

ULTRASOUND ASSISTED EXTRACTION FOR DETERMINATION OF MOBILE FRACTIONS OF COPPER IN SOIL

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ABSTRACT

Background. For evaluation of the mobility of metals present in soil various approaches based on sequential extraction procedures (Tessier's, BCR or modified BCR) have been proposed, but they are very laborious and time-consuming.

Objective. The aim of this study was to develop a new fast extraction procedure for the determination of mobile fractions of copper in soil.

Material and methods. The samples of soil that vary in terms of physicochemical properties and certified reference material (BCR-701) were used in this study. The three-stage modified BCR procedure used for fractionation of copper in soil was accelerated by ultrasound irradiation using an ultrasonic probe. The content of copper in soil extracts was determined by electrothermal atomic absorption spectrometry (ETAAS).

Results. The extraction conditions for separation of mobile fractions of copper from soil with the use of ultrasonic probe were optimized. The working parameters of the probe were as follows: the power was 15 W for all fractions while the sonication time was 7 min, 10 min and 6 min for fraction I, fraction II and fraction III, respectively. The developed procedure was validated; linearity of calibration graphs, limit of detection and limit of quantification, accuracy and repeatability of the procedure were evaluated. The content of copper in fraction I-III in soil samples collected from Podlaskie Voivodeship was at the level of 0.09-1.92 mg·kg⁻¹, whereas its pseudo-total content was in the range 2.75-9.75 mg·kg⁻¹.

Conclusions. The developed ultrasound assisted sequential extraction procedure allowed for shortening the total extraction time from 48 h to 27 minutes in comparison to conventional modified BCR procedure. The studied soil contains a low pool of copper bioavailable to plants because a sum of mobile fractions (FI-FIII) represents only 22-46% of its pseudo-total content.

Key words: *copper in soil, mobile fractions of copper, ultrasound probe, modified BCR sequential extraction, electrothermal atomic absorption spectrometry, certified reference material BCR 701,*

STRESZCZENIE

Wprowadzenie. Ocena zawartości mobilnych form metali w glebie najczęściej prowadzona jest z wykorzystaniem praco- i czasochłonnych procedur ekstrakcji sekwencyjnej wg Tessiera, BCR oraz zmodyfikowanej procedury BCR.

Cel badań. Opracowanie nowej szybkiej procedury ekstrakcji sekwencyjnej wspomaganą ultradźwiękami do oznaczania mobilnych frakcji miedzi w glebie.

Materiały i metody. Badania prowadzono wykorzystując próbki gleb różniące się właściwościami fizykochemicznymi oraz certyfikowany materiał odniesienia (BCR-701). Trój etapowa zmodyfikowana procedura BCR zastosowana do frakcjonowania miedzi w glebie została przyspieszona przez wykorzystanie ultradźwięków. Do oznaczania analitu w otrzymanych ekstraktach zastosowano technikę atomowej spektrometrii absorpcyjnej z atomizacją elektrotermiczną (ETAAS).

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Wyniki. W pracy zoptymalizowano warunki ekstrakcji mobilnych frakcji miedzi z gleby z wykorzystaniem sondy ultradźwiękowej. Jako optymalne warunki pracy sondy ultradźwiękowej przyjęto: moc sondy 15 W dla wszystkich frakcji oraz czas 7 min dla Frakcji I, 10 min dla Frakcji II i 6 min dla Frakcji III. Opracowaną procedurę zwalidowano wyznaczając: linowość, granice wykrywalności i oznaczalności, dokładność i powtarzalność wyników. Zawartość miedzi we frakcjach I-III wyekstrahowanych z gleb pobranych z terenu województwa podlaskiego była na poziomie 0,09-1,92 mg·kg⁻¹, natomiast jej całkowita zawartość wynosiła od 2,75 do 9,75 mg·kg⁻¹.

