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Microwave assisted EDTA extraction—determination of pseudo total contents of distinct trace elements in solid environmental matrices

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ABSTRACT

Quantitative determination of metals in environmental matrices became important in the past few decades because of increasing pollutant concentrations in aquatic and terrestrial ecosystems. The extraction phase in the process of determining metals is crucial but very time-consuming. Consequently, new extraction techniques for extractable metals have been developed which probably will substitute conventional procedures in the future. The aim of this study was to improve a closed vessel microwave assisted extraction (MAE) by using EDTA as an exclusive extraction agent (MAE-EDTA) for the determination of pseudo total metal contents in solid environmental samples. For this purpose, a large set of soil and compost samples were analyzed.

MAE-EDTA was compared with both closed vessel microwave assisted aqua regia extraction (MAE-AR) and a conventional aqua regia extraction (AR) method for the determination of pseudo total Cd, Cu, Mn, and Pb contents of soil and compost samples. Certified reference materials were used for comparison of recovery rates from different extraction protocols. Metal concentrations in soil and compost extracts were determined by ICP-OES.

MAE-AR which was considered as a reference MAE method for further steps of the study, showed the same extraction yields in the determination of pseudo total metal contents of the investigated elements (As, Ba, Be, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, Zn) in soil and compost samples as the conventional AR.

MAE-EDTA gave similar values as the reference methods in the determination of Cd, Cu, and Pb amounts in soil samples and Cd, Mn, and Pb amounts in compost samples. The recovery rates ranged between 89.0–117.1% for soil samples and 93.5–104.0% for compost samples. MAE-EDTA provides fast processing of the samples that is less than one hour, including time for cooling of the samples. Apart from significantly less processing time, minimal consumption of sample and reagent chemicals is a strategic characteristic of MAE-EDTA procedure which has advantages including accuracy and reduction of contamination. The drawback of MAE-EDTA was that the optimized conditions for the metals Cd, Cu, Mn and Pb may not be generally applicable for the other metals. Therefore, the analytical parameters available in MAE-EDTA should be further investigated for the metals of interest.

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1. Introduction

Increasing concentrations of heavy metals in the environment result in continuously growing importance for elemental analysis in environmental samples [1,2]. Elevated metal loads in soil result in increased uptake by plants and thereby accumulate in the food chain. Thus any soil amendment and fertilizer such as compost, which are used as fertilizer [3], should be monitored for their elemental concentrations. Though total concentrations of metals in soil is used to characterize its base-line elemental composition (parent geological material), pseudo total metal concentration analysis allows to assess soil pollution and to ascertain heavy metal and other pollutant contents in the soil. Although different procedures have been described to determine pseudo total metal contents, the most widely adopted method is aqua regia digestion [4]. Sastre et al. [5] compared microwave-fluoric acid assisted total extraction with this conventional aqua regia extraction for Cd, Zn, Cu, and Pb in sediments, soils, sludge and plant materials and reported that both methods show similar results. Therefore, aqua regia offers a good choice for monitoring samples with low organic matter content. Conventional procedures for metal analysis such as extraction of soil samples using hot plate and concentrated strong acids are usually time-consuming and labor intensive [6]. In addition, it may result in corruption of analytes [7].

Abbreviations: MAE, microwave assisted extraction; MAE-EDTA, microwave assisted edta extraction; MAE-AR, microwave assisted aqua regia extraction; ICP-OES, Inductively coupled plasma-optical emission spectrometer; TMAH, tetramethylammonium hydroxide; C_{org}, organic carbon; O.M, organic matter.

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Abu-Samra et al. [8] first described microwave heating for the impetuous wet acid digestion of biological samples. From then on, the microwave digestion technique has been accepted as a rapid, effective sample preparation method for trace metal determination [9,10] to enhance elemental recoveries [11]. The closed vessel microwave assisted acid digestion system which is also useful for volatile elements has proven to be a fast and accurate method for the decomposition of several environmental and biological matrices [12]. Consequently, MAE has been applied to many fields of analytical chemistry [13–15] and is used as an effective extraction method for biological, environmental, geological, and metallic matrices.

The main advantage of MAE is the reduction of the processing time [16–18] due to direct transition of the microwave energy to the sample by absorptive polarization and concurrent heating of samples [19,20]. In conventional heating methods, a finite period of time is needed to heat the vessel before the heat is transferred to the solution [16]. Moreover, the heat is only transferred to parts of the solution that are in contact with the heat source [21]. Other advantages of MAE over traditional methods include lower reagent and sample usage, enhanced operator safety, reduced contamination, reproducible sample preparation and environmental compatibility [2,7,17,20–23].

Chelating agents contain varying numbers of functional groups that are capable of forming complexes with heavy metals [24]. EDTA (ethylenediaminetetraacetic acid) is a non-specific chelating agent and it may react with metal ions [25]. Wanekaya et al. [26] used pressure assisted chelating extraction (PACE) as a novel approach to extract metals in solid matrices and showed that EDTA is able to solubilize Pb more than water and acetic acid. The authors mentioned that the increased temperatures decrease the surface tension of the medium and matrix, allowing the medium to wet the sample matrix. Furthermore, for resulting in a clean and less hazardous metal dissolution, the usage of EDTA as an extracting medium instead of concentrated acids is attractive [27].

Our present study reports an innovative method, the use of EDTA as an exclusive extraction agent within a closed vessel MAE for the determination of pseudo total metal concentrations of selected elements in soil and compost samples.

The aim of this study is to demonstrate that closed vessel MAE-EDTA could be a good alternative to both conventional AR extraction and MAE-AR for certain metals (Cd, Cu, Mn, and Pb) for soil and compost samples. To prove this, a large set of soil and compost samples with a broad range of physical and chemical properties as well as certified reference materials were chosen to validate the method. Thereby, the applicability of MAE-AR as a reference method for MAE procedures and accuracy of MAE-EDTA was to be demonstrated.

