony (Sb) is a rare element in the earth's crust, occurring at 0.2 – 0.3 mg kg^{-1} (Rish 2004). Common minerals are sulphides (in particular stibnite Sb_2S_3) and oxides (valentinite Sb_2O_3 and cervantite Sb_2O_4). However, it has become widespread in the environment because of its industrial uses in fire retardants, semiconductors, and as an agent for metal hardening (Filella et al. 2002). Antimony consumption has increased with global economic growth, resulting in elevated Sb concentrations in soils and natural waters, where it may affect plants, animals and humans.

In Switzerland, high levels of Sb occur in soils associated with the ca. 2000 shooting ranges scattered throughout the country (Gresch and Wettstein 2002). On average, new bullets and pellets consist of over 90% Pb, 1-7% Sb, <2% arsenic (As) and <0.5% nickel (Ni) (Rooney et al. 1999). Johnson et al. (2005) reported that Sb was the most soluble and mobile of all the

trace element contaminants in Swiss shooting ranges. Wersin et al. (2002) reported elevated Sb concentrations in receiving waters near a Swiss shooting range.

Antimony is a non-essential element for both plants and animals. Antimony is toxic to humans at chronic uptake rates exceeding 100 mg day^{-1} (Bowen 1979); rats are susceptible to an intake of 11-75 mg day^{-1}. Due to its toxicity and potential carcinogenic nature, there are regulations regarding the human exposure to Sb in the workplace (Rish 2004). The EU limit for Sb in drinking water is 5 μg L^{-1} (Filella et al. 2002). The European Commission has set a threshold of 0.04 mg Sb kg^{-1} for plastic materials and articles intended to come in contact with food (EU 2005). To date, there are no Swiss limits for antimony in soil and food. The Dutch intervention values for soil and groundwater are 15 mg kg^{-1}, and 20 μg L^{-1}, respectively (Swartjes 1999).

The biogeochemical behaviour of Sb is in similar to the other group VI elements arsenic (As) and phosphorous (P). It is a metalloid that exists in four oxidation states: -III, 0, +III, and +V. In the environment, Sb usually occurs as Sb(III) (antimonite) and Sb(V) (antimonate) in reducing and oxidizing conditions respectively. In soils, Sb(III) is oxidized within hours to Sb(V) (Krachler et al. 2001).

Compared to other trace elements, Sb is relatively mobile in soils, increasingly the likelihood of its entry into the food chain via plant uptake (Gresch and Wettstein 2002). Ainsworth et al. (1990a) measured Sb concentrations in the leaves of several grasses growing adjacent to an Sb smelter in Northeast England, where soil Sb concentrations reached 400 mg kg^{-1}. They found leaf Sb concentrations of over 300 mg kg^{-1} in some samples. Grasses grown near the smelter in pots that contained non-contaminated soil had similar Sb concentrations to plants growing in contaminated soil. This indicated that leaf Sb burden came from surface deposition, rather than plant uptake. Nevertheless, invertebrates and mammals that feed on these plants had elevated Sb concentrations in various tissues (Ainsworth et al. 1990b).

Baroni et al. (2000) reported over 1000 mg kg^{-1} Sb in the basal leaves of *Achillea ageratum* growing in a mine soil containing 9000 mg kg^{-1} Sb. Other species growing in the same soil also had foliar Sb concentrations greater than 100 mg kg^{-1}. Conversely, Pratas et al. (2005) reported maximum stem concentrations of less than 5 mg kg^{-1} Sb for species on growing in a

Portuguese mine soil with an average soil concentration of 663 mg kg^{-1}. There is a lack of information on possible mechanisms of Sb toxicity in plants.

The aforementioned studies indicate that there is large inter-specific variability in the plant uptake of Sb and that surface deposition can account for a large proportion of total Sb content of plant stems and foliage collected in the field.

Given that Sb has similar chemical properties to As and P, plants may take it up by the same mechanism. It has been shown that the addition of P to soil affects the plant uptake of As by competing for binding sites in the soil, thus enhancing solubility, and competing for transporters into the plants, thus reducing uptake of the soluble As (Woolson et al. 1973). However, there are no comparable studies on the possible effects of P on plant Sb uptake.

This study aimed to determine the extent of Sb translocation into the shoots of two crop plants, *Zea mays* (L.) and *Helianthus annuus* (L.) and to reveal whether phosphate addition affects the plant uptake of Sb. Our experiments used antimonate (Sb^V), because it is the most common chemical species in water and soil solution (Filella et al. 2002). We chose a hydroponic system for our experiments to eliminate surface contamination and the influence of soil parameters.

6.2 Material and Methods

We grew maize (*Zea mays* (L.) cv. Magister, a monocotyledon) and sunflower (*Helianthus annuus* (L.) cv. Iregi, a dicotyledon) in a climate chamber (photoperiod 16h, with day temperature 22/14°C (day/night) and light intensity of 11000 lux. Seeds were germinated in quartz sand. After 2 weeks, they were transferred to 30 L plastic boxes containing a modified Hoagland's nutrient solution. The nutrient solution consisted of 0.4 mM $Ca(NO_3)_2$, 0.2 mM $MgSO_4$, 0.1 mM KH_2PO_4**,** 0.5 mM KNO_3, 0.01 mM NaFe(III)EDTA, 0.01 mM H_3BO_3, 2 µM $MnSO_4$, 0.2 µM $ZnSO_4$, 0.2 µM $CuSO_4$, 0.1 µM Na_2MoO_4 and 0.02 mM NaCl (Krämer et al. 1996).

Two weeks later, single plants were transferred to 1 L plastic vessels containing treatment solutions. These comprised modified Hoagland's solution and added Sb in absence or presence of phosphate (PO_4^{3-}) in the solution. Antimony was added (as $KSb(OH)_6$) to the

nutrient solution at concentrations of 0, 3, 6, 12, 18 to 24 mg L^{-1}, in combination with either 0 mg L^{-1} P or 3 mg L^{-1} P. Each treatment had two replicates. Solutions were adjusted to pH 6 with NaOH. continually aerated, and replaced weekly.

After one week in the treatment solutions, plants were harvested, the roots and shoots separated, and dried at 65°C until constant weight was obtained. Dried shoots were digested by aqua regia in closed Teflon vessels in microwave (MLS). The total Sb concentrations were measured using Hydride Generation Atomic Fluorescence Spectroscopy (HG-AFS) (PSAnalytical). We analysed a reference material (Virginia tobacco leaves IC-CTA-VTL2) and obtained a recovery of 94.5%. Statistical analyses (ANOVA and regression) were performed with SPSS 13.0 (SPSS 2004).

6.3 Results

Antimony produced no toxicity symptoms in the plants even at the highest Sb concentrations. There was no significant decrease in the biomass of plants treated with Sb compared to the untreated controls. The average dry biomass of the plants at the end of the experiment was 2.4 ± 0.11 g for maize and 1.8 ± 0.06 g for sunflowers. The water use during the one week treatment was 0.22 ± 0.01 L for maize and 0.22 ± 0.006 L for sunflowers. In previously identical experiments using As, short term phytotoxicity appeared at concentrations of 3 mg L^{-1} (Tschan and Schulin 2006). The experiments indicate that Sb is less phytotoxic than As. However, the one-week length of our treatment period precludes any conclusions regarding Sb toxicity over the life cycle of the plant.

Antimony uptake by both species was significantly and positively correlated with the Sb concentration in the nutrient solution (Fig. 1 and 2). The maximum shoot Sb concentrations for *Z. mays* and *H. annuus* were 41 mg kg^{-1} and 77 mg kg^{-1} dry wt, respectively. These occurred at respective solution concentrations of 18 and 30 mg L^{-1}. There was no significant difference in Sb uptake between species. The average bioaccumulation coefficients, defined here as the plant / solution concentration quotients, were 0.93 and 1.33 for *Z. mays* and *H.annuus*, respectively in absence of P. Phosphate addition had no significant effect on the uptake of Sb (Fig. 1 and 2). It neither increased growth nor induced phytotoxicity.

