

The effect of soil physicochemical characteristics on zinc analysis methods

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Abstract: Zn is an essential micronutrient involved in a wide variety of physiological processes. Soils are tested for zinc in many countries with several extractants. Each country has its validated methods, best-suited for its soils. The current study was designed to compare different zinc content measuring methods with seventy-one samples from Hungary. The data were first compared for the whole dataset and then in certain categories such as CaCO₃-content, pH, texture and clay content. The zinc content was determined by the water extraction, KCl-EDTA (ethylenediaminetetraacetic acid), Mehlich 3, CoHex (cobalt hexamine trichloride), and XRF (X-ray fluorescence) methods. Based on the analyses of all the data, we can conclude that all the methods are different. However, further analyses during the comparison of the methods based on the influencing factors, such as the pH, lime content, texture class, and clay content proved that, in some of the cases, there are similarities among the methods and, this way, we can get more knowledge on the measurements and the results provided. Farmers can gain extra knowledge from the comparison of the influencing factors to know where intervention is needed to use extra Zn for the proper fertilisation of their plants.

Keywords: comparative analyses; extraction methods; soil test

Zinc (Zn) is essential for plant growth; it is taken up as zinc ions (Zn²⁺). The average Zn concentration in uncontaminated soils is in the range of 17 to 160 µg Zn/g soil (Reed & Martens 1996). Most of the zinc in soils exists in biologically unavailable forms. According to Viets (1962), zinc may be present in the soil as water-soluble, easily exchangeable, adsorbed,

precipitated with secondary minerals and bound to primary minerals. The amount of various forms of Zn depends on the soil texture, pH, calcium carbonate content, organic matter content, and other soil characteristics (Sharma et al. 2004)

The main soil properties controlling the amounts of plant-available forms of Zn in soils include the

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total Zn content, pH and redox conditions, calcite (CaCO₃) and organic matter contents, concentrations of all the ligands capable of forming organo-Zn complexes, the microbial activity in the rhizosphere, concentrations of other trace elements, concentrations of macro-nutrients (especially P) and the soil moisture status (Alloway 2009).

Soils are tested for zinc in many countries with several extractants. Each country has its validated methods, best-suited for its soils. It is important to understand the background of the different methods to compare and interpret the results. Furthermore, as remote sensing methodology is improving very fast, it is of high importance to know the comparability of the different methods for the calibration of remote sensing devices. Mehlich (1953) introduced the Mehlich 1 (double acid, M1) procedure for the evaluation of acid sandy soils. This method has been widely used since its introduction, particularly in North America and Latin America (Matejovic & Durackova 1994; van Raij 1994; Tucker et al. 1996). The M1 procedure was updated in 1978 (M2, Mehlich 1978) to try to extend its use to a wider range of soils. Mehlich 2 (Mehlich 1978) was the standard extractant for assessing the fertiliser and liming requirements of crops in the Czech Republic/Slovakia up to 1994 (Matejovic & Durackova 1994). Mehlich 3 (M3, Mehlich 1984) replaced this procedure in 1981 for two reasons: (1) The chloride in NH₄Cl and HCl was highly corrosive to laboratory instrumentation and (2) EDTA (ethylenediaminetetraacetic acid) was added to Mehlich 3 to enhance the extraction of Mn, Zn and particularly Cu (Mehlich 1984; Tucker 1988). Although Mehlich 3 was introduced initially for acid soils, its use has been extended to include alkaline soils (Tran et al. 1990; Alva 1993; Mamo et al. 1996; Schmisek et al. 1998). M3 is used in the Czech Republic, Slovakia, and Estonia (Fotyma & Dobers 2008).

In Hungary, the Hungarian Standard has used the KCl-EDTA (0.05 M EDTA + 0.1 M KCl) extract as a soil test method for zinc (MÉM-NAK 1978;

Baranyai et al. 1987) since 1978. This extract is not used outside of Hungary; therefore, it is a hard task to compare the results of this method to those of other extractants.

The operating protocol of the cobalt hexamine trichloride (CoHex) method has been described in the study of Ciesielski et al. (1997) to determine the cation exchange capacity (CEC) and the number of exchangeable cations. Today the CoHex method is based on the ISO 23470:2007 Standard wherein the exchangeable cations in the sample are replaced by trivalent cobalt hexamine ions. The CEC is calculated from the difference between the initial and final concentrations of the cobalt solution which are determined using the analytical method of absorption colorimetry.

X-ray fluorescence (XRF) spectrometry nowadays is being given much attention as an upcoming proximal soil sensing (PSS) technique. XRF is a quick method for the determination of the total elemental compositions of soil samples (Weindorf et al. 2014).

