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Sorption parameters as a predictor of arsenic phytotoxicity in Australian soils

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ABSTRACT

Arsenic (As) is a mobile and ecotoxic metalloid that is of serious concern to the environment. In this study, As phytotoxicity was studied using a dose–response approach for seven contrasting soils considering 3 end-points (shoot biomass, root elongation and chlorophyll content) and focusing on predictors of toxicity. Root elongation study was carried out for 4 days using both *Cucumis sativus* L. (cucumber) and *Triticum aestivum* L. (wheat) and shoot end-points with a 4 week a pot study using cucumber only. Root elongation of cucumber was a substantially less sensitive indicator to As than data from the 4 weeks pot study. Effective concentrations (50%)(EC_{50}) from cucumber root elongation studies were overall 1.6 times higher than the 4 week shoot data. Cucumber was however considerably more sensitive to wheat. Given the large discrepancy in phytotoxicity end points for 7 soils, root elongation data for ecotoxicological assessment should be treated with some caution. Arsenic phytotoxicity was strongly related to the sorption constants of each of the seven soils in our study. Both root elongation and shoot data were related strongly to Freundlich partitioning constants (K_f) (L/kg). Wheat and cucumber root elongation had R^2 values 0.90 and 0.91 respectively, while cucumber shoot data was 0.79. The K_f values were related to soil pH and also EC_{50} data and, thus, shows that As phytotoxicity in our study was primarily controlled by sorption reactions. The rate of As bioaccumulation to cucumber shoots depended heavily on the soil under consideration. Chlorophyll and carotenoid content of cucumber shoots increased with As content in 3 soils and decreased in other soils.

1. Introduction

Arsenic is a naturally occurring metalloid in soil that is toxic to human and ecological receptors at excessive levels. Elevated levels of metal(loid)s in soil occur from numerous anthropogenic activities, such as pesticide application, landfill, smelting of metal ore bodies (Lamb et al., 2009) animal husbandry, wood preservative use and defoliants (Lamb et al., 2013; Smith et al., 1998; Song et al., 2006). Cattle dips using arsenical insecticides in the past have resulted in large numbers of As contaminated sites. Typical As concentrations in soil from geogenic sources range from 5 to 10 mg/kg (Smedley and Kinniburgh, 2002) and 1 to 50 mg/kg (NEPC, 1999), but in some cases have been reported in the range of 90–200 mg/kg (Juhasz et al., 2007). Total concentrations of As exceeding ecological regulatory limits may represent little risk to biota (Juhasz et al., 2009; Naidu et al., 2008), due to As being occluded in minerals of low solubility. Metal(loid)s from anthropogenic sources are generally more bioavailable than from geogenic sources, but must be assessed to determine if a risk exists (Juhasz et al., 2007; Lamb et al., 2009).

Arsenic occurs in the soil environment primarily as anionic inorganic species of arsenate (As V) and arsenite (As III), however organo-arsenic species are produced by microorganisms (Cullen and Reimer, 1989; Smedley and Kinniburgh, 2002). The oxidation state of As is believed to be a key factor determining ecotoxicity in soils. Arsenite is typically more toxic to humans and ecological receptors. However, in aerobic soil environments arsenate species are the most likely to dominate As speciation. In upper soil horizons, As transformation between oxidation states occurs from arsenite to arsenate, but the transformation from arsenate to arsenite is not chemically favoured (Cullen and Reimer, 1989; Smedley and Kinniburgh, 2002).

Regulatory and phytotoxicity threshold limits for As range markedly between jurisdictions, soil types and test species. Nevertheless, despite advances between soil-specific guidance for metals (Heemsbergen et al., 2009; Sheppard, 1992; Song et al., 2006), As guidance is still based on a single total As concentration for all soils. Sheppard (1992) reviewed phytotoxicity data available and concluded that soil type was the most important factor controlling As phytotoxicity and generic guidelines should be categorised within soil types. Factors generically known to influence As toxicity include soil pH and iron oxyhyroxide contents. However, this is not always the case. Song et al. (2006) studied As phytotoxicity in European soils using a 4 day barley root elongation

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procedure. Arsenic phytotoxicity, represented by dose–response parameters, suggested that clay content and amorphous Mn oxide minerals were the best explanatory factors for their data set.

Assessment of As phytotoxicity in soil is most typically performed by greenhouse studies, where plants are grown under approximately natural conditions. This type of study is relatively time consuming, typically ranging from 4 to 8 weeks in duration (Wong and Bradshaw, 1982). Root elongation is a more rapid method that is also used to assess inhibition of plant function by metalloid contamination in soil (Wong and Bradshaw, 1982). Short term root elongation studies are typically very short ranging from 2 to 7 days in length, depending on the procedure and plant species. The toxicity of metalloids is believed to vary with plant age, and different methodologies may yield different phytotoxicological end-point values (De Koe et al., 1992; Sneller et al., 1999a,b).

In this study, As phytotoxicity over a wide concentration range was studied in a range of soils using both greenhouse (4 week) and root elongation (4 d) studies using the test species cucumber and wheat. The results from a variety of phytotoxicity endpoints were compared. The relationship between toxic thresholds and soil properties and solid-solution partitioning are reported.

