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Journal
Environmental Science and Pollution Research, 24(33)

ISSN
0944-1344

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Publication Date
2017-11-01

DOI
10.1007/s11356-017-0177-x

Peer reviewed

Published in final edited form as:

Environ Sci Pollut Res Int. 2017 November ; 24(33): 25912–25922. doi:10.1007/s11356-017-0177-x.

Framework for Assessment and Phytoremediation of Asbestos-Contaminated Sites

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Abstract

We examine the feasibility of phytoremediation as an alternative strategy to limit the exposure of asbestos in site with asbestos-containing materials. We collected soils from four locations from two sites—one with naturally occurring asbestos, and another, a superfund site, where asbestos containing materials were disposed over decades—and performed ecotoxicology tests. We also performed two experiments with crop cultivar and two grasses from serpentine ecotype and cultivar to determined best choice for phytoremediation. Asbestos concentrations in different size fractions of soils varied by orders of magnitude. However, different asbestos concentrations had little effect on germination and root growth. Presence of co-contaminants such as heavy metals and lack of nutrients affected plant growth to different extents, indicating several of these limiting factors should be considered instead of the primary contaminant of concern. Crop cultivar survived on asbestos contaminated soil. Grasses from serpentine ecotype did not show higher biomass than the cultivar. Overall, these results showed that soil conditions play a critical role in screening different crop species for phytoremediation, and that asbestos concentration has limited to no effect on plant growth. Our study provided a framework for phytoremediation of asbestos-contaminated sites to limit long-term asbestos exposure.

Keywords

Asbestos; ASTM method; crop cultivar, heavy metal; native grass; phytostabilization; size fraction

Introduction

Asbestos is a group of six naturally occurring fibrous minerals, whose properties include resistance to heat or fire and high tensile strength (Schreier 1989; Dodson and Hammar

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2011; Kumar et al. 2016). Because of these useful properties, asbestos has been mined and once used extensively in many commercial applications, including the construction and automobile industries (Morrison and Murphy 2010). Despite restrictions on asbestos use in recent decades in United State, nearly 2.5 million metric tons of asbestos are produced annually worldwide, and asbestos products are still widely used in India and China—home to more than one-third of the world's population (Virta 2006). Mining of asbestos minerals and the production of asbestos-containing materials generate waste that itself can pose serious health hazards (Van Gosen 2007a; Boulanger et al. 2014). Exposure to asbestos fibers can cause asbestosis, mesothelioma, lung and stomach cancer (Cunningham and Pontefract 1971; 2006; Fortunato and Rushton 2015). Therefore, it is critical to assess the extent of asbestos contamination and improve remediation design to minimize asbestos exposure.

Asbestos fibers are found at two main types of sites, where it naturally occurs (referred to as naturally occurring asbestos or NOA) and in asbestos containing material (ACM) from waste disposal (Schreier 1989). NOA refers to asbestiform minerals occurring within rocks or soils that can be released by human activities or weathering processes. NOA is mostly found in ultramafic rock and especially serpentinite (Lee et al. 2008). These sites include many serpentine sites with specific vegetation, serpentinophytes, adapted to particular soil properties. Other NOA localities include mines or quarries for heavy metals or other materials, such as several chromite mines in the Eastern US (Pearre and Heyl Jr 1960; Van Gosen 2007a) and a vermiculite mine contaminated with amphibole asbestos in Libby, MT (Bandli and Gunter 2006). Vegetation may be absent or reduced in some mining areas (Meyer 1980), in part due to the presence of other pollutants such as Ni and Cr (Levitan et al. 2015). Indeed, asbestos pollution is found in many sites with asbestos and heavy metal gradients. The disposal sites where ACM is found, on the other hand, do not necessarily share common geological properties.

Determining the concentration and form of asbestos in contaminated soil or waste material is the necessary first step to designing the remediation plan as it helps in risk assessment and risk based decisions for brownfields and Superfund sites (Wroble et al. 2017). Based on the Framework for Investigating Asbestos-Contaminated Superfund Sites (US EPA), asbestos concentration in contaminated soil, storage piles, waste materials is estimated by the CARB Method 435. This method involves grinding a representative bulk soil sample and quantifying the amount of asbestos fiber in the ground sample using Polarized Light Microscopy (PLM). Consequently, this method does not account for the distribution of asbestos based on the grain size of contaminated soil or waste materials. Because the size of asbestos fibers controls their exposure pathways (Boulanger et al. 2014), it is critical to develop a screening method that accounts for the fractionation of asbestos based on the particle size of contaminated soil or waste materials. To overcome this limitation, the ASTM D7521-13 method has been proposed, which requires measuring asbestos concentrations in different size fractions of contaminated soil or waste (D22 Committee 2013). Although EPA is evaluating this sampling method for its applicability to Superfund site characterization (US EPA), a comparison of the two methods for assessing asbestos contamination is lacking.

Most asbestos mines or sites contaminated with asbestos materials have low vegetation cover and/or diversity, indicating low colonization of plants from surrounding vegetated areas. In this case, assisted phytostabilization with soil amendments could be a cost-effective solution (Brown and Chaney 2016). Although phytostabilization has been used to treat soils containing heavy metals, such as cadmium or lead, in many Brownfield sites or chromite mines (Kumar and Maiti 2015), its utility to treat asbestos-contaminated sites has not been examined to date. Plants are known to stabilize the topsoil and minimize erosion (Alkorta et al. 2010; Brown and Chaney 2016), which could limit asbestos exposure via air. Furthermore, recent laboratory studies show that organic acids typically released from plant roots or soil microbe can leach out elements, alter surface charge of fibers, and induce a lower toxicity (Daghino et al. 2006; Favero-Longo et al. 2013; Holmes and Lavkulich 2014; Mohanty et al. 2017). Thus, phytostabilization and phytoremediation, more broadly, could be viable technology for asbestos-contaminated sites.

