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Extractability of nutrients using Mehlich 3 and ammonium bicarbonate-DTPA methods for selected grassland soils of China

Chunping ZHANG[1], Decao NIU[1,*], Yuntao REN[1,2], Hua FU[1]

[1]*State Key Laboratory of Grassland Agro-ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, P.R. China*
[2]*Urumqi City Agricultural and Pastoral Bureau, Urumqi, P.R. China*
**Corresponding author: xiaocao0373@163.com*

ABSTRACT

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This study aimed to obtain a simple and efficient soil extraction method as an exhaustive and systematic technology guideline for the determination of potentially available phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) in various grassland soils covering different regions. In this study, 25 soil samples from 5 different grassland types of China were collected to measure the amounts of potentially available nutrients and to compare the results of Mehlich 3, the ammonium bicarbonate-diethylenetriamine-pentaacetic acid (AB-DTPA) and other four methods which are widely used in China (i.e. Olsen, Bray 1, 1 mol/L NH_4OAc, $CaCl_2$-DTPA, called conventional methods). The results showed that the amounts of potentially available nutrients extracted by different methods were significantly different. Moreover, a positively significant correlation was obtained between the amounts of potentially available nutrients extracted by conventional methods and those extracted by Mehlich 3 and AB-DTPA. However, for Mg and Mn, the results from AB-DTPA appeared to have a stronger relationship with the results from the conventional method than with the results from Mehlich 3. These differences might be caused by the effect of soil pH. Our results suggested that both Mehlich 3 and AB-DTPA can be effectively used to measure nutrients availability in grassland soils, while the AB-DTPA will be more recommended to measure the amounts of potentially available nutrients in alkaline soils.

Keywords: nutrition; soil testing; heavy metals; extractant; multi-element analysis

Soil potentially available nutrients (P, K, Mg, Fe, Cu, Mn and Zn) determine soil fertility and plant productivity and affect the healthy development of grassland animal husbandry (Grzebisz 2013). Therefore, the measurement of potentially available nutrients is crucial to evaluate soil quality and instruct animal husbandry production. Conventionally, in China, soil potentially available phosphorus (P) is analysed using Bray 1 for acid soils (Bray and Kurtz 1945), while Olsen is used for weakly acidic, neutral and alkaline soils (Olsen et al. 1954). Soil potentially available potassium (K) is analysed with 1.0 mol/L NH_4OAc (Jackson 1958). Soil metal elements (e.g. Fe, Cu, Mn, Zn, Ni) are analysed with $CaCl_2$-DTPA (Lindsay and Norvell 1978). Nevertheless, these conventional methods (i.e. Olsen, Bray 1, 1 mol/L NH_4OAc, $CaCl_2$-DTPA) are only used to analyse specific

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Table 1. Main characteristics of the investigated areas

Investigated area	pH	Soil type	Grassland type	Longitude and latitude
MaQu	5.9	Phaeozems	alpine meadow	37°23'N, 98°41'E
QingHai	6.1	Chernozems	alpine steppe	23°75'N, 106°90'E
Hulun Buir	6.8	Podzols	temperate steppe	50°10'N, 119°22'E
Loess Plateau	8.5	Kastanozems	typical steppe	35°57'N, 104°09'E
Alashan	9.1	Solonetz	desert steppe	39°08'N, 105°36'E

elements with special requirements of soil pH, which is mainly caused by the difference of acidity-basicity, ion complexation and exchange capacity of different extractants (Sparks 1996). In addition, these conventional methods are inefficient as they consume more labour force and reagents for analysing multiple elements of a great number of samples (Alva et al. 1990).

At present, the ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) (Soltanpour and Schwab 1977) and Mehlich 3 (Mehlich 1984) are attractive to soil testing laboratories, since these methods can extract multiple elements (macro- and micronutrients) simultaneously and greatly improve extraction efficiency (Takrattanasaran et al. 2010). Elrashidi et al. (2003) found that both Mehlich 3 and AB-DTPA were closely related to the conventional methods for extractable P and K. Moreover, Pradhan et al. (2015) compared the results with different methods for extractable Zn and Cu in acid soils from agroecological areas of India. They found that the results of Mehlich 3 and AB-DTPA were strongly correlated to the results of $CaCl_2$-DTPA. Although there are studies giving the results of comparison between the Mehlich 3 or AB-DTPA and the conventional methods for the measurement of amounts of potentially available nutrients (Takrattanasaran et al. 2010), those studies are mostly limited to potentially available P, K and a limited number of micronutrients. Additionally, these works were done only with soil samples in certain regions, except the grassland in China. Moreover, there is still a lack of studies about providing the results of comparison and correlation analysis between Mehlich 3 or AB-DTPA and four conventional methods (Olsen, Bray 1, 1 mol/L NH_4OAc, $CaCl_2$-DTPA) for determining multi-elements in soils.

