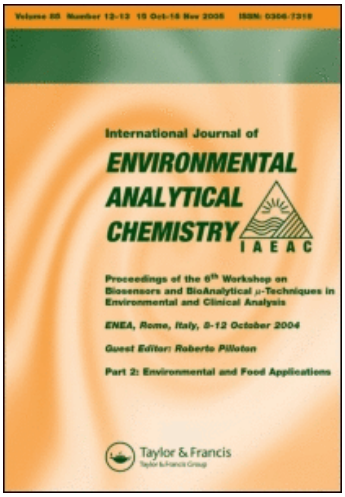

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Speciation of Heavy Metals in Soils and Sediments. An Account of the Improvement and Harmonization of Extraction Techniques Undertaken Under the Auspices of the BCR of the Commission of the European Communities

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SPECIATION OF HEAVY METALS IN SOILS AND SEDIMENTS. AN ACCOUNT OF THE IMPROVEMENT AND HARMONIZATION OF EXTRACTION TECHNIQUES UNDERTAKEN UNDER THE AUSPICES OF THE BCR OF THE COMMISSION OF THE EUROPEAN COMMUNITIES

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An account is presented of a series of investigations and collaborative studies, initiated by BCR, on current methods of metal speciation by extraction of soils and sediments with chemical reagents. It was established by extensive consultation with European experts that the diverse procedures used could be harmonized into agreed methods. These methods, including both single extractant and sequential extraction procedures were subjected to collaborative, interlaboratory trials and the results, presented briefly here, showed that it was both possible and desirable that reference soils and sediments, characterised by certified values for extractable contents, be prepared. As a consequence of these studies two soils have been prepared and will shortly be the subject of interlaboratory analysis with a view to certification of their EDTA and acetic acid extractable contents of some heavy metals. Following this workshop a feasibility study of the agreed sequential extraction procedure will, it is believed, shortly lead to certification of sediments for contents extractable by a defined sequential extraction procedure.

KEY WORDS: Speciation, heavy metals, extractable contents, single extraction methods, sequential extraction methods, soils, sediments, reference materials, BCR.

INTRODUCTION

Some thirty years ago, the Macaulay Institute for Soil Research in Aberdeen was approached by NIST, then the National Bureau of Standards, as to the feasibility of preparing a reference soil certified for its extractable contents as distinct from its total contents. In view of A.

Ure's opinion then that the difficulties of preparation, stability and analysis were too great, it is ironic that he should now be describing the approach to doing precisely that. The difficulties foreseen then were real enough but the power of the collaborative approach adopted by BCR in these current studies is evidenced by the considerable progress now made in overcoming them.

An initial study of the literature on the speciation of metals in soils and sediments, mainly by chemical extraction procedures, and a consultation with European experts was carried out by the first author on behalf of BCR. The objectives of this study were to determine whether or not there was a need for a BCR involvement in improving and harmonizing the methodology for speciation in these materials. This study was discussed at a meeting of representatives of leading European soil and sediment laboratories with BCR in Brussels in 1987 and the following recommendations were made for action under the auspices of BCR:

1) that an interlaboratory trial analysis of soil and sediment extracts be carried out using both single and sequential extraction procedures, but mainly single extraction for soil and sequential extraction for sediment,

2) the elements to be Cd, Cr, Cu, Ni, Pb and Zn

3) the soil to be a sludge-amended, <2 mm, air-dried unground soil,

4) the sediment to be selected from materials available from the CEC Joint Research Centre in Ispra, Italy

5) a long and a short sequential extraction scheme be tried,

6) provided that adequate methodologies were available or were made so by these studies, there was both a need and a demand for soil and sediment reference materials whose extractable contents were certified by such extraction procedure.

SPECIATION DEFINITION

One of the first tasks was the clarification of the term "speciation" whose use had become widespread with little attempt to define its meaning [1]. Chemical speciation, in the context of soils and sediments was defined in the past [2] as:

1) the *process* of identifying and quantifying the different *defined* species, forms or phases present in a material. The "species" are further defined:

a) functionally,

b) operationally or

c) as specific compounds or oxidation states of an element.

2) speciation can also be defined in a similar way as the *description* of the amounts and kinds of species, forms and phases present.

The "species" can be defined, as above,

a) functionally, as for example as "plant-available species" as "mobile forms" or as "exchangeable cations", or defined,

b) operationally, by the procedures, reagents or extractants used to isolate them; examples include the physical isolation of a soil solution or a particle size fraction, or the use of acid ammonium oxalate to extract metals associated with "moderately reducible" soil or sediment components.

More recently, the term "speciation" has been defined as the determination of a specific form (monoatomic or molecular) or configuration in which an element can occur or to a distinct group of atoms consistently present in different matrices [3]. In this paper, the term speciation will be used although it is understood that the term "extractable trace metals" related to a defined reagent should be used instead.