For evaluation of the MAE techniques, ICP-OES (inductively coupled plasma-optical emission spectrometer) was chosen, due to its countenance to rapid, sensitive and simultaneous multi-element capability (over 70 elements, including all the investigated elements) [28–30].

2. Experimental

2.1. Samples

Soil samples ($n=158$), collected from the topsoil layer (0–30 cm), were taken from different locations in Hesse, Germany and from contaminated sites in Mashavera Valley, Georgia. In addition to soil samples, samples from compost ($n=27$) were also analyzed as another solid environmental matrix.

Soil samples with a broad range of physical and chemical properties were used to prove the effectiveness of both extraction procedures. The characteristics of soil such as soil pH (DIN ISO 10390) [31], soil carbonate content (DIN ISO 10693) [32], organic carbon by using C–N–S element analyzer (Elementar) and soil particle size distribution (DIN EN ISO 14688) [33] were determined for the samples collected. Additionally, characteristics of compost samples were tested according to the Federal Compost Quality Assurance Organization, Germany (FCQAO) [34]. The characteristics of soil and compost samples are shown in Tables 1 and 2.

Table 1
Characteristics of soil samples ($n=158$).

Properties	Mean	Minimum	Maximum	Median
pH [$CaCl_2$]	5.92	4.08	8.00	5.55
C_{org} (%)	2.66	0.11	6.13	2.34
Sand (%)	19.81	0.00	81.23	13.54
Silt (%)	44.68	0.00	80.62	45.78
Clay (%)	35.5	5.76	100.00	35.01

C_{org}: Organic carbon.

Table 2
Characteristics of compost samples ($n=27$).

Properties	Mean	Minimum	Maximum	Median
pH [$CaCl_2$]	7.43	4.4	8.5	7.4
O.M (%)	43.04	28.37	52.67	41.37

O.M: Organic matter.

2.2. Reagents and reference materials

The reagents used were all of analytical-reagent grade certified for the impurities. 69% (w/v) nitric acid (HNO_3) (Merck, Germany), 35% (w/v) hydrochloric acid (HCl) (Carl Roth, Germany), and solid ethylenediaminetetraacetic acid (EDTA) (Merck, Germany) were used for the extraction procedures. Distilled and deionized water, purified with a Milli-Q plus system (Millipore), was used for the experiments. Element solutions were prepared by appropriate dilution of ICP standards (Carl Roth, Germany) of the investigated elements.

The accuracy of extraction methods for soil samples were examined by analyzing two certified reference materials (CRMs), “7001” (light sandy soil) and “7004” (loam), from Analytika Co. Ltd, Prague, Czech Republic, and for compost samples by one interlaboratory test reference material (RM). To designate the amount of possible cross contamination, blank values were determined within each sample series.

2.3. ICP-OES instrumentation

Metal concentrations in soil and compost extracts were determined by an inductively coupled plasma-optical emission spectrometer (ICP-OES; Agilent 720ES, Darmstadt, Germany) with axial torch and echelle optic configuration, Charge Couple Device (CCD) detection system and full wavelength coverage from 167 nm to 785 nm (for operating parameters see Table 3).

2.4. Extraction techniques

2.4.1. Conventional aqua regia extraction procedure (AR)

The conventional AR extraction procedure was based on the International Organization for Standardization (ISO) 11466 method [35]: 3 g of soil and compost samples were extracted, respectively, with a mixture of 21 ml HCl (32%) and 7 ml HNO_3 (69%) in 250 ml Pyrex extraction tubes.

Table 3
ICP-OES Operating parameters.

Incident power (kW)	1.20
Plasma gas flow (l min^{-1})	16.5
Auxiliary gas flow (l min^{-1})	1.50
Sample uptake time (s)	45
Test time, repetition (s)	30

Table 4
Operating conditions for MAE-AR and MAE-EDTA extraction procedures for soil and compost samples.

Step	MAE-AR procedure			MAE-EDTA procedure		
	Power (W)	**Limit Temp. (°C)**	**Hold time (min)**	**Power (W)**	**Limit Temp. (°C)**	**Hold time (min)**
1	250	–	1	500	150	1
2	250	–	15	1000	150	7
3	500	–	10	1200	150	10
Ventilation			30			30

2.4.2. Microwave assisted extraction system (MAE)

MAE-AR and MAE-EDTA were performed with a StarT-1500 microwave oven (MLS GmbH Cooperation, Leutkirch, Germany), equipped with a rotary table on which a maximum of 10 polytetrafluorethylene (PTFE) digestion vessels (100 ml each) can be placed and which includes a temperature and pressure sensor providing monitoring and controlling of temperature and pressure of the present conditions.

2.4.2.1. MAE-AR and MAE-EDTA extraction procedures.

The US EPA Method 3051A [36], which provides the analyst with options to perform either a HNO_3-only or a HNO_3–HCl mixed acid, was modified for the MAE-AR procedure which was taken into consideration as a reference validation MAE method for further steps of the study. For MAE-AR extraction 0.3 g of soil and compost sample, respectively, was weighed directly into the PTFE extraction vessels and 6 ml HCl and 2 ml HNO_3 was added. The modified microwave assisted extraction program for MAE-AR can be seen in Table 4.

For MAE-EDTA procedure 0.3 g of soil and compost sample, respectively, was weighed directly into the PTFE extraction vessels as it is like in MAE-AR and 8 ml 0.02 M EDTA solution was added. The MAE-EDTA program with a processing time of less than 1 h, is given in Table 4. After extraction and cooling of the samples, the PTFE vessels were opened and the extracts were transferred into 50 ml HNO_3 treated polypropylene calibrated flasks. The solutions were then made up to volume with deionized H_2O. The final solution was filtered (185 mm Macherey-Nagel MN 280 1/4) and stored in HNO_3 treated polyethylene bottles at 4 °C until analyzed. Blanks were treated in the same way as the samples.

