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Comparison of magnesium determination methods on Hungarian soils

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Abstract: Magnesium is one of the most important nutrient elements. Soils are tested for magnesium in many countries with several extractants. Each country has its own validated methods, best-suited for its soils. The current study was designed to compare different magnesium content measuring methods with 80 Hungarian samples. The magnesium content was determined by the potassium chloride (1 M KCl 1:10), Mehlich 3 and CoHex (cobalt hexamine trichloride) methods. The maximum, mean and median values resulting from all the Mg determination methods showed the following order of measured magnitude: KCl < CoHex < M3.

Keywords: comparative analyses; extraction methods; soil nutrients

Soil is an ever decreasing and finite resource (Lal 2008). Its value is still not appreciated in many countries, while the majority of the human population suffers from malnutrition or simply lack sufficient food for survival. There are various efforts from many scientists to develop agriculture into producing the necessary amount of food by mapping the distribution of soil properties (e.g., soil thickness, soil organic matter content, soil nutrients (Behera & Shukla 2015), soil moisture content (Milics et al. 2017), etc.) and, thus, provide enough information for precision farming. Besides the necessity of having enough water, soil nutrients are the other important element in plant production. In this study, magnesium (Mg) is under scope.

Mg is an essential nutrient element for plant growth and reproduction (Marschner 1995; Koch *et al*. 2018). Its deficiency in soils has also been investigated by many authors (Yan & Hou 2018; Li et al. 2019). Magnesium in soil includes (Metson & Brooks 1975):
– soluble (Mg-sol),
– readily exchangeable (Mg-rex),
– slowly exchangeable (Mg-sex) and
– structural forms.

Water-soluble Mg forms account for the soil Mg present in the soil solution and in water soluble precipitates. The readily exchangeable Mg forms, comprise cationic Mg pieces in the diffuse layer, electrostatically adsorbed to negatively charged soil particles (Van Erp 2002).

The slowly exchangeable Mg fraction, includes Mg specifically adsorbed to humic substances (Salmon 1963; Murray & Linder 1984), (hydr)oxides (Chan et al. 1979) and clay minerals. The structural Mg forms, include the Mg present in the lattices of clay minerals, in carbonates, etc. (Burns & Burns 1974; Hunsaker & Pratt 1970).

Generally, readily exchangeable magnesium accounts for 3–20% of the total soil Mg content (Schroeder & Zahiroleslam 1962). However, the concentration of Mg in the soil solution is buffered by the readily available Mg that, in turn, is gradually replenished by the slowly exchangeable Mg and the structural Mg forms (Marschner 1995).

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Pot experiments in which soils were depleted, and long-term field experiments of Mg-balance sheet studies have shown that a plant's uptake of Mg is related to the size of the readily exchangeable magnesium (Schroeder et al. 1962; Rice & Kamprath 1968).

Mg is one of the main nutrients in plant nutrition, therefore, in many countries, soils are tested for its Mg content to make sure whether or not it is necessary to apply a Mg fertiliser (Ristimaki 2007; Roemheld & Kirkby 2007). Several extractants are used in routine soil testing to determine the soil Mg status, each country has its own validated methods which are best-suited for its soils.

Mg soil testing programmes use salt solutions, acidified salt solutions or acid solutions as extractant to assess the "plant-available Mg". The cations or protons added via these extractants replace (part of) the Mg (Mg-rex) resulting in an increased Mg concentration in the solution immediately after the addition (Thomas 1977). Depending on the extraction time and the affinity of the (specific) adsorption site(s) for Mg and the added cations, the slowly exchangeable Mg (Mg-sex) can also be extracted. The acidified extractants may promote the dissolution of the structural forms like Mg containing carbonates and minerals (Sposito 1994). The extent of the dissolution strongly depends on the procedural aspects like the proton activity, ionic strength, extraction time and soil-solution ratio. When it is assumed that Mg dissolves completely in the extractant (Mg-sol) during the soil extraction, irrespective of the extraction procedure, then the total amount of Mg in the extractant solution (Mg-ext), should equal the sum of the Mg-sol and the changes in the other soil Mg fractions (Van Erp 2002) (Figure 1).