SINGLE EXTRACTANTS—FUNCTIONALLY DEFINED SPECIATION

Speciation, in the functionally defined sense, of plant-available species, or at least of species that are correlated with plant content or uptake, has been widely used in agriculture long before the term speciation was invented. Its role was mainly in the prediction and assessment of trace element deficiency or toxicity in crops or in animals eating them by the analysis of soil extracts.

A large number of single extractants for soils have been evolved. These have mostly been empirically derived but validated by field experiments correlating plant contents with extractable soil contents. These include water (hot) for B, EDTA and DPTA for Cu and Zn, acetic acid for Co and Ni, mixed ammonium acetate/EDTA for Cu and Zn, ammonium acetate for Mo, and weak neutral salt solutions such as calcium chloride, sodium and ammonium nitrate for Cd and Pb. While there are very many other extractants, perhaps mention should be made of the soil solution, or sediment pore water, itself which could be included in this category but which suffers, like the weak neutral salt extracting solutions, from analytical difficulties because of the low element concentrations present.

While it is apparent that many of these extractants quantify species that correlate with the plant-available forms they tend to be element-or crop-specific. In addition significant methodological variations exist within nominally identical procedures as they are applied in different laboratories and countries. Consultation with expert opinion in Europe indicated that the most generally acceptable of these functionally defined extractants were:

a) EDTA 0.05 mol/l or DTPA 0.005 mol/l with similar roles but with EDTA preferred as it extracted greater amounts and was simpler to prepare and use,

b) ammonium acetate 1 mol/l at pH 7 and

c) calcium chloride 0.05 mol/l.

It was considered, too, that a standardisation procedure for these reagents could readily be agreed.

OPERATIONALLY DEFINED SPECIATION

Operationally defined speciation is often, and conveniently, equated with the quantification of the elements in a specific phase of a soil or sediment, despite the fact that the phase may be ill-defined or the procedure insufficiently specific. Single extractants can be used, as was the case for functionally defined speciation, or the procedure can be made more selective by employing several extractants in a defined sequence. Some of these sequential extraction procedures are discussed later.

TEMPORAL STABILITY OF EXTRACTABLE CONTENTS

One of the major hurdles to be surmounted before the preparation of a reference material certified for extractable trace metal contents could be contemplated is the question of their temporal stability. This problem has been studied by Salomons and Scheltens [4], who repeated, in 1987, the sequential extraction and analysis of freshwater sediments first carried out in 1975. While those authors were somewhat disappointed in that significant changes did occur in the measured species concentrations after 12 years, it is likely that the temporal stability was sufficient for the sediments to be characterised on each occasion in such a way that decisions on the management or use of the sediments would be unchanged.

A test of the stability of the extractable contents of a single soil was carried for EDTA, acetic acid, ammonium acetate and calcium chloride extracts and the results are shown in Table 1. It can be seen that, with the possible exception of Cr, the EDTA extractable contents are stable over both 1 and 3 year intervals within about 10% for the elements Cd, Cu, Fe, Mn, Ni, Pb and Zn. For acetic acid extracts the 1 year changes for Cr, Fe, Mn and Zn are poorer than for EDTA but for Cd, Cu, Ni and Pb the extracts are reasonably stable. For ammonium acetate and calcium chloride extracts, however, the results, apart from Cd and Ni in ammonium acetate, are useless. This failure in ammonium acetate and calcium chloride, however, may not be related to temporal instability but, more probably, to the fact

Table 1 Temporal stability of soil extracts. Temporal stability over 1 year and 3 years of EDTA-extractable contents of sludge-amended soil.

EDTA	*PERCENTAGE CHANGE 1989-1990*	*PERCENTAGE CHANGE 1987-1990*
Cd	- 3.5	+ 19
Cr	- 31 -	2.8
Cu	+ 5.0	+ 2.6
Fe	+ 0.2	—
Mn	- 8.7	- 0.3
Ni	+ 5.2	—
Pb	+ 6.5	- 12.8
Zn	+ 14	- 3.0

Temporal stability over 1 year of acetic acid, ammonium acetate and calcium chloride extractable contents of sludge-amended soil.

PERCENTAGE 1989-1990	*HOAc* %	*NH_4OAc* %	*$CaCl_2$* %
Cd	+ 7.4	+ 7.5	+ 118
Cr	+ 36	+ 101	—
Cu	+ 21	+ 77	+ 158
Fe	- 18	+ 131	—
Mn	+ 63	+ 242	+ 1120
Ni	+ 10	- 11	+ 53
Pb	+ 1.9	- 19	—
Zn	+ 19	- 27	+ 100

that the solution concentrations measured are too low for reliable determination by the FAAS of ICPOES methods used.

