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Phytotoxicity and heavy metals speciation of stabilised sewage sludges

Ana Fuentes, Mercedes Lloréns*, José Sáez, Mª Isabel Aguilar,
Juan F. Ortuño, Victor F. Meseguer

Department of Chemical Engineering, University of Murcia, Campus Espinardo, 30071 Murcia, Spain

Abstract

The presence of heavy metals in the sludges produced in wastewater treatment restricts plants growth and hence their use for agricultural purposes. This study looks at different types of sludges (aerobic, anaerobic, unstabilised and sludge from a waste stabilisation pond) and compares the distribution of the heavy metals that they contain according to the treatment that they have undergone. The sewage sludges were subjected to chemical characterisation and phytotoxicity testing (in absence of substrate) to provide a preliminary assessment of their suitability for land application. In addition, the total quantity of metals (Cd, Cr, Cu, Ca, K, Fe, Mg, Ni, Na, Pb and Zn) was determined. The Community Bureau of Reference (BCR) method for heavy metal speciation was followed. It was confirmed that the total concentration of heavy metals did not exceed the limits set out by European legislation and that the stabilisation treatment undergone by the sludges strongly influenced the heavy metal distribution and the phases to which they were associated. The sludge extracts did not exert any significant adverse effect on the relative seed germination (RSG) of barley (*Hordeum vulgare* L.) or cress (*Lepidium sativum* L.), although the reduction in germination index (GI) indicates that some characteristics existed did have an adverse effect on root growth.
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1. Introduction

The accumulation of sewage sludges from urban wastewater treatment plants is a growing environmental problem. The use of such sludges as fertilisers or as organic soil regenerators seems an attractive possibility because it would enable valuable components (organic matter, N, P and other nutrients necessary for plant growth) to be recycled [1–3]. However, this practice represents a potential threat to the environment because of the possible high heavy metal content, a problem that may be aggravated if the toxic metals are mobilised in the soil to be taken up by plants or transported in drainage waters [4–6].

To evaluate the environmental impact of these metals it is not sufficient to determine their total content since their behaviour in a given medium and their capacity for mobilisation are equally important. These factors will depend on their chemical form in the sludge [3,7,8].

Chemical speciation can be defined as the process of identifying and quantifying different species, forms or phases present in a material.

During recent decades, a great variety of extraction schemes, both simple and sequential, have been developed and, although some methods have been widely used [9,10] none has been unreservedly accepted by the scientific community. Indeed, given the wide range of procedures used, the results obtained are seldom comparable since they present important variations that depend on the extraction method used [11–15]. For this reason the Community Bureau of Reference (BCR) in 1987 began a programme to harmonise the methodology used in the sequential extraction schemes used for determining metals in soils and sediments [16] and also to define certified reference materials. This procedure has been successfully applied to a variety of matrices, including lake, lagoon and marine sediments, sewage sludge, soil and industrially contaminated made-up ground [17–21]. Some workers, however, report difficulties with the scheme, including a lack of phase selectivity, redistribution of analytes between phases and variability between operators, which was attributed to small variations in the pH of the hydroxylamine solution [22,23].

* Corresponding author. Tel.: +34-968-3673-49;
fax: +34-968-3641-48.
E-mail address: llorens@um.es (M. Lloréns).

The phytotoxic effects produced by organic wastes are the result of a combination of several factors, rather than one [24,25]. These factors include the presence of heavy metals [26,27], ammonia [28], salts [29] and low molecular weight organic acids [30] all of which have been shown to have inhibitory effects. The evaluation of sewage sludge toxicity by chemical characterisation and biological testing is therefore extremely important for screening the suitability of sludge for land application.

