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Evaluation of extractability of different extractants for zinc and copper in soils under long-term fertilization

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ABSTRACT

We aimed to evaluate the extractability of different extractants for zinc (Zn) and copper (Cu) in soils of long-term field experiments covering different agro-ecological zones of India. The relationships between the amounts of Zn and Cu extracted by Mehlich 3, 0.1 mol/L HCl and AB-DTPA (ammonium bicarbonate-diethylenetriaminepenta-acetic acid) with those extracted by DTPA, the conventional extraction method widely used in soil testing laboratories in India, were elucidated. The treatments of the long-term experiments included control (no fertilizer), 100% NPK, 50%NPK + 50% N through FYM (farm yard manure) and a fallow soil. Some important physico-chemical properties of soil like pH, organic carbon, textural class, $CaCO_3$ content, etc. were analyzed. The NPK + FYM treatment was found to be the most effective treatment in terms of increased content of Zn and Cu in soils. The results showed that the amount of Zn and Cu extracted by Mehlich 3 were significantly correlated with that extracted by 0.1 mol/L HCl (r = 0.970** for Zn and r = 0.914** for Cu). Accordingly, Mehlich 3 and 0.1 mol/L HCl could be used effectively for estimating Zn and Cu availability in soils of India. However, Mehlich 3 was superior to all the other extractants used for the study.

Keywords: micronutrients; trace elements; crop nutrition; plant-availability; chelating agents

With the advent of modern technology and intensification of crop production there has been an awareness of the need for supplying one or more of the micronutrients along with other fertilizers. One of the major advances in micronutrient soil testing was the development of extracting solutions that contain chelating agents, primarily DTPA (diethylene triamine pentaacetic acid) and EDTA (ethylenediaminetetraacetic acid) (Sims and Johnson 1991). The long-term studies provide a unique opportunity to evaluate the influence of different management practices and agro-climatic conditions on crop nutrition. Several methods for assessment of micronutrient status in soils are available. Again, many of the extractants in routine analyses are used for multiple elements, in general developed for other nutrients and used for determination of copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) due to the operational ease in laboratories for routine soil testing. However, the lack of standardization of extraction methods can affect the reliability of the analytical results, since the success of this practice is associated with the choice of an extractant suited for the specific agricultural conditions in the region under study. Different chemical extractants are used to determine plant-available Cu, Zn, Fe, and Mn, particularly with dilute acid solutions, e.g., 0.1 mol/L HCl

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(Wear and Sommer 1948) and Mehlich 1 (0.05 mol/L HCl and 0.00625 mol/L H_2SO_4) (Mehlich 1953), and the chelates, such as DTPA and EDTA (Lindsay and Norvell 1978). The extraction principles of the methods vary greatly. Acid extractants are based on lowering the pH and the consequent solubilization of some compounds containing these elements. In turn, chelating extractants, have the capacity of reducing the activity of dissolved metals, resulting in release of more soluble compounds in buffered pH (Motta et al. 2007). The present work is an attempt to evaluate the extractable content of Zn and Cu in soils of a few selected long-term experiments in India. The efficiency of extractants like AB-DTPA, Mehlich 3 and 0.1 mol/L HCl for predicting extractable zinc and copper in soils was also evaluated. DTPA being the most commonly used extractant was used for comparison.

MATERIAL AND METHODS

Soils used. Soil samples were collected from 12 selected long-term experimental sites in India covering 7 agro-ecological zones (Table 1). Experimental design followed at each site was randomized block design with 4 treatments viz., control (T1); 100% NPK (T2); 50% NPK + 50% N through FYM (T3) and an uncultivated fallow (T4), each having 3 replications (the notation NPK refers to the dose of nitrogen, phosphorus and potassium recommended for the specific crop and region). The soil samples were collected depth-wise (0–15, 15–30 and 30–45 cm) from the specific long-term experiments after harvesting of one crop and before sowing/transplanting of subsequent crop. Some important physico-chemical properties of soil like pH, organic carbon, textural class, $CaCO_3$ content, etc. were analyzed following the standard protocols. Mechanical analysis of the soil was done using the Bouyoucos hydrometer method as described by Black (1965). Organic carbon was determined by wet digestion method of Walkley and Black (1934) as described by Jackson (1973). $CaCO_3$ content was determined by rapid titration method as described by Black (1965). Amorphous Fe and Mn were extracted using the ammonium oxalate-oxalic acid extraction method (McKeague and Day 1966). Estimation of Zn and Cu was done using four different extractants (Table 2) and their concentration was measured in atomic absorption spectrophotometer (GBC Avanta-Model No. 912, Victoria, Australia). Concentrations of standard samples taken were of 0.5, 1.0, 2.5, 5.0 mg/kg.

