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.

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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-

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:

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

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$_4$NO$_3$ 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$_4$NO$_3$ 0.02 M pH 8	10	Constant	5.0
		5	Linear decay	0
		5	Constant	0

Outflow 0.8 mL min^{-1}.

Table 2
ICP-MS instrumental and data acquisition parameters.

Instrumental parameters		
RF power	1200 W	
Argon gas flow rate		
Plasma	15 L min^{-1}	
Auxiliary	1.2 L min^{-1}	
Nebulizer	0.9 L min^{-1}	
Data acquisition parameters	AsFlFFF	Continuous
Measuring mode	Peak hopping	Peak hopping
Points per spectral peak	1	1
Dead time	60 ns	60 ns
Sweeps	100	1000
Dwell time	5 ms	10 ms
Integration time	500 ms	10,000 ms
Settle time	0.3 ms	0.3 ms
Isotope ratios	^{63}Cu/^{65}Cu	^{63}Cu/^{65}Cu
	^{206}Pb/^{208}Pb	^{206}Pb/^{208}Pb
Isotope ratio acquisition frequency	60 min^{-1}	3 min^{-1}

The AsFlFFF system was coupled to an UV-vis diode array detector (Shimadzu, Duisburg, Germany), for detection of size/mass calibration standards, followed by an ICP-mass spectrometer (ELAN DRC-e, PerkinElmer, Germany) for on-line isotope ratio measurements. An automatic six-way injection valve (sample loop of 100 μL) was placed between the diode array detector and the ICP-MS for injection of the isotopic standards. The sample introduction system of the ICP-MS consisted of a glass concentric Slurry nebulizer and a cyclonic spray chamber (Glass Expansion, Melbourne, Australia). Instrumental and data acquisitions parameters are summarized in Table 2.

3.2. Standards and reagents

Stock lead isotopic standard reference solution (1000 mg L^{-1}) was prepared by dissolving 1 g of NIST SRM-981 (National Institute of Standards and Technology, Gaithersburg, MD, USA) in nitric acid (J.T. Baker, Holland). Copper isotopic standard reference material ERMAE633 (Institute for Reference Materials and Measurements, Geel, Belgium) was provided in nitric acid solution (551 mg L^{-1}). Standard solutions were prepared from the stock solutions in 1% (v/v) nitric acid.

The enriched ^{206}Pb (98.41%) and ^{65}Cu (99.9%) stock solutions (10 mg L^{-1}) were obtained from SpexCertiPrep (Metuchen, NJ, USA).

3.3. Samples

The composts used in this work were provided by a Spanish composting plant, they were produced from municipal solid wastes (MSW). Compost A was obtained through the anaerobic digestion of the biodegradable MSW fraction followed by the aerobic post-treatment by composting of the solid digestate, whereas compost B was obtained directly by composting. A summary of their physicochemical properties is given in Table 3.

3.4. Procedures

3.4.1. Determination of the isotopic exchangeable fraction

A fixed amount of compost (40 g), together with 400 mL of ultrapure water were placed into a precleaned polyethylene bottle and agitated in a rotary tumbler at a speed of 28 ± 2 rpm at room temperature for 24 h. The suspension was then spiked with a small volume of solution containing enriched ^{65}Cu or ^{206}Pb, equivalent to approximately 1% of the total metal content in the composts [9], and returned to the rotary tumbler for another 24 h. At the end of this equilibration period, the compost suspension was settled and the supernatant centrifuged at 4000 rpm for 30 min. The centrifugation conditions were fixed in order to remove those particles larger than 1 μm. Centrifuged samples were kept at 4 °C until analysis.

3.4.2. Determination of E-values

Aliquots of the centrifuged samples were digested with nitric acid in a microwave oven (Mars, CEM, Matthews NC, USA), to avoid potential interferent effects of organic matter. 10 mL of each solution were mixed with 5 mL of concentrated HNO$_3$ into XP-1500 Plus vessels. The digested samples were diluted with 1% (v/v) HNO$_3$ to give a concentration of 50–100 μg L^{-1}, if possible, so that the detector remained in pulse count mode. Samples were introduced in continuous mode for isotope ratio measurements.

The natural isotope ratio of copper and lead in the original samples was determined by following the procedures described above, but without spiking with enriched isotopes. E-values were obtained from Eq. (1). Determinations were performed in duplicate.