The success of this ‘green’ strategy depends on the survival and performance of plants in the presence of asbestos—a basic premise that has not been tested systematically. It seems important to adjust soil properties if necessary and/or choose the best plants as phytoremediation agents. Among different phytoremediation strategies (Ali et al. 2013), assisted phytostabilization with soil amendments and planting of crop cultivars seems an alternative choice (Trivedi and Ahmad 2011). In chromite asbestos mines in India, several studies showed that using suitable plants with metal low shoot metal uptake, such as grasses or legumes, could act as a potential barrier for metal transport in food chain (Trivedi and Ahmad 2011; Kumar and Maiti 2015; Kumar et al. 2017). In a Vermont asbestos site, biomass production of grass and clover with two different compost mixture showed higher compared to the control (Chaney et al. 2011). Serpentinophytes species from ultramafic substrates may represent another alternative due to their tolerance for high concentrations of heavy metals, such as Ni and Cr, and low levels of essential nutrients, namely nitrogen, phosphorus, potassium, and calcium (Brady et al. 2005).

In the U.S., 1312 sites are contaminated with either NOA or ACM (Van Gosen 2005, 2007b, 2008). In some of these sites, including the Ambler, PA Superfund site disposal waste site studied here, asbestos is the primary contaminant of concern. For these sites, *ex-situ* treatment is typically not preferred because asbestos fibers may be emitted when large quantities of contaminated material must be relocated (Paik et al. 1983; Brown 1987). For *in situ* treatment, EPA recommends capping with uncontaminated soil at least 2 m thick, which could be expensive (Lee and Jones-Lee 1997; Millano 1998). Brownfield sites or other uncategorized asbestos-contaminated sites are typically left untreated because alternative, cost-effective remediation methods are lacking, despite the possibility of significant health risk associated with long-term asbestos exposure.

Here, we compared two methods to test asbestos contamination in soils and examined the feasibility of phytoremediation to treat asbestos-contaminated sites. We hypothesized that asbestos itself would not pose any risk to plants that are typically utilized for phytoremediation. To test this hypothesis, we collected soils from two locations at each of two known asbestos-contaminated sites in Pennsylvania, the Superfund site with ACM and a site with NOA. We compared the asbestos concentration as determined using the current

standard method (CARB 435) and an alternative method under consideration by EPA (ASTM D7521-13). To examine toxicity of asbestos for plants, we conducted an ecotoxicology test using three species commonly employed for phytoremediation. We examined germination and root growth for each of the species. Finally, we performed a large screening of plants and soil microbes suitable for phytoremediation of the Superfund site in two complementary experiments, with (i) commercial crop species classically used for heavy metal remediation and (ii) two grasses, including one serpentine ecotype of each species. We also examined whether soil microbial inoculum collected at the serpentine site facilitated plant growth in soils from the Superfund site. In addition to assessing plant growth, we also measured elemental concentrations in aboveground tissues to detect any major nutrient deficiency or heavy metal toxicity.

Materials and methods

Soil Sampling

In Spring 2015, we collected soil samples from two sites. One is the BoRit Superfund site (Ambler, PA; 40°09'18.7"N 75°13'49.6"W), where ACM were discarded from a nearby manufacturing plant over several decades. The other, located in Nottingham Park (Chester County, PA, 39°44'8.53"N 76°2'9.94"W), is a serpentine site, containing NOA in the serpentinite bedrock (Smith and Barnes 1998). The site has a long history of mining for the extraction of sodium feldspar, chromium, and building stone (Lookingbill et al. 2007). The area was once the world's leading source of chromite (Pearre and Heyl Jr 1960). We collected soil with asbestos containing materials from two locations at BoRit: from what had been a reservoir before the removal of water and along the banks of a stream. We collected bulk soil and rock also from two locations at Nottingham: a grassland area with native C_4 grasses as the main constituents of the plant community (Gonneau et al. 2017), and at the waste pile of a former chromite mine. Soil samples were collected in zip-lock plastic bags, and a well-mixed portion of soil was stored at 4°C for mineralogical studies whereas another portion was air-dried for 48 h. The latter was used to characterize soil elemental content and for performing an ecotoxicology test

Asbestos quantification

We measured asbestos concentrations using two methods: California Air Resource Board 435 (CARB 435) and ASTM D7521-13. CARB 435 is included in the EPA framework to assess asbestos contamination in rocks and soils including serpentine aggregate storage piles (US EPA). For this analysis, the bulk sample was crushed in a mill (Mill 8000M, SPEX, USA) and sieved to retain only the particle size <75 μm. The ground samples were analyzed for asbestos under polarized light microscopy based on a 400-point count technique with a detection limit of 0.25% (or 1 count). In contrast to the CARB 435 method that uses only the finely crushed part of the bulk sample, the ASTM D7521-13 method requires analysis of asbestos in different grain size fractions. Briefly, for the latter, soil samples were dried and sieved to separate factions according to the following size ranges: >19 mm, 2–19 mm (coarse), 0.106–2 mm (medium), and <0.106 mm (fine). Masses for each fraction are recorded and the presence of asbestos was measured first by stereomicroscopy, followed by polarized light microscopy (PLM), to determine whether fibers were observed in matrices or

as isolated material. If asbestos was not detected by the PLM results in any fraction, then only the fine fraction of the sample was re-analyzed for detection of asbestos fibers using transmission electron microscopy (TEM), a more precise technique.

Soil characterization

For soil characterization, air-dried soil samples were sieved to obtain soil with particle size < 2.0 mm. Soil pH was measured by making a slurry consisting of a 1:5 ratio of soil to ultrapure water. Phosphorus was estimated by the Bray-1 method (Bray and Kurtz 1945). Briefly, 1.0 g of soil was suspended in 10.0 mL solution containing 0.025 M HCl and 0.03 M NH_4F and shaken vigorously for 1 min before centrifuging at 6000 rpm for 5 min. The supernatant was transferred to a spectrophotometer cuvette and analyzed at 880 nm (Hach, DR 6000, USA). Pseudo-total concentrations of major elements (Al, Ca, Fe, K and Mg) and trace elements (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) were determined by digesting 0.5 g of ground soil (< 200 μm) in an aqua regia solution: mixture of 10 mL HNO_3 65%, 5 mL H_2O_2 30%, 5 mL HCl 37% in a DigiPrep system (EPA Method 5030).