For optimization of MAE-EDTA procedure for soil and compost samples, the present conditions were controlled through a reference vessel equipped with both temperature and pressure sensor. Extraction conditions inside the vessels are shown in Fig. 1. Decomposition of EDTA begins at 190 °C [37], on this account; the temperature limit of 150 °C was used for MAE-EDTA procedure. Another reason for temperature limitation was the higher input of power during the procedure and possible exothermic reactions especially with samples rich in organics.

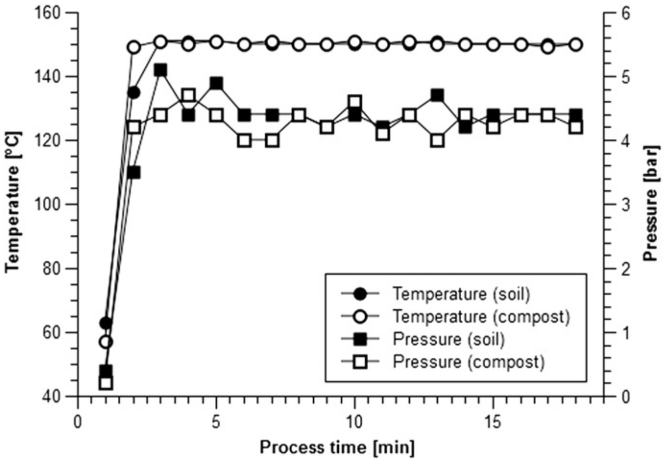

Fig. 1. Course of temperature and pressure during the MAE-EDTA procedure.

Considering poor biodegradability for EDTA in natural environments [38], any rest of EDTA extracts after analysis should be disposed appropriately as other organic solvents used in the laboratory.

2.4.2.2. Cleaning conditions for MAE extraction procedures.

Before each extraction procedure, microwave cleaning was performed to obviate metal cross-contamination of the microwave vessels from previous samples. 10 ml HNO_3 (69%) was used for the cleaning procedure of MAE methods with the same program as MAE-AR. Blanks were performed for monitoring the cleaning effectiveness.

2.4.3. Conventional EDTA-extraction

For better understanding of the effect of microwave assisted conditions within the MAE-EDTA method, the corresponding results were compared with a conventional EDTA extraction for soil samples. Here, such EDTA-extracted heavy metal concentrations (5 g soil with 50 ml 0.02 M EDTA in 1 M NH_4OAc solution was mechanically shaken for 1 h and subsequently filtered) from soil samples represent the termed potentially bioavailable fraction [39].

2.5. Statistical evaluation

For data analysis SPSS 19.0 for Windows was used. Before testing for differences in analyte concentrations, each element across samples were tested for normality by examination of histograms and residual plots. Pearson's correlation test was executed to correlate the extraction methods, and student's *t*-test at a confidence level α: 0.05 was used to assess significant differences between treatments.

3. Results and discussion

3.1. Validation of MAE procedures

For each element and extraction procedure, limit of detection (based on 3σ of the blank) and limit of quantification (based on 9σ of the blank) given in Table 5 were calculated on the basis of DIN-32645 calculation procedure [40].

In order to ascertain accuracy, precision and repeatability, the results from MAE-AR and MAE-EDTA procedures were evaluated with two CRMs (7001 and 7004) for soil samples with five

Table 5
Limits of detection and quantification (mg kg^{-1}) for AR, MAE-AR and MAE-EDTA extraction procedures for soil and compost samples at given wavelengths within ICP-OES analysis.

		Limit of detection (3σ)			Limit of quantification (9σ)		
Elements	**Wavelength (nm)**	**AR (mg kg^{-1})**	**MAE-AR (mg kg^{-1})**	**MAE-EDTA (mg kg^{-1})**	**AR (mg kg^{-1})**	**MAE-AR (mg kg^{-1})**	**MAE-EDTA (mg kg^{-1})**
As	193.696	0.185	0.261	0.341	0.555	0.782	1.023
Ba	455.403	0.029	0.043	0.047	0.086	0.129	0.141
Be	313.042	0.003	0.007	0.005	0.009	0.022	0.015
Cd	228.802	0.014	0.015	0.018	0.042	0.045	0.054
Co	237.863	0.049	0.051	0.072	0.148	0.153	0.217
Cr	267.716	0.040	0.036	0.043	0.120	0.108	0.130
Cu	324.754	0.356	0.567	0.479	1.067	1.701	1.437
Mn	257.610	0.019	0.035	0.107	0.057	0.104	0.322
Ni	231.604	0.036	0.051	0.088	0.108	0.153	0.264
Pb	220.353	0.231	0.240	0.378	0.692	0.719	1.134
V	292.401	0.037	0.047	0.048	0.110	0.140	0.144
Zn	213.857	0.033	0.075	0.065	0.099	0.225	0.195

Table 6
Metal contents in both CRMs and recovery rates (%) obtained by MAE-AR and MAE-EDTA, RSD % values are given in parenthesis (n=number of replicates).