In Hungary, the use of potassium-chloride has been standard practice since the 1980s when the national standard for soil testing was created (Loch 1970). The readily soluble Mg-containing solid constituents and the Mg bound to the soil cation exchange complex is regarded as the plant-available fraction (Loch 1970). Extraction of the soil, with unbuffered soil solutions like 1M KCl, have been used (Mazaeva 1967) to extract the plant-available fraction from soils in Hungary. The contents are assessed according to the soil texture. Potassium chloride extracts are also used for the Mg determination in Russia, Belarus, Ukraine and the Balkans.

Mehlich 3 (M3) is used in Czechia, Slovakia and Estonia (Fotyma & Dobers 2008). The Mehlich method (Mehlich 1978, 1984) was developed as a multi-nutrient extractant, buffered at pH = 2.5, for the determination of the plant-available soil fractions of phosphorous, potassium, calcium, magnesium, sodium, manganese and zinc. Here, the measured Mg content, according to 5 groups of richness, is grouped for light, moderately heavy and heavy soils. For example, in Czechia, the classification of the available Mg for moderately heavy arable soils are as follows: < 105, 106–160, 161–255, 256–330 and >330 mg/kg. However, these classes are different in sites with perennial grasses, orchards, vineyards and hop plantations (Budnakova & Čermak 2009).

The operating protocol of the CoHex method has been described in the study of Ciesielski & Sterckeman (1997). Today, the CoHex method is based on the ISO 23470 Standard wherein the exchangeable cations in the sample are replaced by trivalent cobalt hexamine ions. The cation exchange capacity (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. The objective of this study was to compare the KCl, M3 and CoHex methods for the Mg measurements.

MATERIAL AND METHODS

Sampling

Eighty geo-referenced soil samples (Figure 2) were collected from arable land in Hungary in 2017, differ-

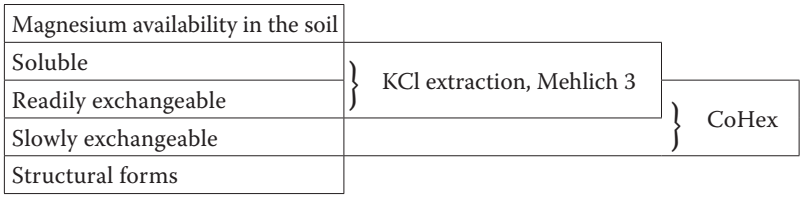

Figure 1. The Mg forms in the soil and extraction methods; the KCl and Mehlich methods are expected to extract the soluble and readily exchangeable Mg forms and the CoHex method is expected to extract the readily and slowly exchangeable forms

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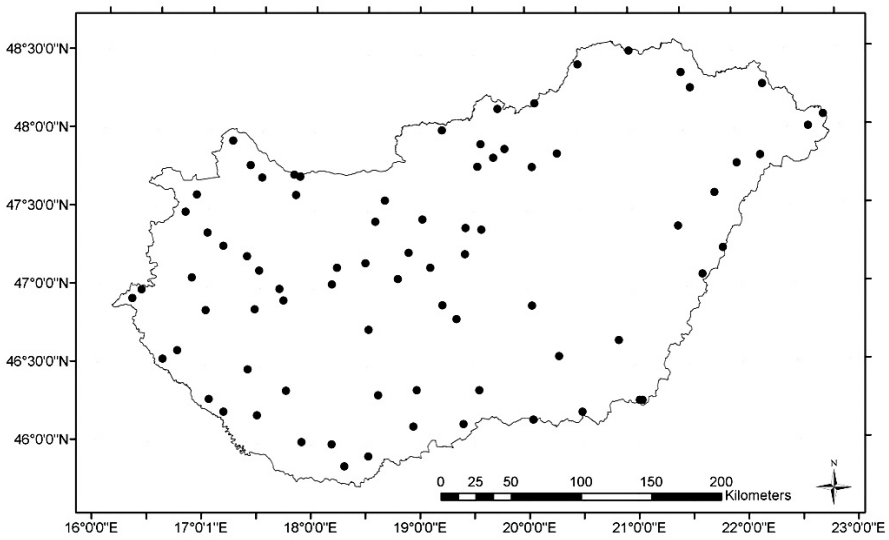

Figure 2. The sampling locations of the 80 soil samples in Hungary, 2017

ing in soil typology, texture and pH. The soil samples were taken from the 0–20 cm layer. The locations of the 80 samples were selected according to Minasny-McBratney (Minasny & McBratney 2006) and Roudier-Hedley (Roudier & Hedley 2013). The factors taken into account in this selection were: land use, soil type, climate data, accessibility and property market value.