2. Methods/experimental

2.1. Soils

Seven uncontaminated soils were sampled with contrasting soil properties from Queensland, South Australia and Victoria, Australia. The soil types include a Ferrosol, Vertosol, Kurosols, Calcarosol, Dermosol and Tenosols. Soils were taken from the top 0.2 m with stainless steel trowels, air-dried and sieved through 4 mm sieves. All soils were spiked with As (V) (Na_2HAsO_4) from 0 to 2000 mg/kg depending on the soil properties. Soils were spiked by spraying As solutions to a thin layer of soil and mixing. Soils were incubated for 4 months prior to experimentation. The soils were incubated at water contents of approximately 60–70% (*w*/*w*) of field capacity at ~22 °C prior to the plant bioassays. Prior to toxicity experiments, soils were dried at 40 °C, sieved and again mixed. Soils were coded using letters from collected place and then added A to mean A-horizon soil.

Sorption of As to all soils was studied using a batch reaction methodology (Smith et al., 1999). In brief, 1 g of soil was reacted with 20 mL of solution containing increasing arsenate concentrations. The background electrolyte was 0.03 M $NaNO_3$. Arsenate was reacted for 24 h, then filtered (0.45 μm) and analysed by inductively coupled plasma mass spectrometry. Sorption curves were modelled with the Freundlich function (Stumm and Morgan, 1996).

2.2. Plant growth

2.2.1. Root elongation study

Air-dried soil (100 g) was transferred to 120 mL plastic pots (108 × 44 mm) for each treatment for every soils in triplicate and high purity water added to achieve approximately 70% field capacity. Seeds of *Cucumis sativus* L. and *Triticum aestivum* L. were treated with 1.25% NaOCl solution for 10 min and rinsed with highly purified water (18.2 μΩ/cm) thoroughly before sowing (7 roots per pot, n = 4, total pots = 252). After 4 d at 25 °C controlled room, maintaining day hour light, plant roots were carefully separated and roots washed for measurements. Root lengths were analysed with WinRHIZO Arabidopsis software after scanning (Epson Perfection V700 Photo, Canada).

2.2.2. Pot study

Cucumber (*Cucumis sativa* L.) seeds were sown to plant pots (120 × 125 × 120 mm) in triplicate for each treatment of all soils in September 2013. Each pot was lined with fine nylon mesh and 300 g of air-dry soil added. Each pot was placed within a collecting tray to collect any excess leachate generated. High purity water was added (18.2 μΩ/cm) for watering. Plants were watered by weight to avoid drainage as much as possible and to reach approximately 70% of field capacity throughout the study. Sufficient seed was added to ensure germination. Plant numbers were later reduced to 4 plants per pot. Each soil treatment was replicated 3 times. Plants were grown for 4 weeks from sowing under greenhouse conditions (~16–25 °C). Plant heights, fresh and dry weights were determined.

Fresh samples of leaf tissue (0.5 g) was collected from the second youngest fully expanded plant leaf of each replicated treatment during harvesting to determine chlorophyll content. These samples were cut into pieces (approximately 1 mm wide) and placed into glass vials with 80%-acetone solution (10 mL) and kept in the dark (4 °C, 24 h) (Oleszczuk, 2008). Samples were centrifuged at 400 *g* for 15 min and the supernatant collected for spectrophotometric analysis at 470, 663 and 645 nm for determination of chlorophyll a, chlorophyll b and carotenoids, respectively (Lichtenthaler and Wellburn, 1983).

After harvesting, plant shoots were washed with tap water and rinsed several times with high purified water. Washed shoot samples were dried at 70 °C for 72 h. Homogenised dry samples were then used for measurement of As content in shoots by acid digestion.

2.2.3. Analysis

The general physicochemical properties of soil samples were determined using standard procedures. The pH and conductivity were measured with purified water and 0.01 M $CaCl_2$ in 1:5(*w*/*v*) soil water suspensions (Rayment and Higginson, 1992). The hydrometer method was used for determination of clay, sand and silt in soil samples (Gee and Bauder, 1986). Soil organic carbon (OC) was determined by the high-temperature loss-on-ignition method after addition of HCl to remove carbonates (Leco TruMac CNS analyser, USA). The Effective Cation Exchange Capacity (ECEC) was estimated according to Amacher et al. (1990). Oxalate extractable, or 'amorphous' Fe and Al oxide content of sample was measured according to Courchesne and Turmel (2008) and Mn oxides according to Gambrell (1996). Arsenic content in soil was determined by microwave assisted acid digestion (concentrated HNO_3) (USEPA method 3051) (see www.caslab.com/EPA-Methods/PDF/EPA-Method-3051.pdf for details of method). Quality control utilised sample blanks and certified reference material (Montana Soil SRM2711) that is available from the National Institute of Standards and Technology (NIST), USA. Recoveries were on average 83% for As.

As and phosphorus content in plant tissue after exposure was conducted by digesting 0.5 g of dried sample in 5 ml of concentrated nitric acid overnight and then heating it under programmed heating to 140 °C to evacuate the acid to approximately 1 ml. The remaining acid was diluted to 20 ml using Milli-Q water. The solution was filtered (0.45 μm) before analysis using inductively coupled plasma-mass spectroscopy (ICP-MS) (Agilent 7500c).

All aqueous samples were analysed by ICP-MS after appropriate dilutions and matrix matching in standards. Quality control during analysis was monitored during analysis by addition of 50 μg/L check samples and blanks every 20 samples. Recovery of check samples was always between 90 and 110%.

2.3. Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference were used to determine significant differences (at the 0.05 significance level) between treatments for specific species using SPSS (version 18) (SPSS, Chicago, IL). Calculation of EC_{50} values was performed by sigmoidal dose - response model using the R programme (https://www.r-project.org) with the logistic function:

$$y = \frac{a}{1 + e^{b(x-c)}} \quad (1)$$