Determination of potentially available nutrients in grassland soils of China is still based on inefficient conventional methods (i.e. for potentially available P it is the Bray 1 method (NY/T 1121.7-2014, 2014); for potentially available K it is NH_4OAc (NY/T 889-2004, 2004); for potentially available metal elements it is $CaCl_2$-DTPA (NY/T 890-2004, 2004); see the details of methods in Table 2). However, Mehlich 3 and AB-DTPA are widely used in Europe and the United States for the determination of potentially available nutrients (Zbíral 2016). To obtain a simple and efficient method of soil potentially available nutrients analysis for grassland management, this study selected 25 soils in different grassland types in China to measure

Table 2. Details of methods employed for the extraction of potentially available nutrients

Extractant	Extractant composition	Soil: extractant	Soil weight (g)	Extractant volume (mL)	Shaking time (min)
Mehlich 3	0.2 mol/L CH_3COOH, 0.5 mol/L NH_4NO_3, 0.015 mol/L NH_4F, 0.013 mol/L HNO_3, 0.001 mol/L EDTA	1:10	5	10	5
AB-DTPA	1 mol/L NH_4HCO_3, 0.005 mol/L DTPA	1:2	10	5	15
Ca_2Cl-DTPA	0.005 mol/L DTPA, 0.01 mol/L $CaCl_2$, 0.1 mol/L TEA	1:2	10	5	120
Olsen	0.5 mol/L $NaHCO_3$	1:20	2.5	10	15
Bray 1	0.03 mol/L NH_4F, 0.05 mol/L HCl	1:7	1	7	15
NH_4OAc	1.0 mol/L NH_4OAc	1:10	5	10	30

TEA – triethanolamine

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Table 3. The analytical results of the amounts of phosphorus (P, mg/kg) extracted by Mehlich 3, AB-DTPA and Bray 1/Olsen (mg/kg soil)

Soil No.	Soil pH	Mehlich 3	AB-DTPA	Conventional method (Bray 1/Olsen)
1	5.9	12.07 ± 0.17^a	1.22 ± 0.06^c	6.60 ± 0.13^b
2	6.1	21.12 ± 0.51^a	9.41 ± 0.371^c	15.87 ± 0.53^b
3	6.8	9.89 ± 0.07^a	2.69 ± 0.06^c	3.41 ± 0.13^b
4	8.5	14.50 ± 0.70^a	8.63 ± 0.03^c	10.64 ± 0.22^b
5	9.1	50.50 ± 0.22^a	10.56 ± 0.37^c	18.65 ± 0.15^b
Range		9.89–50.50	1.22–10.56	3.41–18.65
Mean		16.62	6.50	12.27

Different lowercase letters represent a significant difference of soil potentially available phosphorus values between different methods of the same soil type ($P \leq 0.05$)

Table 5. The analytical results of the amounts of potassium (K, mg/kg) extracted by Mehlich 3, AB-DTPA and NH_4OAc (mg/kg soil)

Soil No.	Soil pH	Mehlich 3	AB-DTPA	NH_4OAc
1	5.9	340.10 ± 0.62^a	218.43 ± 2.22^c	318.16 ± 1.41^b
2	6.1	308.87 ± 1.13^a	190.47 ± 0.42^c	277.70 ± 1.75^b
3	6.8	194.58 ± 0.63^a	117.29 ± 0.24^c	174.20 ± 2.69^b
4	8.5	113.83 ± 0.80^a	87.38 ± 1.78^c	99.74 ± 1.26^b
5	9.1	240.36 ± 12.18^a	162.10 ± 9.90^c	198.82 ± 0.55^b
Range		113.83–340.10	87.38–218.43	99.74–318.16
Mean		239.55	155.13	213.72

Different lowercase letters represent a significant difference of soil potentially available potassium value between different methods of the same soil type ($P \leq 0.05$)

the amounts of potentially available nutrients (P, K, Mg, Fe, Cu, Mn and Zn) and to analyse the relationship between Mehlich 3 or AB-DTPA and the conventional methods.

