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Evaluation of Chelating Agents as Heavy Metals Extractants in Agricultural Soils under Threat of Contamination

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Abstract

Four chelating agents (HEDTA, EDTA, EGTA, and NTA) added to 1M NH_4OAc were compared in order to assess their ability for heavy metals extraction. NH_4OAc alone revealed the lowest efficiency of metals extraction than the remaining chelate-enriched extractants for extracting Cu, Zn, Mn, and Pb. Among chelating agents such as EDTA and HEDTA, solutions of NH_4OAc+EDTA and NH_4OAc+HEDTA were the most effective extractants. The percentage share of extracted Cu, Zn, Mn and Pb varied from 32% to 42% of their total content, irrespective of the added chelating agents and for NRtOAc from 13% to 20% for all metals. Minimizing the direct influence of the parameters set (i. e. extractant concentration, pH, soil/extractant ratio, extraction time) one may suggest that the slight differentiation in the amounts of extracted metals is probably a matter of chelating agent features. Correlations between amounts of all extracted metals and soil properties were relatively low, irrespective of the extractant used. This state may be explained by a prevailing anthropogenic origin of these metals (mainly Cu and Pb) in studied soils.

Keywords: Heavy Metals (Cu, Zn, Mn, Pb), NH_4OAc, EGTA, HEDTA, EDTA, NTA

Introduction

Evaluation of trace element availability to plants is increasingly an important objective in soil testing methods. This need has focused on investigations on the use of chelating agents, for example the DTPA (diethylenetriamine-pentaacetic acid) micronutrient soil tests [6, 12], and has led to a comparison of DTPA and other chelating agents as extractants for several trace metals such as Cu, Zn, Pb, Mn, and Fe [15]. The expression "bound in soil" and "fixed in soil" are concepts depending on the concentration and feature of the extractant used.

A comparison of the expected stability of a number of chelates under a variety of soil conditions was made by Sommers and Lindsay [20]. They reported that the common chelating agent EDTA (ethylene-diaminetetraacetic acid), HEDTA (hydroxyethylethylenediamine-triacetic acid), NTA (nitrilotriacetic acid), and EGTA [ethylene-glycol-bis(2-aminoethylether)tetraacetic acid] would have potential as chelating agents to extract microelements and heavy metals under most expected soil conditions. However the choice among the chelating agents is extremely difficult because they have usually been evaluated under widely incomparable conditions.

This paper is an experimentally based work aimed at an evaluation of chelating agents added to NH_4OAc in order to extract essential as well as toxic metals from soils. A comparison was also made with a chelate-free NH_4OAc commonly used for the determination of exchangeable and weakly bound forms of trace metals in soil.

Materials and Methods

Sixteen soil samples from arable soils were collected in the surroundings of the Glogow copper plant in order to include soil variety and also to represent a wide range of extractable metals. This area is located in the so-called sanitary zone at 700-800 m (South-East) from the main sources of emissions [18]. Soil samples were air-dried and crushed to pass through a 1 mm mesh sieve. The soil particle content was determined by the hydrometer method, organic carbon by Tiurin's method [22], and soil reaction

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(pH) in 0.01M $CaCl_2$ [17] using a glass electrode. Cation exchange capacity (CEC) by the Mehlichg$_{8.2}$ method in Kociatkowski and Ratajczak [10] modification. Some soil physicochemical properties are listed in Table 1.

Table 1. Physical and chemical properties of the studied soils (n = 16).

Statistical summary	Organic carbon (g/kg)	Clay (g/kg)	CEC* (cmol$_c$/kg)	pH (0.01 M $CaCl_2$)
Mean	11.1	250	10.7	–
Minimum	3.0	110	7.9	4.9
Maximum	19.6	480	21.3	7.6
Coefficient of variation (CV %)	37	42	34	–

* CEC - cation exchange capacity

All chelating agents (EDTA [5] with modification, EGTA, HEDTA, and NTA) were prepared to give a concentration of 0.005M of a given reagent. They were buffered by a mixture of 1M of acetic acid and ammonium acetate. A chelate-free extractant, i.e. 1M NH_4OAc (acid ammonium acetate solution), [11] was also prepared and considered as standard. The final solution pH of all extractants was carefully adjusted to 4.65 with diluted CH_3COOH or NH4OH. Table 2 illustrates some extractant features and extraction procedures used.