Laboratory analysis

KCl extract. The Mg content of the soil samples was established at an accredited laboratory in Hungary using the traditional accepted standard (MSZ 20135:1999): the samples were extracted in a 1 M KCl solution, a soil to solvent ratio of 1 : 2.5 and stirred for 1 h.

Mehlich 3 method (M3). The Mehlich 3 method is undertaken in accordance with Chapter 5 of the Recommended Soil Testing Procedures for the Northeastern United States (Wolf & Beegle 2009).

The sample was extracted in a Mehlich 3 solution (0.2 M acetic acid, 0.015 M ammonium fluoride, 0.013 M nitric acid, 0.25 M ammonium nitrate, 0.001 M ethylenediaminetetraacetic acid, a solution was prepared from all the reagents, with pH 2.5), a soil to solvent ratio of 1:10 and stirred for 5 min. The soil extracts were analysed using inductively coupled plasma mass spectrometry (ICP-MS).

CoHex method. The cobalt hexamine method was implemented in accordance with ISO 23470:2007.

The CEC is determined by the difference between the initial quantity of hexamminecobalt in the solution and the quantity remaining in the extract after the exchange reaction. The measurement of the hexamminecobalt concentration in the extract is performed by an ICP-MS measurement of the Co concentration, which is compared to the concentration of a blank solution. The concentrations of the exchanged cations are measured in the same extract, using ICP-MS. The ICP-MS technique was chosen for the analyses of the sample extracts because it allows for the analysis of more elements due to an increased sensitivity and wider dynamic range than ICP-AES (inductively coupled plasma atomic emission spectrometry). The principle cations retained in a soil sample are exchanged with an aqueous solution of the hexamminecobalt ions by shaking the sample solution for 60 minutes at room temperature (20 ± 2 °C). After shaking, the samples are filtered through a syringe filter and measured using ICP-MS. Before the measurement, the extracts are individually diluted with varying dilution factors based on the predicted CEC values.

Measuring the CaCO3-content. The $CaCO_3$-content of the dry soil samples are given as a percentage. It was determined by the Scheibler volumetric method.

Analyses of the measured Mg content in the different $CaCO_3$-content categories. 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 magnesium versus the lime content in a more detailed manner. The carbonate content was divided into five groups in the following way;

– lime free soils < 0.1% of $CaCO_3$ (n = 31 samples),
– very low lime content 0.13–0.84% of $CaCO_3$ (n = 19 samples),

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– low lime content 1.01–3.16% of $CaCO_3$ (n = 10 samples),
– moderate lime content 3.64–8.79% of $CaCO_3$ (n = 10 samples) and
– high lime content 9.66–18.71% of $CaCO_3$ (n = 10 samples).

The basis for creating the categories were the sample size, the minimum was set to 10 per group in order to make a proper statistical analysis possible.

Statistical analysis

The following statistical indicators were used as a basis; arithmetic mean, standard deviation (SD), median (Me), coefficient of variation (CV), maximum (max) and minimum (min) values. 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. The normality of the data series of the different analysis methods was tested with the Kolmogorov-Smirnov test. If the values of the analysis did not have a normal distribution, then a non-parametric Friedmann ANOVA (analysis of variance) test was used. If the measurements of the analysis methods had a 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 Mg determination methods (CoHex, M3, KCl) to assess whether their mean ranks differed.

RESULTS

The descriptive statistics of the soil sample set is presented in Table 1.

The pH of the sample set was between pH 3.4 and pH 8.1. The mean of the pH was 6.3. The carbonate and Ca content were also diverse in the sample set, the mean/median was 2.6/2.3% for $CaCO_3$, and 6 208/3 917 mg/kg for Ca, measured with the Mehlich 3 method.

Comparison of the Mg determination methods with the all-inclusive data analyses. The Wilcoxon signed-rank test showed that the M3 and CoHex measurements are similar, while the values measured by the KCl method are significantly different. The Mg analysed with the KCl showed a significantly lower Mg content compared to the CoHex and M3 method.