The water extraction method is mostly used for the phosphorus determination. It mainly shows the water-soluble forms of each component in the soil.

Figure 1 shows the zinc forms in the soil and extraction methods; the soluble form with the water extraction method, the soluble and readily exchangeable zinc forms with the KCl-EDTA and Mehlich methods, the readily and slowly exchangeable forms are expected to be measured with the CoHex method while the total Zn content are determined with the XRF method.

The present study aims to compare the extraction efficiency of the most widespread methods focusing on the Zn concentration. An additional aim is to quantify the role of soil properties affecting the Zn extraction efficiency and to evaluate the chosen classification method of the properties that can affect the evaluation of the Zn measurements. This comparative analysis study can provide a guide to interpret the different analysis methods by way of harmonisation.

Water-soluble	Water extraction	KCl-EDTA extraction, Mechlich 3	CoHex	XRF
Readily exchangeable				
Slowly exchangeable				
Structural forms				

Figure 1. Zinc forms in the soil and extraction methods

MATERIAL AND METHODS

Sampling

Seventy-one geo-referenced soil samples were collected from arable lands of Hungary in 2017, differing in soil typology, texture, and pH. The soil samples were taken from the 0–20 cm layer. The locations of the seventy-one samples were selected according to Minasny and McBratney (2006) and Roudier and Hedley (2013). The land use, soil type, climate data, accessibility, and property market value were the factors taken into account in this selection. The samples were air-dried and sieved (< 2 mm).

Methods for extracting and determining Zn

The most common analytical procedures, the water extraction method (WA), Mehlich 3 method (M3), KCl-EDTA method (EDTA), Cobalt hexamine method (CoHex), XRF method were selected to determine the different Zn pools in the soil. The soil pH, CaCO₃ content, Arany-type soil texture index, and clay content were analysed to evaluate their effect on the results of the extraction methods.

Water extraction method (WA). 12 grams of air-dried soil was mixed with de-ionised water in a ratio of 1 : 5 (m/V). After 30 min of shaking and filtering, the extract was analysed by ICP-MS (7700 System, Agilent, Japan).

Mehlich 3 method (M3). The Mehlich 3 method was implemented following Chapter 5 of the Recommended Soil Testing Procedures for the Northeastern United States (Wolf & Beegle 2009). The sample was extracted with a Mehlich 3 solution (0.2 mol/dm³ acetic acid, 0.015 mol/dm³ ammonium fluoride, 0.013 mol/dm³ nitric acid, 0.25 mol/dm³ ammonium nitrate, 0.001 mol/dm³ ethylenediaminetetraacetic acid, pH 2.5). The soil to solvent ratio was 1 : 10 (m/V). After 5 min of shaking and filtering, the extract was analysed by ICP-MS (7700 System, Agilent).

KCl-EDTA method (EDTA). The KCl-EDTA method was implemented according to the Hungarian standard (MSZ 20135:1999).

The sample was extracted with a potassium chloride-EDTA-solution (0.05 mol/dm³ EDTA, 0.1 mol/dm³ potassium chloride) with the application of a soil to solvent ratio of 1 : 2 (m/V), was shaken with an overhead shaker for 2 h, then filtered and analysed with an ICP-AES (LabX, Canada).

Cobalt hexamine method (CoHex). The cobalt hexamine method was implemented following ISO 23470:2007. The cations retained by the soil sample were exchanged

with the hexaamminecobalt ions of an aqueous solution (0.0166 mol/dm³) with shaking for 60 min. The CEC was determined by the difference between the initial quantity of hexaamminecobalt in the solution and the quantity remaining in the extract after the exchange reaction. The quantities of exchanged cations (Zn) were determined on the same extract. The measurement of hexaamminecobalt concentration in the extract is performed by the ICP-MS (7700 System, Agilent) measurement of the Co concentration, which was compared to the concentration of a blank solution.

XRF method. The determination of the bulk multi-element concentrations in the dry soil samples with usage of a PANalytical Epsilon 3 ED-XRF (Malvern & PANalytical, The Netherlands) was performed with an in-house method optimised and validated according to ISO18227:2014. The samples were dried at 40 °C, sieved and finely ground to a particle size < 0.2 mm. Due to the fact that a moisture content above 20% was interfering with the XRF results, the samples were dried at 105 °C to remove the water. In order to reduce soil matrix effect, the samples were thoroughly homogenised and mixed with wax in a 10 : 1 ratio using a Fritsch Planetary Micro Mill Pulverisette and pressed with a hydraulic press VANEON 40t (Fluxana, Germany) automatically into an aluminium cup 40 mm in diameter. The pelletised samples were analysed according to a standard operating procedure using a validated method. An Epsilon 3 (Malvern & PANalytical, The Netherlands) is equipped with an Rh anode X-ray tube, besides a window and SDD detector (silicon drift detector). The spectrometer has a carousel (circular rotating sample changer) with ten positions inside the sample chamber since each sample is isolated in an individual sample container, there was almost no chance for cross contamination. The Epsilon software handles all the spectra deconvolutions and elemental qualification and quantification.

