

APPLICATION OF ISOTOPIC DILUTION AND SINGLE-STEP EXTRACTIONS FOR LABILE SOIL ZINC DETERMINATION

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Abstract: Concentration of available zinc from soils is the primary concern in assessment of its toxicity or essentiality for plants. This study evaluates the changes in chemical extractable Zn from three Slovak typical soils with simultaneous extractions as tools of zinc bioavailability. We found out that extractability of binding zinc decreased in order Na₂EDTA, Mehlich 3, Mehlich 2, NH₄NO₃ and CaCl₂ for all soil samples. Using flow-through stripping chronopotentiometry (SCP) and atomic absorption spectrometry (GFAAS) we found out that maximum of soil zinc was removed by organic ligands. Lability of Zn determined by isotopic dilution method using ⁶⁵Zn and γ -spectrometry showed the significant decrease of isotopic exchangeable zinc fraction (*E*-value) with decrease of soil reaction. Obtained *E*-values of uppermost soil horizons showed the zinc lability ranged from 20 to 39%. Our research confirmed the effect of soil reaction, composition and physico-chemical characteristics to Zn lability. For further assessment of zinc bioavailability is needed to find the correlation and effects of structural changes and aging in studied soils.

Key words: soil, Zn, bioavailability, extraction, *E*-value

1. Introduction

Zinc is an essential trace element for the biological systems and is getting into the soil weathering of minerals and rocks forming the subsoil of the environment. Major form is divalent ion Zn²⁺ that is involved into sorption interactions of individual soil components. The increased concentrations of zinc can be potential risk of phytotoxicity. Many elements involved as trace elements at higher than optimal concentrations can be toxic (FRIŠTÁK *et al.*, 2013). Soil contamination is not directly limited but can be reasonably associated with the negative anthropogenic effects as one of the major environmental problems. Generally, the mobility of trace metals in soils is a function of its chemical forms (VIJVER *et al.*, 2003). Bioavailability may be defined as a fraction of metal that is available or can be made available for uptake of organisms (VAN GESTEL, 2008). Mobility and bioavailability of heavy metals in soil depend on their speciation or fractionation. LUOMA and RAINBOW (2005) showed factors of metals bioavailability such as soil characteristics, routes exposure and physiological attributes. Bioavailability can be also affected by the application of fertilizers and soil conditioners, which can significantly change the soil reaction (pH), thus uptake of element by plants acceptors. Generally, the higher value of pH of the soil can cause more mobile and accessible elements forms for plant uptake (FRIŠTÁK *et al.*, 2012).

As a tool for estimation and determination of bioavailable metals fraction and metal fractionation in soils are commonly used simultaneous and sequential extraction

protocols as parts of fraction analysis. Non selectivity of extracting agents and redistribution of elements among phases during extraction are disadvantages of these methods (TESSIER *et al.*, 1979). Despite a weak selectivity, chemical extraction methods are still often used to provide an understanding of mobility and bioavailability of metals (HULLEBUSCH *et al.*, 2005). Direct methods have generally insufficient sensitivity for metal speciation analysis and give the quantity of metal ions in solution together with those in the solid phase which are in equilibrium with the dissolved metal ions. Therefore, determination of exchangeable metal fraction (*E*-value) represents quantification of ion pool from which ambient living organisms may take up the metal (STERCKEMAN *et al.*, 2009).

The main aim of our paper was to describe distribution, leaching and bioavailability of zinc in soil samples from three typical Slovak areas by series of simultaneous extraction protocols. For obtaining of relevant data and comparing the method the isotopic dilution with ^{65}Zn was used.

2. Materials and method

2.1 Experimental

Available fractions of zinc in samples of soil extract were measured using flow-through stripping chronopotentiometry. Experimental measurements were carried out on electrochemical analyser EcaFlow model GLP 150 (Istran, Ltd., Bratislava, Slovakia) equipped with electrochemical cell of type 104 with auxiliary, Ag/AgCl reference and E-104L graphite porous working electrodes. Used parameters are given in Table 1. Obtained results were compared with reference values of GFAAS measurements by atomic absorption spectrophotometer Shimadzu AA-7000 (USA). For correction of background D2 lamp was used, after microwave digestion of the samples by the Multiwave system MW 3000 (Anton Paar GmbH, AUS). To calculate bioavailable zinc and obtain parameters of Paired *t*-test, ORIGIN 8.0 Professional (OriginLab Corporation, Northampton, USA) was used.