To determine fractionation of elements based on their association with different types of minerals or sorption sites, we used a three-step sequential extraction procedure developed by the Commission of the European Communities Bureau of Reference (BCR; Clevenger 1990). Elemental concentrations in the extracted solutions at different steps were determined using Inductive Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Spectro Genesis, Spectro Analytical Instruments, Germany).

In order to evaluate fertility and potential limiting factors for phytoremediation, critical values were assigned to each of 16 physical or chemical soil parameters: ten parameters were based on the method proposed by the Fertility Capability Classification (Sanchez et al. 2003), and six parameters were the main phytotoxic heavy metals (Nagajyoti et al. 2010).

Ecotoxicology test

To measure potential toxicity induced by different soils on plants (Baran and Tarnawski 2013), we employed the Phytotoxkit (MicroBioTest, Belgium). The kit includes three plant species: *Sorghum saccharatum* (*S. saccharatum,* Poaceae), *Lepidium sativum* (*L. sativum*) and *Sinapsis alba* (*S. alba*, Brassicaceae), and based on plant performance allows assessment of toxicity of soil compared to an international standard soil (control). We considered two main parameters of performance that are relevant early in the plant life cycle, germination and root growth. The ecotoxicology test was conducted in accordance with the procedure recommended by the manufacturer. The percent inhibition of seed germination (IG) and inhibition of root growth (IR) for each soil were calculated with the formula: IG or IR=[(A −B)/A]×100, where A and B are the mean seed germination or root length in the control and tested soil, respectively.

Germination tests of crop cultivars on BoRit soil

Seed germination in the two localities from BoRit was also tested for cultivars of eight crops from belonging either to the family Poaceae: *Adropogon gerardii* (*A. gerardii*), *Lolium perenne* (*L. perenne*), *Panicum virgatum* (*P. virgatum*), *Sorghastrum nutans* (*S. nutans*) or

Brassicaceae: *Brassica juncea* (*B. juncea*), *Brassica oleracea* (*B. oleracea*), *L. sativum, S. alba*. Seeds were obtained from Sheffield's Seed Co., Inc. or from Johnny's Selected Seeds (Table S1). Seeds were soaked in 70% ethanol for 15 min and washed twice with deionized water. Thirty seeds per species were placed on soil in a square petri dish (10 cm) containing either 60 g of test soil or control, a mixture of sand and compost (v 1:1). Petri dish were watered every day and placed in an incubator at 25 °C. The number of germinated seeds was recorded daily for 10 days after sowing. Each species × soil type combination was replicated three times.

Experimental design for plant growth

Screening crop cultivars—Six seedlings of five crop species and the three species from the ecotoxicological test, above (Table S1) were grown in the two soils from the BoRit site (Reservoir and Stream Bank) and their growth compared to that in a control soil, a mixture of compost and sand. Tube-shaped Conetainer™ were filled to approximately 16 cm with soil and capped with 2 cm of compost to minimize entrainment of asbestos fibers in air and allow better plant growth. Plants were maintained in the greenhouse and watered daily. After 12 weeks, the aboveground portions were harvested, cleaned with tap water, and dried for 48h at 60 °C before weighing. To determine elemental concentrations, a portion of the dried shoot was ground to small pieces and digested in 1N HNO_3 for 3h at 95 °C in a DigiPREP system (Gonneau et al. 2014). Digested samples were diluted with ultrapure water, filtered with 0.45µ membrane, and analyzed for elemental concentrations using ICP-AES.

Serpentine native soil inoculum experiment with C_4 grasses—A separate experiment was conducted to determine if soil microbes naturally occurring at the Nottingham Serpentine site could facilitate growth of two C_4 grasses in the BoRit asbestos-contaminated soils. Both serpentine ecotypes and commercial cultivars of *Sorghastrum nutans* (*S. nutans*) and *Andropogon gerardii (A. gerardii)*, Holt and Niagara respectively, were grown. Seeds and soil to be used as inoculum were collected in Fall 2015 at Nottingham, Chester County, PA. Inoculum soils were collected under five individuals of the two grasses, brought back to lab in a cooler, and stored at 4° C. Soils were separated into two parts. One part was maintained as fresh inoculum (live) and other part was autoclaved 1h at 121°C (sterile) and used as a control for the abiotic soil fraction in the inoculum. Contaminated soils from each of the two BoRit locations, reservoir and stream bank, were placed in Conetainers™ to approximately 20 cm depth and covered with 5 cm of live or sterile inoculum as it might be in the process of remediating a contaminated site. Seeds were germinated and grown in sterilized sand and seedlings transplanted at two weeks of age. There were six replicates of each soil × seed source × inoculum type for each plant species. Aboveground portions were harvested after 15 weeks, dried and weighed, and the shoots analyzed for elemental concentrations as described above.

Statistical Analysis

All statistical analyses were performed using R v3.2.5. For soil properties, differences between the four soil locations in soil properties were analyzed by the Kruskal-Wallis test (a non-parametric test). For the first seed germination with *S. saccharatum*, *S. alba* and *L. sativum*, the difference in germination and root growth inhibition relative to the control was

analyzed by Kruskal-Wallis test. For the second seed germination test, we analyzed the proportion of germinated seeds (out of 30 seeds planted) on Day 9 using a series of logistic regression models. The three levels of substrate, namely BoRit stream bank, BoRit reservoir and control, were treated as fixed effects and the eight species as a nested factor within the soil substrate factor. Eight species were further combined into two species categories for comparison in a separate analysis: the Poaceae group with *A. gerardii, S. nutans, L. perenne and P. virgatum* (Group 1) and the Brassicaceae group with *B. juncea, B. oleracea, L. sativum and S. alba* (Group 2). Log odds of germination (i.e., log of probability of a seed is germinated over the probability not germinated) for each substrate and species combination were estimated. The significance of the substrate and species factors was tested sequentially by comparing alternative models using a deviance test. Finally, differences in growth and elemental concentrations were analyzed in the cultivar experiment by a one-way ANOVA and in the inoculum experiments by three-way ANOVA including seed source, inoculum and soil.