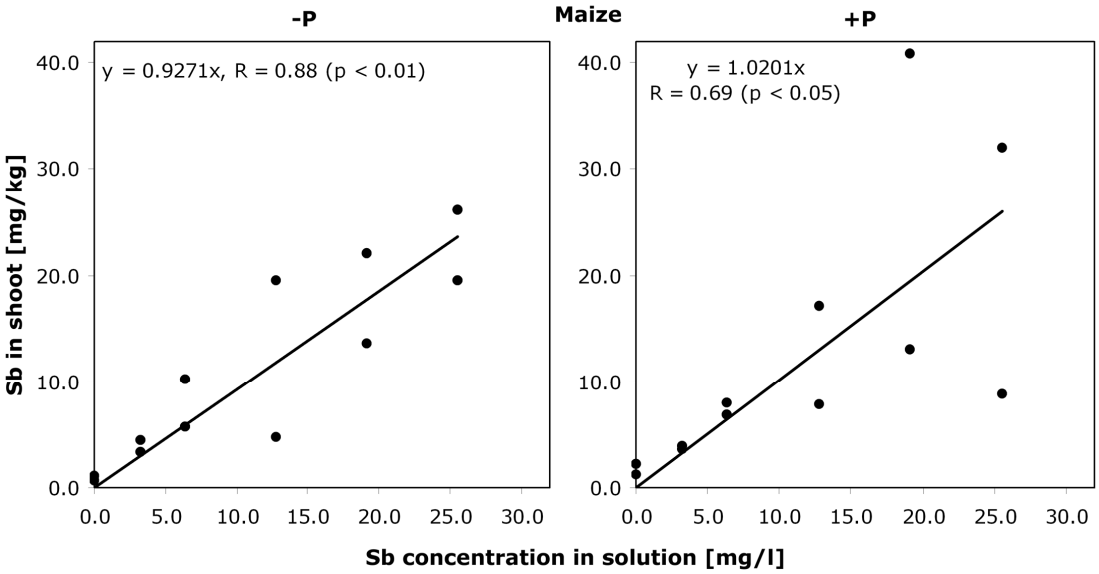

Fig. 1 Antimony concentrations (mg kg^{-1} dry matter) in the shoots of maize and the effect of adding of 3 mg L^{-1} phosphate to the ambient solutions.

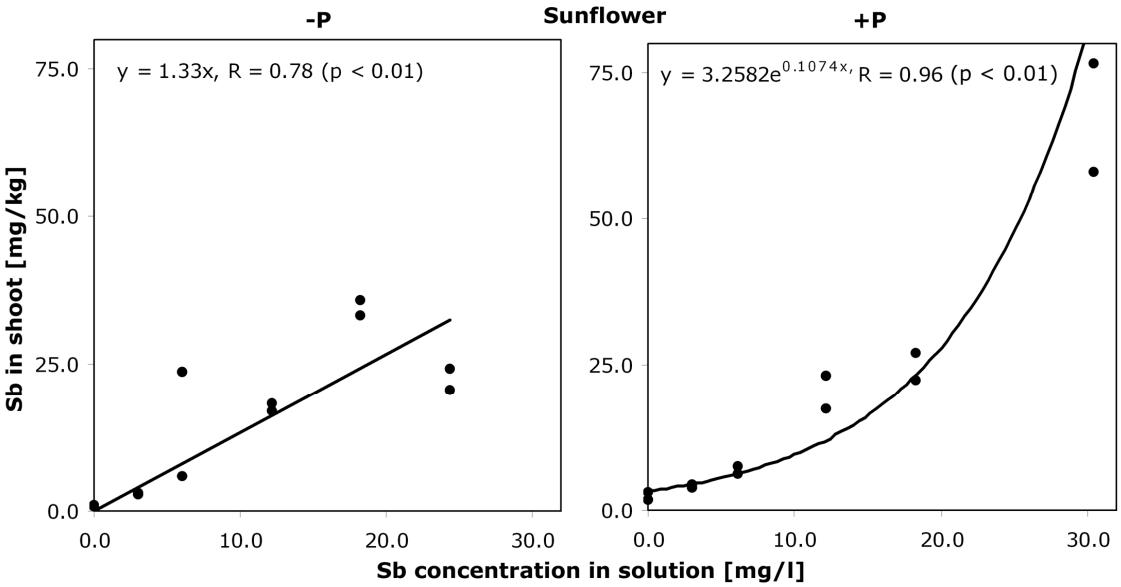

Fig. 2 Antimony concentrations (mg kg^{-1} dry matter) in the shoots of sunflowers and the effect of adding of 3 mg L^{-1} phosphate to the ambient solutions.

6.4 Discussion

Z. mays and *H. annuus* took up Sb from nutrient solution, and transported some into shoots. The extent of Sb translocation, as indicated by the bioaccumulation coefficient, is lower than that found for some other common trace elements. Tandy et al. (2006) found in similar experiments using Cu, Zn and Pb that the bioaccumulation coefficients for sunflowers in hydroponics were: 6.2, 123, and 0.02 for Cu, Zn and Pb respectively. Our experiments reveal the rate of Sb uptake over one week, rather than the total Sb accumulated over the lifetime of

the plant. Therefore, the Sb concentration in mature plants may be different than we report here.

Johnson et al. (2005) found concentrations of up to 6 mg L^{-1} Sb in leachates from shooting range soil samples with total Sb concentrations up to 10 g kg^{-1}. Based on this data and using our bioaccumulation coefficients, the predicted uptake, not allowing for any modifying soil factors, would be 5.6 mg kg^{-1} for maize and 8.0 mg kg^{-1} for sunflowers. Given that 100 mg day^{-1} are chronically toxic (Bowen 1979), an animal would have to consume more than 0.9 kg of maize or 1.3 kg of sunflower a day (assuming concentrations of 77 mg kg^{-1} in sunflowers) over a longer period. The direct consumption of soil (which contained up to 10 mg kg^{-1} Sb) by herbivores or children poses a bigger threat.

That phosphate did not affect the uptake and toxicity of Sb, indicates that Sb, unlike As, is not taken up via the phosphate pathway. This may be due to the different structures of their pentavalent oxyanions. Arsenate (AsO_4^{3-}) is tetrahedral while $Sb(OH)_6^-$ is octahedral (Baes and Mesmer 1986). The linear uptake of Sb by plants in these experiments indicates that plants either take up Sb by another selective pathway, which was not at saturation in our experiment, or via a non-selective apoplastic pathway (Bell et al. 2003).

6.5 Acknowledgements

We gratefully acknowledge A. Grünwald, R. Saladin and Viktor Stadelmann for their continuous help and support in the laboratory. This project was financially supported by the Swiss National Science Foundation (Grants No. 200021-103768).

6.6 References

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .1. Vegetation and Soils, *Environmental Pollution* **1990a**, *65*, 65-77.

Ainsworth, N., Cooke, J. A., Johnson, M. S., Distribution of Antimony in Contaminated Grassland .2. Small Mammals and Invertebrates, *Environmental Pollution* **1990b**, *65*, 79-87.

Baes, C. F., Mesmer, R. E., Arsenic, Antimony, Bismuth. In: *The Hydrolysis of Cations* (Eds **1986**, pp. 366-375 (Krieger: Malabar, FL).

Baroni, F., Boscagli, A., Protano, G., Riccobono, F., Antimony accumulation in *Achillea ageratum*, *Plantago lanceolata* and *Silene vulgaris* growing in an old Sb-mining area, *Environmental Pollution* **2000**, *109*, 347-352.

Bell, P. F., McLaughlin, M. J., Cozens, G., Stevens, D. P., Owens, G., South, H., Plant uptake of ^{14}C-EDTA, ^{14}C-citrate, and ^{14}C-histidine from chelator-buffered and conventional hydroponic solutions, *Plant and Soil* **2003**, *253*, 311-319.

Bowen, H. J. M., *Environmental Chemistry of the Elements* **1979**, Academic Press: London.

EU, *Commission directive 2005/79/EC* **2005**.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters I. Occurence, *Earth-Science Reviews* **2002**, *57*, 125-176.

Gresch, M., Wettstein, B., *Antimon- und Bleibelastung bei Schiessanlagen, Fallbeispiel Eschenbach (SG)* Semester Thesis, **2002**, ETH Zurich.