PRELIMINARY TRIAL OF SEQUENTIAL EXTRACTION

Four laboratories,

(a) The Technical University of Hamburg-Harburg, Germany, (U. Förstner, M. Kersten);

(b) The Institute for Soil Fertility, Haren, The Netherlands, (W. Salomons, M.N. Kerdijk and S. Scheltens);

(c) The Central Highways Laboratory, Bougenais, France, (D. Robbe);

(d) C.E.C. Joint Research Centre, Ispra, Italy, (H. Muntau and M. Van Son),

took part in an interlaboratory trial of 3 different sequential extraction schemes with 7 sediments and 1 soil supplied by the Joint Research Centre in Ispra.

The sequential extraction methods were:

(I) the modified Tessier [5] procedure of Förstner [6],

(II) the short method of Salomons and Förstner [7] and

(III) that of Meguellati [8], shown in Table 2,3, and 4.

Laboratories (a) and (d) used method (I), laboratory (b) used method (II) and laboratory (c) method (III). In order to make comparisons of the three different methods, M.N. Kerdijk evolved a method of grouping steps from the different methods to produce fractions that were directly comparable. Because the method of determining the residual phase was different in the three methods, Kerdijk's procedure was modified so that the residual phase was calculated from the total content. The comparable Fractions 1,2 and 3 thus obtained are shown in Tables 2, 3 and 4 and an example of the bar charts used to display the results is

Table 2 Method (1) Modified tessier scheme.

STEP	*EXTRACTANT*	*PHASE EXTRACTED**
1	$MgCl_2$ 1 mol/l pH 7	EXCHANGEABLE
2	NaOAc 1 mol/l pH 5	CARBONATE
3	$NH_2OH.HCL$ 0.04 mol/l 25% HOAc	Fe/Mn OXIDE
4	H_2O_2 8.8 mol/l/HNO_3 NaOAc EXTRACT	ORGANIC + SULPHIDE
5	HF/$HClO_4$	RESIDUAL:SILICATE

FRACTION 1 = STEP 1 + STEP 2
(EXCHANGEABLE + CARBONATE)
FRACTION 2 = STEP 3 + STEP 4
(REDUCIBLE Fe/Mn OXIDE + ORG/SULPHIDE)
FRACTION 3 = TOTAL - STEPS 1 + 2 + 3 + 4 (RESIDUAL)
(TOTAL = METHOD III STEP 5)

* Reagents may also extract phases partly; this table just refers to commonly used names of "forms" and does not have any scientifically proven meaning.

Table 3 Method (II) Short method of Salomons and Förstner.

Step	*Extractant*	*Phase Extracted**
1	$NH_2OH.HCL$ 0.1 mol/l	ACID REDUCIBLE
2	HH_2O_2 8.8 mol/l; NaOAc EXT.	ORGANIC
3	$HF/HClO_4$	RESIDUAL:SILICATE

FRACTION 1 = STEP 1
FRACTION 2 = STEP 2
FRACTION 3 = TOTAL - STEPS (1 + 2)
* Reagents may also extract phases partly; this table just refers to commonly used names of "forms" and does not have any scientifically proven meaning.

given for one of the sediments in Figure 1 (reproduced from ref. [10]. With the exception of one sediment the results show, as in Figure 1, that the fractional contents by the different procedures are in good enough agreement for the sediments to be sufficiently well characterized for decisions to be made on the management or use of the sediments. There are however serious failures in detail and it can be concluded that, while the results are promising, improvements are necessary if reference material certification were to be attempted. Furthermore, any future study must use a single well specified procedure.

INTERLABORATORY TRIAL OF SOIL EXTRACTION

A sewage sludge-amended soil from Great Billings Sewage farm, Northampton (by courtesy of Anglian Water) was collected, air-dried, sieved and some 12 kg of the <2 mm soil homogenised and bottled in 100 g lots for an interlaboratory trial analysis. The large range of particle sizes in the < 2 mm soil material normally used for extraction necessitates the use of large (5–20 g) subsamples to ensure representative sampling of the bulk material for extraction and analysis. This posed the not trivial problem of homogenisation of the bulk material and the determination by experiment of the minimum sample size required for each extraction and analysis and the verification that each sample bottle was statistically representative of the whole.

Homogenisation of the bulk soil was achieved by rolling it in a polyethylene bag. The

Table 4 Method (III) Meguellatti.

Step	*Extractant*	*Phase Extractant*
1	$BaCl_2$ 1 mol/l, pH 7	EXCHANGEABLE
2	H_2O_2 8.8 mol/l, HNO_3	ORGANIC + SULPHIDE
3	NaOAc 1 mol/l PH 5	CARBONATE
4	$NH_2OH.HCl$ 0.1 mol/l 25% NaOAc	Fe/Mn OXIDE
5	ASH/HF/HCl	RESIDUAL:SILICATE

FRACTION 1 = STEPS (1 + 3 + 4)
FRACTION 2 = STEP 2
FRACTION 3 = TOTAL - STEPS (1 + 2 + 3 + 4)
* Reagents may also extract phases partly; this table just refers to commonly used names of "forms" and does not have any scientifically proven meaning.

