



Assessment of metal(loid)s phytoavailability in intensive agricultural soils by the application of single extractions to rhizosphere soil

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ARTICLE INFO

Article history:

Received 18 September 2014

Received in revised form

12 December 2014

Accepted 15 December 2014

Available online 23 December 2014

Keywords:

Lettuce

Soil

Metal(loid)s

Phytoavailability

Single extraction methods

ABSTRACT

The influence of soil properties on the phytoavailability of metal(loid)s in a soil–plant system was evaluated. The content of extractable metal(loid)s obtained by using different extraction methods was also compared. To perform this study, a test plant (*Lactuca sativa*) and rhizosphere soil were sampled at 5 different time points (2, 4, 6, 8 and 10 weeks of plant growth). Four extraction methods (Mehlich 3, DTPA, NH_4NO_3 and CaCl_2) were used. Significant positive correlations between the soil extractable content and lettuce shoot content were obtained for several metal(loid)s. The extraction with NH_4NO_3 showed the higher number of strong positive correlations indicating the suitability of this method to estimate metal(loid)s phytoavailability. The soil CEC, OM, pH, texture and oxides content significantly influenced the distribution of metal(loid)s between the phytoavailable and non-phytoavailable fractions. A reliable prediction model for Cr, V, Ni, As, Pb, Co, Cd, and Sb phytoavailability was obtained considering the amount of metal(loid) extracted by the NH_4NO_3 method and the main soil properties. This work shows that the analysis of rhizosphere soil by single extractions methods is a reliable approach to estimate metal(loid)s phytoavailability.

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1. Introduction

Metals and metalloids, from now on referred to as metal(loid)s, are ubiquitous components of the lithosphere. Metal(loid)s are distributed in both the solid and aqueous phases of the soil and may be involved in a panoply of interactions ranging from weak electrostatic sorption up to irreversible binding (Fedotov et al., 2012).

According to ISO (2008a), *environmental bioavailability* is defined as the fraction of an element that can be either available or potentially available to living organisms. This definition includes both the actual available fraction (i.e., the sum of dissolved free ions/molecules plus dissolved complexes) and the potentially available fraction (i.e., ions/molecules that can be released from stable organo-mineral soil complexes). When this definition is specifically applied to plants, it is commonly referred as *phytoavailability* (Meers et al., 2007).

The uptake of an element by plant can lead to its depletion at the rhizosphere zone, which usually induces a response from the surrounding soil. When the element uptake is slow, depletion at

the root surface is insignificant and the concentration can be expected to be proportional to the dissolved free ions in solution. In this case, the Free Ion Activity Model (FIAM) can be applied (Lofts et al., 2013). However, when the rate of removal of the element by the plant at the soil–root interface exceeds its diffusional supply, its concentration in the rhizosphere can become depleted. This can lead to resupply of elements from dissociation of complexes in the solution and/or release of elements from the soil particles in contact with the confined zone where depletion in solution occurs. In this case, the dynamic process of (re)supply of elements to a plant is likely to be determined by the concentration of total labile element in solution, its diffusional supply, the concentration of labile element available from solid phase and the rate at which it is released from solid phase to solution (Lehto et al., 2006).

The use of a single well-defined extraction procedure is one of the most common approaches used to assess the phytoavailable fraction of elements in soil. In the last years, single extraction methods have been widely applied to study particular solid-phase associations of metal(loid)s in soils (Feng et al., 2005; Gupta and Sinha, 2007; Hass and Fine, 2010; Meers et al., 2007; Menzies et al., 2007; Minca and Basta, 2013; Pueyo et al., 2004). Several extractants are used and can be classified according to the intrinsic mechanism involved in the release of elements from soil. For instance, salt solutions such as NH_4NO_3 and CaCl_2 can only extract

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elements from the water-soluble and exchangeable phases. However, the DTPA extractant is also able to attack organically-bound elements (Rao et al., 2008). The data generated in these studies are particularly useful for understanding the physicochemical processes that take place in the soil since they allow the elucidation of the mechanisms involved in metal(loid)s binding, transformation and/or release from soil. However, until now, no extraction procedure proved to be suitable to accurately predict the phytoavailability of metal(loid)s (Fedotov et al., 2012). One of the major challenges in field studies has been how to correlate the results from single extraction assays with metal(loid)s uptake and accumulation by plants.

As mentioned before, the actual and potential phytoavailable fractions of metal(loid)s can be considered as the fraction of the total amount of an element present in a specific environmental compartment that, within a defined time period, is either available or can be made available for uptake by plants (Rao et al., 2008). Therefore, the plant metal(loid)s content should closely reflect the metal(loid)s phytoavailability in the rhizosphere zone. Several factors are known to control the phytoavailability of metal(loid)s in the soil, and ultimately, their soil–plant–human transfer and accumulation (Pauget et al., 2012). The soil properties pH, cation exchange capacity (CEC), organic matter (OM) content, electrical conductivity (EC), particle size distribution and oxides content are generally regarded as the main parameters that control the distribution of metal(loid)s between the phytoavailable and non-phytoavailable fractions (Friedrich and Catalano, 2012; Hernandez-Soriano and Jimenez-Lopez, 2012; Luo et al., 2011; Pinto et al., 2014). Moreover, the phytoavailability of soil metal(loid)s is also the result of root–rhizosphere interactions. Reactions that take place at this interface strongly determine the metal(loid)s speciation and uptake by plants, ultimately affecting the accumulation and overall content of these elements in plants (Cheng, 2009; Rajkumar et al., 2012).