According to the basic statistical description, the highest Mg content was measured by using the M3 and the lowest content was determined by the KCl method. The mean and median of the Mg content measured by the three methods resulted in the following order for the measurement accuracy (Table 2); the lowest mean/median was 271/215 mg/kg for the KCl, the middle one was 359/289 mg/kg for the CoHex and the highest was 395/355 mg/kg for the M3 methods.

A correlation regression was used to determine the relationships of the soil Mg content measured by the Mehlich 3, CoHex and KCl methods, as can be seen in Figure 3.

A moderate correlation was established between the Mg content determined by the KCl and M3 methods (R^2 = 0.62). The relationship between the Mg content determined by the M3 and CoHex methods was slightly stronger (R^2 = 0.67). While the relationship of the Mg content between the KCl and CoHex methods was shown to be very strong (R^2 = 0.96).

However, regardless of the strong relationship between the KCl and CoHex methods, the Mg measurements with the KCl method are significantly lower compared to the CoHex method. The CoHex method

Table 1. The basic statistical data of the soils' pH level, $CaCO_3$, humus and Ca (M3) contents (No. of samples (n) = 80)

Indicators	pH (KCl)	$CaCO_3$	Humus	Ca M3
		(%)		(mg/kg)
Mean	6.3	2.6	2.3	6 208
SD	1.3	4.2	1.1	6 829
Me	7.0	0.4	2.3	3 917
CV	20.5	163.1	46.1	110
Min	3.4	0.1	0.5	50
Max	8.1	18.7	6.5	38 441

SD – standard deviation; Me – median; *CV* – coefficient of variation

Table 2. The basic statistical data of the soils' Mg contents determined by the different methods (No. of samples (n) = 80)

Indicators	Mg content (mg/kg)		
	CoHex	M3	KCl
Mean	359	395	271
SD	280	278	200
Me	289	355	215
CV	78	70	74
Min	7	11	13
Max	1 213	1 295	860

SD – standard deviation; Me – median; *CV* – coefficient of variation

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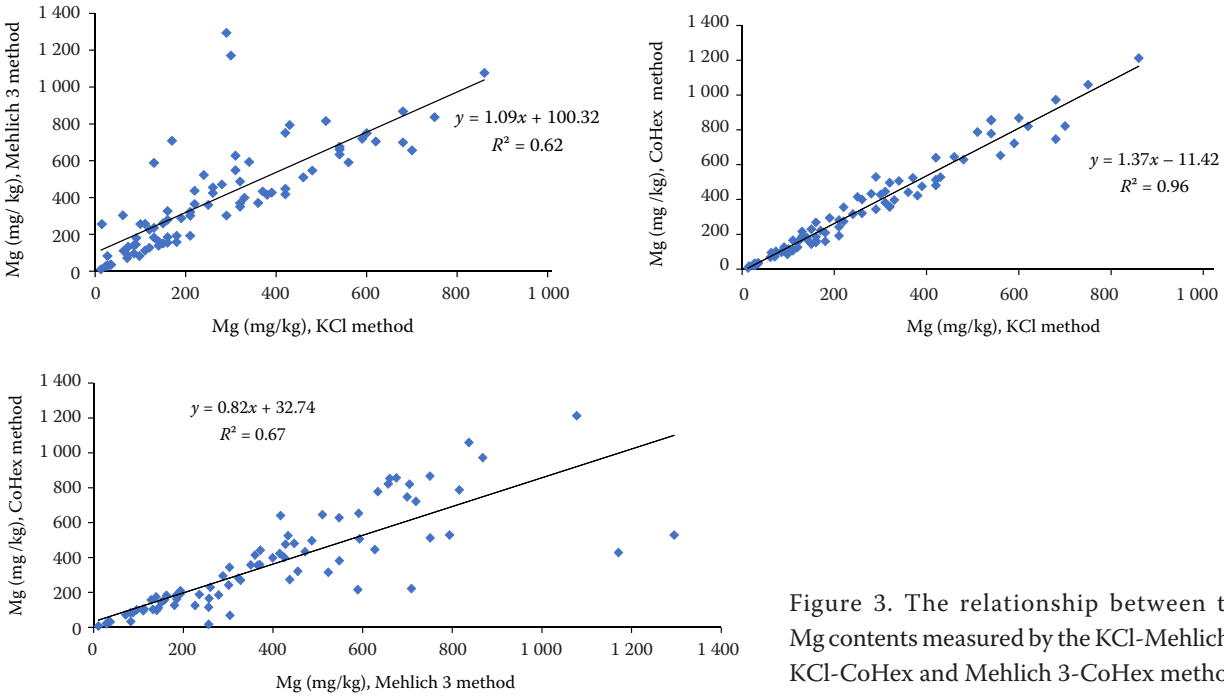