The total metal content was extracted in an "Aqua regia" (A.r.) (HCl/HNO_3; 3:1 ratio) concentrated mixture by heating 2g of soil in 30 cm^3 of A.r. Metals in all chelating extractants were extracted at the ratios of 3 g in 15 cm^3 of appropriate extractant. The extraction time (shaking) lasted one hour and after an overnight decantation extracts were filtered and analyzed for Cu, Zn, Mn and Pb contents by AAS method (Atomic Absorption Spectrophotometry, Varian Spectra 250 plus). Statistical estimations and other computations were made by using Statgraphics (ANOVA) procedures.

Results and Discussion

Extractants were compared according to the amount of each metal extracted. Despite differences in pH, texture, organic matter content and total extractable metals among the 16 studied soils, the relative ranking of extractants was somewhat variable. Consequently, the results are summarized on the basis of means, medians, and ranges of amounts given in Table 3. Expression in percentage of amounts of extracted metals allowed us to set a better estimation of the ability of the extractants to extract the investigated metals.

Table 3. Evaluation of Cu, Zn, Mn, and Pb extractability by different chelating agents and a chelate-free extractant (n = 16).

Extractant*	Statistical summary			
	Metal	Mean	Median	Min. – Max.
Aqua regia	Cu	86.0	28.5	22.3 – 334.0
	Zn	45.2	39.3	26.3 – 90.6
	Mn	311.9	299.2	111.0 – 960.0
	Pb	52.8	36.1	15.2 – 185.6
HEDTA	Cu	36.2	4.7	2.5 – 142.5
	Zn	14.8	12.5	6.2 – 34.3
	Mn	104.7	98.7	55.1 – 220.3
	Pb	20.4	8.9	2.5 – 103.8
EDTA	Cu	34.4	6.5	2.1 – 145.0
	Zn	14.9	11.9	5.5 – 35.1
	Mn	109.3	105.3	52.4 – 262.0
	Pb	20.9	6.5	1.8 – 118.5
EGTA	Cu	33.9	4.6	2.4 – 157.0
	Zn	14.3	11.8	6.7 – 30.0
	Mn	109.0	98.0	52.6 – 200.0
	Pb	16.5	9.2	2.2 – 95.0
NTA	Cu	28.8	4.7	2.6 – 141.5
	Zn	14.6	11.7	6.7 – 31.8
	Mn	108.7	104.3	50.7 – 204.5
	Pb	18.5	8.3	2.5 – 111.5
NH_4OAc	Cu	14.8	2.0	0.9 – 84.9
	Zn	6.1	5.4	1.4 – 12.9
	Mn	63.5	56.8	38.5 – 135.8
	Pb	7.1	3.4	1.4 – 33.7

* For more details. see "Materials and Methods"

Table 2. Chemical characteristics of extractants used in the work.

Feature	Aqua regia	HEDTA* + NH_4OAc	EDTA + NH_4OAc	EGTA + NH_4OAc	NTA + NH_4OAc	only NH_4OAc
Concentration (Mole)	–	0.005 +1.0	0.005 +1.0	0.005 +1.0	0.005 +1.0	+1.0
pH	–	4.65	4.65	4.65	4.65	4.65
Time (h)**	2	1	1	1	1	1
Ratio***	1:15	1:5	1:5	1:5	1:5	1:5

- The concentration of 0.005M was set for all chelating agents + NH_4OAc (+1.0): means 1M NH_4OAc
- ** Extraction time in hours
- *** Soil / Solution ratio

Extraction of Cu

Comparison of the results for Cu showed some dispersions in the amounts of metals extracted by the chelate-free NH$_4$OAc and with added chelating agents. Among the chelating extractants NT A extracted 33% of total Cu content whereas an average of 40% of total Cu was found for HEDTA, EDTA and EGTA being attributed to the very similar pK values of these three chelating agents, [13]. NH$_4$OAc was clearly differentiated from other extractants because it extracted only 17%. These values are graphically presented in Figures 1, 2, 3, 4, and 5. On this basis extractants may be ranged as follows:

HEDTA (42%) > EDTA (40%) ≈ EGTA (39%) >
> NTA (33%) > NH$_4$OAc (17%) (1)

Fig. 1.

