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Study of the size-based environmental availability of metals associated to natural organic matter by stable isotope exchange and quadrupole inductively coupled plasma mass spectrometry coupled to asymmetrical flow field flow fractionation

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ABSTRACT

The determination of the isotopically exchangeable fraction of metals in environmental solid samples (soils, composts, sediments, sludges, etc.) is used to know the amount of metal potentially available (*E*-value). Stable isotopes can be used for determination of *E*-values through the analysis of the aqueous phases from spiked suspensions. However, the presence of isotopically non-exchangeable metal forms in the aqueous phase led to overestimation of the *E*-values. In this paper, a method for monitoring the degree of isotopic exchange in function of the molecular mass and/or size of the metal form has been developed based on the direct coupling of asymmetrical flow field flow fractionation (AsFlFFF) with inductively coupled plasma mass spectrometry (ICP-MS) for on-line isotope ratio measurements. ICP-MS data acquisition parameters were stressed to avoid degradation of isotope ratio precision. Two sets of fractionation conditions were selected: a colloids separation, which allowed the separation of substances up to 1 μm, and a macromolecules separation, designed to resolve small size substances up to 50 kDa. The methodology was applied to study the environmental availability of copper and lead in compost samples, where metals are mainly associated to different forms of organic matter. No significant differences on isotopic exchange were observed over the size range studied, validating the *E*-values determined by direct analysis of the aqueous phases.

1. Introduction

Bioavailability is defined in ISO 11074 as the degree to which chemicals present in the soil may be absorbed or metabolised by human or ecological receptors or be available for interaction with biological systems [1]. Bioavailability is a dynamic process, which depends on a specific target organism and specific contaminants, that can be described by the following steps: (i) availability of the contaminant in the soil (environmental availability), (ii) uptake of the contaminant by the organism (environmental bioavailability or bioaccessibility), and (iii) accumulation and/or effect of the contaminant within the organism (toxicological bioavailability) [2]. Chemical methods are used to measure environmental availability, whereas biological and ecotoxicological methods are applied for measuring environmental and toxicological bioavailability.

The potentially available fraction of an element in a soil or a soil-like material (sediment, compost, sludge, etc.) consists of dissolved ionic and complexed forms of the element, as well as sorbed forms in the soil matrix suitable of being incorporated to the soil solution. Typical methods to measure the environmental available fraction of metals are based on extraction with neutral salts or chelating agents [3]. These methods extract the element that would be present in the soil solution, as well as an indeterminate fraction associated with the solid phase. Isotopic exchange methods have also been used to measure the availability of essential and toxic elements in soils [4]. These methods quantify the amount of a given element that is capable of freely exchanging, over a certain period of time, between the soil solid and solution phases and thus quantify the isotopically exchangeable fraction, also called labile, which is considered to reflect the potentially available pool of the element under equilibrium conditions [4].

Isotopic exchange methods are based on the isotope dilution principle, which establishes that when a small amount of an isotope is introduced into a soil suspension, its distribution will reflect the corresponding available fraction in the soil, which is the sum of the isotopically exchangeable element in the aqueous and solid phases. This distribution can be determined by sampling and analyzing the aqueous phase. The fundamental underlying assumptions of this methodology are [5]: (i) the solid and aqueous phases are in chemical equilibrium; (ii) all the added element remains isotopically exchangeable; and (iii) all forms of the element in the aqueous phase are exchanged with the corresponding spiked iso-

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tope. In the aqueous phase of a soil suspension, metals can be found as free ions, organic or inorganic complexes and/or associated to colloidal forms [5], whose degree of isotopic exchange can be different, unfulfilling the third assumption. Several authors have considered that the presence of colloids in the aqueous extracts can lead to significant overestimation of the exchangeable fraction, because elements associated with colloids might be isotopically non-exchangeable during the isotopic equilibration period [6,7]. To minimize the errors associated with these non-exchangeable forms, a number of procedures have been proposed based on the separation of the free ions of the element, which are by definition fully isotopically exchangeable, by chromatography [6,7] or Donnan dialysis [8].

Typically, radioactive isotopes of metals and metalloids have been used in isotopic exchange methods [4]. However, the availability and performance of ICP-MS instruments for determination of isotope ratios have led to the use of enriched stable metal isotopes for environmental isotope dilution studies [9].