Figure 3. The relationship between the Mg contents measured by the KCl-Mehlich 3, KCl-CoHex and Mehlich 3-CoHex methods

measures the slowly exchangeable cations besides that which the KCl method measures (soluble and readily exchangeable). Still, it seems to extract less – most likely, only the readily exchangeable Mg cations.

Analyses of the measurements based on the comparison of the data in the CaCO3-content groups of the samples. In the lime-free or low calcareous soils, there is strong correlation between all the methods. The increased CaCO3-content showed a regressively weaker correlation between all three methods.

The KCl and CoHex methods showed a strong correlation in all the CaCO3 categories. In the case of the high CaCO3-content, the relationship was a bit weaker (R^2 = 0.85), in all the other cases it was 0.95 or higher.

In the lime-free, very low and low calcium carbonate groups, there was strong correlation between the M3 and CoHex methods. The increased calcium carbonate content showed a regressively weaker correlation between all three methods. In the calcareous soils, the M3 method measured a higher magnesium content compared to the CoHex and KCl methods (Table 3).

DISCUSSION

There are several soil analysis methods used for predicting the phytoavailability of the nutrients, making a direct comparison of the results often difficult (Rice & Kamprath 1968; Gransee & Führs 2013). In this comparable analysis study, we also found difficulties. The data analyses proved that there are significant differences between the methods. Our findings show that a small amount of Mg was measured without significant differences between the methods, while the differences between the methods

Table 3. The correlation between the different analysis methods in the different lime content categories

No. of the group	Lime content (%)	Sample size (n)	R^2 values		
			M3 and KCl methods	CoHex and KCl methods	CoHex and M3 methods
1	< 0.1 (lime free)	31	0.98	0.97	0.97
2	0.1–1 (very low)	19	0.97	0.98	0.98
3	1.01–3.16 (low)	10	0.82	0.95	0.91
4	3.64–8.79 (moderate)	10	0.49	0.99	0.49
5	9.66–18.71(high)	10	0.17	0.85	0.29

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increased with an increase in the measured amounts. A comparison of methods showed an unexpected strong correlation (R^2 = 0.96) between the KCl and CoHex methods based on the R^2 values. However, these two methods should be more dissimilar from one another as the KCl method "only" measures the soluble and the readily exchangeable part of the Mg in the soil, while the CoHex method can also measure the slowly exchangeable part.

The KCl and M3 methods were expected to produce similar results with a high correlation, but showed a very low correlation (R^2 = 0.67), especially compared to that of 0.97 between the KCl and CoHex methods.

The M3 and CoHex methods had a similar low correlation, such as that of the KCl and M3 methods. In this case, this reflected our expectations. Thus, we can conclude that the Wilcoxon signed-rank test and the R^2 values produced opposite results.

The complexity of a soil analysis was proven by Staugaitis and Rutkauskienė (2010) who compared six different Mg extraction and determination methods and found that the extraction procedure strongly impacts the outcome, similarly seen in this study. Nevertheless, all mild extraction procedures including $CaCl_2$, KCl, NH_4OAc and the M3 method showed quite high correlations over all three sampling depths investigated, indicating similar extraction characteristics for the soil Mg. These results do not reflect our findings where the KCl extraction resulted in much lower amounts, compared to those of the M3 method. A comparison of methods showed expected differences between the KCl and CoHex methods (as the KCl method measures significantly lower amounts) with the Wilcoxon signed-rank test, since KCl does not bring the slowly exchangeable Mg into the solution, so it does not measure it. The basic statistical figures of the mean and median values produced the same order of magnitude for the methods; the KCl method measured the smallest, the M3 method the middle range and the CoHex method the largest amount of Mg.

