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The usefulness of Mehlich 3 and 1 M HCl extractant to assess copper deficiency in soil for environmental monitoring purpose

Przydatność ekstrahentów Mehlich 3 i 1 M HCl do oceny niedoborów miedzi w glebie pod kątem monitoringu środowiska

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Słowa kluczowe: testy glebowe, 1 M HCl, Mehlich 3, formy dostępne, miedź, ekstrakcje

Abstract
The aim of this study was to compare two soil tests, 1 M HCl and Mehlich 3, to extract phytoavailable Cu forms from the soil. The evaluation of tests was performed on the basis of the correlation between soil Cu extracted by the studied extractants, and plant Cu or yield of a test plant. Data for the calculation originated from the microplot experiment with winter wheat. The experiment included three soils that differed in texture, pH level and copper content. Each of the soil was fertilized with five doses of Cu: 0, 4, 8, 12 and 16 kg•ha^{-1} against two pH levels. The results showed a strong correlation between the two soil tests and their similar usefulness for the extraction of copper available to plants. On the basis of Pearson correlation coefficients and equation of simple regression, it was found that Mehlich 3 was slightly more useful for heavier soils with higher pH, whereas 1 M HCl was better suitable for acid sandy soil.

Streszczenie
Celem prowadzonych badań było porównanie dwóch testów 1 M HCl i Mehlich 3 do ekstrakcji przyswajalnych dla roślin form miedzi. Podstawą oceny były współczynniki korelacji pomiędzy zawartością Cu podatną na ekstrakcje obydwoma roztworami, a jej koncentracją w roślinie oraz wysokością plonu. Materiał do badań pochodził z doświadczenia mikropoletkowego, założonego na trzech glebach różniących się uziarnieniem, pH oraz zawartością miedzi. Dla każdej z gleb ustalono dwa poziomy pH oraz stosowano pięć różnych dawek Cu: 0, 4, 8, 12 i 16 kg•ha^{-1}. Wyniki wskazują na silną korelację pomiędzy wynikami obu ekstrakcji a także ich podobną przydatność do ekstrakcji fitoprzyswajalnych form miedzi. Osiągnięte współczynniki korelacji Pearsona oraz równania regresji świadczą o nieco lepszej przydatności roztworu Mehlich 3 do ekstrakcji przyswajalnych form miedzi w glebach cięższych o wyższym pH, 1 M HCl natomiast – w glebie lekkiej o kwaśnym odczynie

1. INTRODUCTION

Soil monitoring is a valuable source of information on the state of the environment. The changes of soil properties caused by agricultural and non-agricultural activities are evaluated on it. In the case of trace elements, monitoring is focused mainly on the assessment of soil pollution. However, some heavy metals (Cu, Fe, Mn, Mo, Zn) may be classified as nutritional micronutrients that have a regulating function in plants. From the point of view of plant production, both the presence of excess and deficiency of these elements are undesirable. Obtaining high and good quality yield requires an adequate supply of plants with nutrients. Rational fertilization should be based on a prior assessment of soil fertility. Accurate assessment of macro- and micronutrients in the soil, and hence optimizing fertilizer recommendations, can be achieved by using soil tests [Rodriguez and Ramirez, 2005].

The determination of bioavailable forms of micronutrients requires a lot of effort, because the elements are present in the soil in various forms bound by soil absorption complex [Khan et al. 2005, McLaughlin et al. 2000, Ure 1991]. Only small quantities of the total content are in the available form to the plants. In the case of copper, which is the most important micronutrient for cereals, only 2–21% of the total content is in the mobile form, and thus potentially available to plants [Fotyma et al. 1987]. The level of this element in the soil, determined as plant available, depends on the type of extraction reagent [Ure 1991, McLaughlin et al. 2000].