The accuracy has been defined during validation. As a part of the routine procedure, a multiple of reference materials are analysed on a daily basis to monitor the drift/accuracy along with the in-house prepared soil quality control samples. Fused beads with a known elemental concentration are used to monitor the drift, the soil quality control samples are prepared together with routine samples to monitor the quality of the sample preparation process.

Characterisations of the soil physicochemical properties

pH(KCl). The pH(KCl) was determined with a potentiometric method according to the Hungar-

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ian standard (MSZ-08-0206-2:1978). The pH value was measured in a soil suspension, prepared with a 1 mol/dm³ KCl solution with a soil to a solvent ratio of 1 : 2.5 (m/V). The suspension was left to stand overnight before measuring.

CaCO₃-content. The CaCO₃-content was determined using the gas volumetric method of Scheibler (MSZ-08-0206-2:1978). The carbonates present in the sample were converted into CO₂ by adding an HCl-solution to the sample. The carbonate content was calculated from the volume of the generated gas, the temperature, and the air pressure.

Arany-type soil texture index. The texture was determined by the Arany-type method according to the Hungarian Standard (MSZ-08-0205:1978). This test quantifies the amount of water in cm³ added (by continuous mixing) to 100 g of the air-dried soil sample to obtain a yarn (upper limit of plasticity); the gained value is the Arany-type soil texture index (Stefanovits et al. 1999). The more water the soil absorbs at the upper limit of plasticity, the more clay the soil contains (Table 1). The value ranges for the Arany-type texture coefficient are summarised in Table 1.

The evaluation of the plant-available Zn content in the Hungarian advisory system (MÉM-NAK) is based on three Arany-type texture indexes (Table 2).

Clay particle size fraction. The particle size distribution was measured using laser diffractometry (Fritsch Analysette 22 Microtech Plus). To break down the aggregates, the organic matter and CaCO₃ content were removed from the samples using H₂O₂ and 10% HCl, respectively. For the complete disaggregation, a 0.5 mol/dm³ sodium-pyrophosphate addition and ultrasonic treatment were applied during the measurement. To calculate the size distribution, the Mie theory was used applying a 1.54 refractive index value.

Table 1. Arany-type texture index and the corresponding soil texture class

Soil textures	Arany-type texture index ranges
Coarse sand	< 25
Sand	25–30
Sandy loam	30–37
Loam/silt	37–42
Clayey loam	42–50
Clay	50–60
Heavy clay	> 60

Table 2. Arany-type texture index

Arany-type texture index	Zn (mg/kg)	
	weak	good
< 38 (sand)	< 1.0	> 1.0
38–50 (loam)	< 2.5	> 2.5
> 50 (clay)	< 3.5	> 3.5

Data analyses of the influencing factors

To evaluate the role of the soil properties affecting the Zn extraction efficiency, the samples were grouped according to the pH, CaCO₃ content, Arany-type texture index, and clay content.

Grouping of the samples based on the pH. The samples were grouped differently from the categories used in Hungary to investigate the dependence of zinc versus pH(KCl) more accurately. The soils were divided into five groups by pH, the more detailed groups were based on the original categories as a starting point, and sample numbers for sound statistical analyses (all groups have a minimum of 11 samples) were the other input for the creation of the analysed groups (Table 3). To provide detailed information about the size of the groups, the exact data from the pH(KCl) measurements were given for the limits of the group, so where there is a gap between the groups, there is no soil with the given pH(KCl). The original groups are as follows: < 4.5, 4.5–5.5, 5.5–6.8, 6.8–7.1, 7.1–8.0, > 8.

Grouping of the samples based on the CaCO₃-content. Most of the samples tested in our study were in the lime-free or low-lime categories, so, the samples were grouped differently from the categories used in Hungary to investigate the dependence of zinc versus lime content in a more detailed manner. The carbonate content was divided into five groups (Table 4). Sample numbers were the basis for creating the groups, the minimum sample number was seven.