3.4.3. Isotope ratio measurements

Data acquisitions parameters, both for continuous and AsFlFFF sample introduction, are summarized in Table 2. Prior to ratioing, the measured signals were automatically corrected for signal losses due to the detector dead time. The detector dead time was determined according to the manufacturer procedure and loaded into the instrument software. The signals were further corrected for the procedural blank for continuous sample introduction or the baseline signal for AsFlFFF sample introduction.

Mass discrimination correction was performed by external correction using the lead and copper isotopic reference materials NIST SRM-981 and ERMAE633, respectively. A bracketing procedure, whereby the measurement of each sample is preceded and followed by the measurement of the isotopic standard, was used. Averaged mass-discrimination factors were calculated by ratioing the certified and experimental isotope ratios from both measurements. Isotope ratio fractograms were obtained by point-to-point ratioing followed by the mass discrimination correction procedure cited above. Standards were injected through the supplementary injection valve located at the exit of the AsFlFFF system.

3.4.4. AsFlFFF size/mass calibration

Microparticles monodisperse size standards based on polystyrene (0.1 ± 0.03, 0.2 ± 0.03, 0.5 ± 0.05 and 1 ± 0.05 μm) were used for AsFlFFF calibration using the *colloids* program (crossflow program 1). The equation of the calibration curve obtained with these standards was:

$$\log R = -0.702 - 0.332 \log d, \quad r = 0.998$$

Table 3
Selected chemical properties of the composts studied.

Compost	Origin	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Total C (% (w/w))	DOC[a] (mg L^{-1})	pH[b]
A	Anaerobic digestion + composting	342	111	27	770	8.4
B	Composting	440	160	29	1870	8.7

[a] Aqueous phase concentration from suspensions 1:10 (solid:water).
[b] Norm UNE-EN 13037:2001.

where d is the particle diameter (μm) and $R = (t_0/t_r)$, being t_0 the elution time corresponding to the void volume and t_r the retention time for a given particle.

In order to correct the lack of linearity in the relationship established between R and retention parameter λ for those species eluted at high λ ($\lambda > 0,1$), which corresponds to poorly retained species, the following equation has been used instead [17]:

$$R = 6\lambda \ \coth\left(\frac{1}{2\lambda}\right) - 12\lambda^2 \qquad (2)$$

The relationship between λ and d (μm) has been obtained experimentally based on the retention value of the 0.1 μm size standard.

Polystyrene sulfonates (PSS) are appropriate standards for calibrating with respect to the molecular mass in the analysis of humic substances by FlFFF [18]; PSS standards of 1.4, 4.3, 6.8, 17, 32, 77, 158 and 976 kDa were used for molecular mass calibration using the *macromolecules* program (crossflow program 2). The equation of the calibration curve obtained was:

$$\log(t_r - t_0) = 0.0654 \log M, \quad r = 0.999$$

All these standards were purchased from Sigma–Aldrich (Sigma–Aldrich Chemie, Buchs, Switzerland). Diluted solutions (20 mg L^{-1}) of these standards were prepared by further dilution with the corresponding mobile phase. Elution was monitored by UV absorption at 254 nm.

4. Results and discussion

4.1. Data acquisition parameters for isotope ratio measurement from transient signals

The measurement of isotope ratios along a fractogram implies the selection of optimized ICP-MS acquisition conditions for obtaining a correct description of the fractogram profile, as well as isotope ratio measurements of sufficient precision. In quadrupole ICP-MS, data acquisition is controlled by the number of points per spectral peak, the number of sweeps (the number of scans along the mass spectrum to get a single reading) and the dwell time (the time spent counting ions per isotope). In order to get a reliable profile of a transient signal, it is common practice to work in peak hopping mode using a single point per mass, the integration time (number of sweep $\times$ dwell time) per reading along the transient signals is selected depending on the number of isotopes monitorized and the width of the signal profile [19]. The precision of isotope ratio measurements is limited by counting statistics, being proportional to the square root of the total counts measured. With quadrupole instruments, precisions below 0.1% can be achieved measuring at high integration times, with high number of sweeps and short dwell times [20].