Wnioski. Opracowana procedura ekstrakcji sekwencyjnej wspomagananej ultradźwiękami pozwala na skrócenie czasu ekstrakcji mobilnych frakcji miedzi z 48 godzin do 27 minut w stosunku do tradycyjnej zmodyfikowanej procedury BCR. Badane gleby zawierają niewiele miedzi biodostępnej dla roślin, a suma mobilnych frakcji miedzi (frakcje I-III) stanowi tylko od 22 do 46% jej całkowitej zawartości.

Słowa kluczowe: miedź w glebie, mobilne frakcje miedzi, sonda ultradźwiękowa, ekstrakcja sekwencyjna wg zmodyfikowanej procedury BCR, atomowa spektrometria absorpcyjna z atomizacją elektrotermiczną, certyfikowany materiał odniesienia BCR 701

INTRODUCTION

Copper is an essential micronutrient for plant development, which is involved in various electron transport reactions in photosynthesis and respiration process, as well as in lipid metabolism and fatty acid synthesis [7, 9]. Copper plays a significant role in carbohydrate distribution, nitrogen reduction and fixation, protein metabolism, and cell wall metabolism. It controls the production of DNA and RNA and is involved in the mechanisms of disease resistance. Copper deficiency affects physiological processes and greatly inhibits the reproduction of plant (i.e. reduces seed production), while its excess causes some toxic symptoms such as chlorosis, reduction of root growth, photosynthesis and chlorophyll production and often is accumulated in plant tissues [9, 12].

Soil is the first component of the trophic chain: soil - plant - animal - the man, thus its contamination with copper can lead to bioaccumulation of metal in the food chain and induce adverse health effects. Copper occurs in soil at very wide concentration range of 1-140 mg·kg⁻¹, with average values in the range of 13-24 mg·kg⁻¹ in dependence of the type of soil [9]. The wide use of fungicides, fertilizers, pesticides and herbicides containing copper in agriculture leads to further increase of its content in soil [5, 9, 12]. The total metals content in soil is a poor indicator of their bioavailability, mobility or toxicity [7, 9], as they are present in different chemical forms and binding state. The uptake of metals by plants is controlled by physical, chemical and biological processes depending on their chemical form and properties as well as properties of soil: granulometric composition, content of organic matter, content and type of clay minerals and Al, Fe and Mn oxides; pH, and redox potential [9]. Changes in environmental conditions, such as e.g. acidification, can cause mobilization of an element from the solid to the liquid phase and shift in its bioavailability.

Copper is a rather immobile element in soil. A small amount of copper is present in readily soluble and exchangeable form, which is considered as bioavailable to plant. This element is also strongly bound to organic matter and clay minerals, and can form sulphates, sulphides and carbonates [9]. From those phases of soil copper can be released only in specific environmental conditions. The pH is often considered as the most critical factor influencing the solubility of copper in soil solution [6-9], but the mobility of copper depends also on the type and content of organic matter as well as on the concentration of other elements that could compete with copper in adsorption and/or absorption processes [6, 9]. Therefore, lowering of soil pH, content of organic matter and cation exchange capacity increases the mobility of copper.

In order to evaluate the abundance of bioavailable fraction of copper in soil and its mobility different single-step or sequential extraction procedures have been used. Single-step extraction, which is based on using one specific extraction reagent (e.g. ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), ammonium acetate (NH₄OAc), calcium chloride (CaCl₂), ammonium nitrate (NH₄NO₃)), allows defining pools of extractable metals in particular chemical conditions [6, 7, 11, 12]. Sequential extraction methods allow to isolate copper bound to main chemical phases of soil, what can help to understand its behavior in soils and subsequently its mobility and availability to the environment. These methods suffer from non-selectivity as well as redistribution of trace elements among fractions during extraction. Nevertheless, sequential extraction procedures, e.g. Tessier's [14, 17, 20], BCR [4, 5, 13, 14, 16, 21] or modified BCR [1, 3, 13, 15, 18, 20], have been recommended for the fractionation of metals in soil. Because such procedures are laborious and time consuming, several attempts to speed up metal extraction by application of microwave [1, 4, 20] and ultrasonic treatments [1, 4, 5, 11, 16-18], have been made.