Statistical analysis was performed by the latest version of CPCS-1 (Steel and Torrie 1980) for analysis of variance (ANOVA) to determine the statistical significance of treatments. They were also subjected to analysis of correlation (Gomez and Gomez 1984) through the requisite statistical computations to predict the cause and effect relationship of various extractants.

Table 1. Details of sampling sites

Soil No.	Location	GPS coordinates	Year of initiation (age in years)	Cropping system	Agro-ecological zone No.	Soil type
1	RSpura (J & K)	32°05'N, 74°04'E	1985 (27)	rice-wheat	14	Inceptisols
2	Rakh Dhiansar (J & K)	32°38'N, 74°55'E	1971 (41)	maize-cowpea	14	Inceptisols
3	Junagarh (Gujarat)	21°30'N, 70°27'E	1996 (16)	groundnut-wheat	5	Inceptisols
4	Parbhani (Maharashtra)	19°15'N, 76°48'E	1983 (29)	sorghum-wheat	6	Vertisols
5	Raipur (Chhattisgarh)	21°14'N, 81°42'E	1983 (29)	rice-wheat	11	Alfisols
6	Bhubaneswar (Odisha)	20°16'N, 85°49'E	1983 (29)	rice-rice	12	Alfisols
7	Chiplima (Odisha)	21°22'N, 83°53'E	1998 (14)	rice-rice	12	Entisols
8	Mohanpur (West Bengal)	23°40'N, 88°52'E	1998 (14)	rice-wheat	15	Inceptisols
9	Gayeshpur (West Bengal)	22°57'N, 88°29'E	1972 (40)	rice-mustard-sesame	15	Inceptisols
10	Varanasi (Uttar Pradesh)	25°18'N, 83°03'E	1985 (27)	rice-lentil	4	Inceptisols
11	Anand (Gujarat)	22°32'N, 72°58'E	1983 (29)	pearl millet-wheat-cowpea	4	Inceptisol
12	Indore (Madhya Pradesh)	22°40'N, 75°54'E	1991 (21)	soybean-chickpea	5	Vertisols

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Table 2. Extractable contents of zinc and copper were estimated using four different extractants as given below

Methods	Reagents	Reference
DTPA	0.005 mol/L DTPA + 0.1 mol/L TEA + 0.01 mol/L $CaCl_2$ (pH 7.3)	Lindsay and Norvell (1978)
AB-DTPA	1.0 mol/L NH_4HCO_3 + 0.005 mol/L DTPA (pH 7.6)	Soltanpour and Schwab (1977)
Hydrochloric acid	0.1 mol/L HCl	Ponnamperuma et al. (1981)
Mehlich 3	Mehlich 3	Mehlich (1984)

RESULTS AND DISCUSSION

The different treatments under consideration had a significant effect on the content of extractable Zn and Cu. The highest content of Zn and Cu was recorded in soils under NPK + FYM treatment which was significantly higher than the rest of treatments. Almost comparable or higher content of Zn and Cu in fallow soil was due to high organic carbon content in soils of most of the sites. Continuous cultivation and balanced application of nutrients also resulted in the increase in available status of Zn and Cu. This may be due to the mobilization of the micronutrient cations from native source. None of the soils showed any deficiency of Zn and Cu.

The Mehlich 3 solution demonstrated a greater capacity of extraction of Zn and Cu in comparison to the other extractants, which is in agreement with De Abreu et al. (1996).

Soils of almost all the sites were found to have extractable contents of Zn and Cu above the critical range which shows that they were not deficient in the given micronutrient cations. Regardless of the extractants taken, overall, soils under the experiment at Mohanpur were found to have the highest content of extractable Zn and Cu while Bhubaneswar and Varanasi were found to have the lowest content of Zn and Cu respectively even though no visible deficiency was encountered.