Elements		mg kg^{-1} ± sd			Recovery (%)	
	Certified material	**Certified value**	**MAE-AR [n=5]**	**MAE-EDTA [n=5]**	**MAE-AR [n=5]**	**MAE-EDTA [n=5]**
As	CRM 7001	10.4 ± 1.0	11.2 ± 0.18	7.032 ± 0.22	107.6 (1.6)	67.6 (3.1)
	CRM 7004	42.4 ± 2.2	47.22 ± 0.43	29.52 ± 0.21	111.2 (0.9)	69.5 (0.7)
Ba	CRM 7001	108	118.22 ± 2.19	20.42 ± 4.05	109.4 (1.8)	18.9 (19.9)
	CRM 7004	217	229.72 ± 2.38	16.12 ± 0.95	105.9 (1.0)	7.4 (5.9)
Be	CRM 7001	1.022 ± 0.10	1.222 ± 0.02	0.182 ± 0.05	119.5 (1.7)	17.3 (27.0)
	CRM 7004	2.692 ± 0.21	3.382 ± 0.04	0.432 ± 0.02	125.6 (1.0)	15.9 (3.6)
Cd	CRM 7001	0.292 ± 0.04	0.232 ± 0.03	0.262 ± 0.02	79.3 (8.7)	89.0 (5.0)
	CRM 7004	1.442 ± 0.07	1.482 ± 0.03	1.692 ± 0.03	102.7 (2.0)	117.1 (1.5)
Co	CRM 7001	9.152 ± 0.47	10.02 ± 0.06	5.012 ± 0.22	109.6 (0.6)	54.8 (4.4)
	CRM 7004	17.52 ± 0.9	19.82 ± 0.26	12.02 ± 0.12	113.0 (1.3)	68.7 (1.0)
Cr	CRM 7001	71.92 ± 5.9	82.92 ± 0.55	14.22 ± 1.15	115.3 (0.7)	19.8 (8.1)
	CRM 7004	46.32 ± 3.8	55.92 ± 1.03	13.42 ± 0.51	120.9 (1.8)	28.9 (3.8)
Cu	CRM 7001	28.92 ± 0.8	31.02 ± 1.52	27.42 ± 3.47	107.3 (4.9)	94.9 (12.6)
	CRM 7004	1672 ± 1	185.72 ± 0.86	175.52 ± 3.69	111.2 (0.5)	105.1 (2.1)
Mn	CRM 7001	4792 ± 18	489.62 ± 4.65	446.22 ± 6.75	102.2 (1.0)	93.2 (1.5)
	CRM 7004	7412 ± 36	766.22 ± 6.17	768.12 ± 2.52	103.4 (0.8)	103.7 (0.3)
Ni	CRM 7001	31.82 ± 1.2	34.62 ± 0.14	11.62 ± 0.68	108.9 (0.4)	36.3 (5.9)
	CRM 7004	30.42 ± 1.2	34.52 ± 0.33	17.92 ± 0.25	113.6 (1.0)	59.0 (1.4)
Pb	CRM 7001	24.12 ± 1.7	25.52 ± 0.38	26.92 ± 0.43	105.6 (1.5)	111.6 (1.6)
	CRM 7004	83.12 ± 2.3	84.22 ± 0.66	96.72 ± 0.43	101.4 (0.8)	116.4 (0.6)
V	CRM 7001	52.02 ± 3.4	52.42 ± 0.32	20.32 ± 0.81	100.8 (0.6)	39.0 (4.0)
	CRM 7004	95.12 ± 4.9	99.52 ± 1.00	40.32 ± 0.60	104.6 (1.0)	42.4 (1.5)
Zn	CRM 7001	1082 ± 3.5	114.32 ± 1.12	59.62 ± 6.23	105.8 (1.0)	55.2 (10.4)
	CRM 7004	1982 ± 6	216.62 ± 0.99	150.52 ± 3.15	109.4 (0.5)	76.0 (2.1)

replicates and with one interlaboratory RM with ten replicates for compost samples.

Table 6 presents recovery rates for each element, based on the mean certified value for CRMs, for MAE-AR and MAE-EDTA procedures for soil CRMs 7001 and 7004. MAE-AR shows the same results as the conventional AR digestion (certified values) in the determination of pseudo total amounts of all investigated elements for the two CRMs with a good repeatability as demonstrated by the low relative standard deviations (RSD) ($<9\%$) and by the recovery ranges between 79.3 and 125.6%. In addition, As and Pb are assumed as volatile elements, but certainly no vaporization loss occurred for the both elements during the determination by ICP-OES as it is also predicated by Wang et al. [41] for the element As.

On these grounds MAE-AR was chosen as a reference MAE method in later stages of the study to express the efficiency of MAE-EDTA on real compost samples. Highly linear relationships and statistical results from the correlation of metal concentrations for the real soil samples (n=158) obtained by MAE-AR and conventional AR are given in appendix A and B.

Higher recovery rates for Cd (89.0 and 117.1%) and Pb (111.6 and 116.4%) and nearly the same results for Cu with recoveries within the range 94.9–105.1% were achieved by using MAE-EDTA compared to conventional aqua regia and MAE-AR extractions (Table 6). MAE-EDTA also showed good repeatability for Cd, Cu, and Pb for the soil samples.

MAE-AR resulted nearly in the same concentrations as represented by the reference values (obtained by conventional AR extraction) for the compost RM. However, data scatter was higher in comparison to the soil extractions. MAE-EDTA gave same values for pseudo total Cd, Mn, and Pb concentrations with recovery ranges within 93.5–104.0% with a good repeatability (expressed as RSD %) in compost RM as the reference and MAE-AR values (Table 7).

3.2. *Efficiency of MAE-EDTA procedures on real soil samples*

EDTA, the most commonly used chelating and versatile mobilizing agent [42], is able to dissolve heavy metals with favorable results due to its ability of increasing water solubility of heavy

Table 7
Metal contents for compost RM and recovery rates (%) obtained by MAE-AR and MAE-EDTA methods, RSD % values are given in parenthesis (n=number of replicates).

	Elements	Compost samples (mg kg^{-1} ± sd)			Recovery (%)	
		Reference value $n=3$	**MAE-AR** $n=10$	**MAE-EDTA** $n=10$	**MAE-AR** $n=10$	**MAE-EDTA** $n=10$
Five country interlaboratory test results	Cd	0.42 ± 0.01	0.45 ± 0.02	0.43 ± 0.02	107.1(4.9)	102.4(4.1)
	Cr	89.1 ± 2.4	88.9 ± 6.3	3.99 ± 1.6	99.7(7.1)	4.48(41.0)
	Cu	56.0 ± 2.1	51.7 ± 5.8	33.3 ± 3.5	92.3(11.2)	59.5(10.4)
	Ni	61.9 ± 0.74	59.2 ± 3.8	10.1 ± 1.3	95.6(6.3)	16.3(13.0)
	Pb	38.7 ± 1.7	38.9 ± 5.1	36.2 ± 0.77	100.5(13.2)	93.5(2.1)
	Zn	193 ± 1.7	182.6 ± 6.2	147.7 ± 5.8	94.6(3.4)	76.5(3.9)
Internal test results		**Reference value** $n=4$	**MAE-AR** $n=10$	**MAE-EDTA** $n=10$	**MAE-AR** $n=10$	**MAE-EDTA** $n=10$
	As	3.06 ± 0.27	2.95 ± 0.18	1.12 ± 0.14	96.4(6.1)	36.6(12.7)
	Mn	1042.7 ± 10.9	1013.6 ± 28.7	1084.7 ± 30.1	97.2(2.8)	104.0(2.8)

Table 8
Comparison of element concentrations in soil samples obtained by AR and MAE-EDTA (mg kg^{-1}).