Ultrasound energy facilitates and accelerates some processes, such as dissolution, fusion, extraction or leaching and digestion [2]. Its application to soil sample dispersed in an extraction solution results in fragmentation of soil particles and disintegration of soil aggregates and increasing the surface area available for reactions with the extraction reagent, what have predictable consequence on the availability of metals. The sonication process generally speeds up the extraction procedure. Implementation of ultrasound in pretreatment of soil samples significantly shortens the operation times and enhances "green" aspects of such procedure in terms of solvent consumption and energy requirements. As a sonication source the ultrasonic bath is most often applied [1, 4, 5, 11]. However, ultrasonic baths generally lack sufficient sonication power to achieve fast extractions, what enforce necessity for the application of higher extractants concentration or longer extraction time [2]. Few authors have used a powerful ultrasonic probe for extraction of metals from solid samples [5, 16-18].

In this work a new ultrasound assisted sequential extraction (UASE) procedure with the use of an ultrasonic probe was developed for the determination of mobile fractions of copper in soil. As the goal of our studies was to obtain performance similar to that of a well-established method, the conventional sequential extraction procedure of modified BCR protocol (with the aid of a vertical rotor) was used for comparison. A systematic study of various working conditions of the ultrasonic probe on efficiency of copper extraction from soil was performed and the optimal conditions were established. The developed UASE procedure was validated and successfully applied for determination of mobile fractions of copper in samples of soil collected from Podlaskie Voivodeship.

MATERIALS AND METHODS

Reagents and materials

Ultrapure water obtained from Milli-Q system (Millipore, USA) was used throughout the work. Acetic acid, hydroxylammonium chloride, ammonia acetate and hydrogen peroxide (30%) were pure for analysis (POCh-Gliwice, Poland). Nitric acid and hydrochloric acid were Suprapur (Merck, Germany). Standard solutions were prepared by dilution of stock solution of copper ($1000 \mu\text{g}\cdot\text{mL}^{-1}$; Fluka, Germany).

The samples of soil collected from Podlaskie Voivodeship, various in terms of physicochemical properties (agricultural type, pH, content of organic matrix) were used for developing of a new procedure. Certified reference material of lake sediment BCR-701 (IRMM, Belgium), with certified content of trace elements isolated by the BCR sequential extraction

procedure, and was used for evaluation of accuracy of developed procedure.

Instrumentation

An ultrasonic probe, VCX 130 model (Sonics and Materials, USA) (max. power 130 W, max. frequency 20 kHz) was used in UASE procedure. In order to keep the constant temperature during sonication the system was cooled down with flowing water.

The content of copper in all fractions was determined by means of an atomic absorption spectrometry with an electrothermal atomization (Solaar M6, Thermo Scientific, UK) and a Zeeman-effect background correction, using graphite tubes with integrated L'vov platform. A Cu hollow cathode lamp (CPI International, USA) was operated at 4 mA current. The integrated absorbance signal of Cu was measured at 324.8 nm with a slit width of 0.5 nm. The following optimized atomizer heating program was used for the Cu determination: drying at 120°C for 12 s, ashing at 1000/1100°C for 10 s, and atomization at 2300°C for 3 s.

Extraction procedure

The three-stage modified BCR procedure [13] was used for fractionation of copper in soil. For extraction of fraction I (FI), water soluble, exchangeable and carbonate bound metal, $0.11 \text{ mol}\cdot\text{L}^{-1} \text{CH}_3\text{COOH}$ was used. For extraction of fraction II (FII), metal bound to iron and manganese hydroxides, $0.5 \text{ mol}\cdot\text{L}^{-1} \text{NH}_2\text{OH}\cdot\text{HCl}$ (pH 1.5) was used. The fraction III (FIII), organically bound metal, was released by oxidation of the organic matter using 30% H_2O_2 and re-extraction of mineralization products with $1 \text{ mol}\cdot\text{L}^{-1} \text{CH}_3\text{COONH}_4$ (pH 2). After each step the suspension was centrifuged at 3000 rpm for 15 min and the supernatant was separated from the solid phase and stored at 4°C until analysis. The remaining solid residue was washed with 20 mL of ultrapure water and the washing solution was discarded after centrifugation. Optimized conditions for UASE procedure are outlined in Table 1.