The acid reagents and chelating agents such as EDTA, higher amounts of micronutrients are extracted by the Mehlich 3 solution than by the DTPA-TEA (Vidal-Vázquez et al. 2005) and by diluted acids (e.g. Mehlich 1 and HCl) (De Abreu et al. 2002). The presence of the NH_4^+ ion in Mehlich 3 renders it able to displace exchangeable cations (Fernandez-Marcos et al. 1998). Dilute acid solutions like 0.1 mol/L HCl may only partially solubilize

Table 3. Physico-chemical properties of the soils

Soil No.	Location	pH_{H_2O}	pH_{CaCl_2}	Organic carbon	$CaCO_3$	Amorphous Fe oxide	Amorphous Al oxide	Soil textural class
				(g/kg)				
1	RSpura (J & K)	6.83	6.36	4.10	20.81	0.86	0.70	Loamy sand
2	Rakh Dhiansar (J & K)	7.17	6.36	6.69	18.03	1.53	1.02	Silty loam
3	Junagarh (Gujarat)	8.14	7.94	6.88	53.92	1.15	0.63	Loam
4	Parbhani (Maharashtra)	8.37	7.42	4.27	33.33	1.35	0.92	Loam
5	Raipur (Chhattisgarh)	6.76	6.31	6.03	19.86	1.06	0.64	Loam
6	Bhubaneswar (Odisha)	6.94	6.30	4.20	30.92	1.23	0.82	Silty loam
7	Chiplima (Odisha)	7.77	7.13	6.02	32.61	2.35	1.58	Silty clay loam
8	Mohanpur (West Bengal)	7.86	6.98	5.71	25.94	1.79	1.20	Silty clay loam
9	Gayeshpur (West Bengal)	8.39	7.35	4.53	63.72	1.57	0.97	Silty clay loam
10	Varanasi (Uttar Pradesh)	6.85	6.08	3.32	28.03	1.35	0.96	Loam
11	Anand (Gujarat)	8.16	7.55	5.54	42.64	1.81	1.07	Silty clay
12	Indore (Madhya Pradesh)	7.82	6.78	7.20	32.78	1.56	0.90	Silty clay loam

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Table 4. Effect of the treatments on the extractable content of zinc (Zn) and copper (Cu)

Treatment	Extractable Zn				Extractable Cu			
	DTPA	AB-DTPA	Mehlich 3	HCl	DTPA	AB-DTPA	Mehlich 3	HCl
T1	0.68	0.93	2.84	2.64	1.28	1.53	2.58	1.79
T2	0.81	1.07	3.30	3.02	1.46	1.75	3.01	2.11
T3	1.08	1.28	3.88	3.50	1.70	2.07	3.52	2.59
T4	0.97	1.21	3.94	3.59	1.64	2.03	3.41	2.65

T1 – control; T2 – 100% NPK; T3 – 50% NPK + 50% N through farm yard manure; T4 – fallow

soil Zn and Cu, while chelating agents such as DTPA or EDTA reduce their activity in solution by complexation, causing the dissolution of the labile forms of in soils (De Abreu et al. 1998). Mehlich 3 (M3) yielded the higher concentration of Zn (1.75 times) than DTPA (Garcia et al. 1997). This finding agrees with that reported by Walworth et al. (1992), Volcasek and Friedericks (1994), Elrashidi et al. (2003) and Maftoun et al. (2003). Amounts of Cu extracted by Mehlich 3 are closely related to those obtained using DTPA (R^2 = 0.864), with M3 extracting 2.45 more Cu than DTPA (Tran and Simard 1993). Tucker (1988) reported an *r*-value of 0.97 for M3 compared with DTPA (Lindsay and Norvell 1978) on acid to near neutral soil.

Relationship of extractable Zn with important physico-chemical properties of soil. Reactions of Zn in soil involve pH, organic matter, clay content, Fe oxides, cation exchange capacity (Junus and Cox 1987, Sims and Johnson 1991, Haddad and Evans 1993, Borkert et al. 1998). The amount of Zn extracted by the four extractants showed a significant positive correlation with organic C, but negative correlations with pH and $CaCO_3$ content of the soils. This indicates that the extractable Zn content of the soils would increase with increasing organic C and decrease with increasing soil pH. The Mehlich 3-extractable Zn, unlike others, showed a significant positive correlation with the content of amorphous Fe oxide of soils. This indicated that Mehlich 3 was able to extract Zn bound to the amorphous iron oxides. All other correlations were found to be non-significant.