In the study described in this paper, a sequential extraction method was applied for Cd, Cr, Cu, Fe, Ni, Pb and Zn in accordance with the scheme proposed by the BCR. This sequential extraction scheme consists of three steps, which give rise to four different fractions [16,31] and an additional step:

- *Step 1 (Exchangeable fraction associated with carbonated phases):* In this fraction the metals are adsorbed on the sludges or on their essential components, denominated clays, Fe and Mn hydroxides and humic acids. Metal adsorption is related with changes in the ionic composition of water, which may affect the processes of adsorption–desorption.
- *Step 2 (Reducible fraction or fraction associated with Fe and Mn oxides):* The Fe and Mn oxides act as cement or are present as nodules between particles or cover the same. The heavy metals are strongly bound to these oxides but are thermodynamically unstable in anoxic conditions.
- *Step 3 (Oxidisable fraction bound to organic matter):* That the metals may be complexed or peptised by the natural organic substances is well known. Soluble metallic forms are liberated when organic matter is attacked in oxidant conditions.
- *Step 4 (Residual fraction):* The residual solids mainly contain primary and secondary solids that occlude the metals in their crystalline structures.

The scheme was applied to four types of sludge from wastewater treatment plants that were subsequently stabilised (or not) in different ways: aerobically, anaerobically, in a waste stabilisation pond (WSP) and one unstabilised. The aim was to establish the influence of the stabilisation method on the mobility of the heavy metals associated to each phase.

Finally, the effects on seed germination and primary root growth were determined in cress (*Lepidium sativum* L.) and barley (*Hordeum vulgare* L.) to investigate the effect of the stabilisation strategy on sludge phytotoxicity. Such bioassays are simple and rapid methods to indicate phytotoxicity [24,27]. Barley seeds have been used by many authors for germination tests involving different organic materials [3,32,33] because they are easy to handle and grow quickly. Cress seeds have also been used in phytotoxicity tests [25,34] due to their rapid germination and sensitivity to low salt concentrations and phytotoxic substances. Both types of seeds were used in this case to provide information on different vegetal species that would (or not) confirm the effects of sludges of different degrees of stabilisation.

2. Methods

2.1. Sludges

All the sludges studied came from urban wastewater treatment plants located in the Region of Murcia (SE Spain). Their mineralogical composition was similar mainly being composed of quartz, muscovite, feldspars and calcite.

2.2. Equipment

A Perkin-Elmer AA300 atomic absorption spectrometer with deuterium background corrector was used to determine the total content of Cd, Cr, Cu, Fe, Ni, Pb and Zn. A fuel-lean air-acetylene flame was used for all the analytes except Cr, which requires the stoichiometric conditions of flame gases.

When the sensitivity of FAAS was insufficient to determine Cd, Cu, Ni and Pb in the extracts, electrothermal atomisation AAS (Perkin-Elmer HGA 800 with AS72 autosampler) was used. In the case of Cr, the standard additions method had to be used due to interference on the part of the matrix. $NH_4H_2PO_4$ ($1000 \, mg \, l^{-1}$) was used as matrix modifier for determining Cd.

2.3. Reagents

All the reagents used were of analytical grade. Deionised water was obtained from a Millipore Milli Q system. Certified aqueous standards of the elements (Panreac, Barcelona, Spain) were used for AAS. All the standards, reagent solution and samples were stored in polyethylene containers previously cleaned with $4 \, M \, HNO_3$ and rinsed with deionised water.

2.4. Sample pretreatment

The sludges from the different stabilisation processes were frozen immediately after collecting and then lyophilised in an Edwards 4K freeze dryer. The sample was then ground and sieved to obtain a fraction of less than $150 \, \mu m$. The resulting material was stored in polyethylene containers at room temperature until analysis.

2.5. Characterisation of the sludges

Four replicates of each lyophilised and sieved sample were characterised according to different agronomic parameters: pH, electrical conductivity, moisture content, organic matter content, total nitrogen and total phosphorus. These parameters were determined by standard analytical methods, except in the case of nitrogen, which was determined by elemental analysis. The quantity of the various macroelements was

ascertained by atomic absorption in the case of calcium and magnesium and atomic emission in the case of sodium and potassium.

2.6. Certified reference material

A standard sludge, whose heavy metals concentration is known, has been used to know the reliability of the aqua regia digestion procedure. In this way, it is possible to compare the measured concentration with the indicative concentration and to know the accuracy and precision of the measurement. The standard sludge is a reference material, codified as CRM 145 and certified by the BCR.