Taking into account all the aspects cited above, a compromise integration time of 500 ms per isotope and reading was selected, performing 100 sweeps at dwell times of 5 ms. By using a short quadrupole settle time of 0.3 ms and measuring just two isotopes, the attainable acquisition frequency was one isotope ratio per second. Fig. 1 shows the profile of a transient signal obtained by flow injection of 100 μL of a 100 μg L^{-1} Cu standard. The peak shows more than 20 points over the 10% of maximum height, and the precision calculated for the point-to-point isotope ratio on the same peak width window was 0.5%. A precision of 0.1% was achieved by continuous sample introduction under the same acquisition conditions.

Using the selected acquisition parameters, the attainable precision along a fractogram is constrained by the concentration of the element at the exit of the channel. Fig. 2 shows two typical frac-
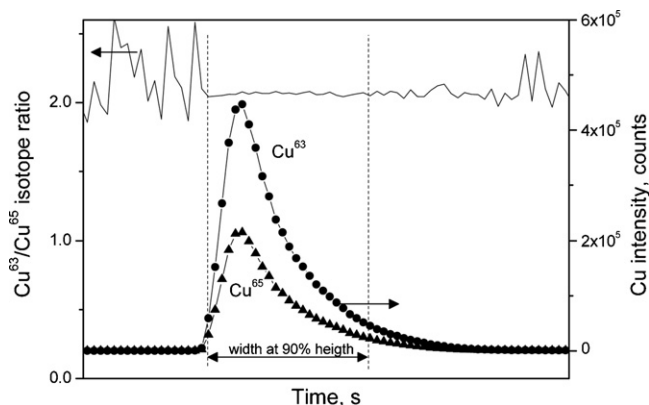

Fig. 1. Typical profile of a transient signal acquired under selected conditions (Table 2) for measuring point-to-point isotope ratios. Injection volume: 100 μL. Cu concentration: 100 μg L^{-1}.

tograms of the soluble phase of compost suspensions for copper and lead. The most abundant isotope is plotted with the point-to-point theoretical relative standard deviation of the measured isotope ratios (RSD$_t$), calculated as:

$$\text{RSD}_t = 100 \times \sqrt{\left(\frac{1}{N_a}\right)^2 + \left(\frac{1}{N_b}\right)^2} \qquad (3)$$

where N_a and N_b are the number of counts measured for the two isotopes involved. Relative standard deviation between 0.5% and 5% were calculated for copper, whereas it ranged from 1.5% up to 6.5% for lead. Differences are related to the higher copper concentrations found in the aqueous phase, about ten times higher than those of lead.
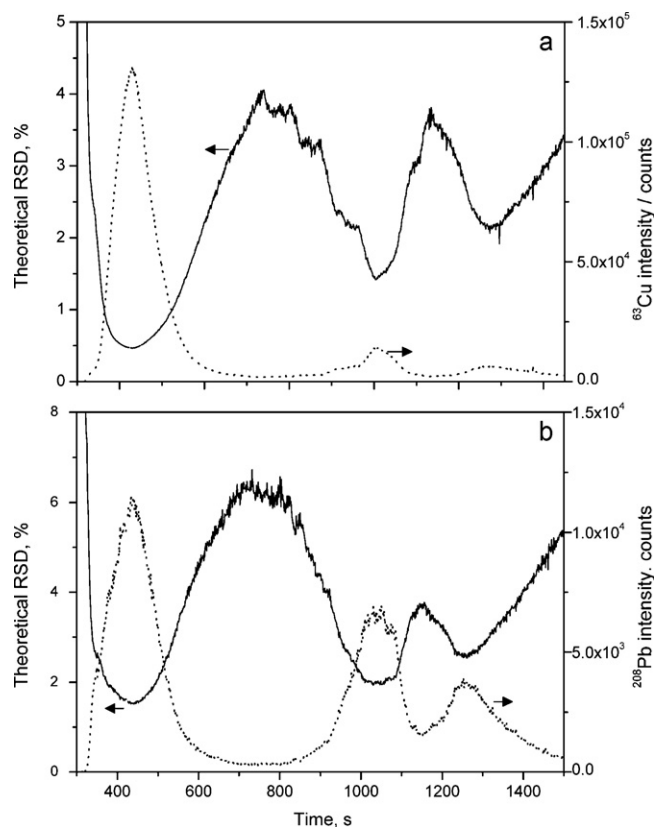

Fig. 2. Theoretical relative standard deviation for the point-to-point isotope ratios measured along fractograms of the soluble phase of compost suspensions for copper (a) and lead (b), using the *macromolecules* program.