Table 3. pH groups with the number of samples analysed

Group	No. of samples	pH(KCl)*
1	11	3.39–4.35
2	11	4.52–5.47
3	12	5.54–6.78
4	13	6.97–7.2
5	24	7.21–8.14

*pH(KCl) groups' upper and lower limits are based on the measured data

Table 4. CaCO₃-groups with the number of samples analysed and with the limits of the formed groups

Groups	No. of samples	CaCO ₃ -content (%)
1	27	< 0.1
2	18	0.11–0.84
3	7	1.01–3.16
4	10	3.64–8.79
5	9	9.66–18.71

Grouping of the samples based on the Arany-type soil texture index. For a better understanding of the effect of the Arany-type texture, the soils were divided into 8 Arany-type texture groups (Table 5).

Grouping of the samples based on the clay particle size fraction. On the triangle for texture identification, the clay content groups are 0–10, 10–20, 20–30 ... etc. Our smallest figure was 6.8%, the biggest was 24.89%, so we created the analysed categories accordingly (Table 6), keeping in mind to have a minimum of five samples per group for the statistical analyses and a close to equal range in each group (2.6–2.88). The clay particles were in the 0–0.002 mm fraction.

Statistical analysis

The XRF method was applied to determine the total Zn contents, based on its results, the percentages of the total Zn (XRF) with the different analysis methods were calculated. These results were described using descriptive statistics with the following statistical indicators: arithmetic mean, median, coefficient of variation (CV), Standard deviation (SD), maximum (Max), minimum (Min) value.

A correlation regression was used to determine the relationship between the Mg determination methods, where R^2 presents a measure to match the relationship of the different methods.

Table 5. Arany-type texture groups with the number of samples analysed

Groups	No. of samples	Arany-type texture
1	11	32–34
2	11	35–37
3	8	38–38
4	14	39–40
5	10	41–41
6	5	42–42
7	6	43–44
8	6	45–51

Table 6. Clay particle size groups and sample numbers

Groups	No. of samples	Clay particle size (%)
1	8	6.82–9.64
2	24	9.91–12.74
3	14	12.99–15.69
4	13	15.99–18.59
5	6	19.16–21.82
6	5	22.01–24.89

The normality of the data series of the different analysis methods was tested with the Kolmogorov-Smirnov test. If the data of the analysis methods were not normally distributed, then a non-parametric Friedmann analysis of variance (ANOVA) test was used. If the data of the analysis methods showed normal distribution, then a parametric, Repeated Measures ANOVA test was used.

The Wilcoxon signed-rank test, a non-parametric statistical hypothesis test was used to compare the Zn analysis methods (WA, EDTA, M3, CoHex) to assess whether their mean ranks differed. The box plot non-parametric method was used to display the variation in the zinc determination methods in the specific groups of the pH(KCl), CaCO₃-content, Arany-type texture index, and clay content.

Investigating the pH(KCl), CaCO₃-content, Arany-type texture index, clay content dependence, a pairwise analyses test, a type of location test that is used to compare measurements of the four zinc analyses methods, was used to assess whether their means differed. The box plot non-parametric method was used to display the variation in the zinc determination methods in the specific groups of the pH(KCl), CaCO₃-content, Arany-type texture index, and clay content.

RESULTS

Comparison of all the values measured by the five different methods

Firstly, a correlation regression was used to determine the relationships of the soil Zn content measured by the Water, Mehlich 3, CoHex, KCl-EDTA and XRF methods, as can be seen in Table 7.

A strong correlation was established between the Zn content determined by the EDTA and M3 methods ($R^2 = 0.71$). The relationship between the Zn content determined by the EDTA and WA methods was weak ($R^2 = 0.21$). While all other correlations

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Table 7. The relationship between the Zn contents measured by the WA, M3, CoHex, EDTA, XRF methods

Methods	R^2 values
EDTA vs M3	0.71
EDTA vs WA	0.21
WA vs M3	0.19
EDTA vs CoHex	0.14
EDTA vs XRF	0.09
CoHex vs XRF	0.06
M3 vs XRF	0.06
WA vs CoHex	0.05
WA vs XRF	0.05
M3 vs CoHex	0.01

WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl; XRF – X-ray fluorescence

between the different analysis methods were very weak ($R^2 < 0.19$).

Comparison of all the measured values by the four different methods

As the XRF method was applied to determine the total contents, based on its results, how much percentage of the total Zn (XRF) could be measured with the different analysis methods was calculated. Table 8 shows the percentages that each method could be measured from the total amount of Zn (XRF).

The mean, median, min and max percentage values resulting from all the Zn determination methods showed the following order of measured magnitude: CoHex < WA < EDTA < M3.