Precision was based on analyses of five extracts obtained from the reference sludge. This parameter, relative standard deviation, was defined as follows [35]:

$$\text{precision} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Accuracy was based on measured analyte concentration relative to indicative values for CRM 145 as soluble in aqua regia. Accuracy was computed as follows [35]:

$$Accuracy = \frac{Measured\ concentration - Indicative\ concentration}{Indicative\ concentration} \times 100$$

2.7. Concentration of elements

The pseudo-total concentration of the metals was determined by FAAS after digestion of the samples with aqua regia [19,21,36,37]. For this, 5 g of sludge samples were weighed into a 500 ml round-bottomed flask and 7.5 ml concentrated HCl and 2.5 ml concentration HNO_3 per gram of dry matter were added. The attack was allowed to proceed for 16 h at room temperature and then for 2 h in open reflux condition at 180 °C. After digestion, the solution was filtered into a 100 ml flask and the filter paper and residue were rinsed several times with several millilitres of warm (50 °C) 2 M nitric acid, making the solution up to the mark with the same acid solution once cold.

2.8. Sequential extraction

The BCR three-step sequential extraction procedure described by Ure et al. [16] was followed. Analysis was performed on four parallel samples of different sewage sludges. For an internal check on the procedure [38] an additional step was applied. After the sequential extraction steps, the residual metal content was determined by digestion with aqua regia in accordance with ISO norm (ISO 11466).

2.9. Seed germination test

Sludge extracts (three replicates for each type of sludge) were prepared by shaking 3 g of lyophilised sludges with 30 ml of distilled water. The suspension was then centrifuged

and filtered before being introduced into a polyethylene tube and kept at 4 °C.

For the germination tests, cress (*L. sativum* L.) and barley (*H. vulgare* L.) seeds were used. A 5 ml aliquot or extract was added to a petri dish with a Whatman no.1 ashless filter paper. Eight seeds were placed in each dish (three replicates for each sample). The plates were incubated at 25 °C in the dark at 75% of humidity. Distilled water was used as a control. Seed germination and root length in each plate were measured at 72 h (cress seeds) and 6 days (barley seeds). In both germination tests, the percentages of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) after exposure to sludge extracts were calculated as follows [24,25]:

$$RSG(\%) = \frac{number\ of\ seeds\ germinated\ in\ sludge\ extract}{number\ of\ seeds\ germinated\ in\ control} \times 100$$

$$RRG(\%) = \frac{\text{mean root length in sludge extract}}{\text{mean root length in control}} \times 100$$

$$GI(\%) = \frac{RSG \times RRG}{100}$$

The effects of sludge type on GI were analysed using the SPSS statistical package. One-way ANOVA was carried out to compare the means of different treatments; where significant *F*-values were obtained, differences between individual means and control mean were tested using the Dunnett test.

3. Results and discussion

3.1. Characterisation of the sludges

Table 1 shows the different agronomic parameters obtained during the characterisation of the four types of sludge studied. As can be seen, these materials have a high percentage of organic matter and nitrogen, which decreases as the degree of mineralisation increases. The pH value of sewage sludges varies from 6.0 to 8.2 and the phosphorus content depends to a large extent on the stabilisation process used. As regards macroelements, calcium is the most abundant, followed by magnesium, potassium and sodium, in all the sludges. All the parameters closely reflect those found in the literature for sludges of similar characteristics, some of which have been used for soil amendment [3,6,39,40].

3.2. Certified reference material

Table 2 shows the results obtained for Cd, Cu, Ni, Pb and Zn using direct calibration with aqueous standards, and for Cr using the standard addition method. A statistic treatment of these data (Student's *t*-test for one sample, with a 95% confidence level) shows that measured concentrations