Table 5. Extractable Zn and Cu in soils of agro-ecological regions under consideration

Soil No.	Location	Extractable Zn				Extractable Cu			
		DTPA	AB-DTPA	Mehlich 3	0.1 mol/L HCl	DTPA	AB-DTPA	Mehlich 3	0.1 mol/L HCl
1	RSpura (J & K)	1.11	1.16	3.39	3.16	1.48	1.67	2.51	2.10
2	Rakh Dhiansar (J & K)	1.51	3.00	2.94	2.73	1.05	1.49	2.23	1.70
3	Junagarh (Gujarat)	0.66	0.86	3.13	2.90	1.59	2.59	3.63	2.52
4	Parbhani (Maharashtra)	0.72	0.96	3.47	3.26	1.22	1.46	4.89	3.10
5	Raipur (Chhattisgarh)	1.10	1.18	3.50	3.34	1.60	1.72	3.53	2.50
6	Bhubaneswar (Odisha)	0.77	0.83	3.00	2.68	1.68	1.81	2.67	2.12
7	Chiplima (Odisha)	0.86	0.93	3.37	3.22	1.58	1.99	1.91	1.90
8	Mohanpur (West Bengal)	0.85	1.00	5.30	4.97	2.20	2.53	5.00	3.19
9	Gayeshpur (West Bengal)	0.98	1.10	4.18	3.02	1.58	1.76	3.24	2.32
10	Varanasi (Uttar Pradesh)	0.73	0.85	3.34	3.16	1.31	1.52	1.54	1.43
11	Anand (Gujarat)	0.69	0.79	3.32	2.90	1.49	1.98	3.27	2.46
12	Indore (Madhya Pradesh)	0.68	0.83	2.95	2.91	1.44	1.63	3.16	2.11

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Table 6. Relationship of extractable Zn with important physico-chemical properties of soil

	pH_{H_2O}	pH_{CaCl_2}	Organic C	$CaCO_3$	Amorphous Fe	Amorphous Al	Clay
DTPA-Zn	–0.466**	–0.422**	0.454**	–0.238	0.033	–0.043	–0.018
AB-DTPA Zn	–0.427**	–0.325*	0.362**	–0.164	–0.245	–0.243	–0.124
Mehlich 3-Zn	–0.110	–0.117	0.565**	–0.126	0.451**	0.185	0.246
0.1 mol/L HCl-Zn	–0.076	–0.143	0.405**	–0.206	0.339	0.059	–0.003

*$P \le 0.05$; **$P \le 0.01$

Table 7. Dynamics within different forms of extractable Zn

	DTPA-Zn	ABDTPA-Zn	Mehlich 3-Zn	0.1 mol/L HCl-Zn
DTPA-Zn	1.000			
AB-DTPA-Zn	0.894**	1.000		
Mehlich 3-Zn	0.674**	0.501**	1.000	
0.1 mol/L HCl-Zn	0.639**	0.473**	0.970**	1.000

*$P \le 0.05$; **$P \le 0.01$

Table 8. Relationship of extractable Cu with important physico-chemical properties of soil

	pH_{H_2O}	pH_{CaCl_2}	Organic C	$CaCO_3$	Amorphous Fe	Amorphous Al	Clay
DTPA-Cu	–0.376*	–0.372*	0.687**	0.008	0.364*	0.250	0.258
AB-DTPA-Cu	–0.281	–0.223	0.659**	0.327**	0.165	0.079	0.153
Mehlich 3-Cu	0.353*	0.383*	0.649**	0.116	0.475**	0.502**	0.626**
0.1 mol/L HCl-Cu	0.162	0.204	0.713**	0.066	0.453**	0.436**	0.486**

*$P \le 0.05$; **$P \le 0.01$

Table 9. Dynamics within different forms of extractable Cu

	DTPA Cu	AB-DTPA-Cu	Mehlich 3	0.1 mol/L HCl
DTPA-Cu	1.000			
AB-DTPA-Cu	0.844**	1.000		
Mehlich 3-Cu	0.539**	0.542**	1.000	
0.1 mol/L HCl-Cu	0.621**	0.623**	0.914**	1.000

*$P \le 0.05$; **$P \le 0.01$

Dynamics within different forms of extractable Zn. All forms of extractable Zn were highly and significantly correlated with each other indicating that they could extract Zn from more or less similar pools from soil. Mehlich 3 Zn and 0.1 mol/L HCl-Zn showed the highest correlation (0.974**); while AB-DTPA-Zn and 0.1 mol/L HCl-Zn showed the least (0.473**).

