

Sequential Chemical Extraction: A Useful Scheme for Evaluating Heavy Metal Mobilization

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ABSTRACT

The metal partitioning of sludge by the SCE was investigated. It was observed that about 25% of Cu and 30% of Pb were bound to organic matter fraction, while 31% Mn and 23% Cd were also bound to Fe–Mn oxides and the residual fractions respectively. Cu was mainly found in the organic matter fraction, though, 22% and 19% of Ni predominated in the fraction bound to carbonates and the exchangeable phase respectively. With regards to the fractions bound to Fe–Mn oxides, the extraction percentage for Mn (31%) was the overall highest in the present SCE scheme, while Mn (12%), and Pb (12%) in the exchangeable phase, together with Mn (12%) in the residual phase respectively were the overall lowest metals extracted. Pb (30%) bound to organic matter fraction appears to have mobilized significantly more than most metals studied.

Keywords: carbonate, exchangeable, leachate, phase, oxidizable, reducible, residual, soil

INTRODUCTION

Sequential chemical extraction (SCE) is applied as an important process to assess the level of heavy metal (HM) mobilization in sludge matrix. SCE application to sludge, soil or sediment materials is a useful procedure to provide information about the availability of metals. The procedure comprises the use of a series of chemical extractants in a sequence of reagents of increasing harshness. A particular chemical form of the HM is extracted for each extraction sequence. The SCE process is very flexible, though manipulatively complex. It fractionates metals in sludge into about five extraction steps. These steps include (Tessier *et al.* 1979; Perez-Cid *et al.* 1999);

- (i) Exchangeable phase: extraction by an electrolyte solution at a pH of 7.0.
- (ii) Carbonate phase: extraction using sodium acetate and hydrogen acetate at a pH of 5.0.
- (iii) Reducible phase: extraction is usually done using reducing agents at pH 2.0 to 4.0. The extractive process incorporates metals absorbed by iron and manganese oxides.
- (iv) Oxidizable phase: extraction process makes use of hot hydrogen peroxide in a nitric acid medium at pH 2.0, incorporating metals bound to organic matter and sulphides.
- (v) Residual phase: this consists of metals held within the crystal lattices of minerals. Digestion with a mixture of strong acids is used for the extraction.

Tessier *et al.* (1979) developed an SCE scheme that was employed by Perez-Cid *et al.* (1999) and Zufiurre *et al.* (1998) in extraction of HM from soils and sediments. Perez-Cid *et al.* (1999) reported that similar extractable metal contents exist between the five-stage Tessier scheme and the four-stage SCE scheme for metals partitioning in sewage sludge proposed by the European Community Bureau of Reference. Veeken (1999) had demonstrated that the Tessier scheme is the most effective scheme for determining the chemical distribution of HM in biowaste. Biowaste compose largely of organic matter, and Veeken (1999) included an additional step to the Tessier scheme to distinguish between metals adsorbed to organic matter and metals incorporated in organic matter.

The SCE scheme, though, relatively experimentally simple has associated problems with the application of the technique in wastewater matrices and soils. These drawbacks include (Lombardi and Garcia 1999; Marchioretto *et al.* 2002):

- (a) The diversity of reagents used to extract specific metal forms, as well as the variety of extraction procedures employed.
- (b) Lack of specificity; extraction reagents cannot destroy one phase without solubilizing the other phases.
- (c) Absence of selectivity; extraction reagents cannot release metals bound to one specific phase and not to other phases.
- (d) Readsorption; HM that are solubilized by an extracting agent can be readsorbed to the remaining solid phases.
- (e) Dependence on many factors, such as type of sample, size of particulates, pH, temperature, contact time, concentration of extractant, and solid-to-liquid ratio.

Besides these set-backs, SCE techniques provide qualitative information that may allow for the prediction of the biological and physical chemical availability, mobilization and transportation of HM.

The extractive removal of HM from sewage sludge is one available approach to reduce HM concentration in sludge. HM can be present in sewage sludge in a number of forms. They can be dissolved in the water phase either as ions, as organic and inorganic complexes, precipitated as salts or hydroxides, co-precipitated with metal oxides, adsorbed to suspended solids, or associated with biological residues. The distribution of the metals over these forms is a function of the chemical properties of the specific metals and on the sludge characteristics. Determining the most effective speciation technique for metals distribution in sludge is a very tasking job. SCE procedures may be used as a useful tool to evaluate metal distribution and mobility in sludge. A recent study in determining the variations of HM distribution (Cu, Mn, Pb and Zn) during the sewage sludge composting process using the SCE procedures showed that the total content of Cu and Zn in the composting mixture increased while Mn and Zn were mainly found in the mobile fraction (exchangeable fraction, carbonate