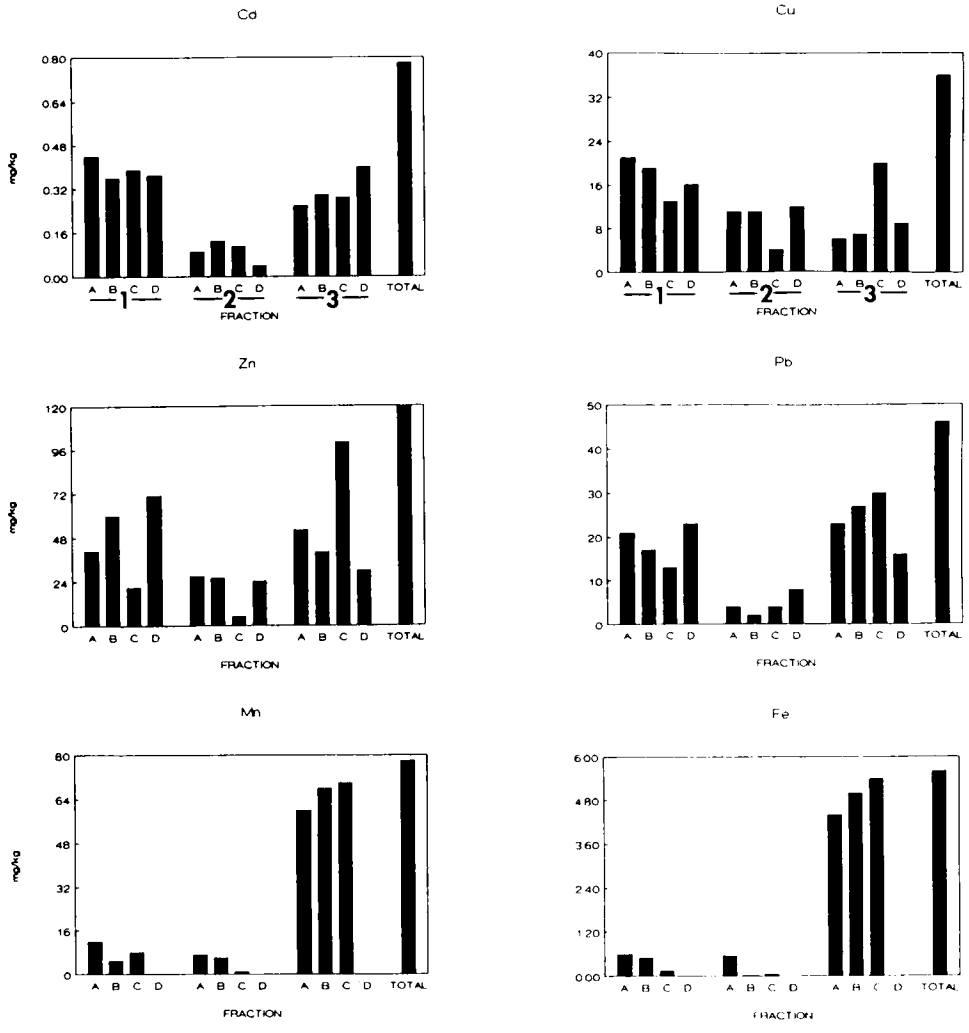

Figure 1 Bar-chart comparison of Fractions 1, 2 and 3 (and total contents) calculated for three different sequential extraction procedures, Methods (I), (II) and (III), applied to sediments 1, 2, 3, 4, 6, 7 and 8. Results for four laboratories A, B, C and D are presented. Laboratories A and D used Method (I), B used Method (III) and C used Method (II).

whole material was subsampled into 100 bottles, each containing approximately 100 g of soil, by coning and quartering. It was determined that a 5 g subsample, taken from each bottle by coning and quartering the whole contents of the bottle was representative of the whole material. By analysing the EDTA 0.05 mol/l and the ammonium acetate 1 mol/l extractable trace metal soil contents from a number of subsamples taken from different bottles and the same number from a single bottle the "between bottle" and the "within bottle" CVs respectively were obtained as shown in Table 5. Only a small decrease in reproduc-

Table 5 Homogeneity test on Great Billings Soil.