Table 1
Physical-chemical characteristics of the sludges

	Aerobic	Anaerobic	WSP sludge	Non-stabilised
pH	6.6 ± 0.1	7.9 ± 0.3	8.2 ± 0.1	6.0 ± 0.1
Conductivity (mS cm^{-1})	2.27 ± 0.06	2.81 ± 0.04	1.25 ± 0.04	2.3 ± 0.1
Moisture (%)	80.0 ± 0.4	71.2 ± 0.1	87 ± 1	82.3 ± 0.1
Organic matter (%)	64 ± 1	43.5 ± 0.8	26.3 ± 0.7	65 ± 1
Total N (%)	4.40	3.27	1.63	4.56
Total P (%)	0.77 ± 0.04	1.67 ± 0.04	0.42 ± 0.02	1.1 ± 0.2
Ca (mg kg^{-1})	42,713 ± 3,092	67,710 ± 1,377	173,874 ± 4,018	40,638 ± 693
K (mg kg^{-1})	3,733 ± 97	2,844 ± 53	3,341 ± 208	3,527 ± 236
Mg (mg kg^{-1})	5,387 ± 139	9,558 ± 636	8,935 ± 139	6,401 ± 163
Na (mg kg^{-1})	1,877 ± 48	1,538 ± 90	3,283 ± 796	1,694 ± 83

The results are expressed as mean ± standard deviation.

Table 2
Results of the measurement of the metals studied in a certified material

Heavy metal	Content* ± standard deviation (μg g^{-1})		Precision	Accuracy	Statistic signification (P)
	Indicated concentration	Measured concentration			
Cu	416 ± 10	402 ± 10	1.9	−3.3	0.063
Cr	85 ± 8	82 ± 2	2.5	−3.6	0.058
Ni	38 ± 2	37 ± 2	3.2	−2.9	1.203
Zn	2772 ± 64	2714 ± 86	3.2	−2.1	0.209
Pb	332 ± 15	322 ± 12	3.7	−3.0	0.136
Cd	17 ± 1	16.8 ± 0.4	2.2	−0.95	0.388

* Mean for four extracts with three replicates.

do not differ significantly from indicative values of these six analytes. Precision is very satisfactory for the metals studied with all values <5% (Table 2). Accuracy shows similar results (Table 2).

3.3. Pseudo-total heavy metal contents

Whether the sewage sludges from treatment plants should be used as fertilisers or not depends on their heavy metal content. The limits are established by European legislation (Directive 86/278/EEC) and represent the maximum permitted concentration of heavy metals in sludge destined for agricultural use, as can be seen in Table 3.

It can be seen that all the heavy metal values recorded for the four types of sludge (Table 4) were within the maximum permitted levels mentioned in the Directive. However, the quantity of Cr found in the anaerobic sludge was very high

Table 3
Threshold values of heavy metals established in Directive 86/278/EEC

	Threshold value (mg kg^{-1} dry matter)
Cd	20–40
Cu	1000–1750
Ni	300–400
Pb	750–1200
Zn	2500–4000
Cr*	1000–1500

* Values stipulated by Spanish law.

and although the above-mentioned directive does not cover this metal, Spanish legislation (Royal Decree 1310/1990) establishes a limit of 1000 mg kg^{-1} for soils of pH <7 and 1500 mg kg^{-1} for soils of pH >7. The proposals currently under discussion at EU level [41] foresee a maximum of 1000 mg kg^{-1}, which means that this sludge would exceed the limit for agricultural use.

3.4. Application of sequential extraction scheme

Table 5 shows the results obtained after application of the sequential extraction scheme proposed by the BCR to all the sludges obtained and the percentages of each metal recovered. As can be seen, the sum of the four fractions is reasonably similar to the total contents obtained after diges-

Table 4
Total heavy metal content of each sludge

	Aerobic	Anaerobic	WSP sludge	Non-stabilised
Cu	204 ± 5	337 ± 10	167 ± 7	146 ± 7
Cr	38 ± 3	3,809 ± 70	71 ± 5	61 ± 1
Ni	17 ± 2	29 ± 2	15 ± 1	25 ± 1
Fe	5583 ± 331	25,208 ± 545	8915 ± 211	5475 ± 44
Zn	487 ± 28	871 ± 37	697 ± 23	458 ± 11
Pb	58 ± 1	167 ± 3	250 ± 10	87 ± 1
Cd	1.10 ± 0.07	18.3 ± 0.5	11.4 ± 0.4	1.14 ± 0.06

The results are expressed as mean ± standard deviation in mg kg^{-1} of dry matter.