Results and Discussion

Size-dependent asbestos concentration

Our results showed that the ASTM method provides better insight than the CARB 435 method regarding asbestos concentration and distribution among different soil particle size fractions. The results from the ASTM method showed that the coarse and medium fractions of soil from BoRit contained orders of magnitude higher concentrations of asbestos (10–12% and 6–8%, respectively) than the fine fraction of soil (<1%), while the concentration in the fine fraction alone matched the asbestos concentration measured by the CARB method (Table 1). In contrast to the ASTM method, the CARB method resulted in an asbestos percentage consistently lower than 1% in both BoRit locations and in both locations in the grassland in Nottingham (Table 1). Among these three locations, soil from the stream bank (0.75%) contained slightly more asbestos than the soil from the reservoir (0.5%).

Microscopic analysis indicates that the asbestos form differed between the BoRit site sampling locations and the grassland at Nottingham. Both locations at BoRit contained chrysotile whereas the grassland soil at Nottingham contained anthophyllite (amphibole). Based on the ASTM method, the BoRit site contained a higher concentration of asbestos than the Nottingham site. All soil fractions at the grassland at Nottingham were similar in asbestos concentration (2.0 %). The asbestos concentration in the waste pile at the chromite mine location at Nottingham was below the detection limit (0.25%). In the chromite mine location, some minerals, such as chromite, lizardite and dolomite (Smith and Barnes 1998), were present that are rare or absent in most soils. Lizardite is a non-fibrous mineral, which belongs to the serpentine-group with a composition similar to chrysotile.

Our results indicate that the current framework that evaluates the level of soil asbestos using only the CARB method likely underestimates the contribution of medium and coarse soil fractions to overall asbestos contamination. Because asbestos fibers are more likely to be airborne when they are present as fine particles, the presence of asbestos in medium or coarse fractions could limit their mobility or exposure route in the environment. In these sites, exposure to asbestos can be minimized by implementing a remediation plan that

lowers soil erosion or abrasion of asbestos-containing material. This result also gives credence to the recent EPA initiative to evaluate the utility of the ASTM method in Superfund site.

Other soil properties and fertility

Soil properties and fertility that affect plant growth are important factors that can determine the types of vegetation and possibly soil amendments needed to implement a phytoremediation strategy at a contaminated site. Soil physical and chemical properties varied mostly between sites but also between locations within a site (Table 2). Soil pH was near neutral for the Grassland (6.72) at Nottingham, whereas pH was alkaline for the Stream Bank (8.13) at BoRit. Elemental concentrations of Co, Cr, Mn and Ni were higher in Nottingham than in BoRit whereas P and Ca:Mg ratio were higher in BoRit. The Ca:Mg ratio, a major indicator of soil fertility in serpentine soils (Brady et al. 2005), was above 1.0 in BoRit and below 1.0 at Nottingham. These differences in Ca:Mg ratio were attributed to a difference in exchangeable Ca and Mg between BoRit and Nottingham sites. The phosphorus concentration in soil from the reservoir at BoRit was higher (33.4 mg kg^{-1}) than at the two locations at Nottingham and in the stream bank at BoRit (all below 20 mg kg^{-1}). Concentrations of heavy metals in BoRit are in the range of values typically found in soils in the conterminous United States (Smith et al. 2013). Indeed, the BoRit site showed lower concentrations than the threshold value for a metalliferous site (Burt et al. 2003).

Among 16 parameters considered, limiting soil factors, as determined from Fertility Capability Classification, varied between the four locations. Limiting factors include: (1) percentage of gravel in the stream bank sample at BoRit; (2) pH for both sites at BoRit and the chromite mine at Nottingham; (3) nutrient reserves (K) at the chromite mine at Nottingham; (4) lower than threshold P (P-Bray) concentrations and higher than threshold Ni and Cr total concentrations for both locations at Nottingham (Table S2). Nevertheless, the Ni and Cr exchangeable fractions, an indicator for their bioavailability, were low, mainly due to high soil pH (Fig. S1). The presence of asbestos in soil induces high pH values, which represent the main limitation for plant growth. At asbestos mine tailing sites, for example, the pH is mostly alkaline (pH~10). High pH is a limiting factor because it makes elements less mobile and thus lowers nutrient availability for plants (Meyer 1980). In BoRit, the pH is within or close to the range of values recommended for crop growth (pH 6.5–8; USDA), indicating plants should grow well in this soil. In contrast to BoRit, the Nottingham soil has a greater number of limiting factors (Table S2).

Ecotoxicology tests

By monitoring seed germination and early stage root growth in three common species used for phytoremediation, we were able to evaluate limiting factors and fertility described above (Fig. 1). In all the soils from four localities, seed germination for both Brassicaceae species were inhibited by 0 to 15% compared to control soil (Fig. 1a) while germination for the *Sorghum saccharatum* species (Poaceae) was inhibited to a greater extent: 15 to 35 %. Compared to seed germination inhibition, root growth inhibition varied to a greater extent between soil types and plant species, and the variability was more pronounced for *S. saccharatum*. For the two Brassicaceae species, root growth inhibition was less apparent in

BoRit compared to Nottingham. Especially, chromite mine soil inhibited root growth more than any other soil for *L. sativum* and *S. alba*. Root growth inhibition of *S. saccharatum* was similar in all four soils compared to controls (Fig. 1b).

The pattern of germination is not related to the concentration of asbestos, which suggests that asbestos does not directly pose any threat to plants. Interestingly, BoRit soils only weakly affected both germination and root growth of *L. sativum* and *S. alba*. Indeed, the BoRit site presented a lower number of limiting factors and better soil for plant growth compared to Nottingham. This could be partly explained by the slightly alkaline pH at BoRit, which lowers the solubility of heavy metals. Between all plants tested here, *Sorghum saccharatum* appears to be the most sensitive. *Sorghum saccharatum* was also found to be less tolerant than two other species when tested on three highly contaminated sediments with heavy metal (Baran and Tarnawski 2013). Similarly, the BoRit soil did not inhibit root growth as much as did soil from Nottingham with its higher levels of heavy metals.