RESULTS

In order to obtain the reliable results of mobile fractions of copper in soil the conditions of extraction step as well as measurement step were optimized carefully.

Initially, the temperature programmes for determination of mobile fraction of copper in soil obtained with different extraction reagents (listed in Table 1) by ETAAS technique were optimized. Due to very corrosive properties of hydroxylamine chloride towards graphite tubes, the extracts of fraction II were evaporated and the residues were dissolved in $0.1 \text{ mol}\cdot\text{L}^{-1}$ nitric acid. The optimal pyrolysis temperature for soil

Table 1. Chemical reagents and conditions for the conventional modified BCR extraction and the developed ultrasound assisted extraction for 1 g of soil sample

Fraction	Reagents	Extraction conditions	
		Conventional modified BCR procedure	Ultrasound assisted procedure
F I: Water-, acid-soluble, exchangeable	40 mL of 0.11 mol·L ⁻¹ CH ₃ COOH	shaking for 16 h, room temp.	7 min at 15 W
F II: Reducible	40 mL of 0.5 mol·L ⁻¹ NH ₂ OH·HCl (pH 1.5)	shaking for 16 h, room temp.	10 min at 15 W
F III: Oxidisable	10 mL of 30% H ₂ O ₂ (pH 2) 10 mL of 30% H ₂ O ₂ (pH 2) 50 mL of 1 mol·L ⁻¹ CH ₃ COONH ₄ (pH 2)	shaking for 1 h, room temp. and 1 h at 85°C; 1 h at 85°C, cooling; shaking for 16 h, room temp.	4 min at 15 W, 1 h at 85°C 6 min at 15 W

Table 2. Analytical characteristic of copper determination by ETAAS in extraction solutions

Extraction solution	Linear range of calibration graph, ng·mL ⁻¹	Detection limit ^a , ng·mL ⁻¹ (mg·kg ⁻¹)	Quantification limit ^b , ng·mL ⁻¹	Precision as RSD ^c , %, n=6
0.11 mol·L ⁻¹ CH ₃ COOH	0.8-40	0.38 (0.015)	0.75	5.2
0.5 mol·L ⁻¹ NH ₂ OH HCl ^d	0.6-15	0.28 (0.011)	0.60	5.1
1 mol·L ⁻¹ CH ₃ COONH ₄	1.2-35	0.32 (0.016)	1.20	4.6

^a detection limit corresponding to blank + 3SD_{blank}

^b quantification limit corresponding to blank + 6SD_{blank}

^c for 5 ng·mL⁻¹ of Cu

^d after evaporation and dissolution of residue in 0.1 mol·L⁻¹ HNO₃

extracts in acetic acid and hydroxylamine chloride was 1000°C, while for soil extracts in ammonium acetate was 1100°C. The optimal atomization temperature of copper in all fractions was 2300°C. The influence of the used extraction solutions as well as the matrix of soil samples on the atomization of copper and its determination in mobile fractions was also examined. For this reason the results of Cu determination in all fractions by two calibration techniques: external calibration graph and standard addition method were compared. For standard addition method the extracts of soil were spiked with increasing amounts of copper. It was found that the regression coefficients of both calibration graphs were the same in the range of analytical error. The performed studies showed insignificant influence of matrix components of soil on the analytical signal of copper, therefore the external calibration procedure based on reagent-matched standard solutions was used for copper quantification. The characteristic of copper determination in extraction solutions by ETAAS is summarized in Table 2.

A major inconvenience of the BCR sequential extraction procedure is the lengthy sample processing time (overall operation time is about 51 h). In order to speed up the extraction process of copper ultrasonic assistance with an ultrasonic probe has been exploited in our studies. The parameters influencing the efficiency of copper extraction by ultrasounds, such as a sonication time and a power of the ultrasonic probe, were optimized for each step of procedure individually. All results obtained by UASE were compared with those obtained

by the conventional modified BCR procedure for the same soil sample. For these studies the samples of soil that vary in category and pH (sample L - light soil, pH 4.3; sample M - medium soil, pH 7.1; sample H - heavy soil, pH 4.3) were used (Figure 1). The recovery of copper in fractions obtained by UASE was calculated assuming the content of copper in fractions obtained by conventional modified BCR extraction as 100%.