Table 5
Metal concentrations in the sludge extracts for the different steps of BCR scheme

Metal	Step 1	Step 2	Step 3	Step 4	Sum	Percentage recovered
Aerobic						
Cu	2.2 ± 0.2	1.9 ± 0.3	97 ± 8	70 ± 9	171	83.8
Cr	0.11 ± 0.01	0.14 ± 0.01	9.6 ± 0.4	20 ± 2	30.4	80.7
Ni	2.4 ± 0.2	0.9 ± 0.1	3.6 ± 0.5	9.8 ± 0.9	16.8	99.6
Fe	83 ± 20	332 ± 40	$1,440 \pm 140$	$2,994 \pm 290$	4,849	86.9
Zn	114 ± 20	84 ± 3	177 ± 20	34 ± 5	409	84
Pb	0.94 ± 0.04	0.63 ± 0.03	45 ± 3	15 ± 1	61.3	106
Cd	0.115 ± 0.004	0.17 ± 0.07	0.43 ± 0.03	0.299 ± 0.008	1.023	93.5
Anaerobic						
Cu	10 ± 1	2.2 ± 0.3	214 ± 12	61 ± 10	287	85.1
Cr	1.5 ± 0.1	0.83 ± 0.04	$2,824 \pm 207$	511 ± 54	3,337	87.6
Ni	5.9 ± 0.8	1.6 ± 0.1	6.4 ± 0.4	10 ± 1	23.3	79.6
Fe	67 ± 6	63 ± 4	$2,450 \pm 256$	$19,381 \pm 1,330$	21,960	87.1
Zn	94 ± 10	77 ± 7	431 ± 38	177 ± 18	778	89.2
Pb	0.225 ± 0.006	0.9 ± 0.1	14 ± 1	125 ± 9	140	83.7
Cd	2.7 ± 0.4	0.79 ± 0.08	13 ± 1	5.3 ± 0.6	21.4	103.3
WSP sludge						
Cu	1.9 ± 0.2	1.1 ± 0.1	120 ± 9	30 ± 2	152	91.1
Cr	nd	nd	41 ± 1	24 ± 2	65	90.3
Ni	2.1 ± 0.2	nd	3.6 ± 0.3	10 ± 1	15.9	104.9
Fe	3.5 ± 0.2	18 ± 2	778 ± 48	8044 ± 326	8843	99.2
Zn	53 ± 5	24.8 ± 0.5	421 ± 25	74 ± 4	572	82
Pb	0.81 ± 0.07	1.1 ± 0.1	104 ± 18	156 ± 5	261.7	104.7
Cd	0.39 ± 0.06	0.39 ± 0.05	8.0 ± 0.8	2.5 ± 0.4	11.2	98.4
Unstabilised						
Cu	2.7 ± 0.2	2 ± 0.2	109 ± 5	26 ± 2	139	94.9
Cr	1.3 ± 0.1	0.51 ± 0.08	26 ± 1	24 ± 4	51	84.3
Ni	4.1 ± 0.3	2.0 ± 0.1	5.3 ± 0.4	5.1 ± 0.5	16.4	64.5
Fe	34 ± 3	176 ± 15	1161 ± 46	3698 ± 72	5069	92.6
Zn	105 ± 6	99 ± 15	174 ± 18	31 ± 7	408.2	89.1
Pb	0.80 ± 0.08	18 ± 2	33 ± 5	35 ± 6	85.5	98.8
Cd	0.169 ± 0.009	0.127 ± 0.004	0.53 ± 0.05	0.312 ± 0.007	1.138	99.6

The results are expressed as the mean $\pm$ standard deviation in $mg\,kg^{-1}$ of dry matter.

tion of the original samples with aqua regia. Recoveries of 80–100% were reached in most cases, which are very similar to those recorded in the literature for the same extraction scheme [31,41,42].