Germination test

Germination of eight species, collectively, was not affected by the BoRit soil compared to the control (Fig. 2). The logistic regression model with only the substrate as a factor suggested no difference across the three substrates (p-value=0.66). The model with both the species and substrate factors indicated that the proportion of germination at Day 9 differed across the species within each substrate (p-value=0.01). When eight species were combined into two plant families, seeds in the Brassicaceae had a significantly higher odds (probability) of germination than seeds in the Poaceae ($p<0.01$, Fig. S2). Seed germination is an important stage since new seedlings represent first stage of a remediation strategy (Kranner and Colville 2011), and seed dormancy can interfere (Finch-Savage and Leubner-Metzger 2006). Here, any effect of soil properties affects seed germination. Many species in the Brassicaceae are known to tolerate high levels of heavy metals (Baker 1987; Krämer 2010), although it would not be a factor in our study because heavy metal concentrations in BoRit are low (Table 2). For Poaceae, the germination level was lower than 50% after 9 days. Overall, these results suggest that all four of the tested species Brassicaceae can be used for phytoremediation of soils contaminated with high asbestos and low metal content. In Poaceae, however, it may be necessary to determine the best conditions for germination and sowing. That is, seeds may need stratification or chemical scarification (Clarke and French 2005; Finch-Savage and Leubner-Metzger 2006).

Growth and elemental concentrations of cultivar in inoculum experiment

Among the eight species tested (Fig. 3), three species, namely *B. oleraceae*, *A. gerardii* and *S. bicolor,* showed significantly different biomass among the three soils (Control and both BoRit locations). Biomass of all species was higher in BoRit soil than in the control (compost and sand mixture), although the biomass differed between the two localities within BoRit (Fig. 3). For instance, biomass of *S. bicolor* was greater on reservoir soil than on soil from the stream bank. Elemental concentrations varied mostly with species but also slightly with substrate (Table S3). For major elements (Ca, K, Mg, P), concentrations were mostly in the sufficient range of concentrations expected in plant species (Munson 1997). The lowest concentrations of Ca were found in some species of Poaceae, with Ca concentrations below

2,000 mg kg^{-1} compared to four species of Brassicaceae. The low concentrations of Ca in Poaceae are typical due to their particular cell wall type 2 (Vogel 2008). The concentration of K (threshold=10,000) was found to be deficient in five species when grown on Stream Bank soil, *B. oleraceae*, *L. perenne*, *S. alba*, *S. bicolor* and *S. saccharatum*, and also in Reservoir for *S. bicolor* and *S. saccharatum*. Concentrations of Mg were always > 2,000 mg kg^{-1}. P deficiency was observed in *A. gerardii* and *S. saccharatum* in all soils, with concentrations < 2,000 mg kg^{1}. For micronutrients (Fe, Ni and Zn), the concentrations of Fe for all species and in three soils were < 100 mg kg^{-1}, which is considered deficient. For all soils, Zn concentrations were in the range of normal, between 20 and 300 mg kg^{-1} (Kabata-Pendias 2000; Marschner 2012). The same results were found for Ni with concentrations < 15 mg kg^{-1}. Concentrations of other toxic heavy metals, such as Cd, Co and Pb, were below their detection limit. Overall, with soil amendments, plant growth and plant nutrition are favorable without any important ecophysiological impacts of the presence of 10 % of asbestos in soil.

In the inoculum experiment with C_4 grasses, biomass varied between species and there was a significant species × seed source interaction. Biomass was greater for *S. nutans* cv. Holt than the three other species × seed source combinations (Fig. 4). There was no effect of live inoculum from Nottingham on biomass for either species in these soils, but the inoculum did affect elemental uptake by the plants. Plants grown in reservoir soil had higher Mg and P concentrations than the plants grown in stream bank soil, and concentrations of these elements were typically higher when plants were cultivated with live inoculum (Table S4). Live inoculum potentially includes pathogens but also beneficial micro-organisms, including arbuscular mycorhizal fungi, which can be indispensable for plant nutrition (Smith and Read 2010). Both species are known to be mycotrophic (Casper et al. 2008; Ji et al. 2012). Interestingly for both species, the commercial cultivar and the native ecotype responded similarly in biomass to the live inoculum (Table S4). Other elemental concentrations showed differences based on the interaction of inoculum with species, soil origin or seed source, i.e. commercial or native. K and Zn differed significantly by interaction species and inoculum with higher concentrations for *S. nutans* with presence of live inoculum. The K tissue concentration was also higher for *S. nutans* from Nottingham (species × seeds effect) with higher concentrations in reservoir soil. Finally, concentrations of K and Zn could be slightly deficient with concentrations lower than 10,000 and 20 mg kg^{-1} respectively (Marschner 2012). Concentrations of Ca and Ni in plant tissues did not differ with inoculum or seed source.

Conclusions

Our results provide a framework for testing asbestos contamination and designing phytoremediation plans to limit asbestos exposure in the affected areas. We showed that, compared to the conventional method included in EPA framework, the ASTM method provides a better estimation of asbestos concentration in a range of soil size fractions. More importantly, it allowed detection of non-friable asbestos contaminated material potentially not detected with a whole soil milling process. We refer to the class of asbestos containing material that cannot be crumbled, pulverized, or reduced to powder by pressure (Perkins et

al. 2007). Currently, EPA is evaluating this sampling method for its applicability to Superfund site characterization.

We showed also that asbestos itself did not affect plant health, although insufficient nutrients or high levels of other phytotoxic contaminants could. Thus, it is important to consider all limiting parameters in soils, not just the primary pollutant of contaminated sites, such as asbestos or heavy metals.

Based on biomass production from different plant species tested here, crops may be adequate for phytoremediation in most sites with low and moderate concentrations of heavy metals in soils. Alternatively, plants making up one particular vegetation type, called serpentinophytes, may be more appropriate with higher concentrations of heavy metal in soils because of their tolerance to particular edaphic properties of serpentine soil (Brady et al. 2005). They did not prove advantageous in the BoRit soil.