The influence of a power of ultrasound probe on the recovery of copper in fraction I was studied in the range 10 W to 26 W at sonication time of 5 min (Figure 1a). It was found that the recovery of analyte from tested samples was from 60% to 120%. The lower copper recovery (~60%) from samples L and H (acidic soil) may be caused by re-adsorption of analyte, what was also reported in [18]. Based on these results and an analogous research performed for other metals (Cd and Pb) (not published), as well as on our efforts to develop the common extraction conditions for all metals, the ultrasound power of 15 W was chosen for further experiments. The influence of sonication time on the recovery of copper in fractions I was studied in the range from 1 to 12 minutes. The recovery of analyte from acidic soil (sample L and H) for sonication time from 1 min to 7 min was at the level 65-77% and decreased to 35-45% when sonication was prolonged. Quantitative recovery of analyte from neutral soil (sample M) was achieved for 3-7 min of sonication time. In order to provide efficient extraction of copper and to avoid its re-adsorption or liberation from the next phase of soil the sonication was performed for 7 min.

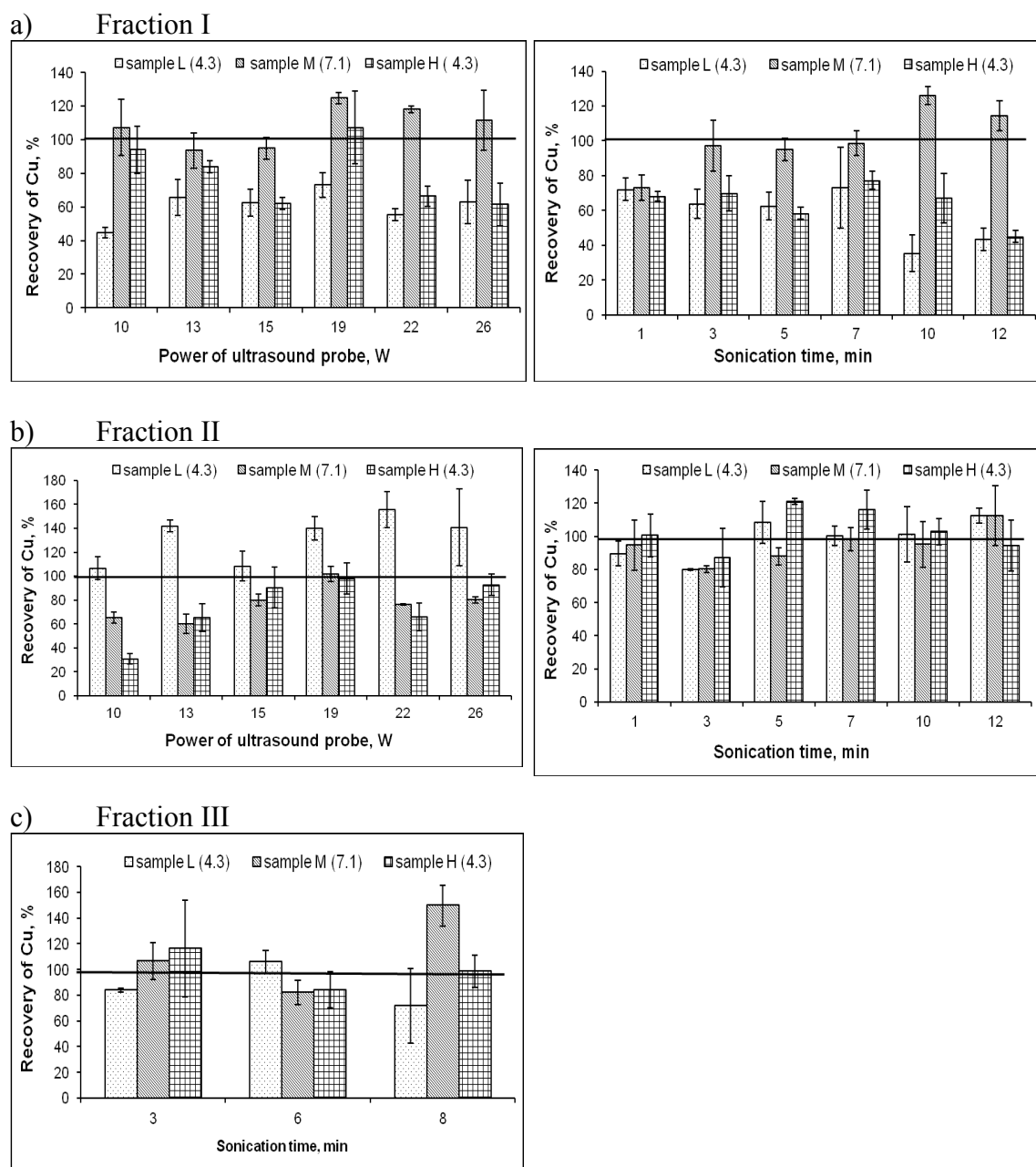


Figure 1. Recovery of Cu in fractions extracted from soil in dependence of the power of ultrasonic probe (fixed sonication time 5 min) and the sonication time (fixed sonication power 15 W): a) fraction I, b) fraction II, c) fraction III; (sample L - light soil, sample M - medium soil, sample H - heavy soil; in brackets value of soil pH)

The optimization of the extraction conditions of copper in fraction II was performed in the same way. The significant influence of the power of ultrasonic probe on the recovery of copper was observed (Figure 1b). Higher recovery of analyte (from 105% to 155%) from sample L (light acidic soil) was probably a result of its re-adsorption in the first step of extraction. The good recovery of analyte (from 80% to 110%) for all tested sonication times was obtained. The sonication of samples for 10 min at 15 W was chosen as it provided quantitative recovery of copper and good repeatability of the results.