MATERIAL AND METHODS

Soil properties. Soil samples were taken from 5 investigated areas in China covering 5 different grassland types. Details of sampling locations are listed in Table 1. In each area, 5 soil samples from the surface layer (0–10 cm) were collected. These soil samples were distinct in physical and chemical properties including 10 acidic, 5 neutral and 10 alkaline soils. In addition, all samples were saved after full air-drying, mixing and passing 2-mm sieves.

Experiment procedure. To assess Mehlich 3 and AB-DTPA, seven elements (P, K, Mg, Cu, Fe, Mn and Zn) were investigated. These elements were divided into three categories: potentially available P, exchangeable K and potentially available metal elements (Mg, Cu, Fe, Mn and Zn). For these three categories, different conventional methods (Bray 1/Olsen (NY/T 1121.7-2014) for potentially available P, NH_4OAc (NY/T 889-2004) for potentially available K, $CaCl_2$-DTPA (NY/T 890-2004) for potentially available metal elements) were employed to investigate the correlation between Mehlich 3 or AB-DTPA and these methods (Table 2).

Statistical analysis. All statistical tests were run using the SPSS (v. 22.0, Chicago, USA). A one-way analysis of variance (ANOVA) was conducted for the amounts of potentially available nutrients and the post-hoc Tukey´s tests were used for the comparison of differences among methods for the same element. The effects of soil pH, methods and their interaction on soil potentially available nutrients data were analysed with two-way ANOVA. Simple linear regression was conducted to compare the results between Mehlich 3 or AB-DTPA and conventional methods and to estimate their substitutability.

Table 4. Regression equations and correlation coefficient (r value) for relationships between the amounts of phosphorus (P) extracted by Mehlich 3 or AB-DTPA and Bray 1/Olsen

Independent variable	Regression model	R^2	Correlation coefficient (r value)
Bray 1/Olsen-P	Mehlich 3-P = 1.950 (Bray 1/Olsen-P) – 4.743	0.962	0.980**
	AB-DTPA-P = 0.802 (Bray 1/Olsen-P) + 6.770	0.811	0.900**

**$P \leq 0.01$

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Table 6. Regression equations relating Mehlich 3 or AB-DTPA to NH_4OAc

Independent variable	Regression model	R^2	Correlation coefficient (r value)
NH_4OAc-K	Mehlich 3-K = 1.041 (NH_4OAc-K) + 16.96	0.962	0.985**
	AB-DTPA-K = 0.604 (NH_4OAc-K) + 26.04	0.811	0.962**

**$P \leq 0.01$

RESULTS AND DISCUSSION

Soil potentially available phosphorus. The amounts of soil potentially available P in the same soil type showed greatly significant differences between different methods (Table 3) and had an interaction between soil pH value and extraction method (Table 10). The range and mean of soil potentially available P were also given in Table 3. The mean of extracted P by Mehlich 3 was 1.4 times higher than that extracted by Bray 1/Olsen. However, the mean of extracted P by AB-DTPA only accounted for 60% of that extracted by Bray 1/Olsen. In acid soil, hydrogen and F^- are the main ions used by Mehlich 3 and Bray 1 to dissolve phosphate rock in soil. However, Bray 1 does not contain ethylenediamine tetraacetic acid (EDTA) which exists in Mehlich 3 and has a very strong ability to displace phosphate from soil solid. However, both AB-DTPA and Olsen remove soil P with HCO_3^- ion and principally from Ca-phosphates (Olsen and Sommers 1882). In addition, hydrogen and F^- ions are stronger than HCO_3^- ion in dissolving phosphate rock (Elrashidi et al. 2003) and the chelating agent diethylenetriaminepentaacetic acid (DTPA) contained in AB-DTPA causes interference in the colorimetric determination of P (Reed and Martens 1996). These differences demonstrated that the amounts of extracted P followed the order: Mehlich 3 > Bray 1/Olsen > AB-DTPA.

A positively significant correlation was obtained between the amounts of P extracted by Mehlich 3 or AB-DTPA and those extracted by Bray 1/Olsen (Table 4). The results suggested that both Mehlich 3 and AB-DTPA can be considered as appropriate P extractants for soil investigations.

