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SHORT COMMUNICATION

COMPARISON OF TWO EXTRACTION PROCEDURES FOR DETERMINATION OF TRACE METALS IN SOIL BY ATOMIC ABSORPTION SPECTROMETRY

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The study compares nitric acid and ethylenediaminetetraacetic acid (EDTA) extraction of forest soils for the analysis of trace metals Fe, Mn, Zn, Cu, Pb, and Cd. Fifty forest soil samples from ten different locations were extracted using both methods. The two extraction procedures were compared through regression analysis for each element. All elements were analysed by flame atomic absorption spectrometry method (AAS). The results show that both extracting procedures are only partially effective for most of the measured trace elements in soil. The extraction with diluted HNO_3 was incomplete, as large amounts of siliceous material remained undigested. Recoveries obtained by this method for Fe, Mn, Zn, Cu, Pb, and Cd on certified reference material (San Joaquin Soil) were 34, 79, 47, 56, 71, and 102%, respectively. EDTA extraction was even less effective and is considered to reflect the quantity of bioavailable metals. The respective recoveries were 2, 45, 7, 20, 38 and 74%. The regression analyses performed for EDTA-extractable vs. HNO_3-extractable metals of forest soils showed high and significant correlation for all examined metals, except Fe.

Key words:
cadmium, copper, EDTA, HNO_3, iron, lead, manganese, zinc

Different extraction procedures are used to determine trace metals in soil. The most aggressive extraction procedures using mixtures of concentrated acids reflect the total concentration of inorganic elements. Other extraction procedures (like diluted acids, EDTA, and/or NH_4Ac) only partly extract metals from soil. Usually, EDTA/NH_4Ac reflect mobilisable metals which can be used and metabolised by plants (1, 2). Therefore, before the analyte is quantitatively determined, it is of utmost importance to know the percentage of extracted element and how reliable and reproducible the extraction method is.

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The aim of this study was to estimate the quantity of extracted trace elements from soil and to compare two extraction procedures, one with diluted nitric acid and the other with EDTA. The efficiency of extraction was determined by applying the same extraction procedures to certified reference standard for soil. The analysis of several trace metals was carried out by atomic absorption spectrometry (AAS).

MATERIALS AND METHODS

Reagents and equipment

Reagents used for the extraction and measurement were distilled nitric acid (65%, p.a. Kemika, Croatia), disodium ethylenediaminetetraacetic acid (EDTA) 0.05 mol/L (Kemika, Croatia), Spectrosol (BDH, Great Britain) standard metal solutions (Fe, Mn, Zn, Cu, Pb and Cd) of 1 mg/L, and water deionised by ion exchange to 0.06 μS/cm conductivity (Labconco, USA). Certified Standard Reference Material SRM 2709, that is, San Joaquin Soil, was provided by the National Institute of Standards and Technology (NIST, USA).

All the AAS measurements were carried out with Varian® Model AA 375 (Australia). Trace metals Fe, Mn, Zn, Cu, Pb, and Cd were determined in acetylene-air flame with deuterium background correction. Varian® Model SpectrAA-300 electrothermal AAS with pyrolytically coated graphite tubes was used to determine Cd in a Standard Reference Material (San Joaquin Soil). Table 1 shows the conditions for determination of the trace metals.

Table 1 *Experimental conditions for trace metal determinations by flame AAS*

	Fe	Mn	Zn	Cu	Pb	Cd
Wavelength (nm)	248.3	279.5	213.9	324.7	217.0	228.8
Slit width (mm)	0.2	0.2	0.5	0.5	1.0	0.5

Sample preparation

A total of 50 samples were collected from ten different forest locations (2–7 samples from each) in the Varaždin county in Croatia. About 1 kg of soil was taken from a depth of 0–10 cm. Samples were air-dried, ground, and sieved through a double aluminum mesh (∅ 1.2 and 0.8 mm). Each soil sample was extracted in duplicate. A composite sample of all 50 soil samples was also prepared, homogenised, and extracted by both methods.