Relationship of extractable Cu with important physico-chemical properties of soil. The amount of Cu extracted by the 4 extractants showed a significant positive correlation with organic C. This indicated that the extractable Cu content of the soils would increase with increasing organic C. DTPA-Cu showed a significant negative correlation with soil pH while Mehlich 3-Cu had a significantly positive correlation with pH. Only AB-DTPA-extractable Cu showed a significant positive correlation with $CaCO_3$ while Mehlich 3-Cu and 0.1 mol/L HCl-Cu showed a significant

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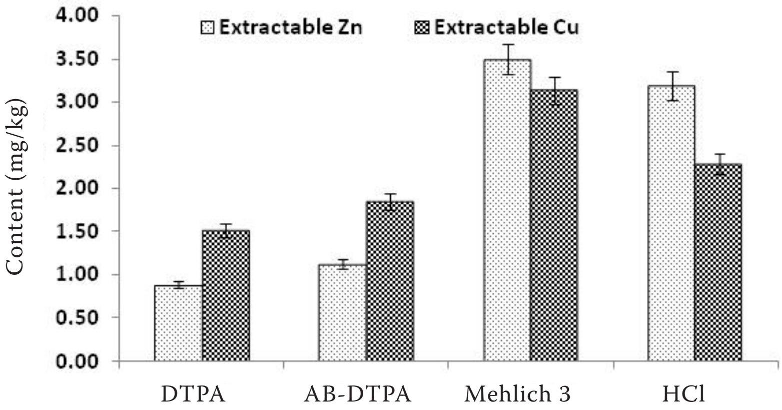

Figure 1. Comparative extractability of extractants for zinc and copper

positive correlation with amorphous oxides of Fe and Al, and also the clay content of the soil which clearly indicates that Mehlich 3 and 0.1 mol/L HCl were able to extract Zn bound to the amorphous iron and aluminium oxides. All other correlations were found to be non-significant.

Dynamics within different forms of extractable Cu. All forms of extractable Cu were found to be highly and significantly correlated with each other indicating that they could extract Cu from more or less similar pools of soils. Mehlich 3-Cu and 0.1 mol/L HCl-Cu showed the highest correlation coefficient (0.914**); while DTPA-Cu and Mehlich 3-Cu showed the least (0.539**).

In conclusion it may be stated that the various treatments taken for the study had a significantly different effect on the extractable content of Zn and Cu in soils. T3 (NPK + FYM) was found to be the most effective treatment in terms of increased content of Zn and Cu in soils. The fallow soils owing to their high organic carbon content had almost comparable values of extractable Zn and Cu. A dynamic equilibrium was found to exist among the extractable forms of all the cationic micronutrients, which is testimony to the fact that they were able to extract the said micronutrients from almost same pools. The overall extractability of the different extractants regardless of the experimental sites for all the cationic micronutrients may be ranked in the order: Mehlich 3 > 0.1 mol/L HCl > AB-DTPA > DTPA.

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REFERENCES

Abreu C.A., Raij B.V., Gabe U., Abreu M.F., Gonzalez A.P. (2002): Efficiency of multinutrient extractants for the determining of available zinc in soil. Communications in Soil Science and Plant Analysis, 33: 3313–3324.

Black C.A. (1965): Methods of Soil Analysis. Part-II. Agronomy Series. No. 9, American Society Agronomy, Inc., Madison.

Borkert C.M., Cox F.R., Tucker M.R. (1998): Zinc and copper toxicity in peanut, rice, and corn in soil mixtures. Communications in Soil Science and Plant Analysis, 29: 2991–3005.

De Abreu C.A., Raij B., Abreu M.F., Santos W.R., Andrade J.C. (1996): Efficiency of multinutrient extractants for the determination of available copper in soils. Communications in Soil Science and Plant Analysis, 27: 763–771.

Elrashidi M.A., Mays M.D., Lee C.W. (2003): Assessment of Mehlich 3 and ammonium Bicarbonate – DTPA extraction for simultaneous measurement of fifteen elements in soils. Communication in Soil Science and Plant Analysis, 34: 2817–2838.