Soil potentially available potassium. The amounts of soil potentially available K in the same soil type showed greatly significant differences between different methods (Table 5) and had an interaction between soil pH value and extraction method (Table 10). The range and mean of soil potentially available K were also given in Table 5. The amounts of ex-

Table 7. The analytical results of amounts of metal elements extracted by Mehlich 3, AB-DTPA and $CaCl_2$-DTPA (mg/kg soil)

Soil No.	Soil pH	Single extraction		
		Mehlich 3	AB-DTPA	Ca_2Cl-DTPA
Magnessium				
1	5.9	247.88 ± 0.81^a	70.33 ± 2.05^b	49.37 ± 0.10^c
2	6.1	263.53 ± 0.34^a	62.58 ± 0.05^b	47.06 ± 0.17^c
3	6.8	300.98 ± 1.11^a	83.67 ± 0.04^b	58.71 ± 0.30^c
4	8.5	303.10 ± 0.11^a	72.40 ± 0.03^b	49.73 ± 0.23^c
5	9.1	270.96 ± 0.55^a	67.64 ± 0.77^b	40.93 ± 0.24^c
Copper				
1	5.9	2.96 ± 0.03^b	5.86 ± 0.02^a	2.28 ± 0.11^c
2	6.1	3.74 ± 0.05^b	6.48 ± 0.05^a	1.74 ± 0.03^c
3	6.8	1.85 ± 0.01^b	2.04 ± 0.02^a	0.56 ± 0.02^c
4	8.5	1.86 ± 0.05^b	4.00 ± 0.01^a	0.89 ± 0.01^c
5	9.1	3.74 ± 0.05^b	6.48 ± 0.05^a	1.74 ± 0.03^c
Iron				
1	5.9	200.88 ± 1.11^a	101.61 ± 0.02^b	66.16 ± 0.11^c
2	6.1	63.19 ± 0.52^a	58.53 ± 0.23^b	22.42 ± 0.52^c
3	6.8	79.93 ± 1.72^a	30.16 ± 0.50^b	13.40 ± 0.49^c
4	8.5	13.44 ± 0.13^a	12.44 ± 0.08^b	3.79 ± 0.21^c
5	9.1	32.95 ± 2.60^a	4.10 ± 0.02^b	2.99 ± 0.41^c
Manganese				
1	5.9	68.06 ± 0.75^a	42.47 ± 3.19^b	29.60 ± 0.17^c
2	6.1	77.87 ± 1.81^a	29.91 ± 0.39^b	12.71 ± 0.32^c
3	6.8	38.41 ± 0.69^a	2.17 ± 0.03^c	7.06 ± 0.49^b
4	8.5	50.68 ± 0.73^a	5.19 ± 0.05^b	2.06 ± 0.03^c
5	9.1	49.89 ± 2.43^a	2.32 ± 0.10^b	1.50 ± 0.12^c
Zinc				
1	5.9	8.07 ± 0.23^a	6.75 ± 0.02^b	4.02 ± 0.02^c
2	6.1	3.00 ± 0.01^a	1.94 ± 0.01^b	1.10 ± 0.02^c
3	6.8	2.52 ± 0.06^a	0.59 ± 0.03^b	0.49 ± 0.06^c
4	8.5	2.59 ± 0.11^a	0.68 ± 0.01^b	0.30 ± 0.01^c
5	9.1	2.64 ± 0.18^a	0.37 ± 0.02^b	0.32 ± 0.04^c

Different lowercase letters represent a significant difference of soil potentially available metal elements value between different methods of the same soil type ($P \leq 0.05$)

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Table 8. Ranges and means of amounts of metal elements removed by different extractants