Table 1. Operation parameters of electrochemical determination of zinc by galvanostatic stripping chronopotentiometry analysis (SCP).

Parameter	Unit	Value
Deposition potential	mV	-1800
Quiescence potential	mV	-1800
Quiescence time 1	s	5
Quiescence potential 2	mV	-1400
Quiescence time 2	s	30
Terminal potential	mV	100
Stripping current	μA	200
Stand by potential	mV	0

2.2 Reagents and Solutions

All the chemicals used were of analytical reagent grade. Deionized water ($<0,05 \mu\text{S}/\text{cm}$) prepared by Simplicity 185 (Millipore, France) was used for the

preparation of all solutions. For electrochemical analysis, carrier electrolyte R-008 (0.01 mol/L CH_3COOH + 0.01 mol/L CH_3COONa + 0.2 mol/L NaCl) and background electrolyte R-013 (0.1 mol/L HCl) were used. The bulk standard solution and calibration solutions of zinc were prepared in the background electrolyte from Certified Reference Material (999 mg/L ZnNO_3 , Sigma-Aldrich, Germany).

2.3 Sampling and Sample preparation

Three typical Slovak soil types were obtained from localities Jaslovské Bohunice (JB, Calcic Phaenozem), Sihla (S, Haplic Cambisol) and Borský Mikuláš (BM, Haplic Arenosol). The related sampling sites are shown in Figure 1. The samples were collected from horizon in depth of 0-20 cm. Before the subsequent analytical procedures the soil samples were dried (at 22 °C), homogenised and sieved through a 2 mm sieve. Samples were stored at dark and dry place.



Fig. 1 Sampling sites of investigated soils.

2.4 Physico-chemical characterisation of soil samples

For actual soil pH, 5 g of air-dried soil samples was allowed to equilibrate in 25 ml of deionised water. Flask was agitated on reciprocal shaker (150 rpm) for 1 h. To determine potential soil pH, 25 ml of 1 mol/L KCl was added to 5 g of air-dried soil samples and allowed to equilibrate for 1 h. After 1 h consolidation, pH values of supernatants were measured using a pH glass electrode (InoLab pH/ION/Cond 750, WTW, France). Total carbonate content in soil samples was obtained by lime-analyser of own construction (GABLOVIČOVÁ *et al.*, 2012). The cation exchange capacity (CEC) of samples was determined according to the STN ISO 11260 modified by FRIŠTÁK *et al.* (2013). Soil samples were suspended (83.3 g/L) in 0.1 mol/L BaCl_2 and mixed on a laboratory shaker for 1 h at 22°C and 150 rpm. Phases were separated after centrifugation (5 min at 5000 rpm). This procedure was repeated twice. The next

step of CEC determination consisted of suspending of soil sediments in 3 ml of 0.025 mol/L BaCl₂ and agitation for 19 h at 22°C. After phases separation, 3 ml of 0.02 mol/L MgSO₄ was added to sediments. After agitation (19 h at 22°C), centrifugation and phases separation, CEC by standardized 0.02 mol/L Na₂EDTA was determined. The CEC values were determined by chelatometric determination of Mg²⁺ ions in liquid phase and calculated according equation:

$$CEC = \frac{(M_0V_0 - MV_v)\epsilon}{10^{-3}} \quad (1)$$

where CEC is cation exchange capacity (meq/100g), M_0 is molar concentration of magnesium added to the sample (mol/L), V_0 is volume of solution of magnesium added to the sample (L), M is molar concentration of the magnesium in the leachate (mol L⁻¹), V_v is volume of obtained extract (L) and ϵ is the conversion factor that has for the bivalent ions and amount 0.25 g of soil value 800 meq (100 g/mol).

2.5 Total (aqua regia extractable) Zn fraction

Pseudo total soil content of zinc was estimated by aqua regia digestion (3:1, v/v, HCl:HNO₃). This digestion procedure is considered adequate for analysing total-recoverable heavy metals in soils (MEERS *et al.*, 2007). Residual elements that are not released by aqua regia digestion are mostly bound to silicate minerals and are considered unimportant for estimating the mobility and behaviour of metals (NISKKAVAARA *et al.*, 1997).