Johnson, C. A., Moench, H., Wersin, P., Kugler, P., Wenger, C., Solubility of antimony and other elements in samples taken from shooting ranges, *J. Environ. Qual.* **2005**, *34*, 248-254.

Krachler, M., Emons, H., Zheng, J., Speciation of antimony for the 21st century: promises and pitfalls, *Trac-Trends in Analytical Chemistry* **2001**, *20*, 79-90.

Krämer, U., Cotter-Howells, J. D., Charnock, J. M., Baker, A. J. M., Smith, J. A. C., Free histidine as a metal chelator in plants that accumulate nickel, *Nature* **1996**, *379*, 635-638.

Pratas, J., Prasad, M. N. V., Freitas, H., Conde, L., Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation, *J. Geochem. Explor.* **2005**, *85*, 99-107.

Rish, M. A., Antimony. In: *Elements and their Compounds in the Environment* (Eds Merian, E., Anke, M., Ihnat, M., Stoeppler, M.) **2004**, pp. 659-670 (Wiley-VHC: Weinheim).

Rooney, C. P., McLaren, R. G., Cresswell, R. J., Distribution and phytoavailability of lead in a soil contaminated with lead shot, *Water Air Soil Pollut.* **1999**, *116*, 535-548.

Swartjes, F. A., Risk-based assessment of soil and groundwater quality in the Netherlands: Standards and remediation urgency, *Risk Analysis* **1999**, *19*, 1235-1249.

Tandy, S., Schulin, R., Nowack, B., The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers, *Chemosphere* **2006**, *62*, 1454-1463.

Tschan, M., Schulin, R. Poster: Phytoavailability and Plant Uptake of Antimony, Workshop: *Bioavailability of Pollutants and Soil Remediation* **2006**, Sevilla, Spain. In; 2006.

Wersin, P., Johnson, C. A., Furrer, G., Antimony contamination in soil and ground water by shooting range activities, *Geochim. Cosmochim. Acta* **2002**, *66*, A829-A829.

Woolson, E. A., Axley, J. H., Kearney, P. C., Chemistry and Phytotoxicity of Arsenic in Soils .2. Effects of Time and Phosphorus, *Soil Science Society of America Journal* **1973**, *37*, 254-259.

7 Conclusion and Outlook

The main objectives of this study were to investigate the uptake of Sb^{III} and Sb^{V} by selected plants from standardized nutrient solutions and soils and its concentration dependence.

7.1 Antimony in the soil-plant system – a review

Our literature review showed a significant linear relationship between Sb in plants and total and extractable Sb in soils over five orders of magnitude. This correlation was significant despite the different plant species, soils, extraction methods and analytical methods used in the cited publications.

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

The plants in our experiments took up Sb from nutrient solution, agar and soil, and transported it into shoots. We can therefore conclude that the Sb found in the shoots entered the plants through the roots. The experiments also showed highly significant correlations between Sb in plants and soluble or soil extractable Sb for all three growth media (nutrient solution, agar and soil) and all six plant species studied.
Uptake was highest in agar, slightly lower in soil and lowest in nutrient solutions. The linear uptake indicates that plants either take up Sb by another selective pathway, which was not at saturation in our experiment, or via a non-selective apoplastic pathway.

7.3 Antimony uptake by sunflower and maize form soil comparing Sb^{III} and Sb^{V} contamination

Antimony was toxic to plants regardless of the oxidation species in which it had been added to the soil. In both treatments the plants took up the antimony and showed toxicity. Sb^{III} is reported to be much more toxic than Sb^{V}, but this was not the case in our treatments. On the contrary Sb^{V} treatment showed trends for higher toxicity, which is probably due to its higher solubility. Also the oxidation behaviour of Sb in soils remains unclear.

7.4 Antimony uptake by Zea mays (L.) and Helianthus annuus (L.) from nutrient solution

Sunflower (*Helianthus annuus*) and maize (*Zea mays*) took up Sb form the solutions, showing a significant linear relationship. They showed reduced dry weight and toxicity symptoms at the highest concentrations in nutrient solution. No difference was found between the treatments with or without phosphate indicating that it does not compete with Sb^V for uptake into plants.

7.5 Outlook

Due to its widespread use, antimony can be found in many locations at enriched concentrations. The risks Sb poses to humans and ecosystems are difficult to estimate because of lacking data about Sb behaviour in soils and biological systems. The low toxicity of Sb to plants presents a risk as it allows for high uptake of Sb by plants on highly contaminated soils. Uptake may particularly high in plants with root damage, e.g. due to feeding by soil organisms of infection by root pathogens. However, the role of soil organisms, including mycorrhizal fungi, on plant Sb accumulation has not been studied so far.
Our experiments showed that uptake rates differed between plant species and that Sb is mostly accumulated in plants leaves. Dicotyledons took up more Sb than monocotyledons.

Plants may look healthy although they contain a substantial amount of Sb in their tissues. This can lead to chronic intoxication, if such plants are consumed over a longer period by humans or animals. The question of the long term risks arising from Sb contaminated soils to human and animals needs much further investigation.

Appendix 1

Influence of phosphate, sulfate and potassium on antimony uptake in two crop species from artificially contaminated soil.

Introduction

We investigated the influence of phosphate, sulfate and potassium on the uptake of antimony by sunflower (*Helianthus annuus* L. cv. Iregi) and maize (*Zea mays* L. cv. Magister) in pot experiments.

Material and Methods

We grew sunflower (*Helianthus annuus* L. cv. Iregi) and maize (*Zea mays* L. cv. Magister) in a climate chamber (photoperiod 16h, with day temperature 22/14°C (day/night) and light intensity of 11000 lux).

The soil used for the pot experiments was a standard potting mix (obtained from Migros Co-operative, consisting of compost, soil with clay minerals, wood and plant fibres, organic carbon: 22.9 ± 2.7 %, pH ($CaCl_2$) 7.0 ± 0.1).

We performed a pot experiment, where we added $KSb(OH)_6$, KH_2PO_4 and K_2SO_4 to test if P, K or S have an effect on Sb uptake by plants, but also to see if they affect Sb solubility in soil. Plants were grown in pots with up to 2500 mg kg^{-1} total soil concentration and 3.2 mg L^{-1} soluble concentration.

At harvest, we separated sunflower shoots into leaves and stems to test for differences in allocation behaviour.

Plant available Sb was determined with a 1:2.5 w/v potassium nitrate extraction modification method on the Ordinance Relating to Impacts on Soils (Swiss Federal Council 1998), that used K instead of Na, since the latter forms insoluble complexes with Sb (Leyva et al. 2001, Massard et al. 2006).

Dried plants were digested using aqua regia in closed Teflon vessels in microwave (MLS). The total Sb concentrations were measured using Hydride Generation Atomic Fluorescence Spectroscopy (HG-AFS) (PSAnalytical). For the experiments in agar ICP-MS (Varian) was used for element measurements. To assure accuracy of analysis we tested reference material and achieved an average certainty of 98 ± 7%. Only total Sb concentrations have been measured, no speciation.

Statistical analyses (ANOVA and regression) were performed with SPSS 13.0 (SPSS 2004). T-test after Sachs (2004) were performed to compare slopes of log-transformed data of different treatments..

Results

In Figure 1 the treatments with K_2SO_4 and KH_2PO_4 added to the soil have higher soluble Sb concentrations than the control. The effect however is not significant, as revealed by comparing the slopes and intercepts of the regression lines of the log-transformed data by a t-test after Sachs (2004).

The different treatments did not significantly affect the dry weights of the plant shoots (data not shown).
In Figure 2 the influence of the two treatments on the uptake of Sb into shoots is shown. In higher Sb concentrations in soil we found a significant difference in maize between the treatments. The uptake was highest in the phosphate treatment, slightly lower in the sulphate treatment and lowest in control.
A t-test after Sachs (2004) revealed no significant differences between the three treatments.
Sunflower shows a trend towards higher uptake in the sulphate treatments, but this is only significant in the stem concentrations with 1847 mg kg^{-1} Sb in soil (Fig. 3). Comparing the leaves and the stems, the Sb concentration in the leaves is significantly higher in both treatments.

