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Humic acids decrease uptake and distribution of trace metals, but not the growth of radish exposed to cadmium toxicity

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ABSTRACT

Naturally-occurring highly-complexed and polymerised organics such as humic acids (HA), due to their large negative charge, play a crucial role in biogeochemistry of trace metals (TM). Toxic (Cd) as well as essential (Zn, Cu, Mn) TM bind strongly to HA, but how these organo-metalic forms influence metal uptake by plants is poorly understood. A solution culture study was conducted to characterize the effects of different concentrations of HA (0–225 mg/L) on the growth and element uptake/distribution in roots, shoots and hypocotyls of radish (*Raphanus sativus* L.) exposed to Cd (0.5 mg/L) contamination. After 10-d-exposure to applied treatments, Cd induced phytotoxicity; in contrast, different concentrations of HA had no influence on biomass, but decreased concentration of most TM in examined tissues (Cu by 4.2-fold, Zn by 2.2-fold, Cd by 1.6-fold and Mn by 34%) and their total plant accumulation (Cu by 73%, Cd by 39%, Zn by 29% and Mn by 22%). HA influenced the transport/distribution of TM, decreasing accumulation in roots and increasing their translocation/deposition in shoots, with no effect on TM content in edible hypocotyls. Chemical speciation modelling of the rooting medium confirmed predominance of free metallic forms in the control (no HA) and the pronounced organo-metal complexation in the HA treatments. The results provide evidence of strong capacity of HA to decrease phytoavailability and uptake of Cd, Zn, Cu and Mn while being non-toxic even at relatively high concentration (225 mg/L). Thus, HA, as naturally present soil components, control mobility and phyto-extraction of most TM as well as their phyto-accumulation.

1. Introduction

Due to intensification of different anthropogenic activities over the past decades (urbanisation, industrialisation, food production), the natural resources, notably soil and water resources, are being loaded with various persistent organic and inorganic contaminants (Savic et al., 2017; Romic et al., 2011). Unsustainable waste management practices (landfill, spreading of wastes on land) leads to a significant build-up of a wide range of soil contaminants (e.g. heavy metals/metalloids) (Seshadri et al., 2015). Contamination of terrestrial ecosystems by metals is recognised as one of the most critical environmental and sustainability issues of this century (Ondrasek and Rengel, 2012), potentially impacting human health.

Some metals (e.g. Cu, Mn, Zn and Fe) are essential nutrients in trace amounts, but become toxic at increased concentrations (e.g. Rengel, 2007). For some trace metals such as cadmium (Cd), no essential/beneficial function in plants and other biota has been confirmed (Yu et al., 2017), although Cd can be taken up by roots relatively easily (exploiting the same/similar routes as some nutrients, such as Zn^{2+}/Fe^{2+} transporters; Clemens, 2006), accumulating in various tissues of plants, including edible tissues (Filipovic et al., 2018; Kwiatkowska-Malina, 2017; Ondrasek et al., 2009). Consuming so-called “metal clean food” (i.e. food with metal levels below the maximum permissible concentrations) might be a sustainable diet strategy for ensuring trace metals homeostasis in humans. Unfortunately, crops grown on metal-enriched growing media may have increased concentration of metal contaminants; consuming such food is the most common way of transferring metals/metalloids (Cd, As, Hg, Pb) into humans, which in long-term may trigger various health disorders (Zhou et al., 2016).

The environmental consequences of trace elements in the biosphere and potential risks to human health (via consumption of food overloaded by metals) depend not only on the total metal content, but also on the metal speciation, concentration of antagonistic elements and various ligands, nature of the rhizosphere matrix, and the pH reaction of surrounding media (e.g. Laurie et al., 1991; Versieren et al., 2013). Presence of biologically-produced, highly-complexed, and relatively stable (resistant to mineralisation) organics such as humic substances, might be of crucial importance in biogeochemistry of numerous metals

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and their dynamics in the soil-plant continuum (e.g. Kwiatkowska, 2006; Ozkutlu et al., 2006). One of the most important property of humics is their huge reactive surface (predominantly negatively charged) that compete with other rhizosphere matrix constituents (inorganic ligands, clays, hydroxides) in metal adsorption/chemosorption reactions (Ondrasek and Rengel, 2012).

Although many humic substances are still not fully characterised, there are numerous hypothetical chemical structures indicating their complexity and heterogeneity (e.g. Schnitzer, 1978). As organic substances polymerised by various functional groups (carboxyl, hydroxyl, aldehyde, ketone, ester, amino, nitro, thiol), humics act as acids and/or bases, i.e. serving as proton (H^+) donors and/or acceptors (also metal acceptors) depending on the pH of the medium (Dobranskyte et al., 2006; Leita et al., 2009). Particular humic fractions may be extracted from the pedosphere after suspension in NaOH and filtering (e.g. XAD-8 resin), where two main adsorbed compounds include humic acids and fulvic acids that are further fractionated by acidification. In strongly acidic solutions (pH < 2), the precipitated fraction represents humic acids that are not soluble in water (but become soluble at higher pH values), whereas fulvic acids remain in solution after acidification and are soluble at all pHs.

Complexation of metals with humics (humic acids/fulvic acids) can be pronounced in water ecosystems (Yang and van den Berg, 2009), which may affect the availability of some metals to (water) biota (Dobranskyte et al., 2006; Winter et al., 2012; Ouyang et al., 2017). Our previous findings (Filipovic et al., 2018; Ondrasek et al., 2012, 2009) and related similar studies conducted in a wide range of environmental conditions (e.g. Halim et al., 2003; Evangelou et al., 2004; Kalis et al., 2006; Kwiatkowska, 2006; Le et al., 2015; Kwiatkowska-Malina, 2017) addressed a possibility that specific components of dissolved soil humics may significantly influence (i) metal biochemistry at the root-soil interface, and thus (ii) transfer of some essential as well as non-essential trace metals from the rhizosphere to plant tissues. For instance, addition of humic acids (HAs) to soil might decrease extractability of the soluble (exchangeable) metal forms (Halim et al., 2003) or have positive effects on metal phytoextraction from the soil environment (Evangelou et al., 2004). Under different root environment matrices (soil vs solution), Kalis et al. (2006) observed that the HA addition decreased adsorption of some metals (Cu, Pb, Fe) at the root surface, and increased adsorption of others (Cd, Zn, Mn).