Extractant		Magnesium	Copper	Iron	Manganese	Zinc
		(mg/kg soil)				
Mehlich 3-extractable metals	max	303.10	3.74	200.88	77.87	8.07
	min	247.88	1.56	13.44	50.68	2.52
	average	277.29	2.40	67.03	51.98	3.76
AB-DTPA-extractable metals	max	83.67	6.48	101.61	42.47	6.75
	min	62.58	1.40	4.10	2.17	0.37
	average	71.32	3.96	36.16	16.41	2.07
$CaCl_2$-DTPA-extractable metals	max	58.71	2.28	66.16	29.60	4.02
	min	40.93	0.37	2.99	1.50	0.30
	average	49.16	1.17	23.80	10.59	1.24

tracted K followed the order: Mehlich 3 ≈ NH_4OAc > AB-DTPA. Although all these three methods use NH_4^+ to displace exchangeable potassium on the surface of soil mineral, the H^+ in Mehlich 3 can effectively dissolve parts of mineral K^+. Moreover, the solution extracted by alkaline AB-DTPA (pH = 7.6) contains a large amount of soluble organic matter, which causes interference with the measurement of K by a flame photometer (UV-1601, Shimadzu, Japan). A similar result was revealed by Elrashidi et al. (2003).

A positively significant correlation was obtained between the amounts of K extracted by Mehlich 3 or AB-DTPA and those extracted by NH_4OAc (Table 6). The regression model could be showed by the following equation: Mehlich 3-K = 1.041 (NH_4OAc-K) + 16.96 (r = 0.985, $P \leq 0.01$) and AB-DTPA-K = 0.604 (NH_4OAc-K) + 26.04 (r = 0.962, $P \leq 0.01$). The results suggested that both Mehlich 3 and AB-DTPA can be considered as an appropriate K extractant for soil investigations.

Soil potentially available metal elements. The amounts of soil potentially available metal elements in the same soil type showed greatly significant differences between different methods (Table 7) and had an interaction between soil pH value and extraction method (Table 10). The range and mean of soil potentially available metal elements were given in Table 8. In general, with the exception of Cu, the amounts of extracted Mg, Fe, Mn and Zn followed the order: Mehlich 3 > AB-DTPA > $CaCl_2$-DTPA, which can probably be due to the existence of the chelating agent EDTA and acid (CH_3COOH and HNO_3) in Mehlich 3 can chelate and dissolve most of the metal minerals containing

Table 9. Regression equations relating Mehlich 3 or AB-DTPA to Ca_2Cl-DTPA

Metal	Regression model	R^2	Correlation coefficient (r value)
Magnesium	Mehlich 3-Mg = 1.918 ($CaCl_2$-DTPA-Mg) + 183.0	0.260	0.510**
	AB-DTPA-Mg = 1.014 ($CaCl_2$-DTPA-Mg) + 21.47	0.642	0.801**
Copper	Mehlich 3-Cu = 0.930 ($CaCl_2$-DTPA-Cu) + 1.307	0.684	0.827**
	AB-DTPA-Cu = 2.443 ($CaCl_2$-DTPA-Cu) + 1.127	0.821	0.913**
Iron	Mehlich 3-Fe = 1.701 ($CaCl_2$-DTPA-Fe) + 7.706	0.841	0.937**
	AB-DTPA-Fe = 1.470 ($CaCl_2$-DTPA-Fe) + 9.392	0.937	0.968**
Manganese	Mehlich 3-Mn = 1.212 ($CaCl_2$-DTPA-Mn) + 39.14	0.450	0.652**
	AB-DTPA-Mn = 1.493 ($CaCl_2$-DTPA-Mn) + 0.610	0.834	0.911**
Zinc	Mehlich 3-Zn = 1.507 ($CaCl_2$-DTPA-Zn) + 1.888	0.963	0.981**
	AB-DTPA-Zn = 1.691 ($CaCl_2$-DTPA-Zn) – 0.038	0.994	0.997**

**$P \leq 0.01$

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Mg^{2+}, Fe^{3+}, Mn^{2+} and Zn^{2+}. Moreover, the AB-DTPA has a higher extraction capacity than the $CaCl_2$-DTPA, which can probably be due to the elution ability of HCO_3^- contained in AB-DTPA to the metal cation which is stronger in original soil structure than the elution ability of Ca^{2+} contained in $CaCl_2$-DTPA. However, with respect to Cu, the extraction amounts followed the order: AB-DTPA > Mehlich 3 > $CaCl_2$-DTPA. The different Cu extraction ability may be caused by the participation of NH_4^+ in the chelating reaction of Cu^{2+} and the concentration of NH_4^+ in AB-DTPA is higher than the concentration of NH_4^+ in Mehlich 3. Besides, the ability of chelating agent DTPA in AB-DTPA to chelate Cu^{2+} is stronger than that EDTA in Mehlich 3. Nevertheless, the extraction ability of Mehlich 3 is stronger than $CaCl_2$-DTPA, which can probably be due to the $CaCl_2$-DTPA that only uses DTPA to chelate Cu^{2+}, whereas Mehlich 3 uses the chelating agents EDTA, NH_4^+ and acid (CH_3COOH and HNO_3) to chelate Cu^{2+} and dissolve metal minerals containing Cu^{2+}.