Table 4
^{63}Cu/^{65}Cu isotope ratios measured in the aqueous phases of compost suspensions after isotopic exchange as function of size and molecular mass by on-line AsFlFFF separation and averaged by continuous introduction without separation. Natural ^{63}Cu/^{65}Cu isotope ratio measured in the aqueous phases of unspiked compost suspensions by continuous introduction.

Compost	Fraction		Cu (%)	AsFlFFF ^{63}Cu/^{65}Cu	Continuous ^{63}Cu/^{65}Cu	Natural ^{63}Cu/^{65}Cu
A	Macromolecules	<5 kDa	55	2.04 ± 0.07	1.998 ± 0.006	2.261 ± 0.001
		>50 kDa	45	2.06 ± 0.05		
	Colloids	<40 nm	66	2.01 ± 0.02		
		40–60 nm	14	2.02 ± 0.08		
		60–115 nm	20	2.01 ± 0.07		
B	Macromolecules	<10 kDa	82	1.99 ± 0.06	2.008 ± 0.005	2.255 ± 0.005
		>50 kDa	18	2.02 ± 0.07		
	Colloids	<60 nm	95	1.98 ± 0.01		
		60–115 nm	5	2.01 ± 0.07		

4.2. AsFlFFF conditions for the characterization of colloids and macromolecules

The aqueous phases obtained by centrifugation, following the procedure described in Section 3.4.1, were injected into the AsFlFFF system under two sets of separation conditions (Table 1). The procedure applied to the suspensions removed particles larger than 1 μm, which corresponds to the steric inversion point found for the membrane used [10], and guaranteed that the whole colloidal pool was injected into the system.

A low crossflow rate of 0.1 mL min^{-1} was selected for separation of the entire size range up to 1 μm (program *colloids* of Table 1). To improve resolution and separate substances up to 50 kDa, a crossflow of 5 mL min^{-1}, the maximum allowable by the channel configuration and pumps pressure, was applied (program *macromolecules*). As can be seen in Figs. 3 and 4, fractograms obtained with the program *colloids* show a first peak, corresponding to sizes lower than 60 nm, and a smaller second one in the range of 60–115 nm, with a maximum around 80 nm. Colloids of sizes bigger than 115 nm were not observed. As cross-reference, the retention time corresponding to 30 nm is equivalent to that of 1000 kDa for polystyrene sulfonate [10]. By using the program *macromolecules*, the resolution of this first peak was improved, showing that the main contribution was due to substances of molecular masses below 10 kDa. Up to two additional peaks at higher elution times were also observed; these peaks appeared at elution times corresponding to the linear decay and the no-crossflow steps, respectively. Under these conditions, substances with molecular mass higher than 50 kDa, which remained into the channel at the end of the separation step, are eluted together. Despite no specific information about their mass or size is available, these substances are not diluted as much as with the *colloids* program, allowing the measurement of isotope ratios under better concentration conditions. Channel recoveries of 40% and 90% were obtained for copper and lead, respectively. The recoveries obtained are justified by the high content of complexes with low molecular

mass organic substances present in the compost leachate (75% of the dissolved organic carbon content), which are lost through the permeation membrane [10].

Tables 4 and 5 show the distribution of copper and lead with respect to the size and molecular mass fractions considered. The percentage distribution was calculated from the integration of the fractograms. The percentage of copper and lead associated to colloids bigger than 60 nm varied between 5% and 20%, depending on the element and the type of compost. These fractions were identified as partially resolved peaks and are shown in the enlarged plots of Figs. 3 and 4. The remaining element was associated to small size species, which also included low molecular mass macromolecules down to 1 kDa, the cut-off diameter of the permeation membrane. Only for copper in compost A, the small size fraction showed a narrower distribution up to 40 nm and a shoulder in the 40–60 nm range. The fractions below 60 nm consist mainly of high molecular mass organic polydispersed substances of humic nature. This macromolecular fraction accounted for about 50% of the copper and lead in both compost studied, except for copper in compost B, which represented more than 80%.