The literature presents a number of extractants used for the extraction of bioavailable forms of elements. They vary in strength of extraction and mechanism of action [Brun et al. 2001, Khan et al. 2005, Liu et al. 2011, McLaughlin et al. 2000, Menzies et al. 2007, Rao et al. 2008, Rodriguez and Ramirez 2005, Stanisławska-Glubiak and Korzeniowska 2010, Ure 1991]. In Poland, since 1987, extraction with 1 M HCl has been used by the agrochemical laboratories. It is a method developed in IUNG-PIB (Institute of Soil Science and Plant Cultivation – State Search Institute) by the team supervised by Prof. Gembarzewski to simplify a former methodology of determination of micronutrient content in soil [Gembarzewski and Korzeniowska 1990, 1996]. Before the introduction of 1 M HCl, evaluation of the content of microelements was carried out with the use of the so-called specific extractants, different for each element. However, 1 M HCl method raises a lot of controversy among experts. This doubt is connected with the high quantity of the extracted elements, which are often uncorrelated with their quantity in the plant.

Due to the above doubts, there have been attempts to introduce a new extractant. It should be characterized by appropriate strength, mimicking the action of plant root exudates and extract only phytoavailable forms of elements. The efficiency of the extractant is best shown by correlation of the extracted quantities of elements from the soil and their quantities in plants [Brun et al. 2000, Rodriguez and Ramirez 2005, Sarto et al. 2011].

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Extraction with Mehlich 3 reagent is a commonly used method for the assessment of soil nutrients in North America and Southeast Europe [Chilimba et al. 1999, Loide et al. 2005, Zbiral and Nemec 2000]. The advantage of this extractant is a high correlation between the content of elements in the soil and in the plants grown on this soil [Khan et al. 2005, Walworth et al. 1992, Sarto et al. 2011]. The use of Mehlich 3 reduces the time and costs of the performed analyses, because it is a multielement extractant for all macro- and micronutrients.

The aim of this study was to evaluate the effectiveness of Mehlich 3 and 1 M HCl solutions for the extraction of the phytoavailable forms of copper from the soil, because this trace element is necessary for the yield of winter wheat [Korzeniowska 2008a, b]. Moreover, the tested extractants were compared in terms of the quantity of copper extracted from the soil.

2. METHODOLOGY

Three microplot experiments were conducted at the Experimental Station of IUNG-PIB in Jelcz-Laskowice near Wroclaw. In total, 120 concrete-framed microplots of the size 1×1×1 m were used, 40 microplots for each experiment. The experiments were established in a randomized block design with four replicates. In each experiment, 10 treatments were tested, 5 levels of copper Cu1 = 0, Cu2 = 4, Cu3 = 8, Cu4 = 12, Cu5 = 16 $kg \cdot ha^{-1}$, and 2 levels of pH: natural and after liming by 1.5 Hh. Test plant was winter wheat, var. Kobra Plus, chosen as a cultivar sensitive to copper deficiency [Korzeniowska 2008a].

Three soils (A, B, C) differing in texture, pH level and content of Cu were used (Table 1). Soil A was classified as sandy soil, had the lowest pH (4.3) and the lowest concentration of Cu (2 $mg \cdot kg^{-1}$). Two others soils (B and C) were classified as loamy soils, characterized by higher pH (respectively 5.3 and 5.7) and higher initial Cu concentration (3 and 4 $mg \cdot kg^{-1}$).Texture of the soils was determined using Bouyoucos's areometric method, modified by Casagrande and Proszynski, and pH was measured in KCl solution (m:v 1:2.5) [Drozd et al. 2002]

The analyses of plant available forms of phosphorus and potassium concentrations were performed by Egner-Riehm's method and organic carbon by Tiurin's method [Drozd et al. 2002]. In the experiments, the same NPK fertilization was used on all the treatments, corresponding to the needs of the test plant (N – 120, P_2O_5 – 80, K_2O – 120 $kg \cdot ha^{-1}$). Soil samples after the harvest were collected. Moreover, samples of aerial parts of wheat in the beginning of stem elongation stage and grain samples were collected too. The absolute and relative yield of wheat grain was determined. The plant samples were analyzed for Cu concentration by FAAS method, after prior dry digestion in the muffle furnace and dissolving in HCl. Cu concentration in soil samples was determined by using two extractants: 1 M HCl and Mehlich 3 (0.001 N EDTA, 0.2 N CH_3COOH, 0.013 N HNO_3, NH_4NO_3, 0.015 NH_4F) [Boreczek et al. 2012, Mehlich 1984]. Also copper concentrations in the extracts were determined by FAAS method.