The results of a joint institutional project in Czechia, Hungary, Poland and the Netherlands on the calibration of the 0.01M $CaCl_2$ soil testing procedure for Mg are presented by Loch et al. (1998). With the relatively weak extractant, 0.01M $CaCl_2$, somewhat lower quantities of Mg were extracted from the soil samples compared to the extraction solutions used in Poland (0.0125M $CaCl_2$), in Hungary (1M KCl) and in the Netherlands (0.5M NaCl). With the relatively strong extractant used in Czechia (Mehlich II), more Mg was extracted. Based on the study of Zbíral and Němec (2000), significant correlations were found between the M2 and M3 methods for all the nutrients (the correlation coefficients were in the range of 0.97–0.99).

This result corresponds with our findings where the M3 method measured more Mg than the KCl method. In general, the amount of Mg extracted increases, i.e., 0.01 M $CaCl_2$ < Schachtschabel < 0.5 M NaCl < 1 M KC1 < Mehlich-2.

The previously mentioned and this study's results show the importance of choosing an appropriate extraction method for the correct evaluation of the soil Mg availability to the crops. The amount of Mg that can be extracted from the soils depends on the chemical composition and concentration of the extracting solution in combination with the ratio of the soil-to-extracting-solution (Loch 1970).

The readily soluble Mg-containing solid constituents and Mg bound to the cation exchange complex of the soil is regarded as the plant available fraction. Extraction of the soil elements with unbuffered soil solutions like 1M KC1 (Mazaeva 1967) is used to extract this plant-available fraction from the soil. The use of acidic extractants is not advised because they also extract part of the Mg reserves of the soil. Despite this, Mehlich (1978; 1984) developed a multinutrient extractant, buffered at pH = 2.5, for the determination of the plant-available soil fractions of Mg (Loch 1970). The applied Mg availability categories are theoretical since Rice & Kamprath (1968) found a large amount of Mg uptake by maize from the nonexchangeable pool. This strengthens the need to re-establish the limit between the readily available Mg and the nonexchangeable Mg pool for agricultural plants.

The Mehlich-2 method also extracts part of the Mg reserves of the soil, especially in soils containing dolomite (Loch 1970). The acidified extractants may promote the dissolution of structural forms like Mg containing carbonates and minerals (Sposito 1994). In our study, it was also visible that the M3 method extracted more Mg compared to the KCl method.

In most comparative studies, the results of the two extraction procedures are related by using statistical techniques like (multiple) linear regression. To increase the explained variance of the relationships, soil characteristics like the soil type, organic matter, clay, and carbonate contents are also arbitrarily included (Baier & Baierova 1981; Matejovic & Durackova 1994; Mamo et al. 1996; Loch et al. 1998). We can

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also conclude that not only the extraction method, but also soil properties like the carbonate content, have an effect on the evaluation of the magnesium measurements. Our results showed that, in the case of the $CaCO_3$ free or low $CaCO_3$-content soils, there was strong correlation between the three methods, but the increased $CaCO_3$ content showed weaker correlations.

Van Erp (2002) compared the relationship between 0.01 M $CaCl_2$, $BaCl_2$ and KCl extractants. An analysis of the difference in the Mg extracted among the $BaCl_2$ and KCl methods showed that the difference was related to the clay content of the soils and not to the organic C content or carbonate content. The Mg was specifically adsorbed at the surface of these (hydr)oxides which is not replaceable by K at a high pH (Chan et al. 1979).

Dontsova and Norton (2002) reported on the degrading effect of a high Mg content on the soil structure and clay dispersion. Contrarily, Wang et al. (2004) found no texture effect on the Mg availability.

Ortas et al. (1999) state that there is often a poor relationship between the plant growth response and extractable nutrients in the soil. A soil analysis just provides a picture of the current situation at a given site; it is not capable of perfectly simulating the plant characteristics on the Mg uptake. Also, other agronomic and environmental factors are not included. Consequently, a soil analysis only gives information on the potential of a soil to provide the respective nutrients (Gransee & Führs 2013). Concerning the comparison and evaluation of the different Mg determination methods, it should be further investigated to find the most appropriate method for the different varieties of influential soil properties.

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