Copper interactions on arsenic bioavailability and phytotoxicity in soil

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ABSTRACT

Arsenic (As) and copper (Cu) are co-contaminants in the environment but little is known about their ecological impact as mixtures in soil. In this study, we investigated the combined As-Cu interactions on toxicity and uptake as binary mixtures in 5 contrasting soils. The study included solubility, contaminant uptake and toxicity in cucumber (*Cucumis sativus* L.) as a model plant species. Soils were spiked individually and as a mixtures at 10 different As levels (2, 4, 8 up to 1024 mg kg^{-1}). Copper was added with As at two effective concentration levels (EC10$_{Cu}$ and EC50$_{Cu}$). Arsenic uptake was significantly reduced in the presence of Cu and a higher effect was demonstrated with increasing pore-water pH. Copper accumulation was not significantly influenced by As. An additive response on plant growth was predominant overall when expressed from pore-water parameters with root mean square errors of 12.6 and 13.2 for EC10$_{Cu}$ and EC50$_{Cu}$ treatments, respectively.

1. Introduction

Arsenic (As) is a co-contaminant with copper (Cu) at a variety of contaminated sites such as around Cu smelters, gold mines and wood preservative sites (Archer and Caldwell, 2004; Gore et al., 2007; Liang et al., 2017). Both As and Cu are highly phytotoxic and pose a significant threat to the integrity of ecological systems when existing in bioavailable forms (Kader et al., 2016a, 2016b). The chemical properties of the two elements are different. Arsenic in the environment is an oxyanion, a nonessential element to plants, its sorption in soil depends strongly on Al- and Fe-(hydro)-oxides, and typically decreases with increasing pH (Meharg and Hartley-Whitaker, 2002). By contrast, Cu is predominantly cationic at environmental pH ranges, an essential micronutrient, and has a relatively high affinity for the soil organic matter (Stumm and Morgan, 1996). Sorption depends strongly on organic matter in the solid and solution phase and sorption typically increases with an increase in pH (Burton et al., 2005; McBride, 1989).

Phytotoxicity data on As and Cu as individual contaminants in soil are available (Kader et al., 2016b; Romero-Freire et al., 2014; Rooney et al., 2006; Song et al., 2006; Warne et al., 2008) but little is known about their combined toxic effect on plants. Phytotoxicity of metal mixtures in soil has been reported based on total metal concentrations (An et al., 2004; Cao et al., 2007) but studies applying the free ion activity model (FIAM) and pore-water based approaches are rare.

Despite As being a common contaminant in soil, sediment and surface waters, its inclusion in semi-mechanistic approaches, such as

the BLM, or empirical studies on mixed contaminant systems, is lacking Similarly, ecotoxicity data detailing effects of As and heavy metals in different soils are rare. Indeed, in a summary of aquatic binary studies, As was one of the least studied metal(loid)s with only 5 out 270 investigations reporting toxicity interactions (Norwood et al., 2003). Unlike metals such as Cu and Zn, As toxicity in nutrient culture does not appear to reveal a strong dependence directly on pH. Furthermore, as there presently are no binding constants included in the WHAM speciation software for As and dissolved organic carbon, there are significant limitations in the application of biotic ligand or the WHAM-based models (Tipping and Lofts, 2015). Arsenic and cadmium interactions have been reported in solution culture (Cao et al., 2007; Sneller et al., 2000) and in a small number of soils (Cao et al., 2007). Arsenic with Cu interaction was demonstrated in gametophytes of *Pteris vittata* L. using the Murashige and Skoog media (Zheng et al., 2008). Fayiga et al. (2004) indicated that the presence of cationic metals (Cd, Ni, Pb and Zn) influenced As accumulation in *Pteris vittata* L. in a mining soil.

The main objective of this study was to investigate the combined effects of As and Cu and their interaction in different soils based on growth and bioaccumulation in cucumber (*Cucumis sativus L.*). Furthermore, we focus on pore-water data to understand As-Cu interactions. A regression-based design was utilized to study the mixture of As and Cu. We included eleven As concentrations (0, 2, 4 to 1024 mg kg^{-1}) and three Cu concentrations (control, EC10$_{Cu}$ and EC50$_{Cu}$ values), where the EC10$_{Cu}$ and EC50$_{Cu}$ values were the effective concentrations resulting in plant growth reduction of 10% and 50%,

Table 1
Properties of experimental soils with Cu spiked concentrations in this study.

Properties	BKA	MGA	PBA	TAA	YEA
Soil Location	Brookland, QLD	Mount Gambier, SA	Port Broughton, SA	Tarrington, Vic	Yenda, NSW
Soil type	Tenosol	Tenosol	Tenosol	Ferrosol	Vertosol
Soil pH-CaCl$_2$	6.29	5.67	7.73	4.91	6.87
Soil pH-water	7.01	6.29	8.72	5.31	8.09
Total As (mg kg^{-1})	0.9	4.5	1.4	2.0	2.6
Total Cu(mg kg^{-1})	14.1	16.4	5.8	7.2	16.8
CEC ((cmol(+)kg +))	20.9	24.1	5.8	11.7	24.2
Clay content (%)	20.0	24.1	6.7	10.0	62.1
Organic carbon (%)	2.99	8.37	1.76	4.98	1.14
Amorphous Fe oxide (mg kg^{-1})	3.49	12.49	0.28	3.12	1.47
Amorphous Al oxide (mg kg^{-1})	1.11	12.60	0.50	3.01	1.23
Amorphous Mn oxide (mg kg^{-1})	0.63	0.30	0.06	0.21	0.30
Cu toxicity					
Cu EC10 level (mg kg^{-1})	626	709	174	410	334
Cu EC50 level (mg kg^{-1})	893	1698	844	873	918

respectively, derived from previous studies (Kader et al., 2016a, 2016b).