Statgraphics 5.1 was used for statistical calculations. Calculations were performed to determine Pearson correlation coefficients between plant available forms of copper extracted from soils by the two studied soil tests and selected indicators of plant (plant Cu and related yield). In addition, simple linear regression (stepwise procedure, backward selection) was performed using the indicators of plant as dependent and soil properties as independent variables. All statistical calculations were performed for each of the soil A, B and C (n = 10) and for the sum of soils A+B+C (n = 30). The variables used in the calculations had a normal distribution according to Kolmogrov–Smirnov test.

3. RESULTS AND DISCUSSION

3.1 The results of copper extractions from soils

The results of the research confirm the high extraction force of 1 M HCl. This extractant caused the release of higher amounts of soluble forms of copper to solution than Mehlich 3 (Fig. 1). This tendency occurred for all three of the analyzed soils. The concentrations of plant available copper in the soil increased with higher doses of fertilizer.

There was a strong correlation between extractable Cu determined by Mehlich 3 and by 1 M HCl method (Table 2). For all three soils, Pearson coefficients were over 0.937 ($\alpha < 0.001$). The highest coefficient was found in the case of soil B – 0.998 ($\alpha < 0.001$). The lowest one was obtained for the sum of A+B+C soils, which indicates the need to evaluate the effectiveness of extractants taking into account different types of soils. The results confirm the high correlation between 1 M HCl and Mehlich 3 tests reported in literature [Santro et al. 2011]. In their study, Loide et al. [2005] obtained 0.893 ($\alpha < 0.05$) coefficient for these extractants.

3.2 Correlations between plant indicators and Cu content in the soil

Assuming that the level of copper concentration in the plant tissue mostly provides information on the availability of this element in soil [Brun et al. 2001, Garcia et al. 1997, Menzies et al. 2007, Sarto et al. 2011], Pearson correlation coefficients between soil Cu and plant Cu, as well as between soil Cu and wheat grain yield, were determined (Table 3).

In the case of B and C soils, there was a stronger relationship between plant Cu and soil Cu for Mehlich 3 compared with 1 M HCl. The coefficients for Mehlich 3 were 0.819 ($\alpha < 0.05$) and 0.779 ($\alpha < 0.01$), respectively, whereas for 1 M HCl they were lower: 0.797 ($\alpha < 0.05$) and 0.630 ($\alpha < 0.05$). The high correlation of soil Cu extracted by Mehlich 3 with plant Cu has been confirmed by many authors [Khan et al. 2005, Walworth et al. 1992]. A strong correlation (0.85, $\alpha < 0.01$) between Cu in tissues of winter wheat and Cu extracted from soil by Mehlich 3 was also indicated by Sarto [2011].

For soil A, and for A+B+C in opposite to soils B and C better results were obtained for 1 M HCl (Table 3). There was no significant correlation between soil Cu extracted by Mehlich 3 and plant Cu. At the same time, a significant Pearson coefficient was obtained for 1 M HCl: 0.674 ($\alpha < 0.05$) for the A soil, and 0.401 ($\alpha < 0.01$) for A+B+C. In the experiment with winter wheat, Loide et al. [2005] obtained a correlation between 1 M HCl and Mehlich 3 and plant

Table 1. Soil properties

Soil	Soil type	Fraction <0.002 mm (%)	pH	C_{org} (%)	P_2O_5	K_2O	Mg	Cu ($mg \cdot kg^{-1}$)
					$mg \cdot 100$ g $soil^{-1}$			
A	Loamy sand	1.4	4.3	0.5	14.3	5.1	3.6	2.0
B	Sandy loam	2.22	5.3	0.8	15.1	20.1	5.9	3.0
C	Sandy loam	2.95	5.7	0.9	27.0	32.7	9.5	4.0