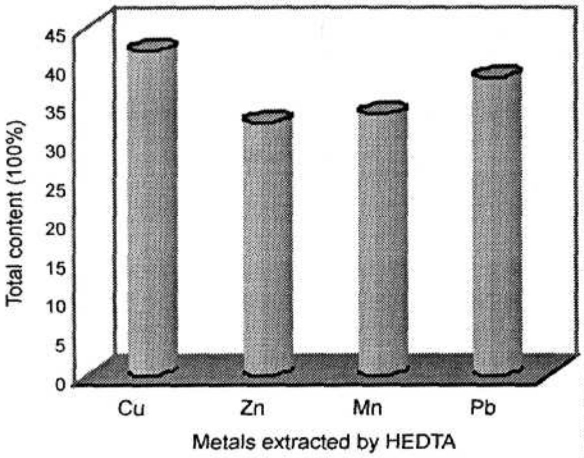


Fig. 2.

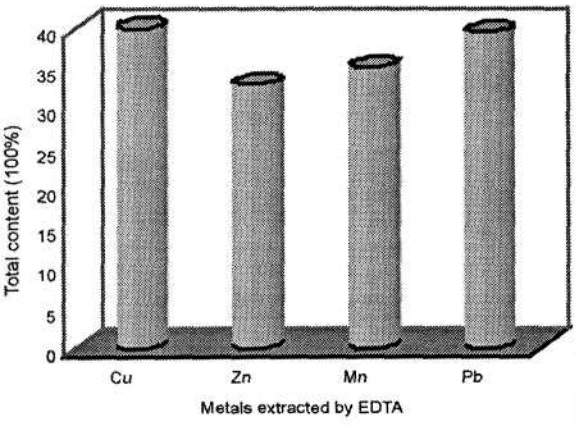


Fig. 3.

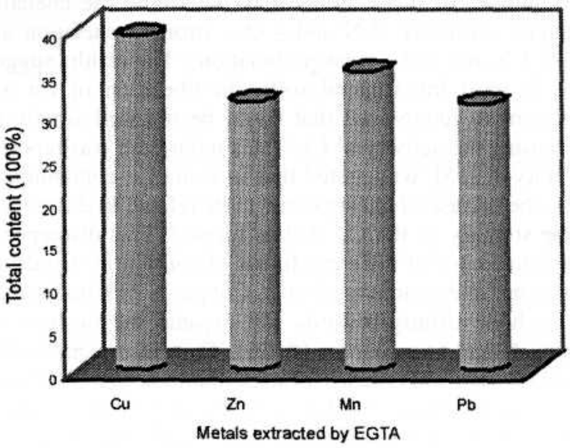
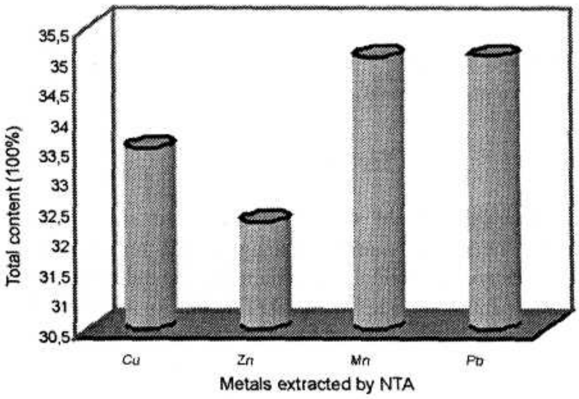


Fig. 4.



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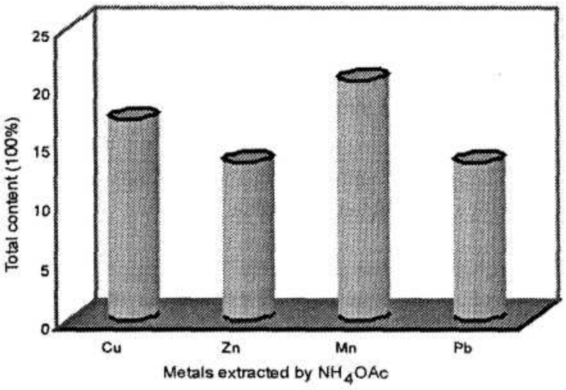


Figs. 1, 2, 3, 4, 5: Illustration of the percentage share of Cu, Zn, Mn, and Pb extracted by HEDTA, EDTA, EGTA, NTA, and NH$_4$OAc in their total content (Aqua regia - 100%).