fraction, and Fe/Mn oxide fraction). Cu and Pb were strongly associated with the stable fractions (organic matter/sulphides fraction and residual fractions). Calculation on the metal mobility (bioavailability) based on the five metals in the sludge and composted mixture revealed that the mobility of Mn, Pb, and Zn increased during the composted process. The metal mobility in the composted mixture ranked in the following order; Mn > Zn > Pb > Cu (Nomedá *et al.* 2008). Using single extractions with distilled water and diethylene triamine pentacetic acid (DTPA), a sequential extraction procedure was conducted to determine the chemical speciation of Cd, Cr, Ni, Pb, and Zn in four municipal sewage sludge. Analytical results indicated that a high percentage of organic carbon was found in all the sludge, and thus the sludge was found beneficial to application on agricultural land. However, Cu and Zn were the most abundant elements in the four sewage sludges. The HM contents with DTPA extraction were much higher than those with water extraction, while the oxide fraction of Zn was the dominant solid phase. Zn, the study reported, was also the most mobile metal in all the municipal sludge. Pb was concentrated in residual fractions, while Cu was the dominant metal in the organic fraction (Kao *et al.* 2007).

The SCE procedure also allows for the determination of the chemical differences between samples and, thus, indicates the differences in availability. From the SCE scheme, it can be deduced whether a large fraction of the sludge materials dissolves in water or acid or whether oxidizing or reducing conditions are necessary. Hence, in predicting metals mobilization in the sludge matrix, the SCE technique is a very useful tool. The presence of HM is one of the main obstacles for agricultural use of dewatered sewage sludge. Electrokinetic treatment and selective sequential extraction have been applied to remove and to determine the fractionation of HM in sewage sludge. Results demonstrated that mobility of HM in sludge significantly increased after its acidification at pH 2.7 and followed the order; Ni, Zn, Cu, As, Cr, and Pb (Wang *et al.* 2005). It is widely accepted that the determination of total elements does not give an accurate estimation of the potential environmental impact. This is so because more and more scientific evidence has revealed that both bioavailability and toxicity are critically dependent on the chemical form of HM (Alvarez *et al.* 2002). When the SCE scheme was applied to study the chemical forms of Ni, Cu, total Cr, Pb, and Zn in sewage sludge, results obtained showed that the metal distribution in the sludge residue showed that the exchangeable fraction represents the dominant part for both Ni and Zn. This fraction, the study revealed, is very unstable and sensitive to environmental conditions. Nevertheless, Pb in the study was found in the residual fraction in a relatively high proportion. Cu and total Cr was mainly associated with organic matter, while the greatest extraction percentage was obtained in the oxidizable fraction. The study further noted that the undesirable solubilization and release of trace metals in the environment could be avoided if the necessary conditions are applied (Stylianou *et al.* 2007). The major limitation of soil application of sewage sludge compost is the total HM contents and their bioavailability to the soil plant system. A research conducted to determine the HM speciation and the influence of changing the physico-chemical forms of Cu, Zn, Pb, and Ni in sewage sludge noted that using a sequential extraction procedure in sludge compost at different steps of treatment, a large proportion of the HM were associated to the residual and more resistant fractions to extraction. Zn, and mainly Cu were present more in the organic and carbonate fractions. Pb was preferentially bound to sulphide forms, while Ni was significantly bound to organic form. The HM distribution and bioavailability showed varying changes during composting depending on the metal itself and the physico-chemical properties of the medium (Amir *et al.* 2005). The luxury of different extraction procedures available in the SCE scheme presents a wide spectrum of results when the SCE scheme is applied to determine metal speciation in sludge. This fact makes SCE a

very difficult technique. As such, it appears that subjecting the sludge to both SCE procedures and single extractions with progressive acidification give better results. The rational combination of these methods gives consistent conclusions about the trace metals profile in the sludge. SCE technique have also frequently been used to help characterize chemical forms of trace elements in soils, which helps determine trace elements environmental availability, toxicity, and hazard to living organism. During fractionation of soils with above background concentrations of trace elements (Cd, Cr, Cu, Pb, Ni, and Zn) from sewage sludge applications, results of analyses revealed that adding amendments to soil help confirm changes in soil-Pb chemistry and these changes were also reflected in changes in Pb concentrations measured in a regulatory relevant testing procedure and Pb technologies as well as decisions on risk assessment (Berti *et al.* 1997). Drying of sludge effectively reduce extractability of fractions that were readily available in the liquid form in SCE. But it is expected that metals such as Cu might be more favorably extracted in dried sludge since the drying process oxidizes organic matter and increase mineralization. Base on this, the size of the minerals particles are reduced and crushing the minerals homogenize it which favors Cu extraction, since Cu predominates in the organic fraction of the sludge.