Nitric acid extraction

Soil samples of 2.5 g were put into an Erlenmeyer flask and added 2.5 ml of concentrated HNO_3 and 5 ml of deionised water. Twenty-four hours later, redistilled water

was added to the mixture to reach 25 g. The extract was filtered through Whatman No. 42 paper. The reference material (0.5 g) was extracted with the same extractant volume in four replicates.

EDTA extraction

For EDTA extraction, soil (2.5 g) was shaken with 10 ml of 0.05 mol/L EDTA for 60 minutes on a rotary shaker. Subsequently, EDTA solution was added to the mixture to reach 25 g. The extract was filtered through Whatman No. 42 paper. The reference material (0.5 g) was prepared with the same extractant volume in four replicates.

Statistical evaluation

All results are presented in mg/kg of dry soil. To correlate two methods for soil extraction, we applied Pearson's correlation at $P<0.01$ and processed the data using Stat for Windows© and Microsoft® Excel.

RESULTS AND DISCUSSION

The first method used was the extraction with diluted nitric acid. Its efficiency was compared with the results of the same extraction procedure applied on Standard Reference Material, SRM 2709 (San Joaquin Soil). The second method was the EDTA extraction procedure which is commonly used to correlate soil extractable metals available to plants (3, 4). Plants make use of loosely bound trace metals, which is just a small portion of their total amount in soil. Of many chelating agents, EDTA proved to be the best extractant (5). Its major advantage is a very short time of sample preparation as compared to extraction with nitric acid.

Table 2 shows the results of the two extraction procedures applied on Certified Standard Reference Material for Fe, Mn, Zn, Cu, Pb, and Cd. The extraction with diluted HNO_3 was incomplete, as large amounts of siliceous material remained undi-

Table 2 *Concentrations of trace elements (mg/kg) in Standard Reference Material 2709, San Joaquin Soil, using various methods of extraction**

Element	Reference value	HNO_3 extraction	Recovery (%)	EDTA extraction	Recovery (%)
Fe	35000 ± 1100	11801 ± 499	34	711 ± 10.8	2
Mn	538 ± 17	429 ± 4.3	79	244 ± 5.22	45
Zn	106 ± 3	49.5 ± 2.56	47	7.92 ± 0.33	7
Cu	34.6 ± 0.7	19.4 ± 0.57	56	7.00 ± 0.07	20
Pb	18.9 ± 0.5	13.4 ± 0.66	71	7.27 ± 0.01	38
Cd	0.38 ± 0.01	0.386 ± 0.014	102	0.283 ± 0.03	74

*Values are expressed as arithmetic means ± standard deviations of four replicates.

gested. Recoveries obtained by this method for Fe, Mn, Zn, Cu, Pb and Cd on San Joaquin Soil were 34, 79, 47, 56, 71, and 102%, respectively. EDTA extraction proved even less efficient. The respective recoveries were 2, 45, 7, 20, 38 and 74%. The regression analyses performed for EDTA-extractable vs. HNO_3-extractable metals of forest soils showed high and significant correlation for all metals, except Fe (Figures 1–6).

The concentration of extractable Fe in composite soil was 5,907 mg/kg for HNO_3 extraction and 1,223 mg/kg for EDTA extraction (Table 3). When comparing 50

Table 3 *Concentrations of trace elements (mg/kg) in composite sample of forest soil from Varaždin county**

Element	Extraction	Mean ± SD	RSD (%)
Fe	HNO_3	5907 ± 281	4.8
	EDTA	1223 ± 60	4.9
Mn	HNO_3	347 ± 16	4.6
	EDTA	188 ± 11	5.7
Zn	HNO_3	24.7 ± 2.0	8.1
	EDTA	10.5 ± 0.45	4.3
Cu	HNO_3	6.18 ± 0.209	3.4
	EDTA	2.46 ± 0.15	6.1
Pb	HNO_3	36.7 ± 3.32	9.0
	EDTA	26.3 ± 1.23	4.7
Cd	HNO_3	0.450 ± 0.070	16
	EDTA	0.475 ± 0.047	22