4.3. Size-based isotopic exchange of copper and lead in aqueous phases of compost suspensions

Figs. 3 and 4 also show the point-to-point isotope ratios measured along the fractograms for copper and lead, respectively. Fluctuations of isotope ratios are inversely related to the intensity of the isotopes measured. Therefore, tailing regions are not suitable for precise isotope ratio measurements. On account of these limitations, better precision was achieved for the first peak of the colloids fractograms than for the higher size region, which showed a progressive tailing. To get more precise isotope ratio measurements on this region, the *macromolecules* program provided a way of concentrating substances of more than 50 kDa by eluting them as one or two peaks when the cross-flow is decaying or stopped. In any

Table 5
^{208}Pb/^{206}Pb isotope ratios measured in the aqueous phases of compost suspensions after isotopic exchange as function of size and molecular mass by on line AsFlFFF separation and averaged by continuous introduction without separation. Natural ^{208}Pb/^{206}Pb isotope ratio measured in the aqueous phases of unspiked compost suspensions by continuous introduction.

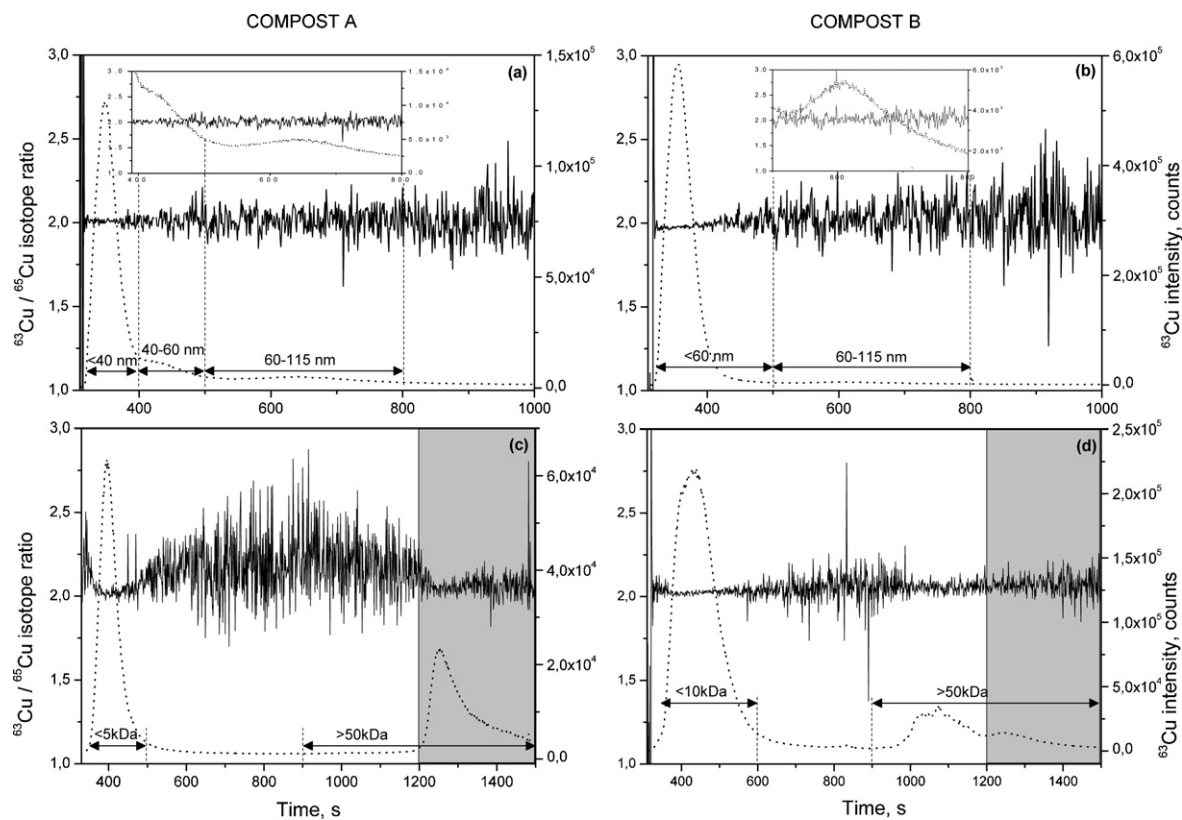| Compost	Fraction		Pb (%)	AsFlFFF ^{208}Pb/^{206}Pb	Continuous ^{208}Pb/^{206}Pb	Natural ^{208}Pb/^{206}Pb
A	Macromolecules	<5 kDa	46	1.58 ± 0.09	1.567 ± 0.006	2.138 ± 0.002
		>50 kDa	54	1.60 ± 0.05		
	Colloids	<60 nm	90	1.58 ± 0.04		
		60–115 nm	10	1.61 ± 0.13		
B	Macromolecules	<10 kDa	47	1.77 ± 0.08	1.784 ± 0.027	2.145 ± 0.004
		>50 kDa	53	1.74 ± 0.08		
	Colloids	<60 nm	85	1.75 ± 0.03		
		60–115 nm	15	1.76 ± 0.10		