Similarly to solutions already used in other Superfund sites contaminated with Pb or Zn, we propose to place soil amendments on top of the soil up to 5 cm thick and use cultivated plant crop cultivars (Dietterich and Casper 2016; Brown and Chaney 2016) or serpentinophytes. This solution seems economically more realizable than capping up to two meters of non-contaminated soils and with placement of a water permeable barrier below the 2 meters of soil. Indeed, phytostabilization could be a viable remediation strategy to minimize asbestos exposure from contaminated sites (e.g. Brownfield sites) that are currently left untreated due to prohibitive cost of other available technologies such as capping. Phytoremediation as a restoration strategy has several benefits: (1) greening of abandoned sites, (2) stabilization of topsoil, thereby limiting dispersion of asbestos fibers via air, and (3) increase in soil fertility when amendments are added with high metal binding capacity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by National Institute Environmental Health Sciences of the National Institutes of Health under award number P42 ES023720 (Penn Superfund Research Program Center Grant). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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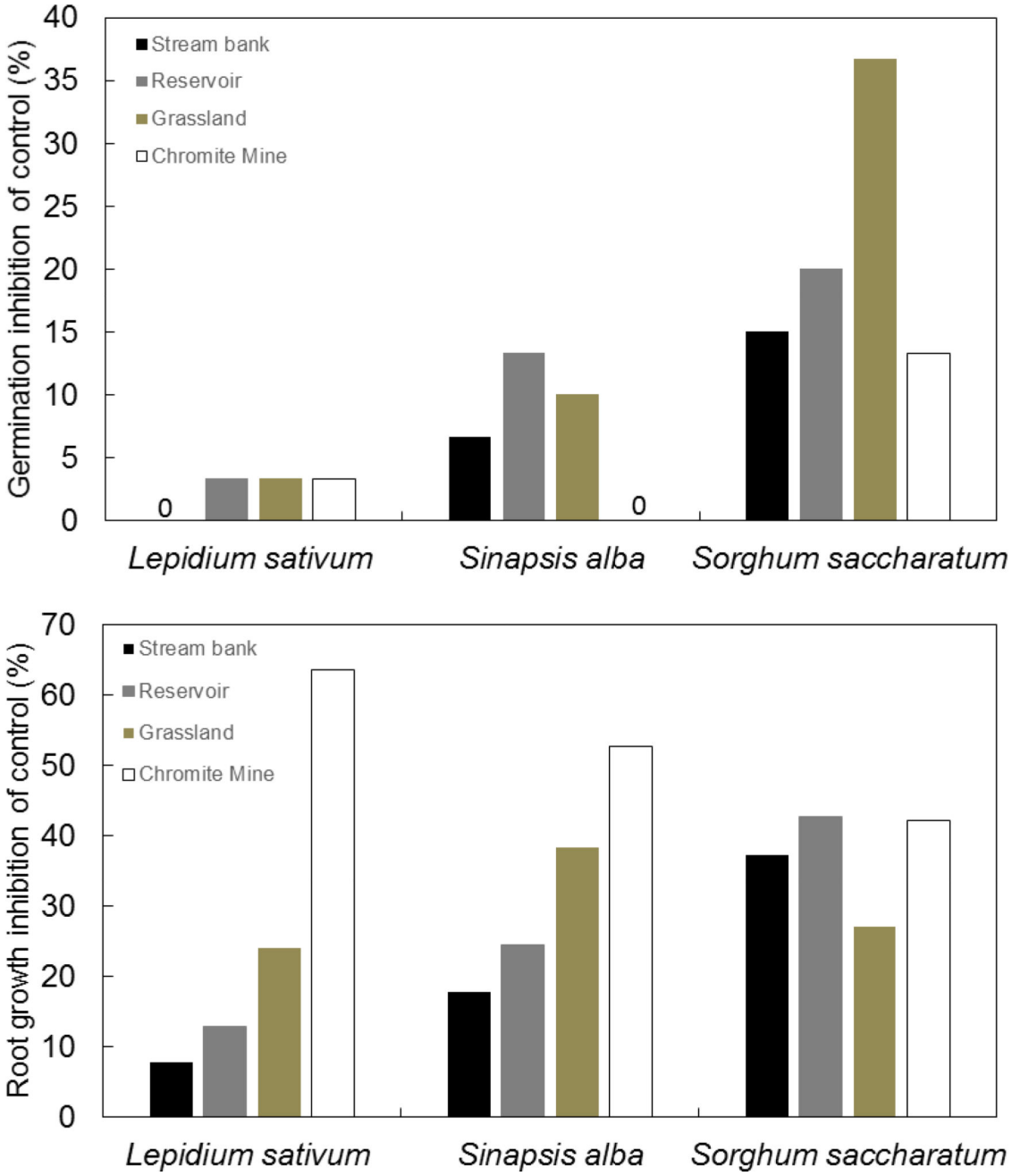

Figure 1.

Percentage of a) germination and b) root growth inhibition relative to the control for three species in different substrates: black: stream bank at Ambler; grey: reservoir at Ambler; olive: grassland at Nottingham; white: chromite mine at Nottingham. 0 indicated no inhibition for *Lepidium sativum* in reservoir and for *Sinapsis alba* in chromite mine soil.