*Each value of trace element concentration is the mean of 10 replicates
RSD – relative standard deviation

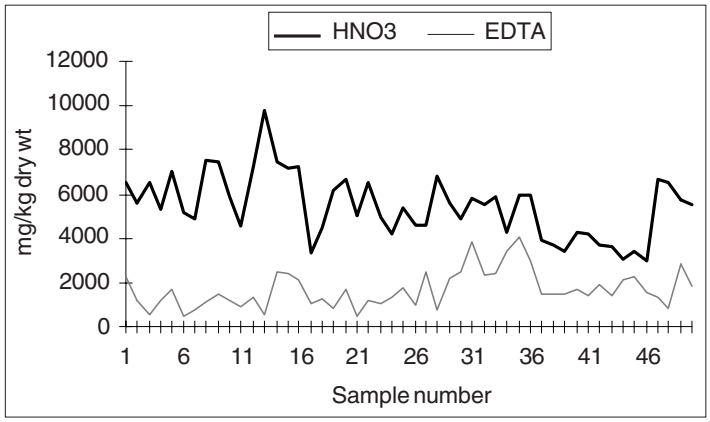

Figure 1 *Concentrations of extractable Fe using HNO_3 and EDTA in 50 soil samples from Varaždin county. Coefficient of correlation HNO_3/EDTA, r=0.076*

individual soil samples, Pearson's correlation factor was 0.076. In other words, the two extraction methods did not correlate (Figure 1). The reason is probably in the fact that Fe in soil mainly takes the oxide form and oxides are dissolved in nitric acid, but not in EDTA. The correlation for all other metals was very high and significant (Figures 2–6). All metal concentrations in HNO_3 extracts, except iron and zinc, were about 1.0–2.8 times higher than in EDTA extracts, both in the composite sample of collected soil and in the certified standard (Tables 2 and 3). These results indicate that the two

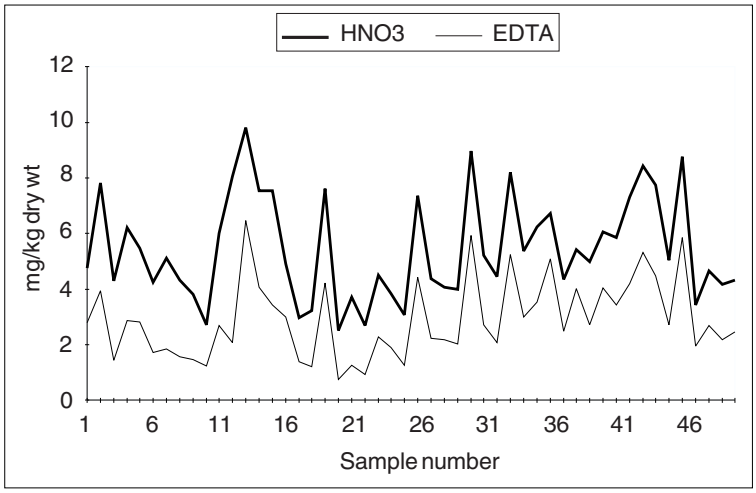

Figure 2 *Concentrations of extractable Mn using HNO_3 and EDTA in 50 soil samples from Varaždin county. Coefficient of correlation HNO_3/EDTA, r=0.958*