We also performed regression analysis with selected elements known to have an influence on the solubility of Sb in the soil. Total chloride concentration has a significant correlation with extractable Sb in soil ($p < 0.05$).

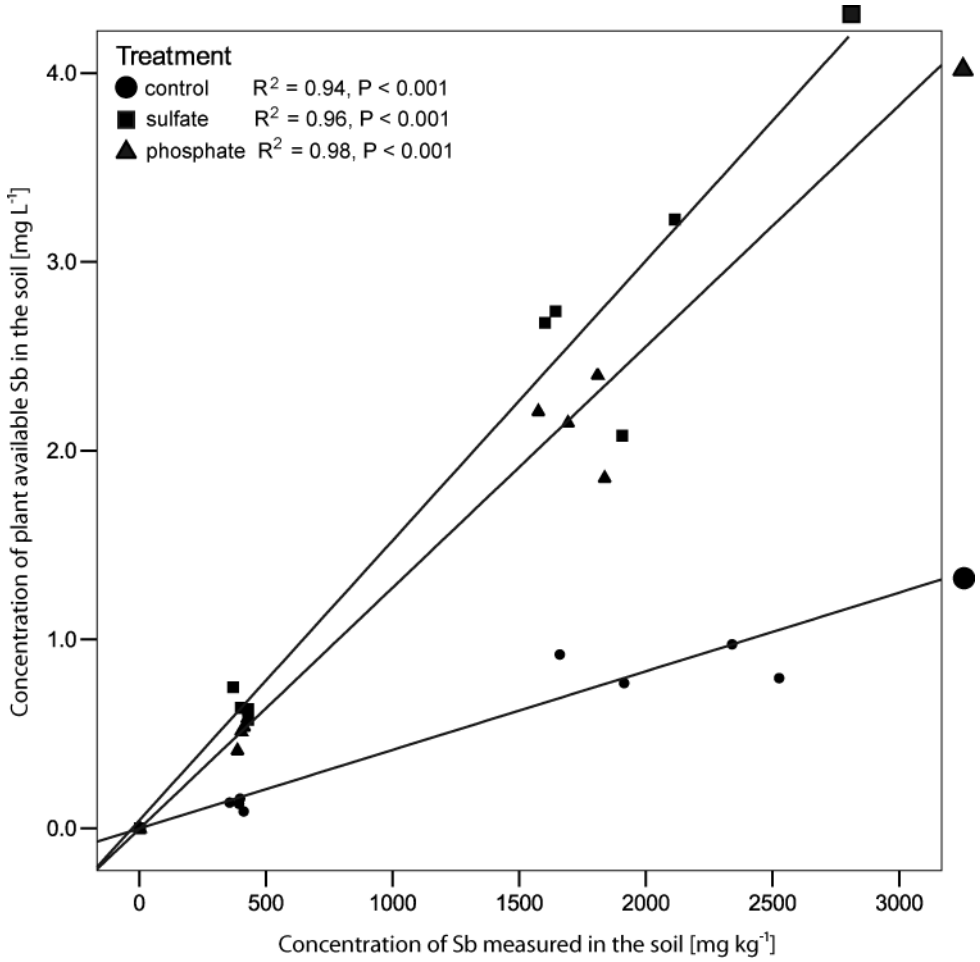

Fig. 1 Soluble Sb plotted against total Sb in soil in the different treatments.

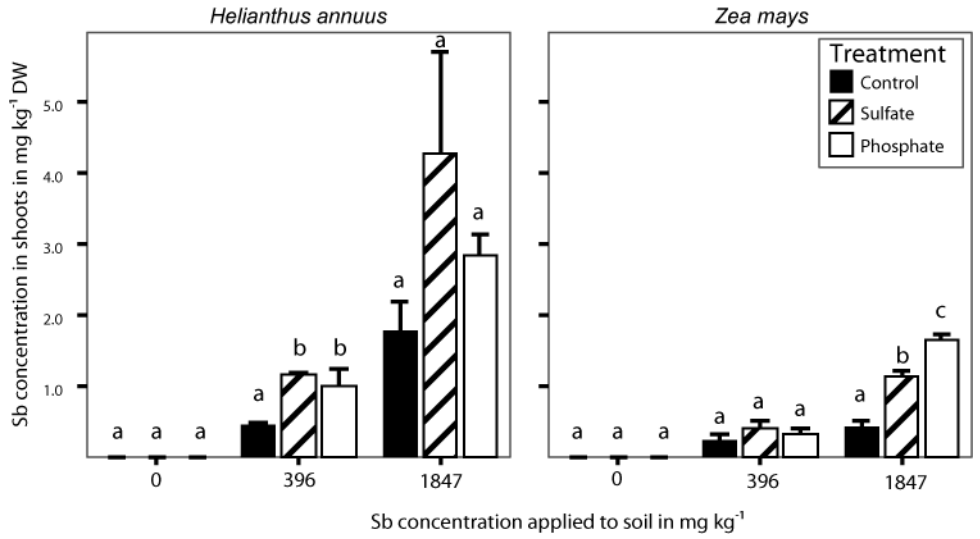

Fig. 2 Comparison of Sb uptake in maize and sunflower in three different treatments. Posthoc tests are LSD tests. Bars show mean and error bars show standard error. Within the same concentrations bars with different letters are significantly different from each other.

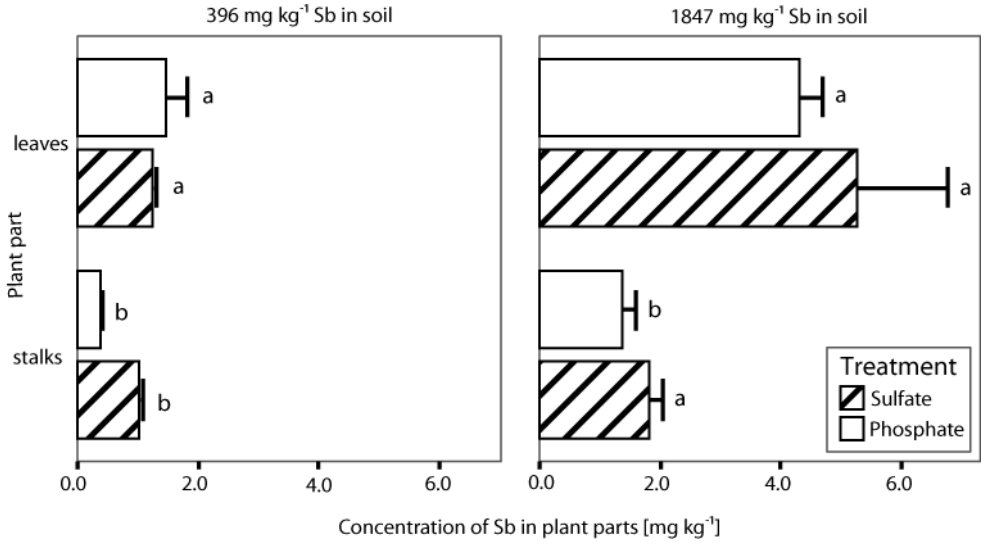

Fig. 3 Allocation in sunflower and effect of different treatments. Bars show mean and error bars show standard error. Bars with different letters are significantly different.

References

Leyva, A. G., Marrero, J., Smichowski, P., Cicerone, D., Sorption of antimony onto hydroxyapatite, *Environmental Science & Technology* **2001**, *35*, 3669-3675.

Massard, G., Reiser, R., Stadelmann, V., Gupta, S. K., *Antimony-Contaminated Soils on Shooting-Ranges* **2006**, Swiss Federal Departement of Economic Affairs.

Sachs, L., *Angewandte Statistik* **2004**, Springer: Berlin, Heidelberg, New York.

Swiss Federal Council, *Ordinance Relating to Impact on the Soil, SR 814.12* **1998**.

Appendix 2

Influence of citric acid on antimony uptake in sunflower (*Helianthus annuus* L.) from nutrient solutions

Introduction

Sb(III) and Sb(V) are both complexed by citric acid (Guy et al. 1998, Hansen and Pergantis 2006). In an extensive literature review, Filella and May (2005) listed equilibrium constants for complexes of Sb(III) with a variety of low molecular mass organic ligands. However, there are only conditional formation constants for complexes of Sb(V) and citric acid reported in the literature (Hansen and Pergantis 2006).