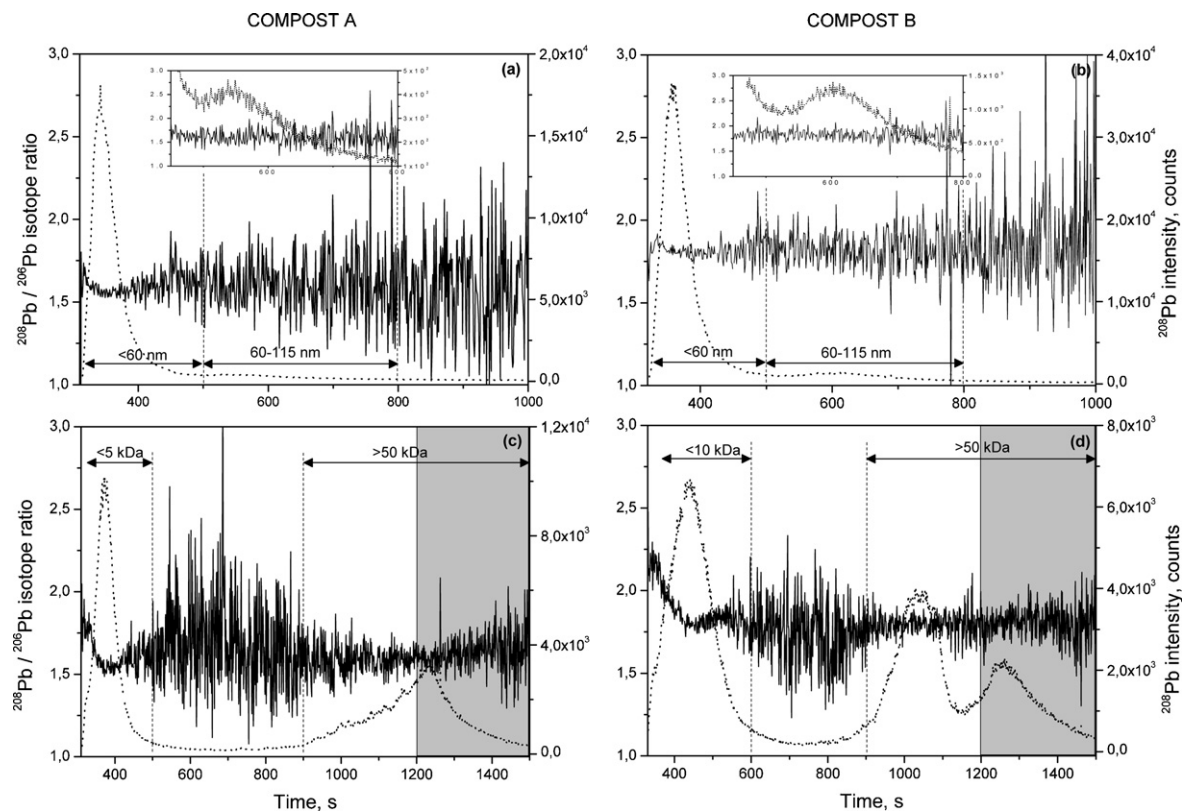**Fig. 3.** Copper fractograms of the soluble phase of compost suspensions A and B, using the *colloids* (a and b) and the *macromolecules* program (c and d). Solid line: $^{63}Cu/^{65}Cu$ point-to-point isotope ratio, dotted line: ^{63}Cu signal. Grey area: elution without cross flow applied.