2.6 Simultaneous extraction protocols

Mobile Zn-fractions were obtained after simultaneous extractions with 0.01 mol/L CaCl₂, 1 mol/L NH₄NO₃, 0.05 mol/L Na₂EDTA, Mehlich 2 (0.2 mol/L CH₃COOH + 0.2 mol/L NH₄Cl + 0.015 mol/L NH₄F + 0.012 mol/L HCl) and Mehlich 3 (0.2 mol/L CH₃COOH + 0.25 mol/L NH₄NO₃ + 0.013 mol/L HNO₃ + 0.015 mol/L NH₄F + 0.001 mol/L EDTA). Extraction conditions of all cationic exchange and complexation procedures are given in Table 2.

Table 2. Extraction conditions of single-step extraction protocols used in experiments

Extraction solution	E:S* ratio	Equilibration time [h]	pH	Reference
0.01 mol/L CaCl ₂	10:1	2	6	DONNER <i>et al.</i> (2010)
1 mol/L NH ₄ NO ₃	10:1	2	6	MEERS <i>et al.</i> (2007)
Mehlich 3	10:1	2	2.5	MEHLICH (1984)
Mehlich 2	10:1	2	3	MEHLICH (1984)
0.05 mol/L Na ₂ EDTA	10:1	2	3	TICA <i>et al.</i> (2011)

*E:S is a ratio between extractant phase and solid phase

Simultaneous extractable fractions were obtained by mixing of soil samples (1 g) with 10 ml of extractant on laboratory shaker (150 rpm) for 2 h at 22 °C. After

centrifugation (10 min, 8000 rpm), 5 ml of supernatant was added to bank with 20-30 ml of reagent solution R-013 and 0.5 ml 0.01 mol/L KMnO_4 . The solution was heated to 80-95 °C for 5-10 min. The oxalic acid was added to reduce the excess of permanganate. The volume of samples was adjusted to 50 ml with the R-013 reagent solution. Solid particles were removed by filtering, centrifugation or sedimentation. Zinc analysis in purified extracts was subsequently performed using stripping chronopotentiometry by electrochemical analyser EcaFlow model GLP 150 (Istran, Ltd., Bratislava, Slovakia). Obtained data were compared with data obtained by GFAAS analyse. All the analyses were carried out in triplicates.

2.7 Isotopic dilution with ^{65}Zn (E -values)

Isotopic dilution with ^{65}Zn was used to monitor changes in the labile Zn fraction over time. The procedure was based on the method of GOLDBERG and SMITH (1984) using 0.05 mol/L CaCl_2 for displacement of soil zinc and top horizons of studied soils. To 2.5 g of soil samples, 25 ml of 0.05 mol/L CaCl_2 was added. To minimize the microbial activity, 0.1 ml of CHCl_3 was added. Flasks were agitated 5 days at 22 ± 2 °C and 150 rpm. Calcium chloride increased the Zn concentration in solution, making its determination more precise. Process of chemical equilibrium was characterized by determination of exchangeable zinc in 5 ml aliquots by galvanostatic stripping chronopotentiometry (SCP). These values were assigned to be the neutral salt extractable Zn concentration. The suspensions were subsequently spiked with 0.1 ml of ^{65}Zn (as ZnCl_2) solution of 25 kBq/ml (Czech Institute of Metrology, Prague, Czech Republic). Spiked suspensions were shaken for 5 days. After centrifugation (5 min, 4000 rpm) radioactivity in 3 ml of solid-free solution was measured by gamma spectrometric assembly using a well-type scintillation detector 54BP54/2-X, NaI(Tl) (Scionix, The Netherlands) and the data processing software Scintivision 32 (ORTEC, USA). All measurements were carried out in triplicates. The quantities of the isotopically exchangeable Zn in soil samples (Zn_E) were calculated according to equation:

$$Zn_E = \left(\frac{R_s}{r_s} \right) \cdot [Zn]_s \quad (2)$$

where R_s is radioactivity introduced in the suspension (Bq/ kg d.w.) and r_s is the radioactivity measured in reaction solution at the end of experiment (Bq/ kg d.w.) and $[Zn]_s$ is the mean concentration of extractable Zn in the soil extract as determined by SCP (mg/kg d.w.).