Many plants excrete citric acid, known to be most efficient phosphate (P) dissolving exudate, under P-deficient conditions to increase P-solubility in soils to increase P-uptake (Dessureault-Rompre et al. 2006, Waisel et al. 1996). Three mechanisms are involved in increasing P solubility: (1) competition with P for common absorption sites, (2) modifications of soil surface characteristics, (3) complexation of cations with which P coprecipitates or forms covalent bonds on adsorbing surfaces (Waisel et al. 1996). We aimed to test whether citrate influences Sb uptake. Our hypothesis was that Sb-citrate complexes are not taken up by plants, because they are too big to be taken up by transporters.
We used antimonate for the hydroponic experiments, because it is the most common chemical species in water and soil solution (Filella et al. 2002).

Material and Methods

We grew sunflower (*Helianthus annuus* L. cv. Iregi) in a climate chamber (photoperiod 16h, with day temperature 22/14°C (day/night) and light intensity of 11000 lux).

The plants (maize, ryegrass, sunflower, wheat) were germinated in quartz sand and after 2 weeks, they were transferred to 30-L plastic boxes containing a modified Hoagland nutrient solution (Hoagland and Arnon 1938). Then, plants were transferred to 1-L bottles containing the treatment solutions. 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) (Krämer et al. 1996). Solutions were adjusted to pH 6 with NaOH, continually aerated, and replaced weekly. Antimony was added as $KSb(OH)_6$ to the nutrient solution, and citric acid as $C_6H_8O_7 \cdot H_2O$.

The influence of citric acid (pK_{a1} = 3.15, pK_{a2} = 4.77, pK_{a3} = 6.40) on Sb(V) uptake was investigated by combining citric acid concentrations varying from 0 to 96.2 mg L^{-1} and Sb concentrations from 0 to 24.4 mg L^{-1}, in the nutrient solutions. In a subsequent experiment, we tested concentrations of citric as from 0 to 192.1 mg L^{-1} and Sb concentrations from 0 to 3.0 mg L^{-1}. This was not in nutrient solution but deionised water to avoid complexation of citric acid with elements of the nutrient solution. Upon completion of experiments, plants were harvested, the roots and shoots separated, and dried at 65°C until constant weight was obtained.

Dried plants were digested using aqua regia in closed Teflon vessels in microwave (MLS). The total Sb concentrations were measured using Hydride Generation Atomic Fluorescence Spectroscopy (HG-AFS) (PSAnalytical). To assure accuracy of analysis we tested reference material and achieved an average certainty of 98 ± 7%.

Statistical analyses (ANOVA and regression) were performed with SPSS 13.0 (SPSS 2004). T-test after Sachs (2004) were performed to compare slopes of log-transformed data of different treatments. ChemEQL v3.0 was used for speciation calculations.

Results

Citric acid had no significant influence on the Sb uptake by sunflowers (Fig. 1 and 2), nor did it significantly change biomass production. There were no significant differences between gradients and intercepts of the controls and the citric acid treatments in both experiments.

Calculations with ChemEQL showed that approximately 35% of the uncomplexed citric acid in solution was completely deprotonated ($C_6H_5O_7^{3-}$) in all solutions despite the of presence of nutrients and varying concentrations of citric acid and Sb(V). Hansen and Pergantis (2006)

assumed that Sb(V) complexes with the deprotonated form of citric acid in a 1:1 ratio. They found conditional logK values of 1.76 at pH 7, 3.75 at pH 5 and >4.6 at pH 3. However, the exact stoichiometry of the Sb-citrate complex is unknown. No stability constants for Sb(V) and low molecular organic ligands are available from the literature (Filella and May 2005, Hansen and Pergantis 2006).

We calculated activity coefficients after Debye-Hückel (25°C). In the experiment with complete nutrient solution, our simulation showed that citric acid was mostly complexed with Ca (45-50%) and Mg (22-25%).

Hansen's logK values decreased with increasing pH so we oriented our range at the pH 7 value (1.76) and took a range of -20 to 20 for logK for a sensitivity analysis (Fig. 3). Hansen and Pergantis (2006) found that their conditional logK was decreasing with increasing pH.

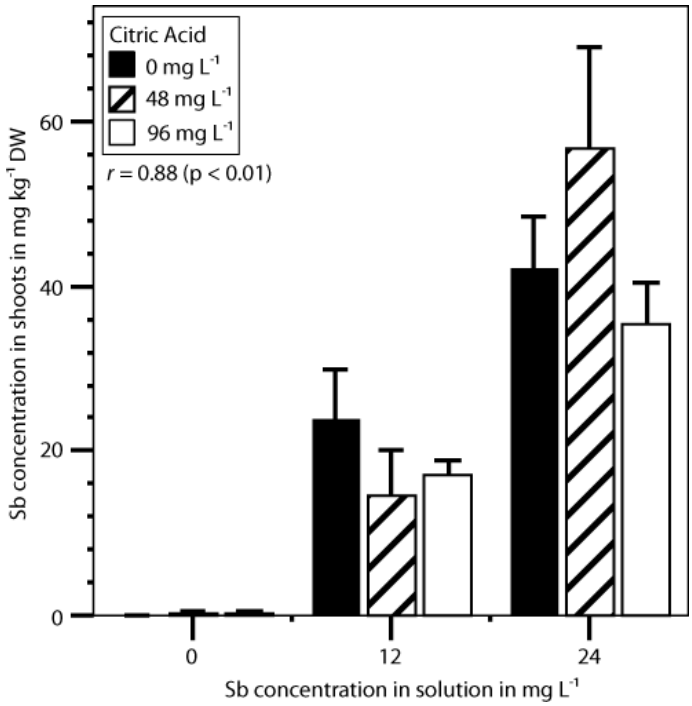

Fig. 1: Influence of citrate on the uptake of Sb into shoots of sunflower from hydroponic solution. Error bars represent the standard error of the mean. There are no significant differences between the different citric acid treatments at the same Sb concentrations.

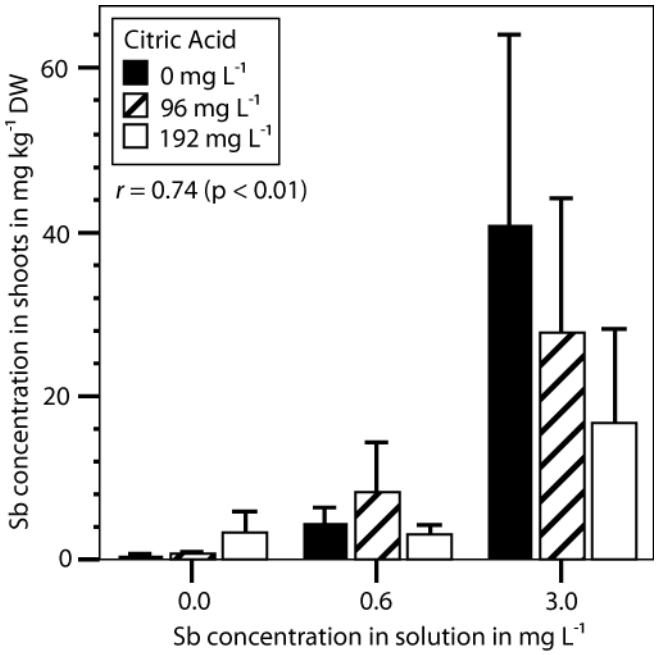

Fig. 2: Influence of citrate on the uptake of Sb into shoots of sunflower from hydroponic solution. Error bars represent the standard error of the mean. There are no significant differences between the different citric acid treatments at the same Sb concentrations.