Of note is the fact that the distribution of the metallic fractions differs with the stabilisation treatment used, although there are similarities in some cases (see Fig. 1). This is the case with Cu, which is mainly associated with the organic matter (OM). The greatest extraction percentage was obtained in the oxidisable fraction of all the sludges, which is to be expected given the affinity of OM for this type of element and the formation of stable complexes [3,19,43]. The sum of the last two fractions accounted for 95% of the total Cu, in all the sludges, which indicated that Cu was associated with strong organic ligands and probably occluded in primary minerals.

In the case of Cr, this element is principally distributed between the third and fourth fraction, as mentioned in the literature [6,31,44]. For the more mineralised sludges with a greater sulphur content, Cr is more likely to be found in the oxidisable fraction than in the residual fraction, since it probably forms sulphides with this type of sludge (obtained anaerobically and by WSP), while in the other types, Cr is mainly associated with the residual fraction. In the first and second fraction, Cr does not exceed 5% in any sludge. With regard to the particular case of the anaerobic sludge, despite its high total Cr content, which makes it unsuitable for agricultural use, the potential mobility of this metal is very low since the sum of the quantity mobilised in the first two fractions does not reach $2.5\,mg\,kg^{-1}$, as can be seen from Table 5.

Ni is extracted in large quantities during the first extraction step, during which the metal is bound to carbonates or exchangeable phases. Quantities vary from 13% of the WSP sludge to 27% in the anaerobic sludge. This metal is widely distributed in the four fractions, the sum of the first two fractions being about 40% in unstabilised sludge and not exceeding 20% in the aerobic and WSP sludges. In these last two, Ni is mainly associated to the residual fraction (58 and 63% extraction, respectively). This high degree of Ni mobility was also found by Alonso et al. [6] in heavy metal speciation of sludges that had been stabilised to different degrees.
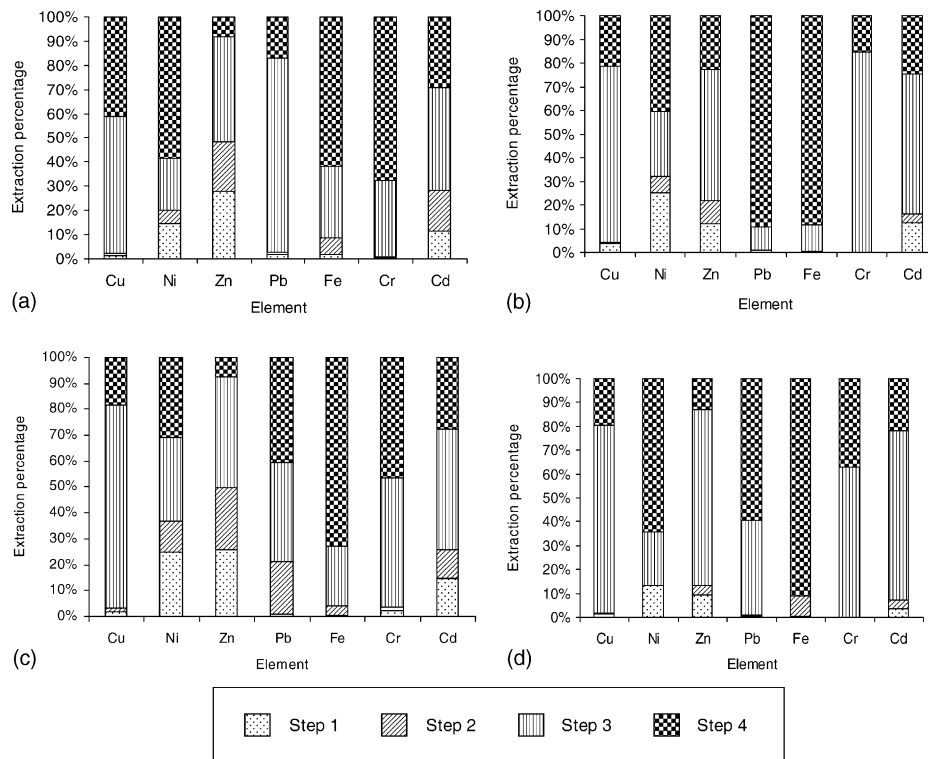
Fig. 1. Fractionation of elements from (a) aerobic, (b) anaerobic, (c) unstabilised and (d) WSP sludges.