3. Results and discussion

The soil samples used in experiments varied widely in soil properties and characteristics (Table 3). Determination of soil pH noted the slightly acidic character of the soil sample S, and low alkaline character of soil sample JB. Soil reaction of soil sample BM varied between 5.9 and 6.8, depending on the method of determination. The sub-alkaline pH of JB sample, likely played a crucial role in reducing the Zn

levels in soil solution. A decrease of Zn solubility with increasing pH has been reported by many authors (FRIŠTÁK *et al.*, 2012; PARDO and GUADALIX, 1994; ZAMBELLA and ADAMO, 2010). Carbonate content was in the range from 0.5 to 0.7 in all soil samples. KIEKENS (1990) studied the reversibility of the exchange reaction between Ca and Zn and found that a key fraction of Zn was irreversibly bound by the soil components and following adsorption of Zn^{2+} and desorption of Ca^{2+} during Zn^{2+}/Ca^{2+} exchange process. Associated with the wide range in soil texture, CEC value varied from 5.9 to 18.1 mmol/100g. Specific adsorption reactions, adsorption sites dependent on pH and reversibility of zinc binding may contribute to selective or major Zn adsorption (MAES, 1973). The determination of pseudo total zinc concentrations in studied soil samples by GFAAS analyses confirmed the increasing trend in order BM < S < JB. The uppermost parts of the rich soil on organic matter and humus contain increased concentration of heavy metals (GABLOVIČOVÁ *et al.*, 2012).

Table 3. Physico-chemical properties of studied soil samples.

Soil sample	Pseudo total Zn [mg/kg]	pH _{H2O}	pH _{KCl}	CEC [mmol/100g]	CaCO ₃ [%]
JB	75	8.3	7.5	18.1	0.7
BM	12	6.8	5.9	5.9	0.5
S	61	4.6	3.8	6.0	0.5

Application of *aqua regia* extraction confirmed the lowest concentration of zinc (12 mg/kg) in BM soil samples obtained from haplic arenosol area with high content of calcium clays, dust, sand, conglomerate, coal beds and seams of lignite. Chemical extraction methods are widely used to assess the release of contaminants from soils, sludges, and sediments. As extracting agents in extraction procedures, strong acid and their mixtures (HNO_3 , H_2SO_4 , HCl , *aqua regia*), neutral solution of salts ($CaCl_2$, $MgCl_2$, $NaCl$), pending puffer and complex-forming agents (Na_2EDTA) can be used (MEERS, 2007). However, no single method is recognized universally (TICA *et al.*, 2011). Available zinc concentrations, expressed as a pore water concentration or concentrations extractable with agent of ion exchange were lower in comparison with other tested extracting agent (Figure 2a). The other extraction protocols can be used for the assessment of potentially toxic metals released from soils to the water recipients and their potential availability to all biological systems (MCLAUGHLIN *et al.*, 2000). For determination of soil zinc mobile fractions 5 extracting agents were used. We tried to quantify the zinc content in extracts using stripping chronopotentiometry. Zinc was deposited on porous electrode from acidic solution with high efficiency dependent on deposition potential.

Obtained data were compared to values from GFAAS as reference method (Figure 2b). Coefficient of determination (r^2) of linear correlation was 0.998 and it showed the comparability of used analytical methods. Paired t-test also confirmed non-significance of differences between used methods and thus statistical significance of obtained data of extractable zinc ($\alpha=0.05$).

The extractability of Zn obtained with the NH_4NO_3 protocols was in general higher than that obtained with the $CaCl_2$ protocols for all soil samples. Low alkaline sample

JB released Zn easily in reaction system of NH_4NO_3 in comparison to studied acidic agents. Effect of this extraction protocol can be attributed to the possible complexation of Zn by NH_3 (LEBOURG *et al.*, 1998) and higher ionic strength of extracting agent. Extraction efficiency of acidic agents increased in order Mehlich 2, Mehlich 3 and Na_2EDTA for all soil samples. Chelating effect of used extraction agent caused significant increasing of extractable zinc from exchange sites, carbohydrate and organic matter [9]. We confirmed the extraction efficiency of organic ligands on wide range of soil components.