During optimization of the extraction conditions of copper in fraction III the oxidation of organic matter

using 30% H_2O_2 was firstly studied. The conventional sample treatment (as listed in Table 1) was compared with shorter procedure applying only one addition of H_2O_2 and sonication of solution for 4 min (at 15 W) followed by evaporation of reagents [16]. As no significant difference in the recovery of copper (79-137% versus 88-133%, $n=3$) was obtained for both tested procedures, the shorter procedure with sonication step was later used. Next, the influence of sonication time on the recovery of copper during re-extraction of mineralization products with CH_3COONH_4 was studied (Figure 1c). The power of ultrasonic probe (15 W) applied in this step, was the same as used before, while the chosen sonication time was 6 min. The optimal working para-

Table 3. Recovery of copper from certified reference material of lake sediment (BCR-701) after conventional modified BCR extraction and ultrasound assisted extraction

Fraction	Certified value \pm SD, mg·kg ⁻¹	Conventional modified BCR extraction, n=3		Ultrasound assisted extraction, n=6	
		Found content \pm SD, mg·kg ⁻¹	Recovery \pm SD, %	Found content \pm SD, mg·kg ⁻¹	Recovery \pm SD, %
F I: 0.11 mol·L ⁻¹ CH ₃ COOH	49.3 \pm 1.7	53.0 \pm 2.5	107.6 \pm 5.0	40.2 \pm 4.8	81.6 \pm 9.8
F II: 0.5 mol·L ⁻¹ NH ₂ OH·HCl	124.0 \pm 3.0	132.5 \pm 4.3	106.8 \pm 3.5	117.2 \pm 10.3	94.5 \pm 8.3
F III: 1 mol·L ⁻¹ CH ₃ COONH ₄	55.2 \pm 4.0	61.5 \pm 3.6	111.4 \pm 6.5	56.1 \pm 4.7	101.6 \pm 8.6

Table 4. Copper content in fractions of soil collected from Podlaskie Voivodeship after ultrasound assisted sequential extraction procedure