Fig. 4. Lead fractograms of the soluble phase of compost suspensions A and B, using the *colloids* (a and b) and the *macromolecules* program (c and d). Solid line: $^{208}Pb/^{206}Pb$ point-to-point isotope ratio, dotted line: ^{208}Pb signal. Grey area: elution without cross flow applied.

Table 6
Exchangeable copper and lead in composts. E-values and percentage with respect to the total metal content (%E). Mean values ± standard deviation ($n = 2$).

Compost	Cu		Pb	
	E (mg kg^{-1})	E (%)	E (mg kg^{-1})	E (%)
A	103.9 ± 0.4	30.5 ± 0.1	12.1 ± 0.02	10.9 ± 0.02
B	96.8 ± 1.6	22.0 ± 0.4	26.7 ± 0.4	16.7 ± 0.2

case, averaged isotope ratio were obtained for each eluted peak by taking the point-to-point isotope ratios in the interval corresponding to 50% of the maximum height of the peak, to avoid regions of high fluctuation due to the low concentration of the isotopes.

Tables 4 and 5 summarize the averaged isotope ratio and the related precision measured on each size or molecular mass region by using the *colloids* and the *macromolecules* programs, respectively. The results from Tables 4 and 5 make clear that differences between the isotope ratios determined on the different fractions were not statistically significant both for copper and lead, and the two compost types studied. Moreover, the isotope ratios measured for each fraction were in agreement with the isotope ratio measured on the digested aqueous sample without any fractionation. Measured natural isotope ratios for copper and lead in the unspiked aqueous phases compost A and B are also included in Tables 4 and 5. If point-to-point isotope ratios along the fractograms had shown ratios closer to the natural ones, then the fractions involved would be isotopically non-exchangeable, leading to an overestimation of the E-values.

4.4. Determination of the isotopically exchangeable copper and lead in composts (E-values)

The isotope ratio results shown in Tables 4 and 5 confirm that the spiked enriched isotopes were exchanged in the same extend over the whole range of substances found in the aqueous phase of the composts suspensions during the duration of the exchange assay. The behaviour of copper and lead in the composts studied allows considering that the conventional procedure to determine the isotopically exchangeable metal content, through the E-value, will not produce biased results due to the presence of non exchangeable colloids.

In comparison to soils, the higher content of organic matter in composts and the aqueous phases of their suspensions implies that metals are mainly found in the aqueous phase associated to different organic substances, forming complexes with low molecular mass organic acids, as well as with fulvic and humic acids [21]. With respect to the colloids, whatever their nature, they should be covered by humic substances, showing a behaviour similar to those of lower mass. The lack of non-labile forms of copper or lead in the aqueous phases of the composts studied may be justified by the rapidness of the forced humification process in composting, 8 weeks, in comparison with the slowness of the natural process in a soil.

Table 6 summarizes the E and %E values measured for copper and lead in the composts under study. It was found that copper was present in forms more environmentally available than lead in the composts. The percentage of available copper ranged up to 30% for the compost from anaerobic pretreatment, in comparison to 22% from the compost obtained directly. This difference can be related to the higher content of humic acids of the former one [21]. For lead, the results obtained, between 11% and 16%, point out that this element is mainly associated to inert forms, non isotopically exchangeable, in the solid.

5. Conclusions

A methodology for studying the isotopic exchangeability of metals with respect to the molecular mass and/or size of their different physicochemical forms has been developed. The use of AsFlFFF on-line coupled to ICP-MS allows measuring isotope ratios in function of mass/size directly, relating their possible variations to the presence of non exchangeable forms of the metal of defined mass or size. The method has been applied to the study of the aqueous phases of compost suspensions previously spiked with enriched stable isotopes for determination of the E-values. Significant isotope ratio variations were not observed in the range from 1 kDa up to 1 μm in the aqueous phases of the materials studied. These results confirm that isotopically non-exchangeable forms of copper or lead were not present in the aqueous phases and hence exchangeability values determined by direct analysis of the aqueous phases provide reliable E-values.

It should be noted that the methodology developed is suitable of being used with other solid environmental samples (soils, sludges, manure, sediments, etc.), as well as other types of tracer experiments with stable isotopes, providing a direct evidence of the variations of isotope ratios with respect to molecular mass or size.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.01.076.

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