Chelating agents are involved in metal complexation by providing N (nitrogen) and O (oxygen) atoms as donors. The number of these atoms may determine the chelating strength, (HEDTA: 2 N and 4 O - stronger chelation and NTA: 1 N and 3 O - weaker chelation). The results suggest that, in most investigated soils, the chelation of Cu was sufficient to remove all that could be released simply by decreasing the activity of Cu^{2+}. A similar case was reported by Norvell [15], who stated that in some Cu-contaminated soils, the release of Cu appeared to be related to differences in the stability of the Cu chelate formed. This discrepancy may be a proof of different forms of soil Cu in metal-free and/or metal-contaminated soils. Copper is reported to present a high affinity towards soil organic substances containing N and O as donors [8, 16]. Then in organic matter relatively rich soils the use of ammonium acetate for assessing even exchangeable forms of Cu may lead to an underestimation of the amounts of Cu being extracted. Correlations among the amounts of extracted Cu for chelating agents were excellent, ($r > 0.96$, $P < 0.05$). Only EGTA showed a high relationship with NH_4OAc ($R^2 = 0.94$), as illustrated in Fig. 6. None of the extractants strongly correlated with soil physicochemical properties (except for HEDTA), but the value of the coefficient of determination was low:

$$\text{Cu-HEDTA} = 66.3 - 14.0\ \text{pH} - 4.2\ \text{CEC} + 5.8\ \text{Corg} - 0.11\ \text{Clay}, \quad R^2 = 0.23\ (P < 0.05) \qquad (2)$$

Extraction of Zn

Extractable Zn of the 16 soils varied slightly (about 15.0 mg Zn/kg) between chelating agents. Notably less Zn was extracted by NH_4OAc indicating its relative ineffectiveness (Table 3). HEDTA, EDTA, NTA, and EGTA extracted on average 33% of total Zn, whereas NH_4OAc alone extracted 14% (Figures 1, 2, 3, 4, 5). Apparently, each of these chelating agents chelated Zn well enough to extract all the Zn that could be in soil solution or in exchangeable forms. Some reports have focussed on the moderate ability of Zn to be bound strongly in soil leading to a possible high Zn extractability [3, 14]. Furthermore, it is worth noting that Zn levels in the soil samples are not high, so weakly-bound forms will be insufficient. This could be more or less expected since the amounts of Zn extracted by NH_4OAC remained very low. Among all extractants (including the chelate-free one) only the amounts of Zn extracted by NTA showed the highest correlations with all soil physicochemical properties:

$$\text{Zn-NTA} = 16.4 - 3.1\ \text{pH} + 0.7\ \text{CEC} - 0.1\ \text{Corg} + 0.03\ \text{Clay}, \quad R^2 = 0.71\ (P<0.05) \qquad (3)$$

Fig. 6.

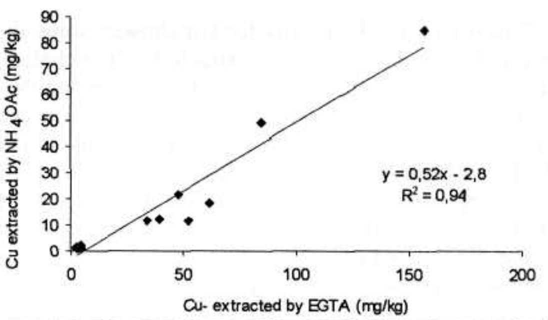


Fig. 7.

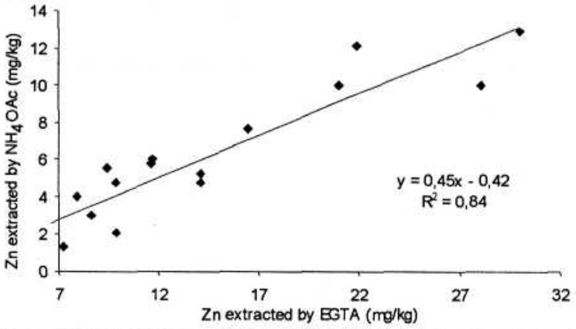


Fig. 8.