The first statistical analysis was based on all the data. According to the Kolmogorov-Smirnov test, the distribution of the data was not normal. This is why the non-parametric, Friedman's two-way analysis of variance by ranks (ANOVA) test was used. The results of the statistical analyses proved that all the applied methods provided different results ($F_r = 181.766$, $df = 3$, $P = 0.000$), so the laboratory measurements measured different amounts of Zn (Figure 2).

According to the pairwise analyses, all the methods differ, the most similar are the M3 and the EDTA methods, but even between them, there is a significant difference.

Evaluating the effect of the soil parameters on the Zn measurements with a pairwise analysis

Investigating the effect of the soil parameters, a pairwise analysis test, a type of location test, was

Table 8. Proportion of the measured Zn from the total amount (in %)

	Total amount of Zn, measured with XRF			
	WA	M3	CoHex	EDTA
Mean	1.46	4.88	0.65	3.95
SD	2.16	4.21	1.39	3.40
Median	0.86	3.98	0.08	3.28
Min	0.34	1.02	0.02	0.76
Max	13.83	27.71	8.81	24.62

WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl; XRF – X-ray fluorescence; SD – standard deviation

used to compare measurements of the four zinc analysis methods to assess whether their means differed. The proportions of the measured Zn from the total amount (XRF) were used in the comparison and they were grouped according to the specified pH, CaCO_3 content, Arany-type texture index, and clay content groups.

Comparison of the measured values in the classic pH groups

Group 1–2. In the first two pH(KCl) groups (3.39 to 4.35 and 4.36–5.47), based on the comparison between the groups M3 was not different from EDTA and WA was not different from CoHex, but the other pairs were significantly different (Table 9).

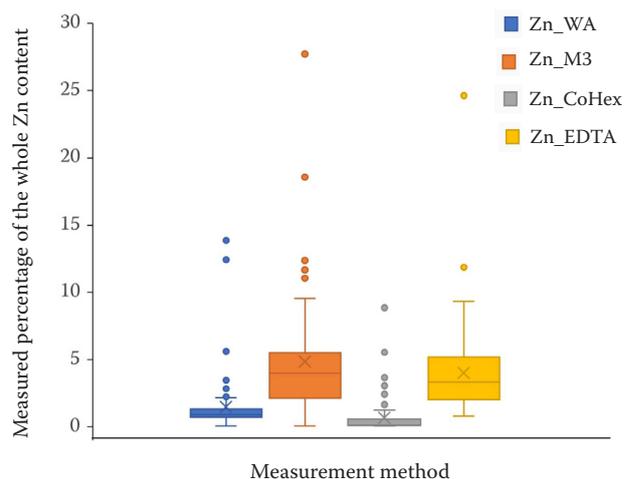


Figure 2. Results of statistical analyses of four different Zn-extraction methods based on the percentage that each method could measure from the total amount of Zn, measured by a fifth method (WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl)

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Table 9. Pairwise analysis of the measured zinc percentages compared with the measured total zinc amounts based on the pH(KCl) groups

	Group 1	Group 2	Group 3	Group 4	Group 5
CoHex vs. WA	$P = 1.000$	$P = 1.000$	$P < 0.347$	$P < 0.290$	$P < \mathbf{0.031}$
CoHex vs. EDTA	$P < \mathbf{0.01}$	$P < \mathbf{0.001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$
CoHex vs. M3	$P < \mathbf{0.0001}$	$P < \mathbf{0.001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$
WA vs. EDTA	$P < \mathbf{0.049}$	$P < \mathbf{0.010}$	$P < \mathbf{0.068}$	$P < 0.201$	$P < \mathbf{0.044}$
WA vs. M3	$P < \mathbf{0.003}$	$P < \mathbf{0.010}$	$P < \mathbf{0.009}$	$P < \mathbf{0.001}$	$P < \mathbf{0.0001}$
EDTA vs. M3	$P = 1.000$	$P = 1.000$	$P = 1.000$	$P < 0.568$	$P < 0.113$

WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl; bold – significant difference

Group 3–4. In the third and fourth pH(KCl) group (5.48–6.78 and 6.79–7.2) there was a new pair that was not significantly different, not only M3 and EDTA, WA and CoHex were different, but also WA and EDTA. All other pairs were different (Table 8).

Group 5. In the fifth pH(KCl) group (7.21–8.14) there was only one pair, that was not significantly different – M3 and EDTA. All the other pairs resulted in significant differences (Table 9).

Based on the pH(KCl) groups, it can be concluded that there was no significant difference between the EDTA and M3 methods, these were similar in all groups. Based on the p values, the differences were the biggest between the CoHex and the M3 methods.