Asymmetrical flow field flow fractionation (AsFlFFF) allows the analysis of complex environmental samples, from particles in the range of micrometers to macromolecules down to a few kDa [10]. In general, environmental studies on colloids and macromolecules using AsFlFFF have been limited to the study of those species present in the “dissolved fraction”, since a prefractionation step by filtering the samples through 0.45 or 0.2 μm pore size membranes is usually included [11–13]. Despite most standard environmental procedures include these filtration step because of its simplicity, removal of substances below the nominal cut-off size of the filters has been reported [10,14]. Focusing on colloids, which are defined as substances having at least one dimension in the 1 nm–1 μm range [15], the use of conventional filtration implies that this fraction will be partially analyzed. Centrifugation procedures have been proposed as an alternative for the study of colloids by FlFFF [10,14].

Soil organic amendments (compost, sludge, manure) are convenient forms of organic matter for agricultural soils or soil remediation, moreover their use contribute to the sustainable management of organic residues. However, their high content on natural organic matter can also contribute to the mobilization of metals from the amendment itself or the soils where they are applied. Metals in leachates from these amendments are not found mainly as free ions but in complexed forms, whose lability depends on the nature of the organic matter involved [16]. Under such conditions, environmental availability studies based on isotopic exchange methods imply that metals in the soluble phase are present as complexes of variable sizes, from macromolecules to colloids. Therefore, depending on the lability of such complexes, the degree of isotopic exchange can be different, invalidating the determination of the exchangeable fraction of the amendments.

The purpose of this work is to study the feasibility of AsFlFFF coupled to ICP-MS for investigating the degree of isotopic exchange of the different physicochemical forms of metals as function of their molecular mass/size, based on the direct measurement of isotope ratios. The methodology developed has been applied to the aqueous phases obtained for the determination of copper and lead exchangeable fractions in composts. Differences in the degree of isotopic exchange with size imply that non- or less-exchangeable forms of the metal are present in the soluble phase, biasing the determination of the exchangeable fraction in the solid samples by using isotopic exchange methods for estimation of the environmental availability of metals.

2. Theory

According to the principle of isotope dilution, when a suspension is spiked with an enriched isotope, it will equilibrate with the exchangeable pool (E (μg g^{-1})). This exchangeable pool is the sum of the isotopically exchangeable element in the solution (C^{sol}) and solid phases (C^{exc}), assumed that all the element in solution is fully isotopically exchangeable, being equal to:

$$E = C^{\text{sol}} + C^{\text{exc}} = \frac{C_y m_y}{m_x} \frac{M_x}{M_y} \frac{R_y - R_{xy}}{R_{xy} - R_x} \frac{a_y^b}{a_x^b} \qquad (1)$$

where C_y is the mass concentration of the element in the spike (μg g^{-1}), m_x and m_y the mass of sample and spike (g), M_x and M_y the atomic mass of the element in the sample and the spike (g mol^{-1}) and a_x^b and a_y^b the atomic abundance of the enriched isotope in the sample and the spike. R_x, R_y and R_{xy} represent the isotope ratio of the element in the unspiked sample, in the spiking solution and the spiked sample, respectively. Eq. (1) resembles the typical expressions used in isotope dilution analysis, although in isotopic exchange the concentration calculated is not the total concentration, because the spiked isotope has not been equilibrated with the total content of the element in the suspension, just with the exchangeable pool. Calculation of E involves the determination of the isotope ratio in the spiked sample R_{xy}, although the determination of the isotope ratio in the unspiked sample (R_x) is also recommended for elements which show a significant natural isotopic variability, like lead. A detailed deduction of Eq. (1) is included in supplementary information.

3. Experimental

3.1. Instrumentation

The asymmetrical flow field-flow fractionation system used was an AF2000 (Postnova Analytics, Landsberg, Germany). The channel dimensions were 27.5 cm in length and from 2 to 0.5 cm in width. The spacer used for all the measurements had 350 μm thickness. The accumulation wall consisted of a 1 kDa polyethersulfone (PES) (Postnova Analytics). Two different crossflow programs were used according to the different mass/size ranges studied (Table 1). Ultrapure water adjusted to pH 8 by addition of KOH 1 M was used as carrier in the *colloids* program (up to 1 μm), whereas a higher ionic strength (NH_4NO_3 0.02 M, pH 8) was selected with the *macromolecules* program (up to 50 kDa) in order to improve resolution [10]. Carriers were degassed prior to use by an on-line vacuum degasser. The out flow was 0.8 mL min^{-1} in both programs. A sample loop of 100 μL was used throughout.

Table 1
Crossflow programs for AsFlFFF separations.

	Carrier	Time (min)	Cross flow type	Cross flow (mL min^{-1})
Program 1 *colloids*	Ultrapure water pH 8	20	Constant	0.1
		5	Linear decay	0
		2	Constant	0
Program 2 *macromolecules*	NH_4NO_3 0.02 M pH 8	10	Constant	5.0
		5	Linear decay	0
		5	Constant	0

Outflow 0.8 mL min^{-1}.