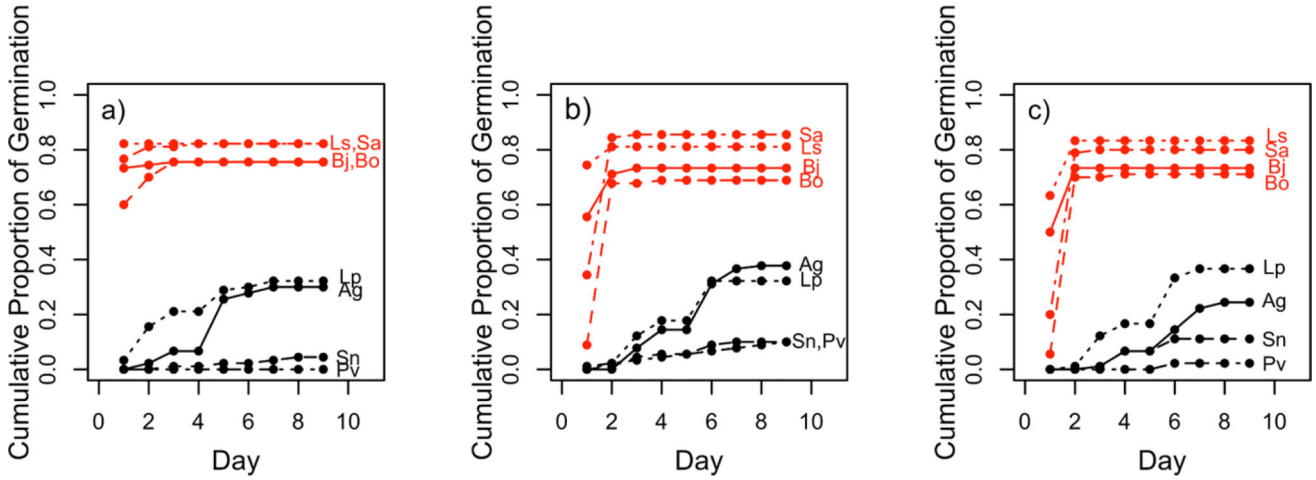

Figure 2.

Cumulative germination of eight species on a) control soil (compost and sand), b) stream bank and c) reservoir soil for 10 days after sowing. Red and black color represent species within Poaceae and Brassicaceae, respectively. Code were given in table S1. Ag: *Adropogon gerardii,* Lp*: Lolium perenne,* Pv*: Panicum virgatum,* Sn*: Sorghastrum nutans*, Bj: *Brassica juncea,* Bo*: Brassica oleracea,* Ls*: Lepidium sativum,* Sa*: Sinapsis alba*.

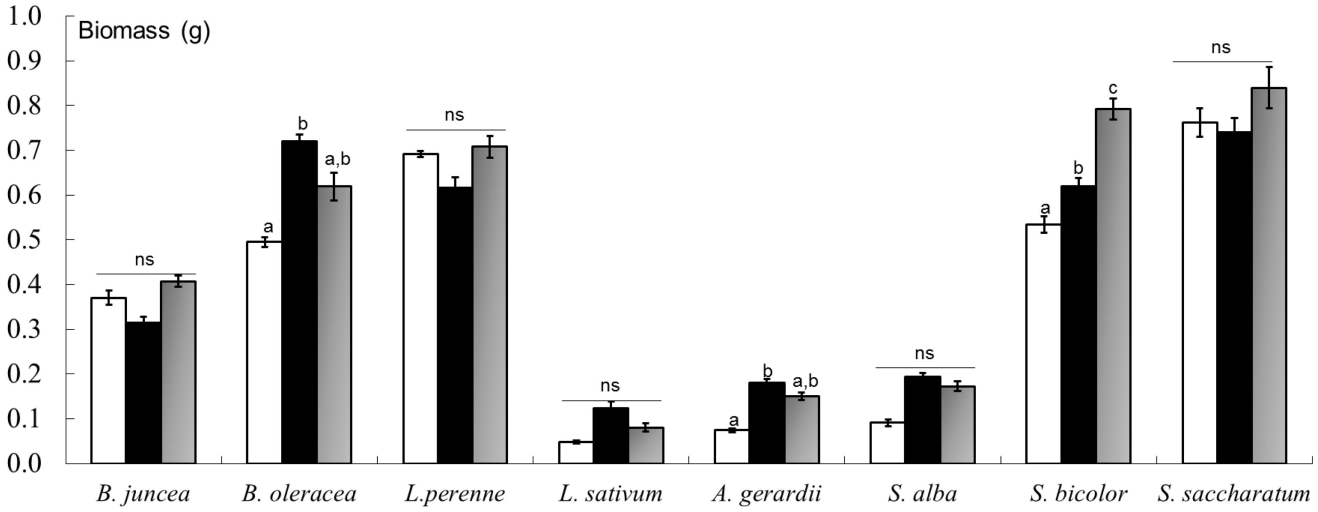

Figure 3.

Shoot biomass of eight crop species cultivated on control soil (compost and sand), (white), stream bank (black) and reservoir (grey). Letters indicate significant differences between soils within a species at $p<0.05$ and ns $p>0.05$. Vertical bars represent standard-error.