On the other hand, there was a clear trend: in the acid groups, the differences between the methods were less obvious compared to the direction of less acid and finally to the more alkaline groups. We can conclude that measurements of the Zn are less different in the case of acid soils and significantly different in almost all of the soils in the 7.21–8.14 pH range. There was one exception, the CoHex and M3 methods showed a very strong significant difference in all the groups. However, the p values decrease towards to higher pH values which also underlines the observed trend even in this case.

The most obvious differences were between CoHex and M3 + CoHex and EDTA.

Comparison of the measured values based on the CaCO₃-content groups

Group 1–2. There was no significant difference between (0–0.84%) EDTA vs M3 and CoHex vs WA, the other methods were significantly different from each other (Table 10).

Group 3–5. (1.01–18.71%) EDTA did not differ from WA, only from the CoHex method. WA and CoHex, M3 and EDTA were not significantly different. The significant differences are listed in Table 10.

The order of differences in the CaCO₃ groups is the same as in the case of the pH(KCl), there is only a slight difference, the strength of the difference between WA and M3 is slightly stronger than between CoHex and EDTA in the case of CaCO₃ than in the case of the pH(KCl).

The reason for this change is that the significance of the difference in the biggest CaCO₃ category (8.8 to 18.71%) between WA and M3 is stronger ($P < 0.006$) than in the case of CoHex and EDTA ($P < 0.021$). Furthermore, the number of non-significant differences is bigger in the case of the comparison of the measured values with the increasing CaCO₃ amounts. So, overall,

Table 10. Pairwise analysis of the measured zinc percentages compared with the measured total zinc amounts based on the CaCO₃ groups

	Group 1	Group 2	Group 3	Group 4	Group 5
CoHex vs. WA	$P < 0.439$	$P < 0.169$	$P = 1.000$	$P < 0.500$	$P < 0.865$
CoHex vs. EDTA	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.023}$	$P < \mathbf{0.003}$	$P < \mathbf{0.021}$
CoHex vs. M3	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$
WA vs. EDTA	$P < \mathbf{0.0001}$	$P < \mathbf{0.040}$	$P < 0.375$	$P < 0.500$	$P < 0.865$
WA vs. M3	$P < \mathbf{0.0001}$	$P < \mathbf{0.0001}$	$P < \mathbf{0.023}$	$P < \mathbf{0.003}$	$P < \mathbf{0.006}$
EDTA vs. M3	$P = 1.000$	$P < 0.933$	$P = 1.000$	$P < 0.500$	$P < 0.407$

WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl; bold – significant difference

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Table 11. Pairwise analysis of the measured zinc percentages compared with the measured total zinc amounts based on the Arany-type texture groups

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
CoHex vs. WA	$P < 0.191$	$P < 0.285$	$P < 0.728$	$P < 0.342$	$P < 0.500$	$P = 1.000$	$P = 1.000$	NS
CoHex vs. EDTA	$P < 0.002$	$P < 0.002$	$P < 0.003$	$P < 0.0001$	$P < 0.001$	$P < 0.165$	$P < 0.083$	$P < 0.05$
CoHex vs. M3	$P < 0.0001$	$P < 0.300$	$P < 0.005$	$P < 0.05$				
WA vs. EDTA	$P < 0.823$	$P < 0.592$	$P < 0.317$	$P < 0.115$	$P < 0.226$	$P < 0.020$	$P < 0.265$	$P < 0.05$
WA vs. M3	$P < 0.018$	$P < 0.006$	$P < 0.040$	$P < 0.002$	$P < 0.011$	$P < 0.042$	$P < 0.022$	$P < 0.05$
EDTA vs. M3	$P < 0.823$	$P < 0.592$	$P = 1.000$	$P = 1.000$	$P = 1.000$	$P = 1.000$	$P = 1.000$	NS

WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl; NS – no significance; bold – significant difference

we can conclude that the methods are more different in the cases of a smaller lime content and becoming less different with an increasing lime content.

Comparison of the measured values based on the Arany-type texture index groups

Group 1–5. In the first five Arany-type texture groups, from sandy loam to a loamy texture (KA = 32–41) M3 vs EDTA, EDTA vs WA, WA vs CoHex were not different. All other pairs are significantly different (Table 11).

Group 6. In the sixth group (KA = 42) M3 did not differ from EDTA and CoHex, EDTA did not differ from CoHex and CoHex did not differ from WA. Only WA differed significantly from M3 and EDTA (Table 11).