With the exception of Mg and Mn, a positively significant correlation was obtained between the amounts of metal elements extracted by Mehlich 3 or AB-DTPA and those extracted by $CaCl_2$-DTPA (Table 9). However, with respect to Mg and Mn, a poor correlation was obtained between Mehlich 3 and $CaCl_2$-DTPA, which may be caused by soil pH. Especially in alkaline soils, Mg^{2+} and Mn^{2+} cannot be completely displaced because of the existence of insoluble $MgCO_3$ and $Mn(OH)_2$. However, H^+ in Mehlich 3 can dissolve a part of $MgCO_3$ and $Mn(OH)_2$, making its measurement results much higher than the results of $CaCl_2$-DTPA and thus affecting the correlation between them (Bao 2000). These results suggested that both Mehlich 3 and AB-DTPA can be considered as an appropriate metal elements extraction. However, AB-DTPA will be more accurate than Mehlich 3 if there is need to measure the amounts of potentially available Mg and Mn in grassland soils.

It is efficient to use the multi-elemental methods (e.g. Mehlich 3 and AB-DTPA method) to estimate the pool of potentially available nutrients in soil, especially because of cost saving in agricultural practice. However, it is still widely suggested to determine soil potentially available nutrients based on other inefficient methods in China (i.e. Olsen, Bray 1, 1 mol/L NH_4OAc, $CaCl_2$-DTPA; NY/T 1121.7-2014, NY/T 890-2004, NY/T 889-2004,

Table 10. The two-way ANOVA analysis for the analytical results of potentially available nutrients

Source of variation	*SS*	*df*	*MS*	*F*
Phosphorus				
Soil pH	1843.997	4	460.999	4993.762**
Methods	1283.420	2	641.710	6951.305**
Soil pH × methods	160.621	8	20.078	217.491**
Error	5.539	60	0.092	
Total variation	13014.549	75		
Potassium				
Soil pH	349920.970	4	87480.243	966.307**
Methods	93540.506	2	46770.253	516.624**
Soil pH × methods	19303.969	8	2412.996	26.654**
Error	5431.828	60	90.530	
Total variation	3552783.162	75		
Magnesium				
Soil pH	7803.990	4	1950.998	796.749**
Methods	791313.184	2	395656.592	161578.334**
Soil pH × methods	5847.395	8	730.924	298.495**
Error	146.922	60	2.449	
Total variation	2123615.190	75		
Iron				
Soil pH	124547.542	4	31136.885	7734.169**
Methods	40870.073	2	20435.037	5075.910**
Soil pH × methods	28015.432	8	3501.929	869.853**
Error	241.553	60	4.026	
Total variation	359818.619	75		
Copper				
Soil pH	100.211	4	25.053	2201.261**
Methods	97.731	2	48.866	4293.576**
Soil pH × methods	31.004	8	3.876	340.524**
Error	0.683	60	0.011	
Total variation	701.009	75		
Mangan				
Soil pH	15337.892	4	3834.473	534.134**
Methods	25107.700	2	12553.850	1748.726**
Soil pH × methods	3316.609	8	414.576	57.750**
Error	430.731	60	7.179	
Total variation	96176.289	75		
Zinc				
Soil pH	296.086	4	74.022	2079.913**
Methods	82.574	2	41.287	1160.105**
Soil pH × methods	14.979	8	1.872	52.613**
Error	2.135	60	0.036	
Total variation	813.059	75		

**$P \leq 0.01$

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Agriculture Industry Standard of China). To verify whether Mehlich 3 and AB-DTPA methods can be widely used for Chinese grassland management, this study was conducted and the results showed there was a positively significant correlation between the amounts of potentially available nutrients extracted by the conventional methods and those extracted by Mehlich 3 or AB-DTPA, which suggested both Mehlich 3 and AB-DTPA can be effectively used to measure the nutrients availability in grassland soils instead of the inefficient methods.

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