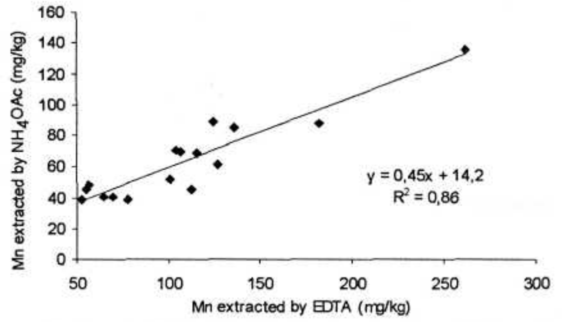
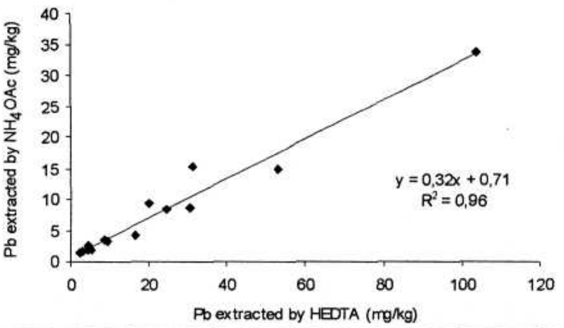


Fig. 9.



Figs. 6, 7, 8, 9: Realtionships between amounts of Cu, Zn, Mn, and Pb extracted by chelating agents and by NH_4OAc ($n = 16$).

The exclusion of the parameter "Clay" improved neither simple correlation values for all extractants nor the multiple regression ones, even a relatively important variation in soil clay content (coefficient of variation 42%). This may suggest a negligible effect of clay on Zn extraction by chelate-free NH_4OAc as well as with chelating agents. The best linear fit for Zn extracted by chelating agents was found for EGTA and NH_4OAc with a coefficient of determination of $R^2 = 0.84$, (Fig. 7). All extractants on the basis of the percentage share of extracted Zn may be ranked accordingly:

$$\text{NTA}\ (33\%) = \text{EDTA}\ (33\%) = \text{HEDTA}\ (32.9\%) \approx \text{EGTA}\ (31.8\%) > NH_4OAc\ (14\%) \qquad (4)$$

Extraction of Mn

Chelating agents extracted almost similar amounts of Mn (about 106.0 mg Mn/kg) representing only 34% of total soil Mn. In the case of NH_4OAc this share averaged 20%, (Table 3 and Figs. 1, 2, 3, 4, 5). Soils used in the present work were almost neutral to moderately basic (except one with pH 4.9). This could partly explain the lower efficiency of all extractants for Mn extraction, since this metal easily forms hydroxides even at moderately neutral soil reaction [9]. It is also important to point out the pK values of some of these chelating agents (HEDTA, pK = **11.4;** EGTA, pK = 13.88). The slight differences, mainly for extractants with chelating agents, may suggest that amounts of Mn reflect the ease with which Mn is released from soils. Figure 8 illustrates only the relationship between EDTA and NH_4OAc since the remaining chelating agents gave lower values of the coefficient of determination ($R^2 < 0.70$). Neither EDTA, EGTA, NTA nor NH_4OAc satisfactorily correlated with soil properties. The multiple regression analysis showed a relatively high influence of the latter ones on Mn extraction by HEDTA:

$$\text{Mn-HEDTA} = 92.0 - 17\ \text{pH} + 3.7\ \text{CEC} - 0.4\ \text{Corg} + 0.22\ \text{Clay}, \quad R^2 = 0.85\ (P < 0.05) \qquad (5)$$

On the basis of the percentage share, extractants may be ranged as follows:

$$\text{EDTA (35\%)} = \text{EGTA (35\%)} = \text{NTA (35\%)} \approx \text{HEDTA (34\%)} > NH_4OAc\ \text{(20\%)} \qquad (6)$$

Extraction of Pb

Lead in comparison with Cu, Zn and Mn is considered a naturally toxic metal. EDTA, HEDTA, and NTA extracted on average 19.0 mg Pb/kg; EGTA was about 16.5 mg Pb/kg. As could be expected the lowest Pb level (7.0 mg/kg) was found for NH_4OAc. In this work it was included in order to make a general evaluation in terms of its physicochemical characteristics as a metal. As listed in Table 3, one may observe the following percentage range of chelating agent ability for Pb extraction.