Group 7. In the seventh group (KA = 43–44) EDTA was not significantly different from the other methods, WA did not differ from CoHex but M3 was significantly different from WA and CoHex. (Table 11).

Group 8. In the eighth group (KA = 45–51) four methods were significantly different: WA vs M3, WA vs EDTA, M3 vs CoHex, EDTA vs CoHex. WA vs CoHex and M3 vs EDTA methods were not significantly different (Table 11).

The influencing factor, the Arany-type texture resulted in less significant differences between the

groups. In this case, there were only significant differences in all the groups between the WA and M3 methods, and all other cases, there was at least one non-significant difference.

This is the first case when the strongest difference is not between the CoHex and M3 methods, but between the WA and M3 methods.

In the Hungarian classification, Group 3–6 belongs to the same category, which is the loamy texture. The null hypothesis was that, in these groups, there will be similar results, but surprisingly there were significant differences between CoHex and EDTA, and CoHex and M3 in Group 3–5, there were none in Group 6, and vice versa. There were no significant differences in the case of WA and EDTA in Group 3–5 while there were in the case of Group 6. So, after all, we cannot conclude that the behaviour of the measurements is the same, even in the same textural classes.

Comparison of the measured values based on the clay-content groups

Group 1. In the first clay group (6.82–9.64%), only CoHex was different from EDTA and CoHex was different from M3, the other pairs were not significantly different (Table 12).

Table 12. Differences and similarities between the Zn measuring methods in the different clay content groups, pairwise analyses, the analyses are based on the measured percentage of the total amount

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
CoHex vs. WA	$P < 0.199$	$P < 0.113$	$P < 0.859$	$P < 0.568$	$P = 1.000$	$P = 1.000$
CoHex vs. EDTA	$P < 0.040$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.022$	$P < 0.518$
CoHex vs. M3	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.005$	$P < 0.020$
WA vs. EDTA	$P = 1.000$	$P < 0.007$	$P < 0.008$	$P < 0.059$	$P < 0.265$	$P < 0.300$
WA vs. M3	$P < 0.121$	$P < 0.0001$	$P < 0.003$	$P < 0.002$	$P < 0.083$	$P < 0.009$
EDTA vs. M3	$P < 0.488$	$P < 0.561$	$P = 1.000$	$P = 1.000$	$P = 1.000$	$P = 1.000$

WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl; bold – significant difference

Group 2–3. In the second and third clay groups (10–12.74% and 12.99–15.69%), the following two pairs were not significantly different: M3 vs EDTA and WA vs CoHex. All the other pairs were significantly different (Table 12).

Group 4. In the fourth clay group (15.99–18.59%), EDTA is no longer different from WA, only there is a significant difference between the results of the M3 and WA methods. The CoHex method still differed significantly from M3 and EDTA (Table 12).

Group 5. In the fifth group (19.16–21.82%), only CoHex differed from EDTA and M3, the other pairs were not significantly different, just like in the first clay group (Table 12).

Group 6. In the sixth clay group (22.01–24.89%), M3 differed from WA and CoHex, the other pairs were not significantly different (Table 12).

Similar to the Arany-type texture groups, there is also only one case when all the groups resulted in significant differences which was is between CoHex and M3. The smallest clay content and the biggest clay content resulted in the smaller differences, while the most numerous significant differences were between 10 and 15.69%. This does not follow the trend that we observed with the texture groups. The biggest number of significant differences was in the most clayey texture group that is clayey-loam.

All-inclusive evaluation of the effect of soil parameters on zinc measurements

The analysis of the differences between the Zn measurement methods allows us to compare the differences and similarities, furthermore, as well as their strength with other soil parameters such as the pH(KCl), texture, clay, and CaCO₃ content. This way we could establish trends or tendencies.

Summarising the effect of the soil parameters, how many percentage of the results of the pairwise analysis were significant along with the four influencing factors (pH(KCl), CaCO₃, Arany-type texture, and clay) was calculated (Table 13).

Based on the average of the significance levels of all the pairwise analysis of the measurements along the four influencing factors (pH(KCl), CaCO₃, Arany-type texture, and clay), the least number of ‘not significant’ results belonged to the EDTA and M3 pairing, there has never been a significant difference between their results in the applied categories and the explanation force of the significant difference is the weakest. So, even though the results of the statistical analysis of the overall, all-inclusive data proved that all the applied methods are statistically different, the one-by-one analyses of the categories of the influencing factors resulted in different outcomes: there are ‘similarities’ not only differences. According to the first statistical pairwise analyses in the result chapter, all the methods differ, the most similar one is between the M3 and the EDTA methods, but even between them, there was a significant difference. Furthermore, there is another pair that was not significantly different, taking into account that there was only one case where a significant difference occurred in the amount of Zn measured: CoHex and WA.