	EDTA 0.05 mol/l				*NH_4OAc 1 mol/l*			
	Between bottle		*Within bottle*		*Between bottle*		*Within bottle*	
	Mean μg/g	*CV* %	*Mean* μg/g	*CV* %	*Mean* μg/g	*CV* %	*Mean* μg/g	*CV* %
Cd	21.4	2.5	20.9	0.4	2.36	3.8	2.44	2.2
Cr	9.9	2.4	9.7	0.4	1.36	13	1.66	5.4
Cu	134	2.4	132	1.0	5.32	6.0	5.76	1.6
Fe	1580	2.7	1580	0.5	6.98	52	9.64	2.4
Mn	87.0	2.4	86.5	0.6	12.7	2.9	13.0	1.2
Ni	13.5	1.4	13.1	1.8	0.88	9.5	0.86	6.4
Pb	205	1.8	202	1.3	0.53	13	0.62	11
Zn	409	1.7	417	0.7	12.0	6.3	13.0	0.9

ibility is seen on going from "within bottle" to "between bottle" CVs and in both cases they are acceptably low for all the elements tested in the two extracts. As both temperature of extraction [9] and the vigour of the mechanical shaking process [10] could be expected to affect the amount of metal extracted, limits for both of these parameters were prescribed in the detailed protocol for extraction and analysis issued to participating laboratories along with the soil sample. As a check on each laboratory's calibration, reference solutions containing 6 analytes, Cd, Cr, Cu, Ni, Pb and Zn were issued with the soil and analysed with the extracts. Three types of single extractant were used, EDTA 0.05 mol/l, acetic acid 0.43 mol/l and ammonium acetate 1 mol/l at pH 7.

The overall mean of the individual laboratory mean extractable contents for six elements in the three extractants are shown in Table 6. It can be concluded that, with the exception of Cr, the CVs for the contents in the EDTA extracts are acceptably low (ca +/- 10%) and that for this, or a similarly contaminated soil, certification of EDTA extractable contents is practicable. While for acetic acid extracts the CVs for Ni and Pb are a little higher than for the EDTA extracts, it is likely that certification of acetic acid extractable contents is also feasible. For ammonium acetate extraction only Cd shows acceptable precision and it must be concluded that certification of ammonium acetate extractable contents is not yet feasible in this or similar soils without significant improvement in the methodology. This failure is almost certainly due to the low element contents extracted by this reagent, as will be discussed briefly later.

Table 6 Overall means of laboratory mean extractable contents (mg/kg air dry soil).

			EDTA EXTRACTS			
Cd	Cr	Cu	Ni	Pb	Zn	
Mean	23.1	8.05	162	16.3	255	492
CV %	9.2	25.88	8.5	13.0	11.8	5.8
			ACETIC ACID EXTRACTS			
Mean	19.3	26.1	29.2	15.7	3.36	522
CV %	7.5	8.2	10.0	18.1	24.6	6.9
			AMMONIUM ACETATE			
Mean	3.43	1.39	5.65	1.42	2.21	18.4
CV %	10.9	40.6	23.4	22.5	26.8	22.5

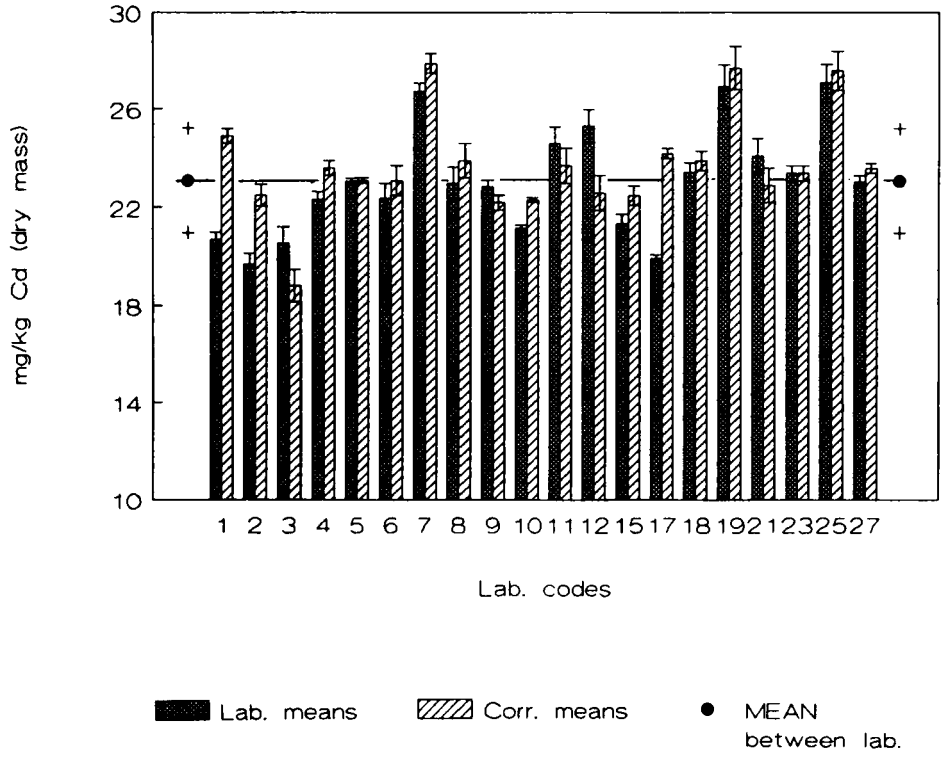

Figure 2 EDTA extractable Cd content in soil (mg/kg dry mass)*Lab. means:* means of five replicate determinations (means of individual laboratories).