$$\text{EDTA (39.5\%)} = \text{HEDTA (39\%)} > \text{NTA (35\%)} > \text{EGTA (31\%)} > NH_4OAc\ \text{(13\%)} \qquad (7)$$

In terms of ability for Pb extraction, EDTA and HEDTA were found to be the most efficient (about 39% of total Pb), whereas NH_4OAc (13%) was less so, (Table 3 and Figs. 1, 2, 3, 4, 5). Our results showed that these two chelate-enriched extractants can be permutated satisfactorily. Amounts of Pb extracted by all chelating agents were among them highly correlated ($r > 0.90$, $P < 0.05$). But in the case of their relationship with the chelate-free extractant only HEDTA-NH_4OAc gave a coefficient of determination $R^2 > 0.95$ (Fig. 9). Amounts of Pb extracted were poorly correlated with soil physicochemical properties, irrespective of the type of extractant. A similar case was reported by Diatta et al. [4], who found a poor relationship between Pb extracted by HCl and HNO_3 and physicochemical properties. The multiple regression analysis established for EDTA gave the best fit, as shown below:

$$\text{Pb-EDTA} = 95.0 - 15.0\ \text{pH} - 2.0\ \text{CEC} + 2.08\ \text{Corg} + 0.05\ \text{Clay}, \quad R^2 = 0.67\ (P < 0.05) \qquad (8)$$

Extraction methods are mainly based on the ratio of a given reagent (extractant) to a given weight of soil sample, and on the chemical feature of the extractant, i. e. with or without chelating agent. Some extractants commonly extract a wide range of soil elements while others are applied for a single element (fosfonic complexones).

Such possibilities have been earlier presented by Combs et al. [2] for extraction of Cd, Cu, and Zn (at different soil/solution). Baron [1] with ammonium acetate (20 g) + ammonium sulphate (66 g) + acetic acid (62.5 g) + water up to 1000ml (pH 4.0) extracted B, Fe, Co, Cu, Mn, Mo and Zn (at soil/solution of 1:25). Chelating agents were widely applied by Norvell [15] to extract Al, Cu, Ni, Fe, Zn, Mn (at soil/extractant of 1:5).

On the other hand extractions were performed for single elements as well. For example Stewart and Berger [21] used 2N $MgCl_2$ (at soil/extractant of 1:5) to extract only Zn. In the case of Cu a 0.43N $HN0_3$ was applied by Henkens [7] and a chelating agent (0.05N EDTA + NH_4Ac; pH 7.0) was introduced by Viro [24] both (at soil/extractant of 15:25) for Cu extraction.

Summary

On the basis of the views presented, one has to take into account the fact that extraction methods vary depending on the conditions and aim. All these methods are partly experimental. Our results showed the importance a comparison of chelating agents on at least three parameters (i. e.: extractant concentration, pH, soil/extractant ratio, extraction time). The availability to plants of soil elements is ruled by several natural factors such as soil reaction, soil physicochemical properties, temperature, and climate. Furthermore, one chelating agent feature is the possibility to chelate metals in solution, avoiding metal resorption by soil organo-mineral particles. Another advantage of chelates over most and neutral salts is that the pH of the extracting medium can be maintained more closely to the normal pH of soils. Undesirable side reactions can be avoided and the extraction procedures can be made more selective for specific elements using complexones [19, 23]. Results of this investigation present a practical approach and may help somewhat in choosing a chelating agent for the extraction of a given metal or group of metals. Furthermore the fractions of metals being extracted may be assigned as "potentially" plant available.

Conclusions

1. The amounts of Cu, Zn, Mn and Pb extracted by chelating agents were not so notably differentiated among the respective extractants.
2. The percentage share of extracted Cu, Zn, Mn and Pb varied from 32 to 42% of their total, irrespective of the chelating agents and for chelate-free extractant NH_4OAc from 13 to 20% for all metals.
3. The best chelating agent for extraction of all metals was EDTA. Copper and lead were both satisfactorily ex tracted by HEDTA as well as EDTA.

4. The amounts of all extracted metals may be attributed more to chelating agents feature than to the soil/extractant ratio, extraction time, extractants concentrations, and extractant pH.

5. Correlations between amounts of all extracted metals and soil properties were relatively low, irrespective of the extractant used. This is due to the prevailance of anthropogenically contaminated soils.

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