Modern agricultural practices largely rely on the application of high amounts of chemical fertilizers, which can be contaminated with several toxic metal(loid)s (Jiao et al., 2012). In this context, studies on the potential transfer of toxic metal(loid)s to the food chain are of major importance. Lettuce (*Lactuca sativa*) is one of the most consumed vegetables worldwide, representing about 6.5% of the total dietary intake of vegetables by humans (WHO, 2003). Besides, lettuce is recommended by the US Environmental Protection Agency (EPA) as a suitable test plant to determine uptake and translocation of toxic substances (USEPA, 2012).

Based on the above background, and in order to contribute for a better understanding of the soil–plant relationship regarding metal(loid)s, the aim of this study was to assess the influence of soil properties on the availability of metal(loid)s to lettuce during its normal growth period and the differences in phytoavailability results obtained by using different extraction methods. To accomplish this, a four-step approach was used: (1) the physicochemical properties of three different soils and the soil metal(loid)s extractable content (as assessed by four different extraction methods) were determined; (2) the metal(loid)s content in the plant shoot at five time points of lettuce growth was measured; (3) the correlation between the metal(loid)s extractable content in the soil and the plant shoot content was performed; and (4) multiple regression analysis was used to identify the most suitable extraction method in order to estimate the metal(loid)s phytoavailability to lettuce and to predict the metal(loid)s content in lettuce shoot.

Table 1

Main physicochemical properties of the bulk soils from the three experimental fields (A₁, A₂ and A₃).

	A ₁	A ₂	A ₃
pH	6.85 ± 0.14a	6.67 ± 0.13b	6.80 ± 0.22a
CEC (cmol/kg)	7.9 ± 0.4a	17.2 ± 0.6b	7.9 ± 0.5a
OM (%)	56.3 ± 3.9a	72.8 ± 2.3b	20.7 ± 1.6c
EC (µS/cm)	283 ± 40a	633 ± 69b	196 ± 27c
Salinity (ng/L)	0.13 ± 0.01a	0.30 ± 0.03b	0.09 ± 0.01a
Al oxides (mg/g)	1.11 ± 0.07a	1.58 ± 0.09b	1.12 ± 0.08a
Mn oxides (mg/g)	0.23 ± 0.01a	0.40 ± 0.02b	0.20 ± 0.01c
Fe oxides (mg/g)	2.20 ± 0.10a	4.77 ± 0.17b	2.16 ± 0.11a
Clay (%)	2.0 ± 0.2a	12.2 ± 0.5b	2.2 ± 0.7a
Silt (%)	2.5 ± 0.2a	12.3 ± 0.1b	2.0 ± 0.5a
Sand (%)	95.5 ± 2.9a	75.5 ± 1.8b	95.8 ± 4.7a
Soil classification	Sandy soil	Loamy sand soil	Sandy soil

Data presented as mean ± SD ($n=15$, i.e., triplicate analysis in each of the 5 sampling time points). Differences were tested according to ANOVA followed by Tukey's test. In a row, different letters (a, b and c) indicate significant differences ($p < 0.05$) between the experimental fields.

2. Material and methods

2.1. Plant cultivation, sampling and sample preparation

Lettuce (*Lactuca sativa* L.) plants ($n=100$) were cultivated in three greenhouse experimental fields: A₁ (41° 26.991 N, 8° 46.335 W), A₂ (41° 25.249 N, 8° 44.936 W) and A₃ (41° 27.435 N, 8° 45.377 W). These fields were chosen because intensive agriculture practices, relying on the use of high amount of chemical fertilizers, have been adopted during the last decade in the growing of high-yield crops. Plants were submitted to similar sunlight exposure (a total of 334 h; photoperiod on average 11.16 h) and temperature conditions (average min temperature: 7.6 °C; average max temperature 20.5 °C) from December to February. Inorganic fertilizers were applied through fertigation in the same way in each experimental field. The nutrient elements provided (per plant and per fertigation) were as follows: 0.127 g N, 0.041 g K, 0.029 g P, 0.212 mg Fe, 0.052 mg Mn and 0.014 mg Zn.

One kilogram (or at least 10 units) of plants were arbitrarily harvested at five time points (2, 4, 6, 8, and 10 weeks, hereafter referred as T₁, T₂, T₃, T₄ and T₅, respectively) of plant growth according to Directive (2002/63/EC). Simultaneously, bulk soil was also sampled from the same locations. Rhizosphere soil was obtained by drying the root system and collecting the soil that falls off when shaking the dried root as well as the soil collected by subsequent brushing of the roots. In order to generate a representative sample, individual samples ($n=10$ for rhizosphere soil and $n=20$ for bulk soil) from each time point were combined to generate a composite sample (Pennock et al., 2007).

For physicochemical analysis, lettuce shoots were brushed, thoroughly washed with deionized water, frozen at −80 °C and then freeze-dried. To obtain a composite sample, dried samples were combined, homogenized by grinding in a blender and sieved through a nylon sieve of 150 µm mesh size.

Pre-treatment of bulk soil samples was performed according to ISO (2006). Briefly, soil samples were spread in plastic trays, oven-dried during 24 h at 40 °C, crushed and sieved through a 2 mm nylon sieve and stored at 4 °C until analysis.

2.2. Reagents and apparatus

Decontaminated polypropylene laboratory ware, pipette tips (VWR, Radnor, PA), volumetric flasks (Kartell, Milan, Italy) and centrifuge tubes (TRP, Trasadingen, Switzerland) were used to perform the study. Calibration solutions were prepared from AccuTrace™ 10 µg/mL multi-element ICP-MS standard solutions

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