2. Materials and methods

2.1. Experimental design

This study involved five soils previously studied for As (Kader et al., 2016a) and Cu (Kader et al., 2016b) individually on *Cucumis sativus* L. (cucumber). Cucumber was chosen based on an unpublished screening study; cucumber is also a recommended test species by the USEPA (Lamb et al., 2016). Methodologies for determination of all soil properties (pH, organic carbon, cation exchange capacity, clay content, oxalate extractable Fe, Al etc) have been described in detail previously (Kader et al., 2016b). Three simultaneous treatment series were used in this study. First, the five soils were treated singly with As at 11 different levels (0, 2, 4 to 1024 mg kg^{-1}). In the case of one alkaline soil (PB), As was spiked up to 128 mg kg^{-1} only. Previous work identified the appropriate toxicity range of each soil. Secondly, individual Cu treatments at EC10$_{Cu}$ and EC50$_{Cu}$ levels were added to each soil without As (Table 1). The previously determined effective inhibition concentrations for Cu (EC10$_{Cu}$ and EC50$_{Cu}$) are presented in Table 1. Thirdly, As and Cu were added to soils as mixtures. All individual As levels were mixed with Cu at EC10$_{Cu}$ and EC50$_{Cu}$ levels. Five replicates were prepared for controls and individual Cu treatments. All other treatments used on plant growth, bioaccumulation and pore-water extraction were replicated twice.

After drying and sieving, soils were spiked with As (V) (Na$_2$HAsO$_4$) and Cu(NO$_3$)$_2$ individually as well as mixtures. Soils were individually spiked with As (V) or Cu in polypropylene trays by spraying solutions onto a thin layer of soil followed by mixing with a trowel. Soils were then transferred to 10 l polypropylene containers and mixed on a mechanical mixer for 5 min and incubated. For mixtures, a 24 h lapse was allowed between Cu and As addition. Arsenic concentrations were made in bulk and separated into three batches. Each As concentration thus received (1) no added Cu, (2) Cu at EC10$_{Cu}$ and (3) Cu at EC50$_{Cu}$ concentrations (see Table 2 for treatment combinations). All containers were incubated for 6 weeks with loosely bound lids below field capacity.

2.2. Phytotoxicity study

Dried soil (300 g) was transferred to each plastic pot (120 × 125 × 120 mm) after sieving (< 2 mm). Pots were watered with deionised water to reach approximately field capacity and the following day 12–15 seeds of cucumber (*Cucumis sativus* L.) were sown to ensure sufficient germination. After one week of seedling emergence, each pot

was thinned to 5 plants. Plants were grown under greenhouse conditions (~ 16–25 °C) (January–February) (Adelaide, South Australia) with randomized placement. Four weeks after seeding, plants were harvested and the fresh weight of shoots determined. Shoots were then washed with tap water and finally rinsed several times with highly purified (Milli Q) water, and then dried at 70 °C for 72 h and dry weights measured.

Soil pore-water from the harvested pots was extracted applying the same methodology for moistening soils as described in our previous study. Pore-water was extracted using Rhizon samplers (Lamb et al., 2013) and separated for measurement of metal concentrations (Na, Mg, Al, K, Ca, Mn, Fe, Cu, and As), dissolved organic carbon (DOC), cupric ion activity (Cu^{2+}) and pH$_{pw}$.

Dried shoots were crushed for measuring metal(loid)s content in shoots. A block digester was used for nitric acid digestion. Briefly, up to 0.5 g of homogenised dried shoot samples were transferred to digester glass tubes for acid digestion using 5 mL of nitric acid overnight. All digested samples were diluted appropriately according to the inductively coupled plasma mass spectrometry measuring range (Kader et al., 2016a).

2.3. Samples analysis

All aqueous samples from pore water, plant samples and microwave digestion were analyzed using ICP-MS (Agilent 7500c). Dissolved organic carbon (DOC) was determined using a total carbon analyzer (1010 OI Analytical). The pH of the extracted soil solution was measured using a pH meter (HACH, USA). Free Cu was measured using a Cu ion selective electrode (ISE) as reported earlier in individual Cu study (Kader et al., 2016b). Total concentrations of metal(loid)s in soil were estimated using XRF (Olympus), calibrated with reference soil (average detection limits for As and Cu were 4.2 and 6.3 mg kg^{-1}, respectively). Quality control was ensured in every run by introducing standards and blanks every 20 samples. Recovery of standards was always between 90 and 110%.

Predicted steady state activities of Cu^{2+}, Ca^{2+} and HAsO$_4^{2-}$ based on equilibrium data for Cu arsenate, Cu(OH)$_2$ and Ca-arsenate (temperature 25 °C, CO$_2$ 0.0032 (g)) were estimated using PHREEQC, version 3 with updated thermodynamic data (Nordstrom et al., 2014; Parkhurst and Appelo, 2013).

2.4. Statistical analysis

Dose-response data were fitted with a logistic function for individual As and As -Cu mixtures. The logistic model used was:

$$Y = \frac{a}{1 + e^{k(x-c)}} \tag{1}$$