It was a notable feature of this trial, and the sediment sequential extraction study carried out at the same time, that calibration by the individual laboratories was frequently in error, often by 10%, and sometimes by extremely large factors, as revealed for example by the range of the value of the factors, F2, required by the analysis of the reference calibrant, to normalize all the laboratory results. These factors ranged, in the case of EDTA extract analysis, from 0.41 to 3.66 instead of the reference value of 1.0. While this calibration error did not significantly effect the overall mean of laboratory means, in individual cases its correction by the F2 factor made rejected values acceptable. This is illustrated for Cd in the EDTA extracts by Figure 2 [10].

INTERLABORATORY TRIAL OF SEQUENTIAL EXTRACTION OF SEDIMENT

For this trial, run concurrently with the above soil extraction trial, a simple 3-step extraction procedure, evolved from that of Salomons and Förstner [7], and outlined in Table 7, was used.

The results obtained in this trial again indicated the importance of checking the laboratory calibration, as systematic errors of 10% from this cause were commonly found and, in a few

Table 7 Three-step sequential extraction procedure for the sediment trial.

Step 1	0.5 g sediment extracted for 5 hours with 20 ml of acetic acid 0.11 mol/l, centrifuged and supernatant decanted for analysis by AAS or ICPOES
Step 2	Residue from step 1 extracted overnight (16 hours) with 20 ml of hydroxylammonium chloride ($NH_2OH.HCl$ 0.1 mol/l) acidified with nitric acid to pH 2, centrifuged and the supernatant decanted for analysis
Step 3	Residue from step 2 treated twice with 10 ml of hydrogen peroxide 8.8 mol/l and the dry residue extracted overnight with 50 ml of ammonium acetate 1 mol/l adjusted to pH 5 with acetic acid. The supernatant, separated by centrifugation, is retained for analysis

cases, major, and quite unacceptable, systematic errors were revealed by the calibrant check analyses. The results for the overall mean of the laboratory mean extractable contents found in each of the steps are summarised in Table 8.

For only 6 of the overall step means, out of a total of 18, are the CVs less than 20%. for Cd the CVs lay between 11 and 20%, for Cr, between 14 and 32%, for Cu all steps had CVs of about 20% while for Zn the CVs fell between 10 and 25%. For Ni and Pb the results were much poorer, with CVs between 26 and 45% and between 50 and 80% respectively.

Table 8 Sediment sequential extraction summary (Overall mean of means mg/kg dry sediment).

Step	*O/A MEAN*	*STD DEV*	*CV%*
	CADMIUM		
1	7.18	0.81	11.3
2	3.41	0.63	18.5
3	1.03	0.196	20.1
	CHROMIUM		
1	1.36	0.20	14.7
2	3.29	1.07	32.5
3	76.3	10.4	13.6
	COPPER		
1	3.69	0.76	20.6
2	3.13	1.96	20.1
3	63.4	13.2	20.8
	NICKEL		
1	9.76	4.36	44.7
2	5.79	1.54	26.6
3	10.2	3.32	32.5
	LEAD		
1	5.06	2.50	49.4
2	11.0	8.87	80.6
3	6.93	4.78	69.0
	ZINC		
1	262	35.1	13.4
2	140	34.2	24.4
3	89.7	9.14	10.2

Table 9 Extract solution concentrations (mg/l) for sediment steps 1 to 3 and for EDTA, acetic acid and ammonium acetate extracts of soil, calculated from mean of laboratory means.

Element	*Step*	*Sediment*	*EDTA soil*	*HOAc soil*	*Amm. Ac. soil*
Cd	1	0.18	4.6	0.48	0.21
	2	0.08			
	3	0.01			
Cr	1	0.03	1.6	0.65	0.09
	2	0.08			
	3	0.76			
Cu	1	0.09	32.4	0.73	0.35
	2	0.08			
	3	0.63			
Ni	1	0.24	0.4	0.4	0.09
	2	0.14			
	3	0.10			
Pb	1	0.12	51	0.08	0.14
	2	0.28			
	3	0.07			
Zn	1	6.6	98	13	1.2
	2	3.5			
	3	0.9			

It can be concluded that the results are much poorer than the results obtained for the EDTA and the acetic acid extracts obtained in the soil trial above and were somewhat poorer even than the inadequate ammonium acetate soil extract results.

While there may be several reasons for this failure in the sediment trial, the principal cause of the poor precision of analysis lies in the very low concentrations found in the extracts of this sediment as shown in Table 9 [10], concentrations in many cases too close to the technique detection limits for reliable determination. The general conclusions can be drawn that for certification of sediment contents obtained by this sequential extraction procedure more sensitive analytical techniques are required, and perhaps, even then, a sediment more contaminated by heavy metals will need to be used.