The nature of the interactions between HAs and metals in the environment is of considerable importance regarding trace metal solubility and thus toxicity to plants and other organisms (e.g. Dobranskyte et al., 2006; Winter et al., 2012) as well as metal entry into the human food chain. However, the physico-chemical basis of such organo-metallic interactions is still poorly understood due to highly complex structure and heterogeneity of HAs (Prado and Airoldi, 2003) as well as strong influence of environmental parameters that may vary markedly on a small scale such as the rhizosphere-root interface (Ondrasek and Rengel, 2012). Hence, the main objectives in this study were to assess the influence of dissolved HAs (0–225 mg/L) and Cd contamination (0.5 mg/L) in solution on: (i) trace metal (Cd, Cu, Zn, Mn) interactions, (ii) metal bioavailability/phytoextraction and distribution, and (iii) vegetative growth parameters of radish.

2. Material and methods

2.1. Environmental conditions and test plant

Selected uniform seeds of radish (*Raphanus sativus* L. cv. Cherry Belle), purchased in the local store, were sterilized by soaking in 80% (v/v) ethanol for 2 min and then in 1% (v/v) sodium hypochlorite for 5 min, washed in ultrapure deionized water (18 mΩ cm^{-1}) obtained from a Milli-Q system (Millipore Corp, Milford, CT, USA) and germinated on the filter paper soaked with Milli-Q water for 48 h in dark. The same Milli-Q water was used for the preparation of all stock and nutrient solutions in this study. Uniformly germinated seeds were positioned on the plastic net floated in 30 L plastic pots filled with ½ strength continuously aerated nutrient solution (Table 1). After 10 days, three uniform plants, supported by inert poly-foam pipe were positioned in holes cut into tight-fitting lids of one plastic pots (4 L) filled with full strength nutrient solution. After additional 4 days, radish plants were exposed to Cd contamination [0.5 mg Cd/L (4.5 μM), as cadmium nitrate, Ajax Chemicals Ltd., Sydney, Australia] and five concentrations of HAs (as sodium humate, Sigma-Aldrich, Saint Louis, USA): 0, 15, 75, 150 and 225 mg/L in four replicates (Table 1). The treatments were completely randomly re-arranged on daily bases and lasted for 10 days.

Table 1
Composition of full-strength nutrient solution (pH 6.0) with the cadmium and humic acid treatments applied 14 days after germination for next 10 days.

Component	Concentration
KNO_3	2.5 mM
KH_2PO_4	0.5 mM
$Ca(NO_3)_2$	2.5 mM
$MgSO_4$	1.0 mM
2-(N-morpholino)ethanesulfonic acid (MES) as a pH buffer	1.0 mM
$FeSO_4$	50 μM
H_3BO_3	5.0 μM
$MnCl_2$	3.70 μM
$ZnSO_4$	0.64 μM
$CuSO_4$	0.52 μM
$NiSO_4$	0.10 μM
Na_2MoO_4	0.02 μM
$Cd(NO_3)_2$	4.5 μM
Humic acid	0; 15; 75; 150 and 225 mg/L

All nutrient solutions were continuously aerated, and pH was maintained at 6.0 ± 0.1 by the addition of 0.1 M KOH or HNO_3. Water lost through evapotranspiration was replaced daily, and solutions were renewed every 2 days. Plants were grown in a controlled environment (12/12 light/dark period, air temperature 24/19 °C, air humidity 60/80%, and 350 μmol m^{-2} s^{-1} photosynthetically active radiation supplied by high-pressure metal-halide lamps) in a growth cabinet at the University of Western Australia in Perth.

The stock solution of humic acid was prepared as described by Brooks et al. (2008). In short, 15 g of humic acid was dissolved in 300 mL 0.1 M NaOH, pH was adjusted to 6.0 by 0.1 *M* HNO_3, filled to 1000 mL by Milli-Q water, and filtered through a 0.45 μm membrane filter.

2.2. Sampling and chemical analyses

Before sampling, plants were rinsed in Milli-Q water for 20 min, followed by 5 m*M* $CaCl_2$ for 20 min and again in Milli-Q water for 20 min. After that, radish organs were separated by the scalpel blade to three main parts: (1) root (taproot with lateral roots), (2) hypocotyl (fleshy thickened edible organ without lateral roots) and (3) shoot. Weighed plant material was dried for 48 h at 70 °C, re-weighed and ground in a ceramic mortar for the chemical analyses. Approximately 0.2 g of ground sample was digested in nitric (HNO_3) and perchloric acid ($HClO_4$). Briefly, plant material was transferred to a 50 mL conical flask and digested firstly in 5 mL of concentrated HNO_3 at around 100 °C for about 45 min. After that, digested mixture was cooled down to room temperature, supplemented with 0.5 mL of concentrated $HClO_4$ and heated to around 150 °C for 30–40 min. Flasks were again cooled down and then heated to 170–180 °C for 10 min to allow dehydration of any silica in the digest. After the digested solutions were cooled down, they were transferred with three rinses by Milli-Q water to 10 mL vials that contained 5 μg of yttrium (serving as an internal standard), and stored for further instrumental analyses. In each analytical batch, at