Elements	AR			MAE-EDTA			AR vs MAE-EDTA	
	Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Correlation (r)**	***t*-test**[b]
Cd (n:158)	0.017	2.91	0.39	0.02	2.85	0.43	0.963[a]	*a*
Cu (n:103)	20.5	462.7	82.5	5.76	434.9	61.2	0.994[a]	*a*
Pb (n:158)	1.50	71.8	25.5	1.49	70.2	23.0	0.960[a]	*a*

[a] Significant at the 0.01 probability level.

[b] Student's t-test (least significant difference) at $\alpha=0.05$ (a=no significant difference at $\alpha=0.05$, b=significant difference at $\alpha=0.05$).

metals [43]. EDTA forms stable complexes with the major ions Ca, Mg and also with heavy metals such as Cd, Pb, and Zn [44].

Our results indicate that MAE-EDTA of soil samples ($n=158$) yields the same concentrations of Cd, Cu and Pb in the determination of pseudo total contents as the conventional AR procedure (Table 8).

Pb is one of the most common anthropogenic contaminant [45]; therefore, it is crucial to find a fast and safe extraction method to determine Pb. Hong et al. [46] tested the extraction with EDTA and applied PACE, to determine Pb in contaminated soil. The results suggest that EDTA is primarily responsible for the chelating removal of Pb from soil. In our work, Pb extraction by MAE-EDTA has proved to be successful both for contaminated and uncontaminated, agricultural soils. By way of addition, to our knowledge, for both Cd and Cu, no data exist for pseudo total contents determined by EDTA-based (EDTA as an exclusive extraction agent) microwave extraction approaches. Regression analysis (Table 8) and highly linear relationships for Cd, Cu, and Pb are given in Fig. 2. For further validation of the analytical procedures, the linear regression was also proved by forcing the fit curve through origin. Consequently as it is seen from the results given in the appendix C, the slope of the fit curve came up to 1, and coefficients of determination became lower.

Comparative results for MAE-EDTA extraction and EDTA-extractable concentrations without microwave assistance, named "potentially bioavailable" of some exemplary elements are given in Fig. 3 to verify MAE-EDTA. Results from the Student's t-test verified that there are significant differences between EDTA-extractable concentrations and MAE-EDTA metal concentrations of Cd, Cu, Mn and Pb at $\alpha=0.05$. It could be deduced from this comparison that MAE-EDTA is sufficient to quantify not only the potentially bioavailable concentrations of Cd, Cu and Pb, but also the pseudo total concentrations of these metals in the soil samples by using minimal amounts of sample and reagent chemical. Düring et al. [47] also suggested that the results of EDTA extractable bioavailable metal concentrations mirrored the concentrations obtained by aqua regia digestion, yet released lower concentrations.

3.3. *Efficiency of MAE-EDTA on real compost samples*

The presence of contaminants in compost may constitute a danger to the environment, and it is the heavy metal content which is the main factor leading to restricted agricultural use of compost [48]. Therefore, in addition to soil samples, samples from compost were also analyzed as another solid environmental matrix.

MAE-EDTA gave same results as MAE-AR in the determination of pseudo total contents of Cd, Mn and Pb for the real compost samples ($n=27$) and no significant difference was determined for the investigated element concentrations (Table 9). Results from our study confirm the finding of Borkowska-Burnecka [49] who investigated the use of water, EDTA and HCl for decomposition and dissolution of plant material with MAE for trace metal determination and established that the use of EDTA provides an adequate determination of B, Cd, Ni, Pb, Sr and Zn in plant samples. Zhou et al. [50] used closed vessel microwave digestion system with combination of tetramethylammonium hydroxide (TMAH) and EDTA to improve the leaching of inorganic constituents from biological samples and reported that with the exception of Fe and Al, good recoveries were obtained for the elemental determination of the samples. Uchida et al. [51] also described the combined use of TMAH and EDTA for elemental determination of botanical samples and expressed that, due to excellent ability of EDTA to form stable complexes with many elements; the recoveries were improved by addition of EDTA.

In our study, highly linear relationships between MAE-EDTA and MAE-AR for Cd, Mn and Pb were found (Fig. 4). Same as the soil samples, the linear regression lines were forced through origin and the slopes approximated 1 for Cd, Mn, and Pb (see appendix D). MAE-EDTA was not applicable for determination of Cu in compost samples, therefore analytical and chemical