CONCLUSION

The collaborative studies outlined here, and presented in more detail elsewhere [10], have succeeded in harmonizing the extractants and procedures for chemical speciation of elements in soils and sediments. The agreed procedural protocols have allowed BCR to commence the process of preparing reference soils for certification of their extractable contents with a good prospect of success in the near future. A similar stage in the preparation of sediments certified for metal contents extracted by a sequential extraction procedure should shortly be reached [11].

Both single and sequential extraction schemes were critically examined by the participating laboratories and amended in order to design common procedures which could be used throughout the EC Member States; these procedures are given in Annex [10].

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ANNEX 1

Single extraction procedure for soil analysis

Extractable contents of the following trace metals shall be determined: Cd, Cr, Cu, Ni, Pb and Zn using 0.05 mol/l EDTA and 0.43 mol/l acetic acid.

The extraction shall be performed in 250 ml pre-cleaned borosilicate glass, polypropylene or PTFE bottles using an end-over-end shaker. All laboratory glassware shall be cleaned with HCl, rinsed with distilled water, cleaned with 0.05 mol/l EDTA and rinsed again with distilled water.

Extractants shall be prepared according to the following procedure:

1) 0.05 mol/l EDTA shall be prepared as an ammonium salt solution by adding in a fume cupboard 146.12 + - 0.05g of EDTA free acid to 800 + - 20 ml distilled water and partially dissolved by stirring in 130 + - 5 ml of saturated ammonia solution (prepared by bubbling ammonia gas into distilled water). The addition of ammonia shall be continued until all the EDTA has dissolved. The obtained solution shall be filtered through a filter paper of porosity 1.4 to 2.0 μm (capable of retaining particles of 8.0 μm size) into a 10 litre polyethylene container and diluted with water to 9.0 + - 0.51. The pH shall be adjusted to 7.00 + - 0.05 by addition of a few drops of either ammonia or hydrochloric acid as appropriate. The solution shall therefore be diluted with distilled water to 10.0 + - 0.11, well mixed and stored in stoppered polyethylene container.

2) 0.43 mol/l acetic acid shall be prepared by adding in a fume cupboard 250 + - 2 ml of redistilled glacial acetic acid to about 5 litres of distilled water in a 10 l polyethylene container. The solution shall be diluted with distilled water to 10 l volume, well mixed and stored in a stoppered polyethylene container.

Extraction shall be batch-wise (e.g. shaking), followed by centrifugation according to the following procedure:

1) a 5 g soil sample shall be transferred to an extraction bottle in which 50 ml of 0.05 mol/l EDTA will be added. The obtained mixture shall be shaken on an end-over-end shaker operating at 30 revolutions per minute for 1 hour in a room at 20 °C.

2) a 5 g soil sample shall be transferred to an extraction bottle to which 200 ml of 0.43 mol/l acetic acid will be added. The mixture shall be mixed by shaking in an end-over-end shaker as described above for 16 hours (e.g. overnight) in a room at 20 °C.

The temperature of the room shall be measured at the beginning and at the end of the extraction as well as the temperature of the extracting solution in the bottle at the end of the

shaking period. The extracts shall be immediately filtered through a filter paper (porosity 0.4 to 1.1 μm capable of retaining particles of 2.7 μm size) previously rinsed with 0.05 mol/l EDTA followed by distilled water. The filtrates shall be collected in polyethylene bottles. Blank extractions (i.e. without soil) shall be carried out for each set of analysis using the same reagents as described above.

The sample for analysis should be taken as it is. Before a bottle is opened it should be manually shaken for 5 min to rehomogenise the content. The results should be corrected for dry mass: this correction must be performed on a separate portion of 1 g taken at the same time from the same bottle by drying in an oven at 105 + - 2 °C for 2–3 hours until constant mass is attained (successive weighings should not differ by more than 1 mg).

ANNEX 2

Protocol for the sequential extraction scheme for sediment analysis

APPARATUS

All laboratory-ware shall be of borosilicate glass, polyethylene, polypropylene or PTFE, except for the centrifuge tubes, which will be of borosilicate glass of PTFE.

Clean vessels in contact with samples or reagents with HNO_3 4 mol/l (overnight) and rinse with distilled water. Determine the blank as follows: to one vessel from each batch, taken through the cleaning procedure, add 40 ml of acetic acid (solution A, see below). Analyse this blank solution along with the sample solutions from step 1 described below. Use a mechanical shaker, preferably of the horizontal rotary or the end-over-end type, at a speed of 30 rpm and record the speed. Carry out the centrifugation at 1500 G.