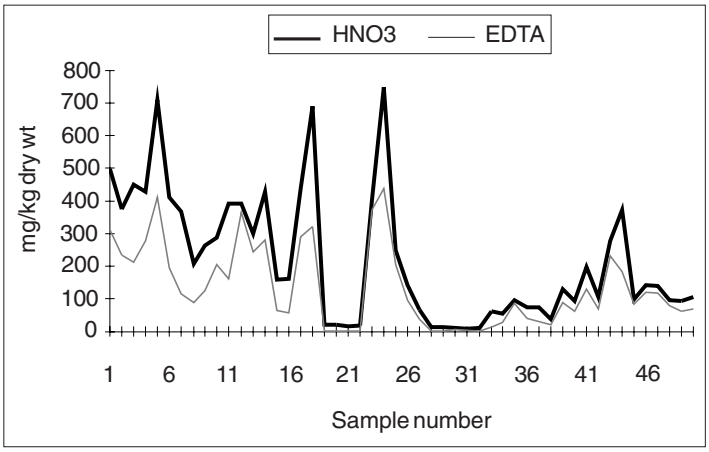

Figure 3 *Concentrations of extractable Zn using HNO_3 and EDTA in 50 soil samples from Varaždin county. Coefficient of correlation HNO_3/EDTA, r=0.779*

soils (Varaždin county and San Joaquin Soil) might have similar chemical composition and could be compared for validation of the extraction method. The within-day repeatability of extraction procedures for different metals, expressed as relative standard deviation, was between 3.4 and 16% for HNO_3 extraction and between 4.3 and 9.9% for EDTA extraction (Table 3).

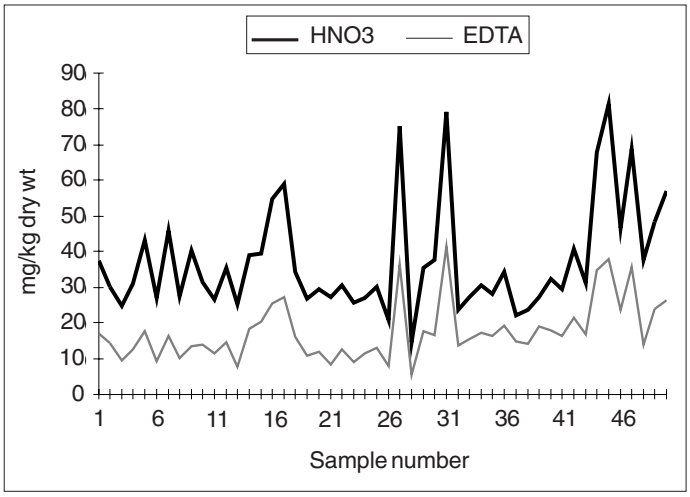

Figure 4 *Concentrations of extractable Cu using HNO_3 and EDTA in 50 soil samples from Varaždin county. Coefficient of correlation HNO_3/EDTA, r=0.909*

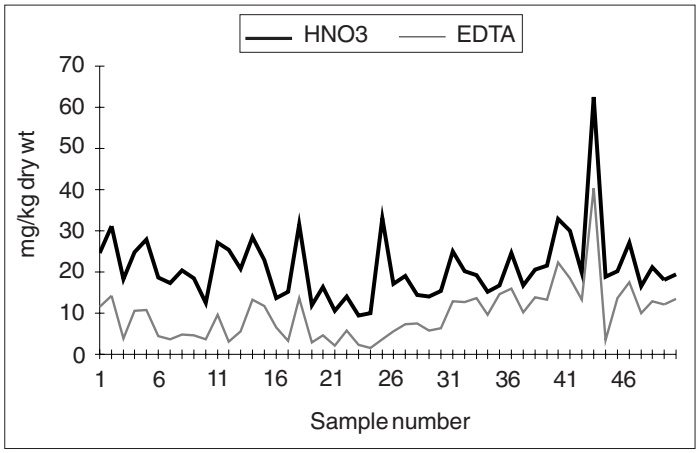

Figure 5 *Concentrations of extractable Pb using HNO_3 and EDTA in 50 soil samples from Varaždin county. Coefficient of correlation HNO_3/EDTA, r=0.936*