Sample	Soil category	Soil pH	Copper content in fraction of soil, mg·kg ⁻¹ , n=3			Pseudo-total content of Cu, mg·kg ⁻¹
			F I	F II	F III	
L1	very light	4.3	0.09 \pm 0.01	0.21 \pm 0.04	0.56 \pm 0.05	2.75
L2	light	4.3	0.21 \pm 0.06	0.17 \pm 0.06	0.63 \pm 0.17	5.00
L3	light	4.6	1.39 \pm 0.10	1.21 \pm 0.03	1.92 \pm 0.11	9.75
M1	medium	4.7	0.25 \pm 0.07	0.66 \pm 0.01	1.03 \pm 0.39	5.50
M2	medium	4.8	0.26 \pm 0.08	0.75 \pm 0.02	0.91 \pm 0.19	8.50
M3	medium	6.0	1.15 \pm 0.03	1.12 \pm 0.09	0.88 \pm 0.10	8.12
M4	medium	7.1	0.11 \pm 0.03	0.68 \pm 0.18	1.31 \pm 0.25	6.75
M5	medium	7.2	0.17 \pm 0.06	1.17 \pm 0.32	1.29 \pm 0.25	8.50
M6	medium	7.3	0.30 \pm 0.03	0.47 \pm 0.05	1.59 \pm 0.09	7.75
H1	heavy	4.3	0.13 \pm 0.03	1.22 \pm 0.22	1.42 \pm 0.27	7.12

soil category: L - light, M - medium, H - heavy

meters of ultrasonic probe for UASE of copper from soil are presented in Table 1.

The developed UASE procedure for determination of mobile fractions of copper in soil was validated. The subsequent parameters were evaluated: linearity of calibration graphs, limit of detection and limit of quantification, precision of results (Table 2). The repeatability of copper determination in fractions I, II and III, expressed as the relative standard deviation (RSD) of six independent analyses of the same soil sample, was in the range of 5% to 8%. The accuracy was evaluated by analysis of certified reference material of lake sediment BCR 701. The content of copper determined in fractions I, II and III was compared with certified values and with its content determined by conventional modified BCR procedure (Table 3). The recovery of copper for both extraction procedures was in the range 82-111%. The repeatability of copper determination in fractions of BCR 701 was below 6% using conventional extraction and below 12% using UASE.

The content of copper in mobile fractions of soil samples collected from Podlaskie Voivodeship was determined (Table 4). The pseudo-total content of analyte was also determined after *aqua regia* digestion.

DISCUSSION

The UASE procedure allows appreciably shortening the total extraction time from 48 h to 27 minutes in comparison to conventional modified BCR procedure. It allows to save the time and energy during preparation of soil extracts. The significant reduction of power consumption by applying of ultrasonic probe instead of vertical rotor is in good agreement with green chemistry concept. The total extraction time of the developed UASE procedure is also considerably shorter than others applying the ultrasonic bath (1.15-5 h) proposed in the literature [1, 5] and similar to those which used the ultrasonic probe [5,16,18]. Shorter time of extraction (17 min or 22 min) was obtained, when higher power of ultrasonic probe (105 and 75W or 20W) was applied [5, 16].

The lower recovery of copper in fraction I of the certified reference material, lake sediment BCR 701, obtained by UASE procedure can be caused by higher re-adsorption of analyte in this step in comparison to conventional one, what was also reported in [18]. It was found that the re-adsorption phenomena mainly had occurred for the most labile fraction, i.e. acid soluble, and was matrix dependent, higher for sediment than for soil samples.

The UASE procedure is characterized by higher RSD values (up to 12%) in comparison to the conventional

modified BCR protocol (up to 6%), as within long time of extraction step (16 h) an equilibrium between solid phase and extrahent is reached. Vigorous running of extraction under ultrasound treatment in UASE procedure induces intensive fragmentation of soil particles and improves solvent penetrability into solid phase. However, the short time of extraction (6-10 min) and in-homogeneity of natural soil samples can lead to bigger difference in the content of extracted analyte.