REAGENTS

Water

Glass-distilled water is normally suitable; simple de-ionised water may contain organically complexed metals and should not be used. Analyse a sample of distilled water with each batch of step 1 extracts.

Solution A (acetic acid 0.11 mol/l)

Add in a fume-cupboard, 25 + - 0.2 ml of redistilled glacial acetic acid (or for example Analar or Suprapur grade acetic acid without distillation) to about 0.5 l of distilled water in a 1 l polyethylene bottle and make up to 1 l with distilled water. Make up 250 ml of this solution (acetic acid 0.43 mol/l) with distilled water to 1 l to obtain an acetic acid solution of 0.11 mol/l. Analyse a sample of each batch of solution A.

Solution B (hydroxylamine hydrochloride or hydroxyammoniumchloride 0.1 mol/l

Dissolve 6.95 g of hydroxylamine hydrochloride in 900 ml of distilled water. Acidify with HNO_3 to pH 2 and make up to 1 l with distilled water. Prepare this solution on the same day as the extraction is carried out. Analyse a sample of each batch of solution B.

Solution C (hydrogen peroxide solution 300 mg/g, i.e. 8.8 mol/l)

Use the H_2O_2 as supplied by the manufacturer, i.e. acid-stabilized to pH 2–3. Analyse a sample of solution C.

Solution D (ammonium acetate 1 mol/l)

Dissolve 77.08g of ammonium acetate in 900 ml of distilled water, adjust to pH 2 with HNO_3 and make up to 1 l with distilled water. Analyse a sample of each batch of solution D.

SEQUENTIAL EXTRACTION PROCEDURE

Determine the extractable contents of the following trace metals, Cd, Cr, Cu, Ni, Pb and Zn using the procedure below.

Carry out all extractions on the sediment as received in the glass bottle.

Before subsampling the sediment, with a suitable plastic (see apparatus above) spatula, shake the contents of the sample bottle, with the PTFE ball supplied in the bottle, for 3 minutes.

Dry a separate 1 g sample of the sediment in a layer of about 1 mm depth in an oven at 105 °C for 2 hours and weigh. From this a correction "to dry mass" is obtained and applied to all analytical values reported (quantity per g dry sediment).

Perform the extractions by shaking in a mechanical shaker at 20 + -2 °C. The temperature of the room shall be measured at the start and at the end of the extraction procedures.

Perform the sequential extraction procedure according to the steps described below:

Step 1 Add 40 ml of solution A to 1 g of sediment (as received) in a 100 ml centrifuge tube and extract by shaking for 16 hours at ambient temperature (overnight). No delay should occur between the addition of the extractant solution and the beginning of the shaking. Separate the extract from the solid residue by centrifugation and decantation of the supernatant liquid into a high pressure polyethylene container. Stopper the container and analyse the extract immediately or store it at 4 °C prior to analysis. Wash the residue by adding 20 ml of distilled water, shaking for 15 minutes and centrifuging. Decant the supernatant and discard, taking care not to discard any of the solid residue.

Break the "cake" obtained upon centrifugation by using a vibrating rod prior to the next step.

Step 2 Add 40 ml of solution B to the residue from step 1 in the centrifuge tube and extract by shaking for 16 hours at ambient temperature (overnight). No delay should occur between the addition of the extractant solution and the beginning of the shaking. Separate the extract from the solid residue by centrifugation and decantation as in step 1. Retain the extract in a stoppered polyethylene tube, as before, for analysis. Wash the residue by adding 20 ml of distilled water, shaking for 15 minutes, and centrifuging. Decant the supernatant liquid and discard, taking care to avoid discarding any of the solid residue. Retain the residue for step 3.

Break the "cake" obtained upon centrifugation by using a vibrating rod prior to the next step.

Step 3 Add carefully, in small aliquots to avoid losses due to violent reaction, 10 ml of solution C to the residue in the centrifuge tube. Cover the vessel with a watch glass and digest at room temperature for 1 hour with occasional manual shaking. Continue the digestion for 1 hour at 85 °C and reduce the volume to a few ml by further heating of the uncovered vessel in a steam bath or equivalent.

Add a further aliquot of 10 ml of solution C. Heat the covered vessel again to 85 °C and digest for 1 hour. Remove the cover and reduce the volume of the liquid to a few ml.

Add 50 ml of extracting solution D to the cool moist residue and shake for 16 hours at ambient temperature (overnight). No delay should occur between the addition of the extractant solution and the beginning of the shaking. Separate the extract by centrifugation and decant into a high pressure polyethylene tube. Stopper and retain as before for analysis.

IMPORTANT:

— The calibrant solutions should be made up with the appropriate extracting solutions. Interlaboratory consistency of calibration will be assessed by the analysis by the participants of simulated extracts.
— The total elemental content (HF method) will be determined by specialist laboratories.
— With each batch of extractions a blank sample (i.e. a vessel with no sediment) shall be carried through the complete procedure.