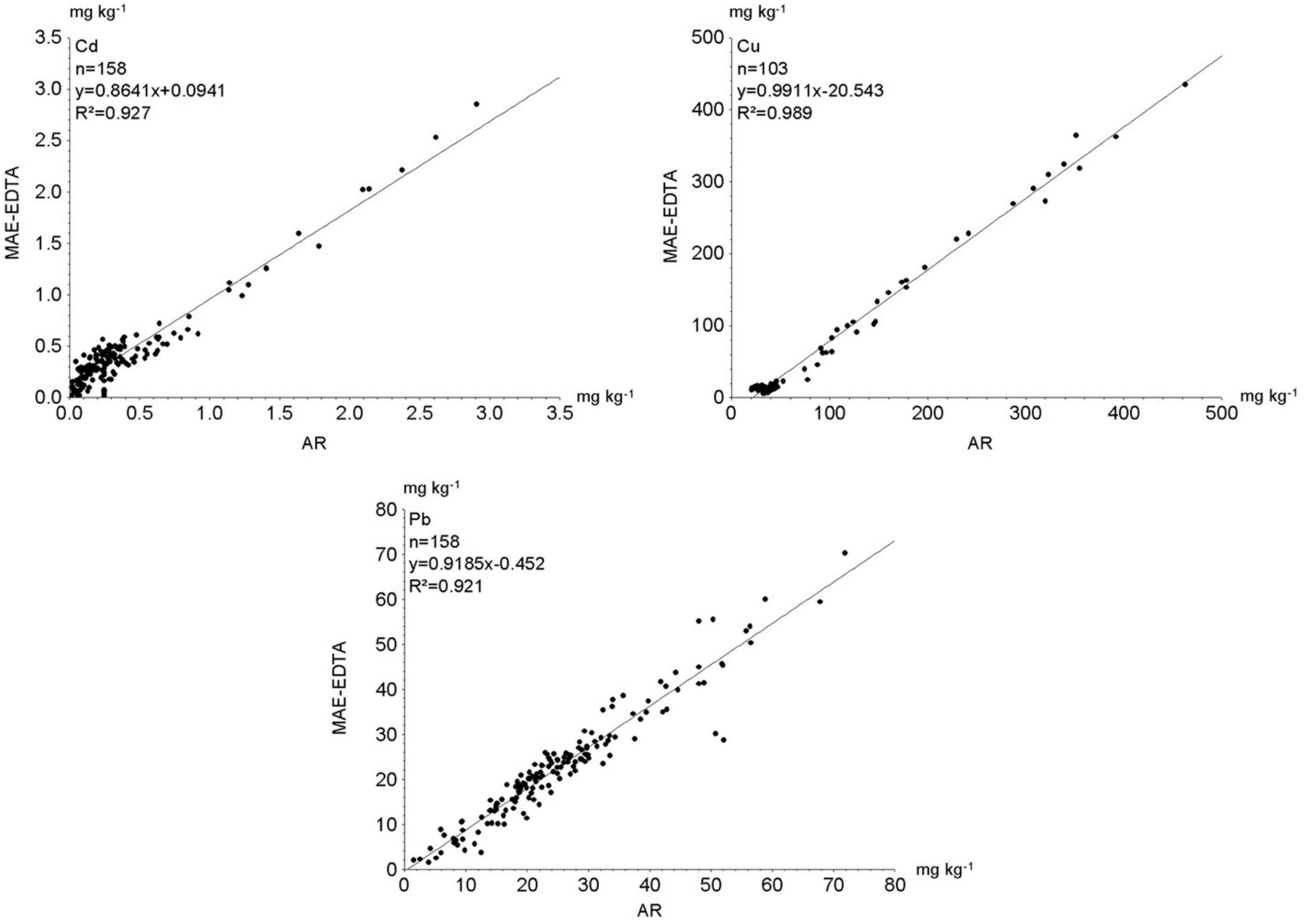

Fig. 2. Correlation between concentrations (mg kg^{-1}) of Cd, Cu, and Pb obtained by MAE-EDTA (*y-axis*) and by conventional AR method (*x-axis*) for soil samples ($y = a + bx$).

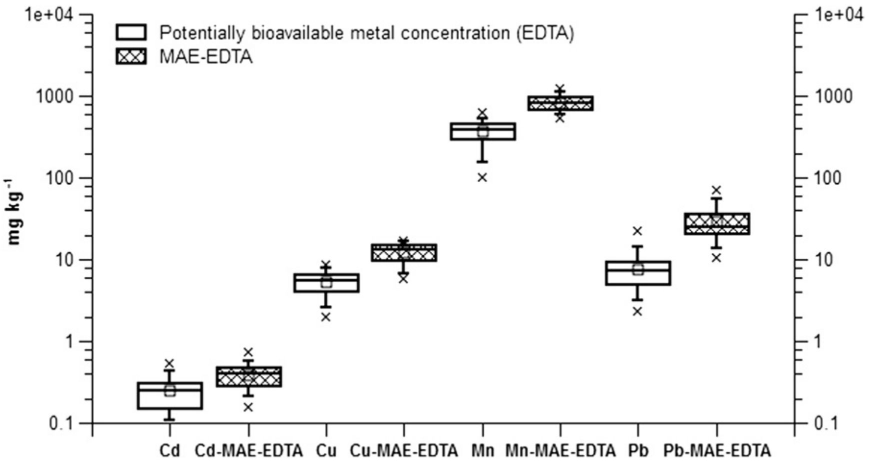

Fig. 3. Comparison of EDTA-extractable metal concentrations of Cd, Cu, Mn, and Pb in soil samples without microwave assistance and MAE-EDTA results ($n=68$).

parameters should be investigated for Cu in samples of compost. Low extraction efficiency may be obtained for certain metals due to the unsuitable element form for chelation [26].

4. Conclusion

On the basis of 158 soil and 27 compost samples and with soil CRMs and with an interlaboratory compost RM, it was shown that MAE-EDTA is a good alternative to both conventional AR and MAE-AR methods for determination of pseudo total Cd, Cu, Mn and Pb contents in soil and compost samples. Advantages include accuracy, reduced contamination, simple sample preparation and significantly less processing time. Total extraction time was reduced from 4 h to 48 min. The availability of MAE systems in all modern laboratories is another advantage of MAE-EDTA.

With our approach, the usage of large amounts of hazardous acids is avoided, whereas with conventional digestion agents such

Table 9
Comparison of element concentrations in compost samples (n=27) obtained by MAE-AR and MAE-EDTA (mg kg^{-1}).

Elements	MAE-AR			MAE-EDTA			MAE-AR vs MAE-EDTA	
	Min.	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**	**Correlation (r)**	***t*-test**[b]
Cd	0.22	1.10	0.43	0.23	1.33	0.50	0.982[a]	*a*
Mn	161.7	1284	723.8	148.8	1459	777.4	0.978[a]	*a*
Pb	2.88	63.9	36.3	0.92	55.5	32.9	0.974[a]	*a*

[a] Significant at the 0.01 probability level.