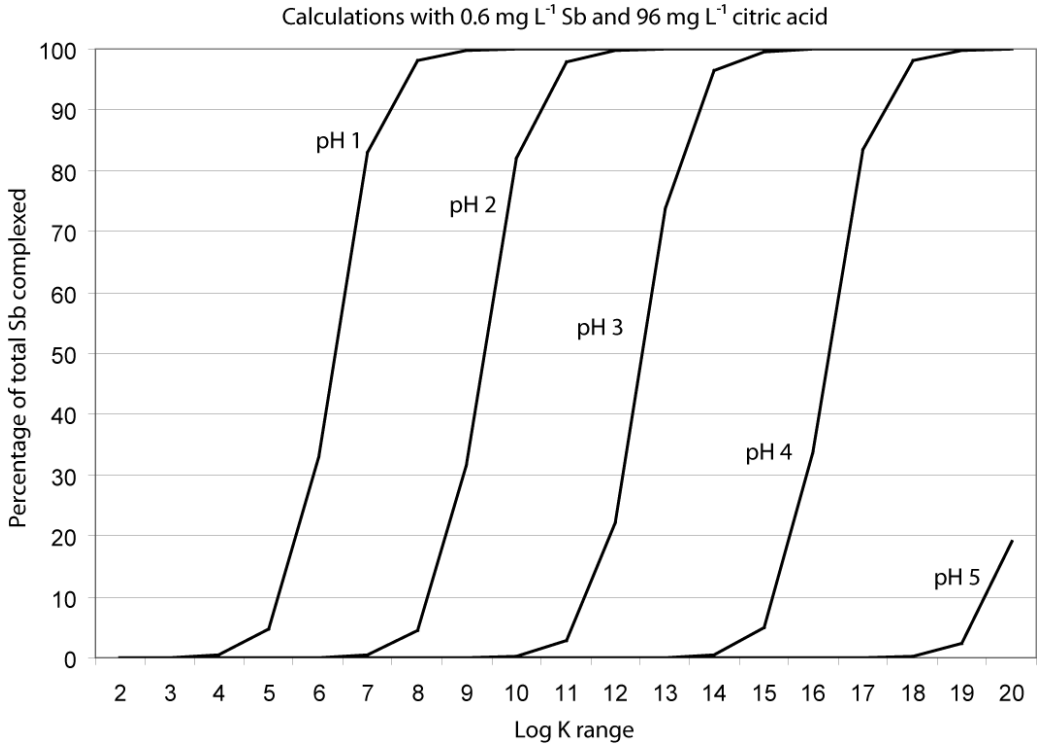

Fig. 3: Amount of Sb complexed in % of total Sb in solution for treatment Sb 0.6 mg L^{-1} citric acid 96 mg L^{-1} in solution in deionised water.

References

Dessureault-Rompre, J., Nowack, B., Schulin, R., Luster, J., Modified micro suction cup/rhizobox approach for the in-situ detection of organic acids in rhizosphere soil solution, **2006**, *286*, 99-107.

Filella, M., Belzile, N., Chen, Y. W., Antimony in the environment: a review focused on natural waters I. Occurence, *Earth-Science Reviews* **2002**, *57*, 125-176.

Filella, M., May, P. M., Critical appraisal of available thermodynamic data for the complexation of antimony(III) and antimony(V) by low molecular mass organic ligands, **2005**, *7*, 1226-1237.

Guy, A., Jones, P., Hill, S. J., Identification and chromatographic separation of antimony species with alpha-hydroxy acids, *Analyst* **1998**, *123*, 1513-1518.

Hansen, H. R., Pergantis, S. A., Investigating the formation of an Sb(v)-citrate complex by HPLC-ICP-MS and HPLC-ES-MS(/MS), *J. Anal. At. Spectrom.* **2006**, *21*, 1240-1248.

Hoagland, D. R., Arnon, D. I., The water-culture method for growing plants without soil, *Circular of the Agricultural experiment station Berkeley, California* **1938**, *347*.

Krämer, U., Cotter-Howells, J. D., Charnock, J. M., Baker, A. J. M., Smith, J. A. C., Free histidine as a metal chelator in plants that accumulate nickel, *Nature* **1996**, *379*, 635-638.

Sachs, L., *Angewandte Statistik* **2004**, Springer: Berlin, Heidelberg, New York.

Waisel, Y., Eshel, A., Kafkafi, U., editors. Plant Roots - The Hidden Half. 2nd ed. New York, Basel, Hong Kong: Marcel Dekker, Inc.; **1996**.

Appendix 3

Influence of Sb^{III} and Sb^{V} contamination on nutrient content in sunflower (*Helianthus annuus*) and maize (*Zea mays*)

Introduction

In the experiments in the potting mix described in Chapter 5, also nutrient contents of sunflower and maize were measured to investigate the effect of Sb^{III} and Sb^{V} contamination on the growth of the plants.

Materials and Methods

We grew sunflower (*Helianthus annuus* L. cv. Iregi) and maize (*Zea mays* L. cv. Magister) in 250-ml pots in a climate chamber (photoperiod 16 h, day/night temperature 22/14°C, light intensity 11000 lux). The soil used was a standard potting mix (obtained from Migros Co-operative, Zürich), consisting of garden soil enriched with compost (organic carbon content: 229 ± 27 g kg^{-1}, pH in $CaCl_2$ extract: 7.0 ± 0.1). Various amounts of either antimonite ("Sb^{III} treatment") or antimonate ("Sb^{V} treatment") were added by mixing granular Sb_2O_3 or $KSb(OH)_6$, respectively, with the dry soil substrates. Added Sb concentrations ranged from 0 (control) up to 10 g kg^{-1} dry soil. Apart from the control treatment, the applied total Sb concentration levels were 156, 313, 625, 1250, 2500, 5000, and 10000 mg kg^{-1} for the potting mix. Regularly watered, the mixtures were left to equilibrate for 2 weeks, then sampled for chemical analysis (one composite sample per batch) and filled into the pots. Three seeds of either maize or sunflower were planted in each pot.

Plants were harvested after four weeks of growth, separated into roots and shoots, oven-dried for 48 h at 65°C, weighed and then digested for chemical analysis using aqua regia in closed Teflon vessels (2 h at room temperature and then for 30 min in a microwave oven (MLS) at 100°C).

Soil samples were oven-dried at 65°C for 1 week, weighed and then stored at 4°C until they were analysed. Extractable soil Sb concentrations were determined by extraction with potassium nitrate as described by Tschan et al. (2009). For this purpose, subsamples of 5 g

each 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 with a frequency of 120 min^{-1} and an amplitude of 55 mm. The resulting slurries were left for 10 min to settle, before 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 containing 0.8 mL of 65% nitric acid.

In the experiment with potting mix plant and soil extracts were analysed for Sb by means of Hydride Generation Atomic Fluorescence Spectroscopy (HG-AFS) (PSAnalytical). Total soil Sb concentrations were measured by means of XRF (X-Ray Fluorescence spectrometry) (Spectro). Nutrient element concentrations in plants were measured with Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Varian).

As reference material for the analysis of plant samples, we used Virginia tobacco leaves (CTA-VTL-2) obtained from LGC Standards. 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 the standards.

Statistical analyses (ANOVA and regression) were performed using SPSS 17.0 (SPSS 2009).

Results

Looking at micronutrients in the potting mix treatment (Table 1 and 2), in sunflower leaves Sb negatively correlates with P in the Sb^{III} treatment, while the correlation is positive in the Sb^{V} treatment. For Sb^{V}, plant Sb also negatively correlates with Ca and K in sunflower leaves. In maize; K negatively correlates with Sb in plants in both treatments.
The same is also the case for calcium which also is in negative relation to plant Sb, but the relation is not significant in the case of maize. All other element concentrations were not significantly correlated with plant Sb.

Table 1 Concentrations (means ± standard errors in mg kg^{-1}) of major nutrient elements in sunflower leaves and maize shoots grown on potting mix. Data were pooled for both Sb treatments as there were no significant differences between the two treatments.