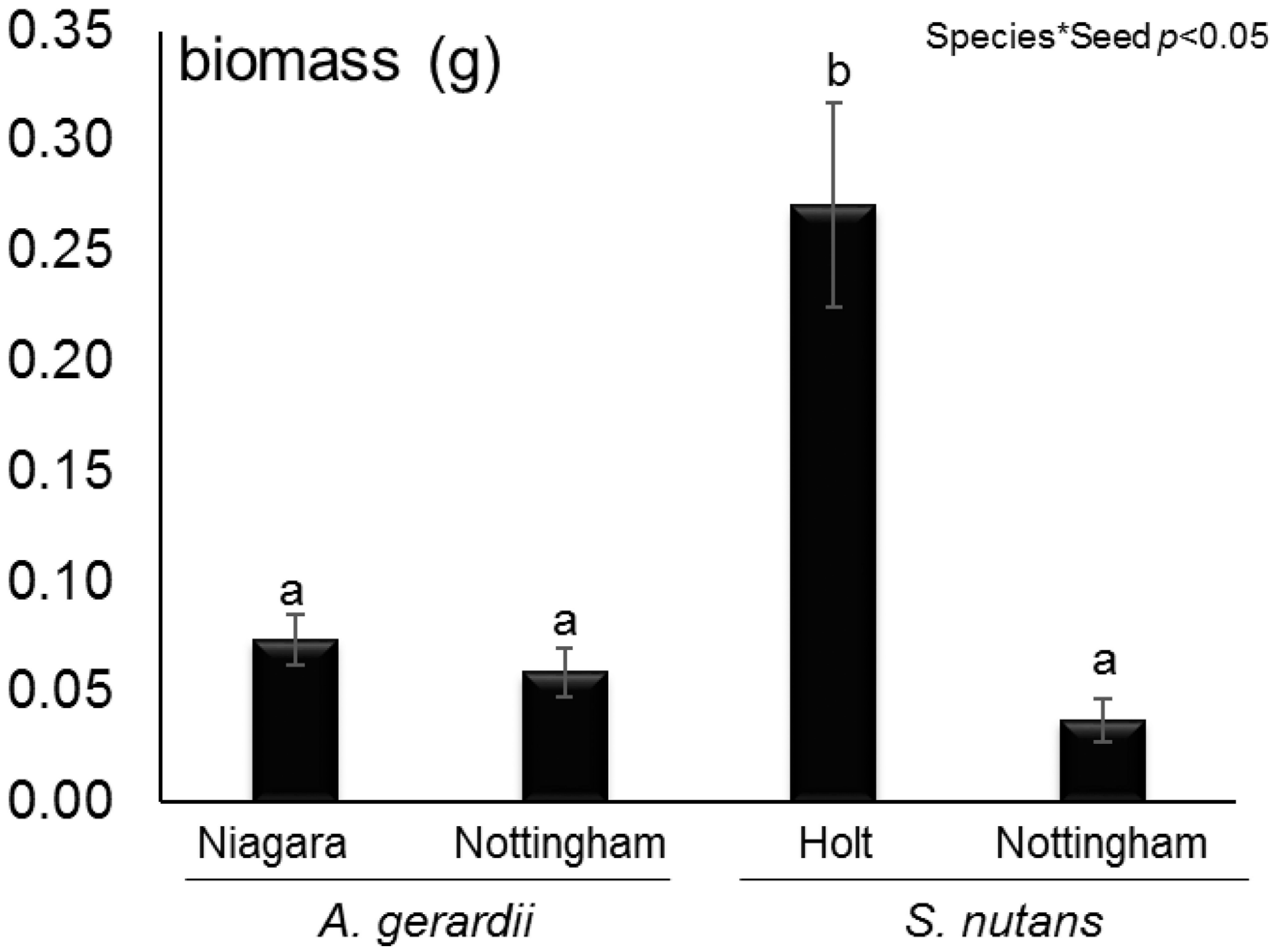

Figure 4.

Shoot biomass of *A. gerardii* and *S. nutans* on BoRit soil according to significant Species * Seed interaction. Letter indicate significant difference at $p<0.05$ for effect of species * seed interaction. Vertical bars represent standard-error. Niagara and Holt are commercial cultivars.

Table 1

Asbestos form and size distribution in two locations at the BoRit contaminated site and Nottingham serpentine soils.

			Method CARB 435			ASTM method D7521-13				
		PLM	PLM Non asbestos		PLM Asbestos	PLM Asbestos Concentration by Sieve Fraction			PLM Weighed	TEM
Site	Locations	Type	% fibrous	% non-fibrous	%	Coarse	Medium	Fine	Bulk (%)	Fine
BoRit	Reservoir	Chrysotile	0	99.5	0.5	12	8	0.75	8.2	
	Stream Bank	Chrysotile	0	99.25	0.75	10	6	0.25	7	
Nottingham	Grassland	Anthophyllite	<1%	98.5	0.5	2	2	0.5	1.9	Chrysotile
	Chromite Mine	nd	0	100	BDL	nd	nd	nd	nd	

BDL: Below detection limit (0.25), nd: non detected. PLM: polarized light microscopy, TEM: transmission electron microscopy. Coarse fraction (>2 mm), medium [100 μm−2 mm], fine (<106 μm).

Table 2

Physical and chemical characteristics of soils collected in two locations at the BoRit contaminated site and Nottingham serpentine soils.

		BoRit		Nottingham	
parameters	**unit**	**Stream Bank**	**Reservoir**	**Grassland**	**Chromite Mine**
Sand	%	62.5	66.9	48.1	67.7
Clay	%	16.6	6.27	19.1	13.2
Silt	%	20.9	26.7	32.8	19.1
Texture USDA		Sandy Loam	Sandy Loam	Loam	Sandy Loam
pH		8.13 a	7.63 b	6.72 c	7.56 b
Ca^{2+}	$cmol^{+}\ kg^{-1}$	5.90 a	5.14 a	2.73 a	0.912 b
Mg^{2+}	$cmol^{+}\ kg^{-1}$	1.9 a	1.8 a	6.7 b	4.1 ab
Ca/Mg		8.24a	10.2a	0.3b	0.12b
K^{+}	$cmol^{+}\ kg^{-1}$	0.122 a	0.136 a	0.317 b	0.149 a
Na^{+}	$cmol^{+}\ kg^{-1}$	0.284 a	0.534 b	0.204 a	0.112 a
Al^{3+}	$cmol^{+}\ kg^{-1}$	0.004 a	0.004 a	0.004 a	0.012 a
Fe^{3+}	$cmol^{+}\ kg^{-1}$	0.002 a	0.002 a	0.003 a	0.004 a
CEC	$cmol^{+}\ kg^{-1}$	10.1 a	10.0 a	25.6 b	5.60 a
Cd	$mg\ kg^{-1}$	< 1	< 1	< 1	< 1
Co	$mg\ kg^{-1}$	0.848 a	0.120 a	79.4 b	15.5 b
Cr	$mg\ kg^{-1}$	9.14 a	9.30 a	284 b	69.9 b
Cu	$mg\ kg^{-1}$	31.5 a	23.4 a	25.3 a	15.5 a
Mn	$mg\ kg^{-1}$	211 a	117 a	984 b	197 b
Ni	$mg\ kg^{-1}$	12.3 a	10.6 a	1284 b	266 b
P	$mg\ kg^{-1}$	17.4 a	33.7 b	3.54 c	2.45 c
Pb	$mg\ kg^{-1}$	< 1	< 1	< 1	< 1
Tl	$mg\ kg^{-1}$	< 1	< 1	< 1	< 1
Zn	$mg\ kg^{-1}$	40.8 a	40.8 a	57.2 a	57.2 a

Different letters represent significant different at $p<0.05$

Environ Sci Pollut Res Int. Author manuscript; available in PMC 2018 November 01.