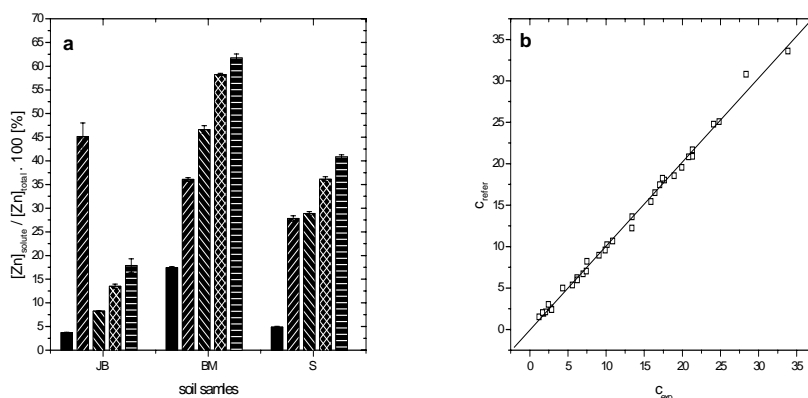


Fig. 2. Efficiency of simultaneous extraction protocols of zinc (mg/kg) from the three soil samples (JB, BM, S). Extraction conditions: 2 h, 22°C, 150 rpm, 100g/L, extracting agents: CaCl_2 (■), NH_4NO_3 (▨), Mehlich 2 (▩), Mehlich 3 (▧), Na_2EDTA (▦). All experiments were carried out in triplicates. Error bars represent standard deviation of the mean ($\pm\text{SD}$) (a). Comparison obtained concentrations of extractable zinc determined by electrochemical analyses (C_{exp}) with reference values determined by GFAAS analyses (C_{ref}) (b).

NAIDU and HARTER (1998) showed that metal extracted by a mixture of organic acids is well-correlated with the mobile metal fraction in the soil solution. Metal fraction extracted by organic acids is more available for biological system compared to other complexing agents. Low molecular weight organic acids such as ethylene diamine tetraacetic acid (EDTA) and their salts (Na_2EDTA) were reported to remove metals organically bound, occluded in oxides, and associated with secondary clay minerals (PAYA-PEREZ *et al.*, 1993). Effect of soil reaction was less significant. We found out that simultaneous extraction protocols had maximal efficiency for low acidic soil sample of Haplic Arenosol (BM). Thus we confirmed the effect of soil composition as a parameter of zinc availability from this typical Slovak soil.

For next investigation of labile or potentially available Zn fraction in the studied soil samples, the isotopic dilution (E -value) was used. The studied samples of uppermost horizons contain the highest pseudo total concentration of zinc. The E -values reported in Table 4 show how much the Zn in soil samples was isotopically exchangeable within experimental equilibrium period. Zinc availability in three soil samples ranged from 20 to 39%. These values were comparable with concentrations of Na_2EDTA extractable zinc. DEGRYSE and SMOLDERS (2006) reported E -values of

zinc equivalent to 32-49 % lability in upper horizons of acid soils (pH 3.3-4.5). On the other hand, DONNER *et al.* (2010) showed the exchangeable fraction of zinc in the range from 12 to 15% for agricultural soils with pH from 5 to 6.6.

Our study confirmed decreasing concentration of labile soil zinc with increased soil reaction of samples in order Haplic Cambisol, Haplic Arenosol and Calcic Phaenzem. Measurements by radioisotope dilution with ^{65}Zn demonstrate a significant of soils pH and variation of Zn bioavailability depending on soil type and physico-chemical characteristics. Further work is needed to assess the importance of soil amendments and long-term ageing in terms of zinc bioavailability.

Table 4. E-values of soil samples JB , BM and S depending on pH and total Zn concentration [mg/kg]. All experiments were carried out in triplicates, mean \pm SD.

	Soil sample		
	JB	BM	S
pH	8.3	6.8	4.6
Total Zn [mg/kg]	75.0 \pm 6.0	12.0 \pm 0.9	61.0 \pm 4.7
Zn E-value [mg/kg]	15.1 \pm 1.9	2.51 \pm 0.2	23.8 \pm 1.3
Labile Zn [%]	20.1	20.9	39.01

4. Conclusions

As tools of available zinc fraction determination, single-step extraction methods can be used. Our paper confirmed the effect of physico-chemical properties (pH) of soil samples and applied extraction protocol to extractable zinc concentration. We found out that extractability of binding zinc decreased in order Na_2EDTA , Mehlich 3, Mehlich 2, NH_4NO_3 and CaCl_2 for studied soil samples. Flow-through stripping chronopotentiometry (SCP) and atomic absorption spectrometry (GFAAS) confirmed the maximum of removable soil zinc by organic ligands. Lability of soil Zn can be determined by isotopic dilution method using radioactive isotope ^{65}Zn and γ -spectrometry. Determination showed the significant decrease of isotopic exchangeable zinc fraction (*E*-value) with decrease of soil reaction. *E*-values of studied soil samples showed the zinc ability in range from 20 to 39%. Our study confirmed the effect of soil reaction, composition and physico-chemical characteristics to Zn lability.

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