	K	Ca	Mg	P	Fe
sunflower leaves	34.5 ± 1.4	25.8 ± 0.9	3.4 ± 0.2	3.2 ± 0.1	0.78 ± 0.08
maize shoots	33.0 ± 1.5	2.3 ± 0.2	1.0 ± 0.0	1.3 ± 0.1	0.56 ± 0.02

Table 2 Pearson coefficients of correlation between the concentrations of major nutrient elements in sunflower leaves and maize shoots and log transformed concentrations of total soil Sb, soluble soil Sb and plant Sb for the two Sb treatments potting mix. * $p < 0.05$, ** $p < 0.01$

Treatment		Ca	Fe	K	Mg	P
		Sunflower leaves				
Sb^{III}	Total Sb in soil	-0.49	-0.25	0.08	0.14	-0.59
	Soluble Sb in soil	-0.44	-0.77	-0.05	-0.04	-0.73
	Sb in plants	-0.46	-0.31	-0.21	-0.03	-0.73**
Sb^{V}	Total Sb in soil	-0.10	-0.32	0.18	0.02	0.09
	Soluble Sb in soil	0.52	0.06	-0.31	0.31	0.44
	Sb in plants	0.51*	0.28	-0.45*	0.17	0.45*
		Maize shoots				
Sb^{III}	Total Sb in soil	-0.73	0.89	-0.76	0.44	0.36
	Soluble Sb in soil	-0.77	0.91	-0.79	0.39	0.31
	Sb in plants	-0.44	0.38	-0.67**	0.20	-0.21
Sb^{V}	Total Sb in soil	-0.13	-0.37	0.02	0.02	0.49
	Soluble Sb in soil	-0.70**	-0.10	-0.75**	-0.09	-0.15
	Sb in plants	-0.35	0.34	-0.63*	0.40	-0.46

References

Tschan, M., Robinson, B. H., Nodari, M., Schulin, R., Antimony uptake by different plant species from nutrient solution, agar and soil, *Environmental Chemistry* **2009**, *6*, 144-152.

Appendix 4

Oxidation kinetics of soluble antimony in a soil spiked with Sb_2O_3

Introduction

As a metalloid Sb can exist in oxidation states -III, 0, +III, and +V. In soils Sb usually occurs as Sb(III) (antimonite) in anaerobic and as Sb(V) (antimonate) and aerobic conditions. Elemental Sb, like it is present for example in the alloy used for bullets, weathers in soil. It is oxidized and released as Sb(III) or Sb(V) into the soil solution.

Trivalent antimony is present as the neutral species $Sb(OH)_3$ over the relevant pH range for soils (Baes and Mesmer 1986). Sb(III) can occur in soil when Sb(0) is oxidized (Johnson et al. 2005), by dust deposition of Sb_2O_3, used as flame retardant, or when Sb(V) is reduced in anaerobic, alkaline solutions with dissolved Fe(II) acting as reductant (Leuz 2002). In acidic conditions however, Sb(III) may be oxidized in the presence of Fe(III)-hydroxides. Sb(III) is also oxidized by Mn-oxides and amorphous Fe-oxides under both oxic and anoxic conditions (Belzile et al. 2001, Blay 2000). Oorts et al. (2008) found more than 70% of Sb in soil solution, added as Sb_2O_3 solution to the soil, to be present as Sb(V) after two days.

We wanted to investigate the amount of oxidized Sb in soil extracts within 8 hours after spiking a soil with $Sb^{III}{}_2O_3$.

Material and Methods

The soil was collected from the plough layer of an agricultural at Birr on the Swiss plateau, organic carbon: 1.51 %, pH ($CaCl_2$) 6.6 ± 0.1. Sb(III) and Sb(V) were added at different concentration by mixing granular Sb_2O_3 ("Sb(III) treatment") with dry soil. Three different initial concentrations of (1044, 2088, 4156 mg kg^{-1} total Sb added to soil) were prepared in pots with 250 g soil. After wetting the mixtures with 100g water, they were left for up to 6 hours in the dark at room temperature. After 0, 1, 2, 4 and 6 hours samples of 10g wet weight were taken and analysed (the analytical procedure is depicted in Fig. 1).

To determine the kinetics of Sb(III) transformation into Sb(V) in the plant-free agricultural soil the soluble Sb fraction was extracted using potassium nitrate as described by Tschan et al. (2009). For this purpose, subsamples of 10 g each were mixed with 25-mL aliquots of a 0.1 mol L^{-1} potassium nitrate solution in polypropylene bottles. The bottles were tightly closed and longitudinally shaken in the dark 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. Then 15 ml citrate 1 mol L^{-1} was added to prevent further oxidation of Sb(III) in one half of the sample, and 3 ml HCl 30% and 12 ml H_2O was added for the total Sb measurements in the other half. The experiment was performed in three replicates.

The speciation analysis was performed using HG-ICP-OES (Hydride Generation Inductively Coupled Plasma Optical Emission Spectrometry) (Varian). Hydride generation was used for the Sb(III) measurements coupled with ICP-OES, whereas the total Sb measurements were performed without hydride generation. Statistical analyses (ANOVA and regression) were performed using SPSS 17.0 (SPSS 2009).

Results

Antimony (III) added to the agricultural soil was quite rapidly oxidized (Figures 2 and 3). As the total Sb extractable concentration increases with time the percentage of Sb(III) decreases. For the three different initial concentrations of (1044, 2088, 4156 mg kg^{-1} total Sb added to soil) the amount of Sb(III) in the extractable concentration dropped by about 5% within 6 hours of measurements.

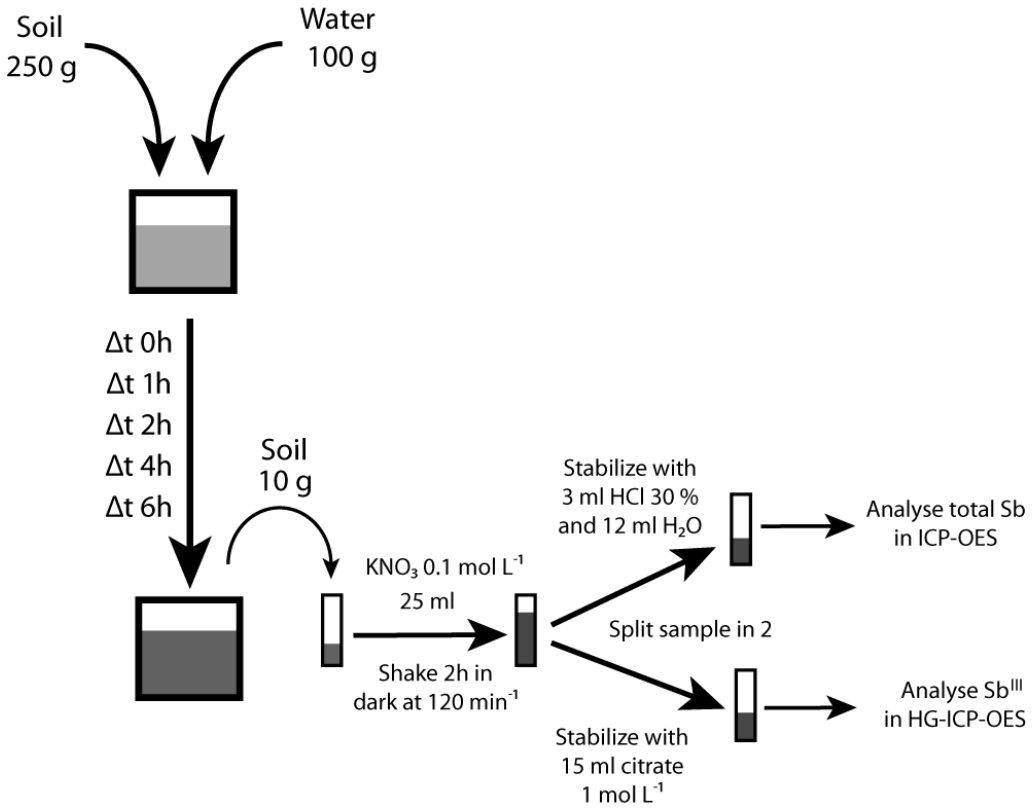

Figure 1 Depiction of the analytical procedure for the extraction and analysis of Sb^{III} and total Sb in soil solution.