The content of copper in all analysed samples did not exceed its maximal tolerance level for agricultural soil in Poland ($150 \text{ mg} \cdot \text{kg}^{-1}$) [19] as well as the recommendation of Polish Chemical and Agricultural Station and the studied soil are suitable for low metal content consumer crops [10]. Figure 2 shows the distribution of copper into three fractions extracted from soil as well as in the residual, named here fraction IV. The content of copper extracted by acetic acid represents only 2-14% of the pseudo-total content in soil. Thus the amount of copper bioavailable to plant is very low in studied soil. The content of copper in fraction II, extracted from phase of iron and manganese hydroxides of soil, was also low and represents only 3-17% of the pseudo-total content. The content of analyte in fraction III was slightly higher than that in first two fractions and represents 17-21% of the total metal content. The results confirmed the affinity of copper to organic matter, in which it can be bound to functional groups of humic and fulvic acids. The fraction IV (residual) made up the largest percentage in copper total content, 54-78%. The other authors also reported the highest concentration of copper in the residual fraction of soil [3,15,21]. As residual fraction is considered as not soluble under natural conditions, therefore copper from this fraction is unavailable to plant. The lower content of copper was observed in fraction III and II, depending on the type of soil and the lowest amount was determined in fraction I [3,15,21]. The percentage share of copper frac-

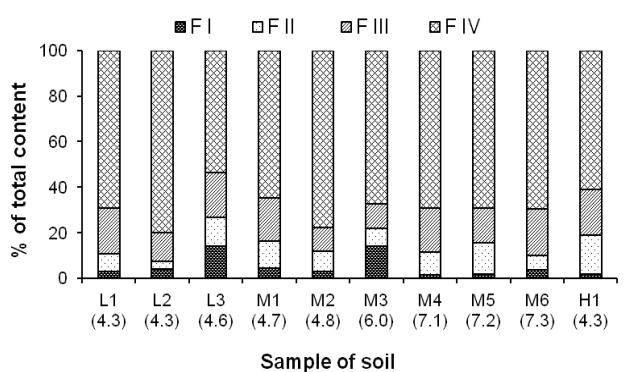


Figure 2. Partitioning of copper in soil collected from Podlaskie Voivodeship after UASE procedure (soil category: L - light, M - medium, H - heavy; in brackets value of soil pH; F IV- residual fraction was calculated as difference between pseudo-total content of Cu and sum of contents of Cu in F I, F II and F III)

tion in the pseudo-total content represents the decreasing order: FVI>FIII>FII>FI for studied soil. A similar order was observed in Rendzic Leptosols, Haplic Arenosols and Haplic Leptosols collected from Lubelska Upland and Sandomierska Valley [21], in Cambisols collected from Siedlce Upland region [15] and soil collected from allotment garden in Koszalin [3]. Slightly different order of percentage share of analyte: FVI>FII>FIII>FI was observed in Luvisols collected from Siedlce Upland region [15], indicating the higher binding of copper with Fe and Mn hydroxides in such type of soil. The obtained results indicate the low mobility of copper in studied soil samples.

CONCLUSIONS

In conclusion, the results obtained from the present studies are as follows:

1. The new extraction procedure based on the modified BCR procedure assisted with ultrasounds generated by ultrasonic probe was successfully developed for determination of mobile fraction of copper in soil.
2. The developed method allows reducing the overall extraction time from 48 h for conventional extraction (with the aid of a vertical rotor) to 27 min for UASE procedure.
3. The recovery of analyte from certified reference material BCR 701 in the range 82-102% proved the accuracy of UASE procedure.
4. The developed procedure fulfills requirements of green analytical chemistry in terms of operation time, safety and energy consumption and can be useful for fast assessment of the mobile fractions of copper in soil.
5. The content of copper in three fractions extracted from soil according to modified BCR UASE procedure indicates a low pool of mobile fraction of copper in soil, what means a low bioavailability of copper to plants and a high risk of its deficits for plants.

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Conflict of interest

The authors declare no conflict of interest.

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