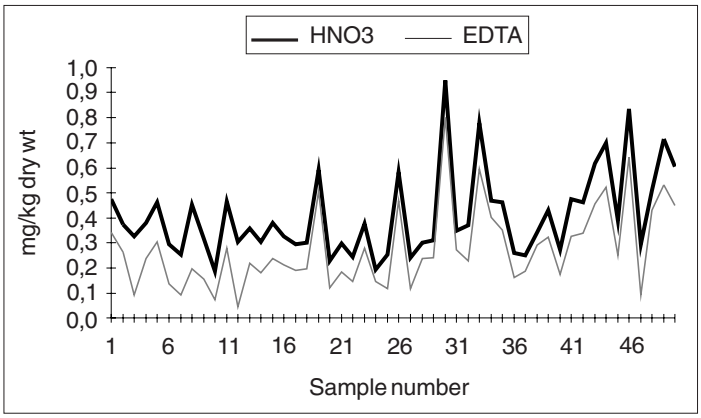

Figure 6 *Concentrations of extractable Cd using HNO_3 and EDTA in 50 soil samples from Varaždin county. Coefficient of correlation HNO_3/EDTA, r=0.959*

CONCLUSION

The results of this study on forest soils point out that the use of EDTA solution as extractant has at least two advantages. Extracts are not corrosive and there is no need for further dilution. Furthermore, short time of sample preparation makes this method very convenient and acceptable. Although the extraction with EDTA is incomplete, it highly correlates with the nitric acid method. The fact suggests that it is likely for the EDTA method also to correlate with the total amount of metals in soil. Since the concentration of metals extractable from soil with EDTA solution reflects the amount of metals which are available to plants, determination of trace metal bioavailability in soil should become quicker and relatively easy with this extraction method.

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Sažetak

USPOREDBA DVAJU POSTUPAKA EKSTRAKCIJE TLA PRI ODREĐIVANJU TRAGOVA METALA ATOMSKOM APSORPCIJSKOM SPEKTROMETRIJOM

Cilj rada bio je usporedba dvaju postupaka ekstrakcije šumskog tla za analizu Fe, Mn, Zn, Cu, Pb i Cd. Ekstrakcija dušičnom kiselinom provedena je s pomoću 20%-tne kiseline stajanjem preko noći na sobnoj temperaturi. U drugom postupku ekstrakcija s pomoću 0,05 mol/L otopine EDTA provedena je tijekom jednog sata na automatskoj tresilici. Kao standardni referentni materijal upotrijebljen je SRM 2709 – San Joaquin Soil. Svi metali, izuzev kadmij, u referentnom uzorku, određivani su metodom atomske apsorpcijske spektrometrije (AAS) plamena, a kadmij je određen metodom elektrotermalne AAS. Pedeset uzoraka šumskog tla skupljeno je na deset različitih lokacija Varaždinske županije. Uzorci su ekstrahirani s pomoću obje metode. Provedena je regresijska analiza između oba postupka ekstrakcije za svaki metal.
Rezultati pokazuju da obje metode samo djelomično ekstrahiraju većinu metala u tragovima iz tla. Dušičnom kiselinom kadmij se može potpuno ekstrahirati iz tla, dok je ekstrakcija ostalih metala nepotpuna. Iz standardnoga referentnog materijala ovom se metodom ekstrahira 34% Fe, 79% Mn, 47% Zn, 56% Cu, 71% Pb i 102% Cd. Ekstrakcija s pomoću otopine EDTA još je manje djelotvorna te se pretpostavlja da ekstrahirana količina metala znači sveukupnu biološku raspoloživost pojedinog elementa u tlu. Ovom metodom ekstrakcije dobiveno je 2, 45, 7, 20, 38 odnosno 74% od ukupne količine gore navedenih metala u tlu. Regresijske analize provedene za koncentracije metala dobivene EDTA ekstrakcijom šumskog tla prema koncentracijama metala dobivenim ekstrakcijom dušičnom kiselinom pokazuju visoku i značajnu korelacijsku povezanost za sve metale osim za željezo.

Ključne riječi:
bakar, cink, EDTA, HNO_3, kadmij, mangan, olovo, željezo

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