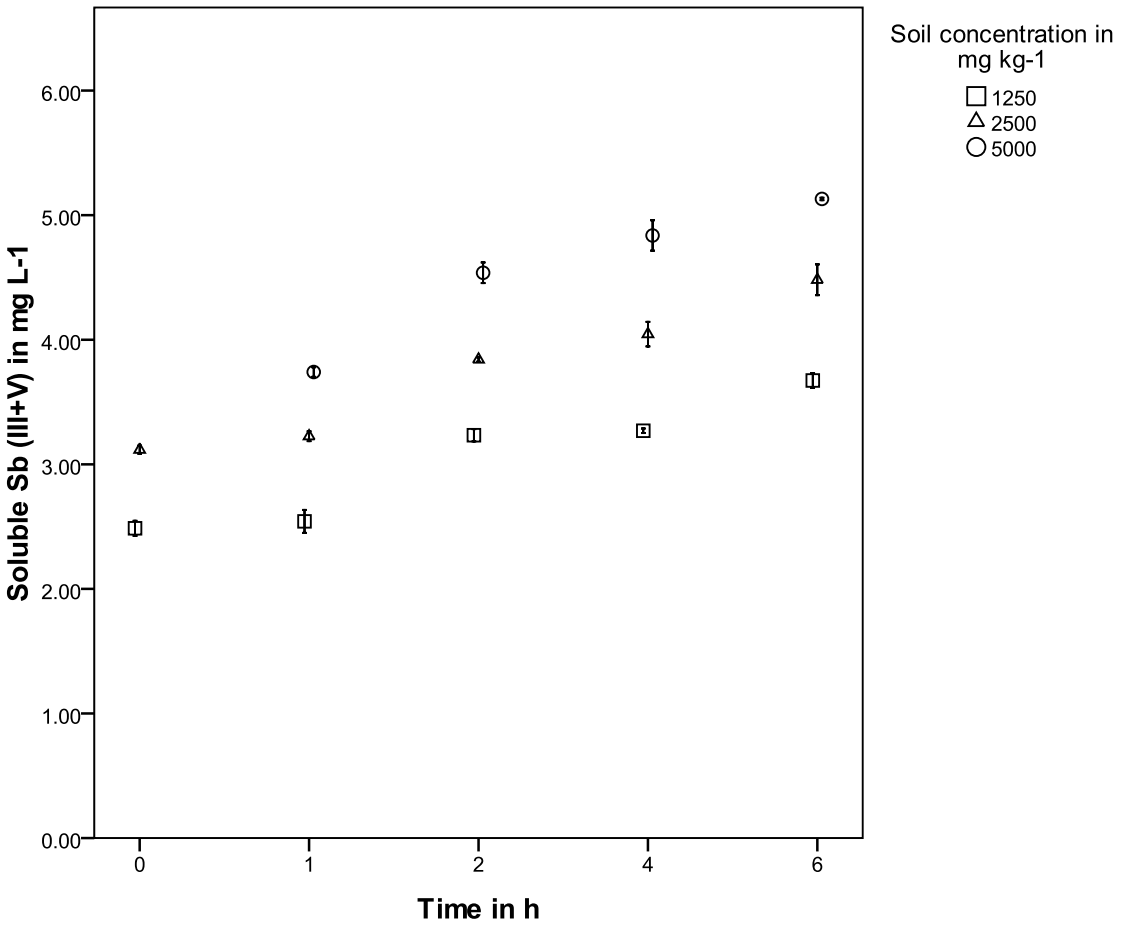

Figure 2 Change in total KNO_3-extractable Sb concentration during 6 hours incubation after addition of various amounts of Sb_2O_3 to the agricultural soil. Error bars show standard error.

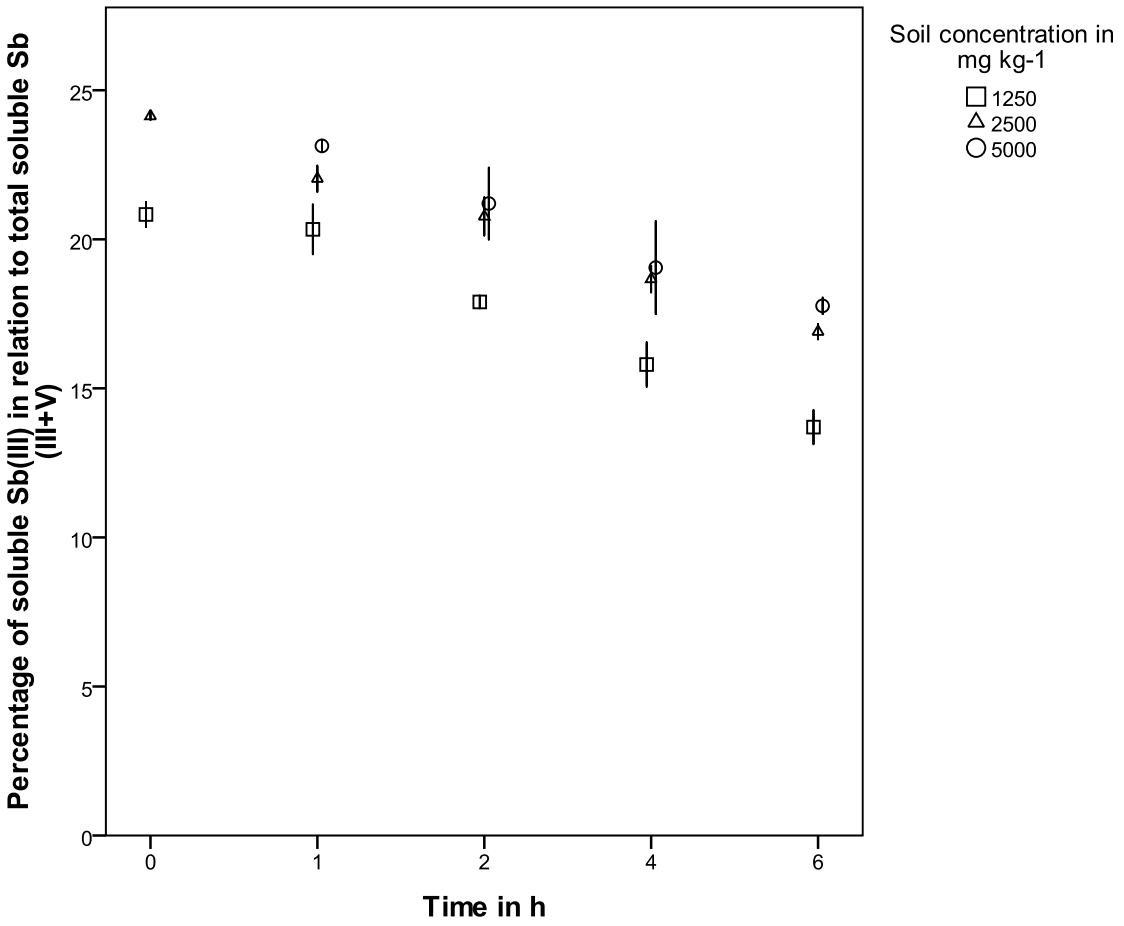

Figure 3 Change in the fraction of Sb(III) in relating to total extractable Sb during 6 hours of incubation in the agricultural soil after adding Sb_2O_3. Error bars show standard error.

References

Baes, C. F., Mesmer, R. E., Arsenic, Antimony, Bismuth. In: *The Hydrolysis of Cations* (Eds **1986**, pp. 366-375 (Krieger: Malabar, FL).

Belzile, N., Chen, Y. W., Wang, Z. J., Oxidation of antimony (III) by amorphous iron and manganese oxyhydroxides, **2001**, *174*, 379-387.

Blay, K., *Sorption wässriger Antimon-Spezies an bodenbildende Festphasen und Remobilisierung durch natürliche Komplexbildner.* PhD Thesis, **2000**, Technische Universität München.

Johnson, C. A., Moench, H., Wersin, P., Kugler, P., Wenger, C., Solubility of antimony and other elements in samples taken from shooting ranges, *J. Environ. Qual.* **2005**, *34*, 248-254.

Leuz, A. K., *Redox Chemistry of Antimony in Aquatic Systems: e.g. Lakes* Diploma, **2002**, Carl von Ossietzky Oldenburg.

Oorts, K., Smolders, E., Degryse, F., Buekers, J., Gasco, G., Cornelis, G., et al., Solubility and toxicity of antimony trioxide (Sb_2O_3) in soil, *Environ. Sci. Technol.* **2008**, *42*, 4378-4383.

Tschan, M., Robinson, B. H., Nodari, M., Schulin, R., Antimony uptake by different plant species from nutrient solution, agar and soil, *Environmental Chemistry* **2009**, *6*, 144-152.