[b] Student's *t*-test (least significant difference) at α=0.05 (*a*=no significant difference at α=0.05, *b*=significant difference at α=0.05).

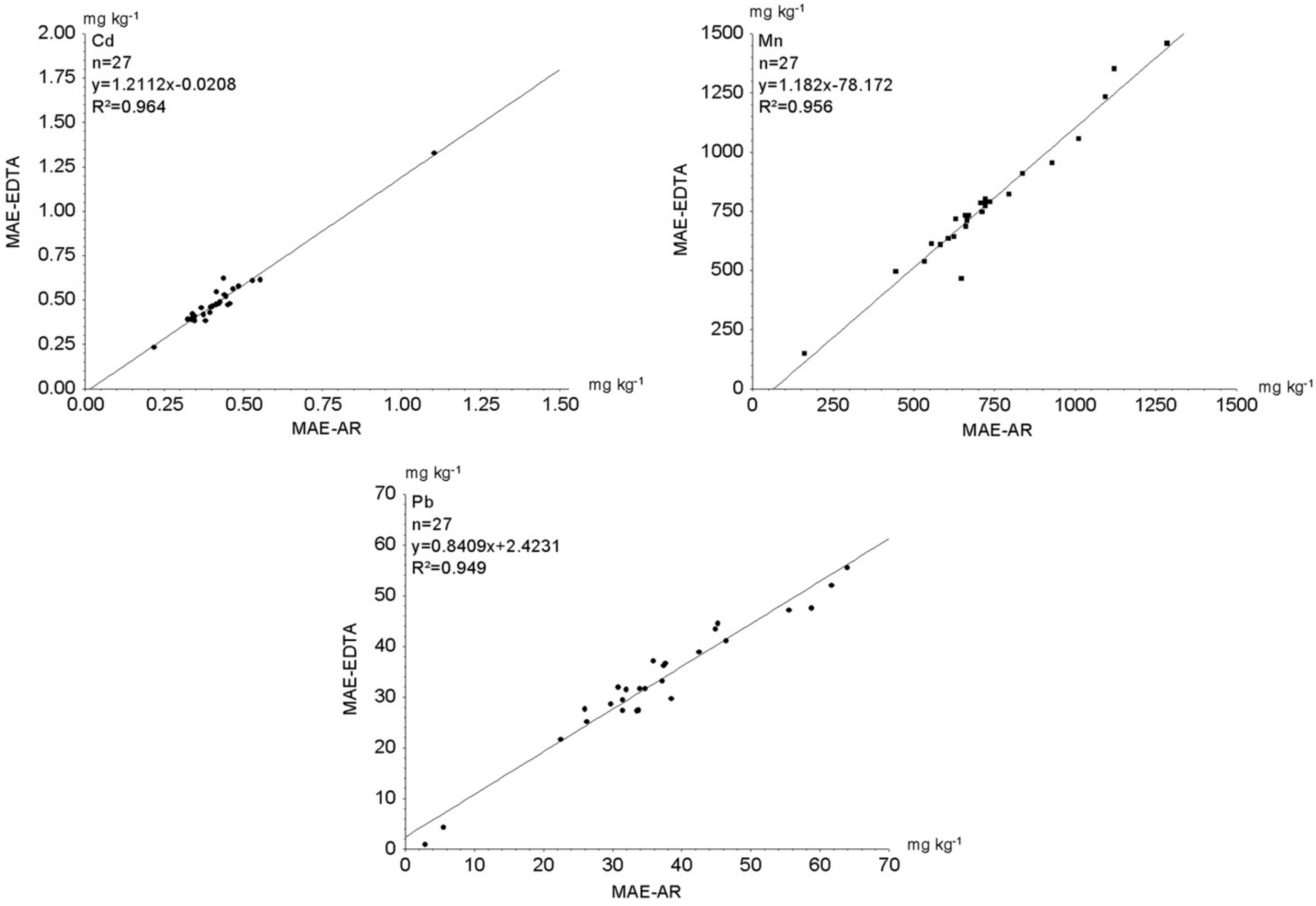

Fig. 4. Correlation between concentrations (mg kg^{-1}) of Cd, Mn, and Pb obtained by MAE-EDTA (*y-axis*) and by MAE-AR method (*x-axis*) for compost samples ($y = a + bx$).

as aqua regia and hydrofluoric acid safety hazards can be associated. Thereto, the use of large amounts of strong acids is contra indicated on environmental grounds. Using minimal amounts of sample and reagent chemicals is a strategic characteristic of the MAE-EDTA procedure presented; i. e. only 0.3 g of sample and 8 ml of extraction solution was needed.

MAE-EDTA gives approximate values in the ascertainment of pseudo total Cd, Cu, and Pb amounts in soil samples as the conventional aqua regia extraction. Additionally, pseudo total amounts of Cd, Mn, and Pb in compost samples were the same for MAE-EDTA and MAE-AR. MAE-EDTA may not be generally applicable for the other metals by reason of optimized conditions for the metals Cd, Cu, Mn and Pb. Therefore, the analytical parameters available in MAE-EDTA should be further investigated for the metals of interest. Additional investigations for MAE are planned to be enforced with other (biodegradable) metal chelating agents.

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Appendix A

See Fig. A1.