The aim of the present study is to assess the distribution of HM in soil leachate using an SCE scheme.

MATERIALS AND METHODS

The soil sample used in this experiment was obtained from the dump-site of the Federal Medical Center, Owerri, Nigeria. About 2 kg of soil sample was leached with deionised water into a 50 L plastic container previously washed and rinsed with deionised water. The plastic container was kept in the chemistry laboratory of the Federal University of Technology, Owerri, Nigeria and allowed to stand for 24 hrs to allow for solid particles to settle. From this stock solution samples were continuously drawn. A quantity of sample containing 2 L of leachate was measured into a 5 L beaker previously washed and rinsed with deionised water. The leachate was centrifuged (Micro Centrifuge Model 5415C) for 2 hrs at 300 rpm at 28°C. The resultant solution was filtered through Whatman No. 42 filter papers. This filtrate (stock) was used in the following SCE steps:

Step 1 Exchangeable phase

Extraction was done by mixing 20 ml of filtrate (stock) with 10 ml of a 1 M MgCl₂ solution in a 100 ml beaker. The pH of the mixture was maintained at 7.8 with the addition of some amount of NaOH solution. The mixture in the beaker was heated at 80°C for 1 hr with intermittent addition of deionised water to prevent drying up of the mixture. The resultant solution was cooled and filtered through Whatman No. 42 filter papers and 10 ml of the solution was made up to 100 ml with deionised water and stirred thoroughly. About 10 ml of this solution was measured into a 10 ml sample holder bottle and the solution analysed for the metals; Cd, Cu, Mn, Ni, and Pb using the Atomic Absorption Spectrophotometer (Model SOLAAR V10). Three replicates analysis were made and the mean concentration of HM was obtained by method described by Ukiwe and Oguzie (2008).

Step 2 Carbonate phase

Extraction was performed by mixing 20 ml of the filtrate (stock) with 10 ml of a 1 M sodium acetate solution and the pH maintained at 5.0 with the addition of NaOH solution. The mixture was stirred and heated for 30 min at 80°C with intermittent addition of deionised water to prevent drying up of the mixture. The resultant solution was cooled and filtered through Whatman No. 42 filter papers and the procedure for HM analyses was performed as in Step 1 beginning with the words "10 ml of the solution was made up ...".

Table 1 Heavy metal partitioning in soil leachate.

(Phase) Metal fraction	Cd		Cu		Mn		Ni		Pb	
	Mean (mg/l) ± SD × 10 ⁻¹	SEM x 10 ⁻²	Mean (mg/l) ± SD × 10 ⁻¹	SEM x 10 ⁻²	Mean (mg/l) ± SD × 10 ⁻¹	SEM x 10 ⁻²	Mean (mg/l) ± SD × 10 ⁻¹	SEM x 10 ⁻²	Mean (mg/l) ± SD × 10 ⁻¹	SEM x 10 ⁻²
Exchangeable phase ^(a)	0.0316 ± 5.3	0.3	0.5088 ± 1.2	6.9	0.8283 ± 2.0	0.1	0.1075 ± 1.0	5.7	0.0793 ± 2.4	0.1
Carbonate phase ^(b) (Bound to carbonates)	0.0380 ± 2.9	0.1	0.5967 ± 0.8	4.6	1.1108 ± 0.4	2.3	0.1227 ± 1.0	5.7	0.1167 ± 1.0	5.7
Reducible phase ^(c) (Bound to Fe-Mn oxides)	0.0345 ± 1.7	9.8	0.5558 ± 0.5	2.8	2.0924 ± 0.1	0.5	0.1149 ± 1.0	5.7	0.1595 ± 1.0	5.7
Oxidizable phase ^(d) (Bound to organic matter)	0.0315 ± 1.2	6.9	0.7727 ± 1.5	8.6	1.7118 ± 0.8	4.6	0.1097 ± 1.0	5.7	0.1970 ± 2.7	0.1
Residual phase ^(e)	0.0413 ± 1.5	8.6	0.6319 ± 1.6	9.2	0.8225 ± 0.0	0.0	0.0970 ± 1.7	9.8	0.0939 ± 1.0	5.7