There was no significant difference between WA and EDTA, but this pair formed a different group. The next group where there was already a significant difference between the methods is WA and M3. CoHex and EDTA followed the previous group in order, while the strongest difference is between CoHex and M3 and we can consider both as having a strong significance. These two pairs can be considered as a separate group.

Table 13. The average of the number of significant results of all the pairwise analysis of the Zn% measurements along with the four influencing factors (pH(KCl), CaCO₃, Arany-type texture, and clay)

Methods	Significant results (%)					average	order
	pH	CaCO ₃	KA%	clay			
EDTA vs M3	0	0	0	0	0	1	
CoHex vs WA	20	0	0	0	5	2	
WA vs EDTA	60	40	25	33.3	39.6	3	
WA vs M3	100	100	100	66.7	91.7	4	
CoHex vs EDTA	100	100	75	100	93.8	5	
CoHex vs M3	100	100	87.5	100	96.9	6	

WA – water soluble; M3 – Mehlich 3; CoHex – cobalt hexamine; EDTA – EDTA-KCl; KA – Arany-type texture; average – the average of the significant results; order – evaluation from 1 to 6 (1 – smallest different, 6 – biggest difference) based on all parameters (pH + CaCO₃ + KA + Clay)

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Evaluating the differences based on all the parameters, the following order can be made (1 – smallest difference, 6 – biggest difference): 1 – EDTA vs M3, 2 – CoHex vs WA, 3 – WA vs EDTA, 4 – CoHex vs EDTA, 5 – WA vs M3, 6 – CoHex vs M3.

DISCUSSION

Comparing the different analysis methods, the Mehlich 3 solution demonstrated a greater capacity of extraction of Zn in comparison to the other extractants, which is in agreement with other researchers like Abreu et al. (2002), Pradhan et al. (2015). The acid reagents and chelating agents such as EDTA result in the higher extraction of Zn (Vidal-Vázquez et al. 2005), which was also seen in our study.

The effect the pH, organic matter, clay content, Fe oxides, cation exchange capacity have on the soil properties has been discussed in several studies (Junus & Cox 1987; Sims & Johnson 1991; Haddad & Evans 1993; Borkert et al. 1998), but there is less information about the effect of the chosen classification method in the evaluation of the zinc measurements.

Diatto and Kocialkowski (1998) reported that the adsorption of Zn by soils is influenced by the soil properties including the texture, calcium carbonate, and organic matter content. In a study conducted on soils with different textures for adsorption reactions of zinc, it is stated that light loam, silty medium loam, and silt loam soils having comparatively higher values for the adsorption maxima, bonding energy constant and differential buffering capacity of the soils will require higher rates of Zn to change in the solution concentration. In our study, the texture was firstly classified based on the Hungarian Arany-type texture index. Until the Arany-type texture index of 44, from a sandy loam to clayey loam texture, a strong correlation in the Zn measurements was shown between the M3 and EDTA method. We can summarise that not only the extraction method, but also some of the soil physicochemical properties and the chosen classification method affect the evaluation of the zinc measurements. From the comparison of the influencing factors, farmers can also gain extra knowledge where intervention is needed to use extra Zn for the proper fertilisation of their plants.

CONCLUSION

There are several methods used worldwide for Zn determination. In this study, we aimed to compare the different soil analysis methods for the zinc measurements. The study is outstanding in the comparison that

the amount of Zn measured with the different analysis methods were compared to the total contents measured with the XRF method. The data were first compared for the whole dataset and then, in certain categories of the CaCO₃-content, pH, Arany-type texture index and clay content. Based on these results, an important conclusion can be made: analysing the all-inclusive data can result in very strong and significant differences between the applied methods, but it can be misleading as an in-depth analysis can prove otherwise. A comparison of the methods based on the influencing factors proved that, in some cases, there are similarities among the methods and we can get more knowledge on the measurements and the results provided this way. The results also guide some possible categories where measurements can provide a new way of forming the categories of, e.g., the available zinc based on the lime content, or clay content or pH, etc. As a result, we can gain extra knowledge from the comparison of the influencing factors to know where intervention is needed to use extra Zn for the proper fertilisation. This possibility of the new targeted measurements, however, is out of the scope of this study.

In conclusion, not only the well-known extraction methods and the soil, but also the chosen classification method of the properties and also, the statistical analysis (measuring all the data or certain classes) affect the evaluation of the Zn measurements. This comparative analysis study can provide a guide to interpret the different analysis methods by way of harmonisation.

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