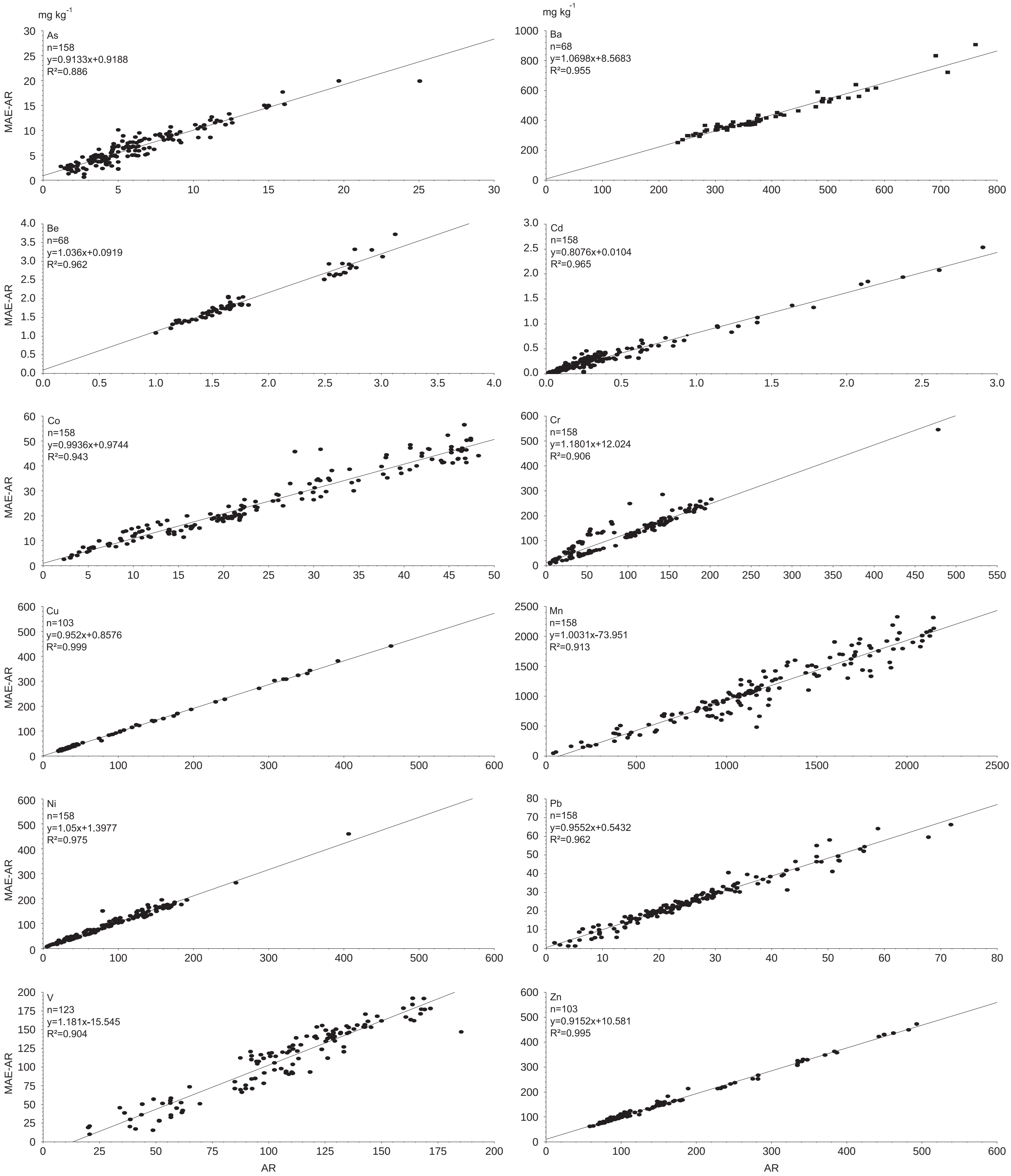

Fig. A1. Correlation between concentrations (mg kg^{-1}) of the 12 investigated elements obtained by MAE-AR (*y*-axis) and by conventional AR method (*x*-axis) for soil samples ($y=a+bx$).

Appendix B

See Table B1.

Appendix C

See Table C1.

Table B1
Element concentrations obtained by AR and MAE-AR methods in soil samples (mg kg^{-1}).

Elements	AR			MAE-AR			AR vs MAE-AR	
	Min.	Max.	Mean	Min.	Max.	Mean	Correlation (*r*)	*t*-test‡
As (n:158)	1.18	25.0	6.16	0.59	19.9	6.54	0.942**	a
Ba (n:68)	233.6	762.0	388.0	251.0	906.1	423.7	0.977**	a
Be (n:68)	1.00	3.12	1.80	1.08	3.72	1.95	0.981**	a
Cd (n:158)	0.017	2.91	0.39	0.017	2.53	0.33	0.982**	a
Co (n:158)	2.31	48.3	24.1	2.52	56.5	24.9	0.971**	a
Cr (n:158)	5.02	478.2	87.8	8.00	545.6	115.7	0.952**	b
Cu (n:103)	20.5	462.7	82.5	18.5	440.5	79.4	1.000**	a
Mn (n:158)	40.0	2149	1179	45.8	2325	1109	0.956**	a
Ni (n:158)	5.21	406.5	82.2	7.64	459.6	87.7	0.988**	a
Pb (n:158)	1.50	71.8	25.5	1.27	66.2	24.9	0.981**	a
V (n:123)	20.0	185.4	106.5	10.0	191.8	110.2	0.951**	a
Zn (n:103)	58.5	493.2	162.5	62.1	472.5	159.3	0.997**	a

** Significant at the 0.01 probability level.

‡ Student's t-test (least significant difference) at $\alpha=0.05$ (a=no significant difference at $\alpha=0.05$, b=significant difference at $\alpha=0.05$).

Table C1
Linear regression by forcing the fit curve through origin for conventional AR and MAE-EDTA for soil samples.

Elements	MAE-EDTA vs AR	
Cd	$y=0.95951x$	$R^2=0.89886$
Cu	$y=0.8857x$	$R^2=0.96211$
Pb	$y=0.9044x$	$R^2=0.92077$

Table D1
Linear regression by forcing the fit curve through origin for MAE-AR and MAE-EDTA for compost samples.

Elements	MAE-EDTA vs MAE-AR	
Cd	$y=1.1680x$	$R^2=0.963$
Mn	$y=1.0829x$	$R^2=0.948$
Pb	$y=0.8991x$	$R^2=0.944$

Appendix D

See Table D1.

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