About 90% of the Fe was recovered in the fourth fraction in the case of the most mineralised sludges (WSP sludge and anaerobic), the amount mobilised in the rest of the fractions being of little significance. However, in the least stabilised (non-stabilised and aerobic sludges), the proportion of Fe present in the third fraction increased, representing about 25% of the total, although the greatest percentage was associated to the residual fraction.

Zn showed the greatest degree of mobility, as seen from the high proportion of metal extracted in the exchangeable and reducible fractions, a result that reflects the findings of Alonso et al. [6]. In this case, the degree of mobility showed the following tendency: aerobic = non-stabilised > anaerobic > WSP sludge.

The high proportion of Pb in reducible form in the unstabilised sludge (40%) should be mentioned (Fig. 1), a finding also described by Scancar et al. [19] in a similar sludge. In the other sludges, the Pb was distributed between the third and fourth fraction, being the highest quantity mobilised (73%) in oxidising conditions for the aerobic sludge (Fig. 1).

In the easily assimilable fractions (exchangeable and reducible) a relatively high percentage of Cd was obtained for the least stabilised sludges, in which the proportion of Cd mobilised reached about 30%. In the case of the WSP and anaerobically treated sludges the Cd was found mainly in its oxidisable form, although it is important to point out that 15% of the total Cd present in the anaerobic sludge was in its exchangeable form, which would represent a potential contamination risk for any soil it was added to due to the high content of this metal.

3.5. Phytotoxicity assays

In general, germination percentages remained at a relatively high level for all four sewage sludges with the RSG tending to be higher in the barley assay than in those involving cress (Fig. 2). In all the cases the percentage of germinated seeds with respect to the control was more than 70%. However, seed germination has been regarded as a less sensitive method than root elongation when used as a bioassay for the evaluation of phytotoxicity [45].

In the case of barley, GI tended to decrease as the degree of mineralisation of the sludges decreased (Fig. 3) perhaps due to the greater content of little mineralised organic matter or to the greater bioavailability of the heavy metals present in less stabilised sludges. When the Dunnett test was applied to the GI obtained with the extracts of the sludges studied and of the control, significant differences were only found between the means obtained with the aerobic and unstabilised sludges with respect to the control.

As regards the cress assays, the GI obtained with the extracts of the four sludges studied were lower than those obtained in barley (Fig. 3), which was to be expected since cress is known to be more sensitive to the toxic effects of ammonium and low molecular weight organic acids than barley. This diminution was more pronounced with the extract from the more stabilised sludges, especially in the anaero-
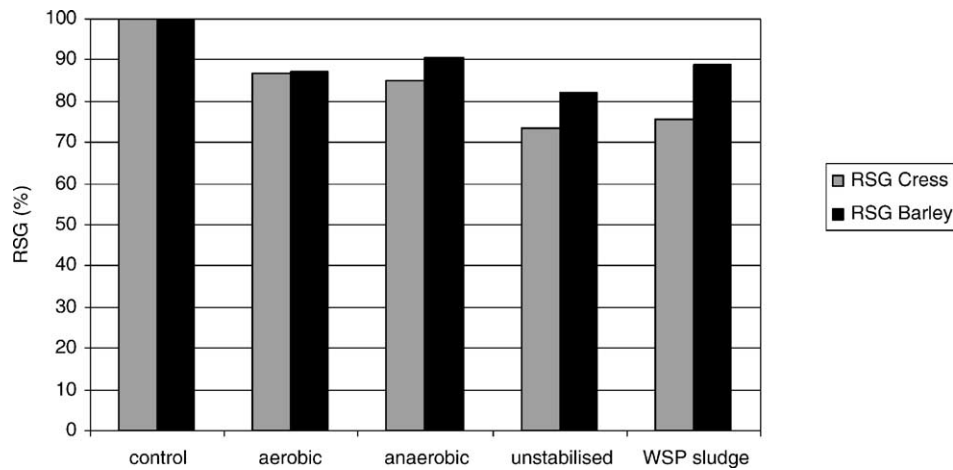
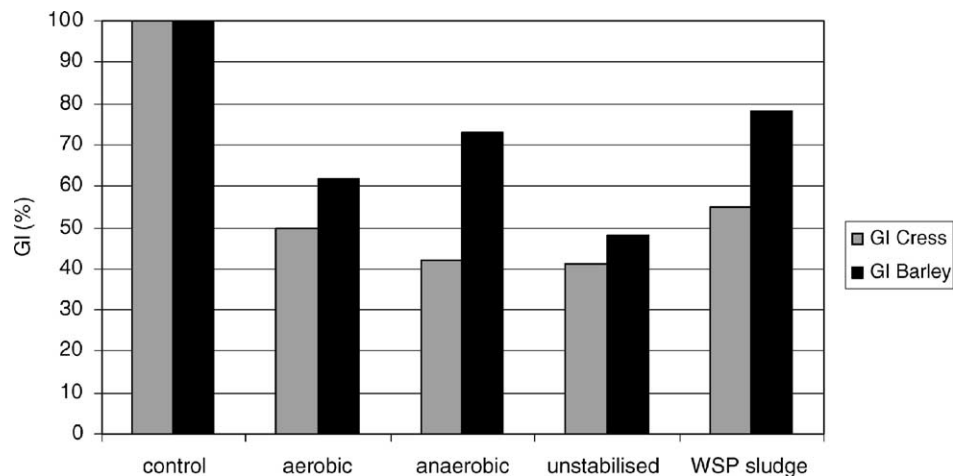Fig. 2. Relative seed germination obtained for barley and cress seeds.