Fernandez-Marcos M.L., Alvarez E., Monterroso C. (1998): Aluminium and iron estimated by Mehlich-3 extractant in mine soils in Galicia, northwest Spain. Communications in Soil Science and Plant Analysis, 29: 599–612.

Garcia A., de Iorio F., Barros M., Bargiela M., Rendina A. (1997): Comparison of soil tests to determine micronutrients status in Argentina soils. Communications in Soil Science and Plant Analysis, 28: 1777–1792.

Gomez K.A., Gomez A.A. (1984): Statistical Procedures in Agricultural Research. 2nd Ed. New York, Chichester, Wiley, 680.

doi: 10.17221/971/2014-PSE

Haddad K.S., Evans J.C. (1993): Assessment of chemical methods for extracting zinc, manganese, copper, and iron from New South Wales soils. Communications in Soil Science and Plant Analysis, 24: 29–44.

Junus M.A., Cox F.R. (1987): A zinc soil test calibration based upon Mehlich 3 extractable Zn, pH, and cation exchange capacity. Soil Science Society of America Journal, 51: 678–683.

Lindsay W.L., Norvell W.A. (1978): Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal, 42: 421–428.

Maftoun M., Haghighat Nia H., Karimian N. (2003): Evaluation of chemical extractants for predicting lowland rice response of zinc in highly calcareous soils. Communications in Soil Science and Plant Analysis, 34: 1269–1280.

McKeague J.A., Day J.H. (1966): Dithionite- and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. Canadian Journal of Soil Science, 46: 13–22.

Mehlich A. (1953): Determination of P, Ca, Mg, K, Na and NH_4 by North Carolina Soil Testing Laboratories. Raleigh, University of North Carolina.

Mehlich A. (1984): Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis, 15: 1409–1416.

Motta A.C.V., Serrat B.M., Reissmann C.B., Dionísio J.A. (2007): Micronutrients in the rock, soil and in the plant. Curitiba, Paraná Federal University, 246.

Ponnamperuma F.N., Cayton M.T., Lantin R.S. (1981): Dilute hydrochloric acid as an extractant for available zinc, copper and boron in rice soils. Plant and Soil, 61: 297–310.

Sims J.T., Johnson C.V. (1991): Micronutrient soil tests. In: Mortvedt J.J., Cox F.R., Shuman L.M., Welch R.M. (eds): Micronutrients in Agriculture. Book series No. 4. Madison, Soil Science Society of America, 427–476.

Soltanpour P.N., Schwab A.P. (1977): A new soil test for simultaneous extraction of macro- and micro-nutrients in alkaline soils. Communications in Soil Science and Plant Analysis, 7: 797–821.

Steel R.G.D., Torrie J.H. (1980): Principles and Procedures of Statistics. 2nd Ed. New York, McGraw-Hill.

Tran T.S, Simard R.R. (1993): Mehlich 3 extractable elements. In: Carter M.R. (ed.): Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, 43–49.

Tucker M.R. (1988): A review and update on the Mehlich 3 extractant. Presented at: Annual meeting of Mid-Atlantic soil test workgroup southern states building, 6506 West Broad St., Richmond, Virginia 23260.

Vidal-Vázquez E., Caridad-Cancela R., Taboada-Castro M.M., Paz-Donzález A., Abreu C.A. (2005): Trace elements extracted by DTPA and Mehlich-3 from agricultural soils with and without compost additions. Communications in Soil Science and Plant Analysis, 36: 717–727.

Vocasek F.F., Friedericks J.B. (1994): Soil micronutrient extraction by Mehlich 3 compared to $CaCl_2$-DTPA. Communications in Soil Science and Plant Analysis, 25: 1583–1593.

Walkley A.J., Black I.A. (1934): Estimation of soil organic carbon by the chromic acid titration method. Soil Science, 37: 29–38.

Walworth J.L., Gavalak R.G., Panciera M.T. (1992): Mehlich 3 extractant for determination of available B, Cu, Fe, Mn, and Zn in cryic Alaskan soils. Canadian Journal of Soil Science, 75: 517–526.

Wear J.I., Sommer A.L. (1948): Acid extractable zinc of soils in relation to the occurrence of zinc deficiency symptoms of corn: A method of analysis. Soil Science Society of America Proceedings, 12: 143–144.

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