SEM: Standard Error of the Mean; F-test between metal fractions (a) and (b) = 1.7; (b) and (c) = 3.5; (c) and (d) = 1.5; (d) and (e) = 3.7

Step 3 Reducible phase

Extraction was done by mixing 20 ml of the filtrate (stock) with 10 ml of a 20% (v/v) solution of 1 M NH₄OH and 0.05 M HCL and the pH maintained at 2.0 with the addition of some amount of HCL solution. The mixture was heated for 2 hrs at 80°C with intermittent addition of deionised water to prevent drying up of the mixture. The resultant solution was cooled and filtered through Whatman No. 42 filter papers and the procedure for HM analyses was performed as in Step 1 beginning with the words “10 of the solution was made up ...”.

Step 4 Oxidizable phase

Extraction was carried out by mixing 20 ml of the filtrate (stock) with 10 ml of a 5% (v/v) solution of 30% (v/v) H₂O₂ and 0.02 M HNO₃ and the pH maintained at 2.0 with the addition of some amount of nitric acid solution. The mixture was heated at 80°C for 1 hr with intermittent addition of deionised water to prevent drying up of the mixture. The resultant solution was cooled and filtered through Whatman No. 42 filter papers and the procedure for HM analyses continued as in Step 1 beginning with the words “10 of the solution was made up ...”.

Step 5 Residual phase

Extraction was by digesting 20 ml of the filtrate (stock) with 12 ml of *aqua regia* (HNO₃ and HCL) solution in the ratio of 1: 3. The mixture was heated at 80°C for 1 hr with intermittent addition of deionised water to prevent the mixture from drying up. The resultant solution was cooled and filtered through Whatman No. 42 filter papers and the procedure for HM analyses continued as in Step 1 beginning with the words “10 of the solution was made up ...”.

Statistical analysis

Data are presented as arithmetic mean, standard deviation and standard error of the mean. The standard error of the difference between mean concentration levels of HM was used to determine the HM difference between fractions. The *F*-test was used to estimate if the mean concentration of HM between metal fractions differs significantly.

RESULTS AND DISCUSSION

Many SCE techniques are applied to assess and profile HM mobilization in the sludge. In-as-much-as there are many SCE schemes, literature abound that indicate that researchers apply only one scheme to sludge, soil, or other sediments materials and the results obtained is used as a unique speciation profile. Nonetheless, given the wide variety in procedure proposed for each extraction step of the SCE scheme, using the result of one scheme as a standard for judging metal speciation in other scheme is vague. For this reason, it is wise to evaluate the SCE technique in association with the experimental variables applied which include; temperature, pH, and reaction time.

The metal partitioning of sludge as determined by the SCE in the present study is given in **Table 1**. It is observed

that most of the content of Cu (0.7727 mg/l or 25%) and Pb (0.1970 mg/l or 30%) are bound to organic matter fraction, but Mn (2.0924 mg/l or 31%), and Cd (0.0413 mg/l or 23%) are bound to Fe–Mn oxides and the residual fraction respectively. Cu was mainly found in the organic matter fraction in the present study which agrees with the result reported by Solis *et al.* (2002). On the other hand, Ni (0.1227 mg/l or 22%) predominated in the fraction bound to carbonates. With regards to the fractions bound to Fe–Mn oxides, the extraction percentage for Mn (31%) was the overall highest in the present SCE scheme, while Mn (12%), Pb (12%) in the exchangeable phase, and Mn (12%) in the residual phase respectively were the overall lowest metals extracted. However, Ni (19%) was the dominant metal extracted in the exchangeable phase. This is a very unstable fraction which is quite sensitive to environmental conditions. Pb (30%), bound to organic matter appears to have mobilized significantly much more than most metals studied. This is in contrast to what Stylianou *et al.* (2007) reported where they demonstrated that Pb was found in large proportion mostly in the residual fraction.

Table 1 also present values of the standard error of the mean concentration of HM in different phases. The *F*-test of the various metal fractions is also indicated, testing these values for 4 and 4 degrees of freedom at *P* < 0.01.

In the recent past, a great variety of extracted schemes have been developed and some of these methods have been widely used. But none of these methods has enjoyed broad acceptance by the scientific community. The point is, given the wide range of SCE procedures available and the variance in the results obtained, it is believed that these results are rarely compared since they contain data that is often a function of the extraction method employed (Ure 1996; Pagnanelli *et al.* 2004). Despite the uncertainty surrounding the SCE method with respect to selectivity and specificity, the scheme is still very resourceful in providing information about metals availability, speciation and mobility.

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