Fig. 3. Germination index obtained for barley and cress seeds.

bic sludge. The Dunnett test confirmed the existence of significant differences between the mean GI values obtained with cress in the anaerobic sludge extracts with respect to the control, significant differences also existing between the GI indices obtained with the aerobic and unstabilised sludge and the control. In certain cases, namely with the anaerobic sludges, a depressive effect on seed germination has been seen, which has been attributed to the release of ammonia [46,47]. Further, greenhouse and field experiments should be performed to confirm the findings of the present experiment.

4. Conclusions

The four types of sludge analysed may be used for soil amendment since they all have a high organic matter content and are rich in nutrients (N, P and K), none having a heavy metal content in excess of the limits laid down by European legislation. However, the anaerobic sludge should not be put

to agricultural use because of its high Cr content, which exceeds the maximum value established by Spanish law and proposed EU limits [41].

The results obtained after applying the sequential extraction scheme proposed by the BCR indicate that Cu and Cr were most abundant in the organic and residual phases, while Zn and Ni showed no dominant chemical phase. It is clear that the stabilisation method used influences the distribution of the metals and the phases to which they are bound. Hence, the WSP sludge, which had undergone a higher degree of mineralisation and stabilisation than the others, showed a lower metal availability index since practically all the heavy metals in it were associated to the oxidisable and residual fractions, which are the least mobile. This was not the case with the unstabilised sludge, which contained the highest accumulations of heavy metals in the most easily assimilable fractions (exchangeable and reducible). In the case of the anaerobic sludge, its high total Cr content and the high degree of Cd availability in the first fraction make it unsuitable for agricultural use.

The same behaviour was seen in the germination assays using barley seeds, on which the extract of the least stabilised sludges had a greater inhibitory effect than the more mineralised sludges, strongly affecting root growth. However, the GI test using cress seeds, which are more sensitive to phytotoxic substances than barley seeds, showed that the extracts from the most mineralised sludges, especially from the anaerobic sludge, also have a significant inhibitory effect on root growth. The effect on the germination of both seed types was less pronounced since the RSG exceeded 70% with all the sludge extracts studied.

Finally, it must be pointed out that only one fraction of the metals present in the sludges and subsequently in the soil is easily assimilable by plants. Furthermore, when the sludges are added to soil, some physical-chemical parameters are modified and such modifications, too, may affect metal bioavailability [3]. Nevertheless, although this study has mainly been concerned with a looking at different types of sludge, it is clear that sequential extraction provides valuable information on the mobility of metals in sludge and helps predict their behaviour if sludges are used for soil amendment.

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