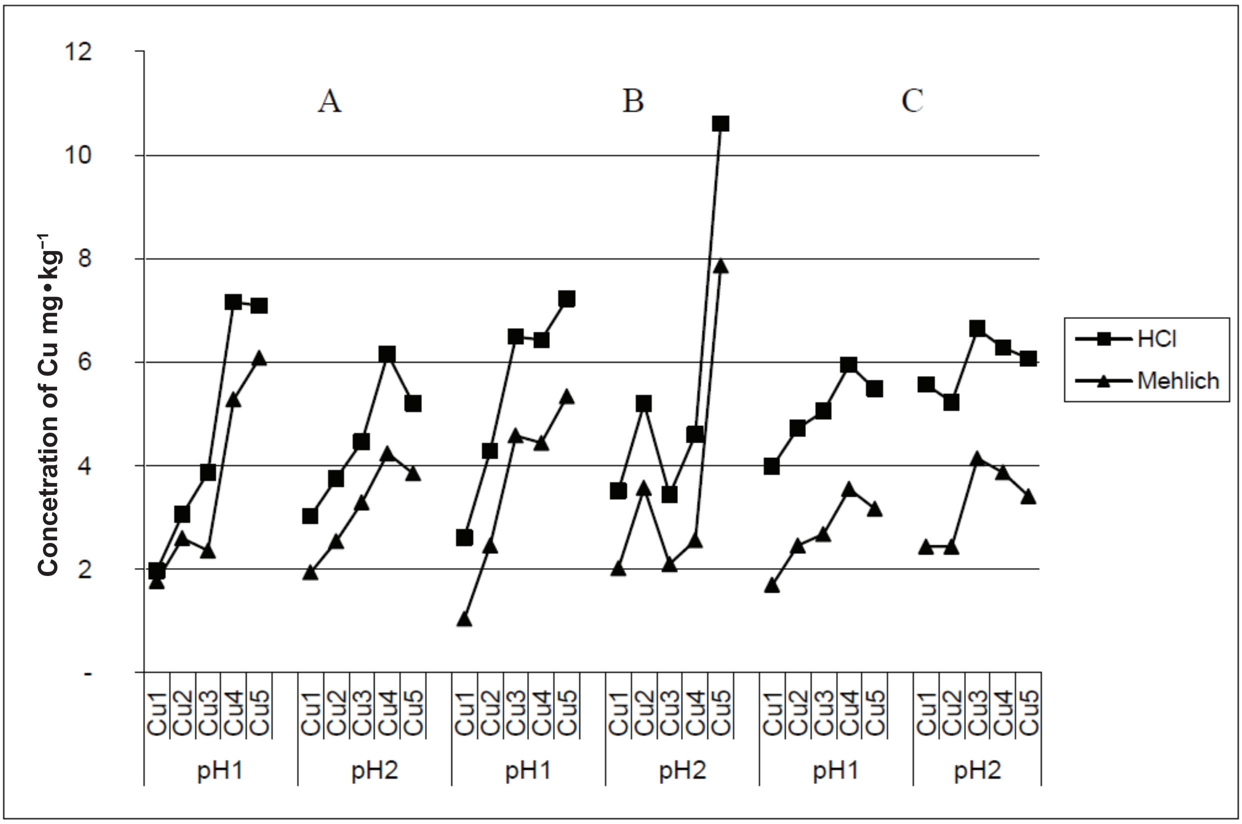

Fig. 1. The amounts of Cu extracted from soils A, B and C with two reagents: 1 M HCl and Mehlich 3. Cu1 to Cu5 – increasing doses of copper; pH 1 – soil without liming, pH 2 – soil after liming

Cu at the levels of 0.710 and 0.838 ($\alpha < 0.05$), respectively. Those results indicated a lower efficacy of 1 M HCl than Mehlich 3 in the extraction of phytoavailable forms of Cu. The lowest level of the coefficient that was obtained for A+B+C soils confirms the need to test the effectiveness of extractants for different types of soil.

Slightly higher correlation between plant Cu and soil Cu for Mehlich 3 than for HCl obtained in our study may be due to the greater suitability of the extractant characterized with chelating activity for the extraction of copper available to plants. A low pH of Mehlich 3 causes transfer of copper ions from the soil to extraction solution. These ions are then complexed by the EDTA molecules contained in this solution [Fonseca et al. 2010, Garcia et al. 1997]. The ability of copper to create chelate complexes has been confirmed by the studies of other authors [Karczewska 2002].

Given the stimulatory effect of copper on wheat yields, the correlation between grain yield and Cu extracted from the soil by both studied extractants was tested. The results showed that Pearson coefficients for soils B and C were slightly higher for Mehlich 3 than for 1 M HCl (Table 3). The strongest correlation between yield and Cu extracted from soil by Mehlich 3 was obtained for soil C – 0.781 ($\alpha < 0.001$), whereas corresponding for 1 M HCl was 0.779 ($\alpha < 0.05$). In the case of soil A, the higher coefficients were obtained for 1 M HCl than for Mehlich 3. These coefficients were higher for 1 M HCl, both for plant Cu and for yield. This indicates a better usefulness of 1 M HCl for the extraction of available forms of copper from acid sandy soils, whereas Mehlich 3 turned out to be more suitable for heavier soils with higher pH level. This tendency is confirmed in the literature. Sarto et al. [2011] stated after Silva et al. [2003] that the increase in clay fraction and soil organic matter caused an increase in the value of Pearson coefficient for the relationship between soil Cu extracted by Mehlich 3 and plant Cu. Different usefulness of the studied soil tests for different soil types was the reason for obtaining lowest correlation coefficients for all three soils together (A+B+C).

3.3 Regression equations

The availability of copper can be affected by a number of properties of the soil. These include pH, organic matter content, total copper and absorption capacity. Since the amount of copper extracted from the soil and its content in plant tissue are connected with the above parameters [Brennan and Bolland 2006, Brun et al. 2001, Fonseca et al. 2010, Rodriguez and Ramirez 2005, Walworth et al. 1992], the attempt was made to determine linear regression taking those factors into account.

The equations describing the relationship between the yield of wheat grain and Cu extracted with studied extractants or/and

Table 2. Correlation between soil Cu extracted by 1 M HCl and by Mehlich 3

Soil	Pearson correlation coefficient
A	0.978***
B	0.998***
C	0.937***
A+B+C	0.922***
Significant at α *<0.05, **<0.01, ***<0.001.	

other soil properties were obtained only for soil C (Table 4). The obtained coefficients of determination (R^2) show that the yield of wheat grain can be more accurately predicted on the basis of the amount of Cu extracted by Mehlich 3 (72.2%) than 1 M HCl (68.8%) ($\alpha < 0.05$).

Regression equations also include soil pH as a significant factor in the availability of Cu. The pH effect on the availability of this micronutrient has been confirmed in the literature [Brennan et al. 2008, Araújo do Nascimento et al. 2003]. Although statistically significant models were not obtained for other soils, they could be obtained in the case of the sum A+B+C soils. The coefficient of determination was in this case higher for 1 M HCl ($R^2 = 46.0$, $\alpha < 0.001$) than for Mehlich 3 ($R^2 = 37.8$, $\alpha < 0.05$).

Regression equations obtained for plant Cu as the dependent variable indicate greater suitability of Mehlich 3 extractant for soils B and C (Table 5).

High R^2 coefficients for soils B (67.0) and C (72.0) show that the copper nutritional status of wheat would be better indicated by soil Cu extracted by Mehlich 3 than 1 M HCl. Better suitability of 1 M HCl for the extraction of available Cu from soil A was confirmed by significant relationships only for this soil. Models calculated for different soils do not include any of the tested soil characteristics. The level of pH and phosphorus content in the soil are included into the equations only for the sum of A+B+C soils.

4. CONCLUSIONS

1) There is a strong, simple correlation between the amounts of Cu extracted from the soils by 1 M HCl and the Mehlich 3 reagent.
2) On the basis of simple correlation between plant Cu and extractable soil Cu and between the yield of wheat and extractable soil Cu, a similar usefulness of 1 M HCl and the Mehlich 3

Table 3. Correlations between soil Cu and selected plant indicators

Plant index	Soil	Pearson correlation coefficient	
		1 M HCl	Mehlich 3
Plant Cu[1)]	A	0.674*	ns
	B	0.797*	0.819*
	C	0.630*	0.779**
	A+B+C	0.401**	ns
Relative yield	A	0.767*	0.717*
	B	0.722*	0.730*
	C	0.779*	0.781***
	A+B+C	0.694***	0.583**

Significant at α: *<0.05, **<0.01, ***<0.001, ns – insignificant.

[1)] Cu concentrations in aerial parts of wheat in the beginning of stem elongation stage.

Table 4. Models for predicting yield of wheat grains on the base of soil Cu and selected soil properties

Soil	Equation	R^2
Mehlich 3		
A		ns
B		ns
C	Relative yield = 6.63 $Cu_{Mehlich3}$ + 20.17 pH – 35.75	72.2*
A+B+C	Relative yield = 4.36 $Cu_{Mehlich3}$ + 5.66 pH – 44.89	37.8*
1 M HCl		
A		ns
B		ns
C	Relative yield = 6.4 Cu_{HCl} + 12.09 pH – 7.6	68.8*
A+B+C	Relative yield = 4.26 Cu_{HCl} + 0.66 pH – 69.47	46.0***

Significant at α: *<0.05, **<0.01, ***<0.001; ns – insignificant; Cu_{HCl}, $Cu_{Mehlich3}$ – Cu concentrations in soil, determined by 1 M HCl and Mehlich 3, respectively, in $mg \cdot kg^{-1}$.

Table 5. Models for predicting Cu content in wheat tissues on the base of soil Cu and selected soil properties

Soil	Equation	R^2
Mehlich 3		
A		ns
B	Plant Cu = 0.24 $Cu_{Mehlich3}$ + 5.83	67.0*
C	Plant Cu = 0.90 $Cu_{Mehlich3}$ + 6.36	72.0*
A+B+C	Plant Cu = 0.52 $Cu_{Mehlich3}$ + 0.26 P_2O_5 – 2.73 pH + 14.52	55.5***
1 M HCl		
A	Plant Cu = 0.83 Cu_{HCl} + 4.06	52.0*
B	Plant Cu = 0.20 Cu_{HCl} + 5.61	63.4*
C	Plant Cu = 0.78 Cu_{HCl} + 4.76	53.7*
A+B+C	Plant Cu = 0.52 Cu_{HCl} + 0.24 P_2O_5 – 3.30 pH + 16.86	61.5***

Significant at α: *<0.05, **<0.01, ***<0.001; ns – insignificant; Cu_{HCl}, $Cu_{Mehlich3}$ – soil Cu determined by 1 M HCl and Mehlich 3, respectively ($mg \cdot kg^{-1}$), plant Cu–Cu concentration in aerial parts of wheat in the beginning of stem elongation stage; P_2O_5 – concentration of available phosphorus in soil ($mg \cdot 100g^{-1}$).

extractants for the extraction of plant available Cu from soil was found. The Mehlich 3 was slightly better for heavier soils with higher pH level, whereas 1 M HCl for acid sandy soil.

3) Better usefulness of Mehlich 3 for soils B and C, and 1 M HCl for A soil, was confirmed by the linear regression equation describing the relationships between the yield or plant Cu considered as dependent variable and extractable soil Cu, pH, TOC and soil P as independent variables.

4) Extractants testing requires the use of diverse soils, especially in terms of pH level and texture.

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