

## ASSESSMENT OF THE COPPER AVAILABILITY IN THE ARABLE SOILS BY DIFFERENT EXTRACTANTS

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**Abstract:** This paper presents an assessment of the copper availability in soil by different single extractions: DTPA (0,005 mol/L), CaCl<sub>2</sub> (0,1 mol/L) and NH<sub>4</sub>NO<sub>3</sub> (1 mol/L, DIN procedure). The research was carried out on 48 samples of the arable soils taken in the north-western part of the Republic of Srpska. Total Cu contents was also determined in the soil samples after the acid digestion (HNO<sub>3</sub>+H<sub>2</sub>O<sub>2</sub>). Copper contents in the extracts was determined by the atomic absorption spectrophotometry. Total soil Cu ranged from 7,7–42,9 mg/kg which corresponded to Cu-unpolluted soils. Low level of Cu mobility and availability was found in the investigated soils. In the average DTPA extracted 10,87%, CaCl<sub>2</sub> 0,31% and NH<sub>4</sub>NO<sub>3</sub> 0,44% of the total soil Cu. High statistically significant correlation ( $r=0,84$ ) between the Cu contents extracted with DTPA and NH<sub>4</sub>NO<sub>3</sub> was found, which implies that maximum allowed level of this element in the soil for the DTPA extraction procedure might be established. That conclusion has to be verified in future research including soils with high total copper content.

**Keywords:** heavy metals, soil, extraction, pollution.

### 1. INTRODUCTION

The importance of heavy metals for the plants and other living beings differs since they are either essential (for example Fe, Mn, Cu, Zn) or toxic (such as Hg, As, Pb). For the essential micronutrients such as copper (Cu), insufficient uptake leads to deficiency-related health problems, while its presence in the large amounts cause toxicity [1].

The soil is the primary source of the heavy metals for the plants, through which they enter in the food chain and determine the health of humans, and animals too [2]. Due to the fact that metals are present in the soil in different chemical forms, their total contents are not sufficient indicator of their availability. Therefore, the data about the total contents of metals, as well as their content in mobile and potentially mobile forms (available content) are necessary for the proper assessment of the degree of soil pollution and environmental risk [3]. For the copper and other microelements, these data are also important for the evaluation of their sufficiency for the cultivated crops [1].

Total contents of metals in the arable soils reflect their geochemical origins as well as anthropogenic inputs such as pollutants from urban-industrial activities and agricultural practice [4]. In case of copper, especially in some arable areas, such as vineyards, orchards or potato fields, the total content is mainly influenced by agrochemicals containing Cu and applied as pesticides and/or fertilizers [5,6].

The extractability of metals from the soil and their mobility to the plants depend on several factors. Among them, the most important are: soil properties (pH, content of organic matter, content of clay, cation exchange capacity/CEC), their origin and environmental conditions, such as acidification, redox-processes and water regime [7]. It is well established that metals originating from geochemical sources (parent material) are present in the soil in the less mobile forms (oxides, sulphides and silicates). On the other side, human activities cause a higher contribution of metals in the forms that are mobile and easily accessed by plants: water-soluble, and easily exchangeable [4,8].

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The copper is generally considered as relatively immobile metal in the soil, because it strongly adsorbs to clay minerals, organic matter and iron and manganese oxides. Therefore, Cu tends to remain in the surface layer (topsoil) with higher content of organic matter [1]. Wei and authors [9] found that the surface layer with depth 0–15 cm contains approximately 41% of the total and 66% of the available Cu originating from the long-term application of the copper fertilizers in the agricultural system.

The most commonly used methods for the determination of the total metal contents, among them copper also, in environmental studies are acid digestions with concentrated  $\text{HNO}_3 + \text{H}_2\text{O}_2$  [10] or  $\text{HNO}_3 + \text{HCl}$  [11]. Nitric and hydrochloric acids do not cause the release of metals from all chemical forms in the soil, nor do they release the metal ions built in the crystal structure of silicate minerals. Therefore, the content of metals obtained after these digestions is actually pseudo-total content. However, metals bound in silicates are not extractable and mobile even under the most extreme conditions in environment and in the environmental researches, as well as in this work, content of metals obtained by the acid digestions is considered as their total content. According to these methods, the maximum allowed concentration of heavy metals in agricultural soils is legally determined in numerous countries, as well as in the scientific literature [4].

The mobility of the copper and other heavy metals in the soil has been generally estimated by procedures of single, selective extractions. These methods include application of various single chemical reagents, such as: neutral salts ( $\text{CaCl}_2$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{NaNO}_3$ ), mild acids (diluted  $\text{HCl}$ ,  $\text{HOAc}$ ) and chelating agents (DTPA/diethylenetriamine pentaacetic acid, EDTA/ethylene diamine tetraacetic acid). [8] The variety of reagents and experimental conditions resulted in numerous single extractions. A lot of effort has been made, especially in Europe, to define the standard method of single extraction, but the predominance of different soil types in various regions resulted in different reference methods in various countries. For example, the reference single extractant in Netherland is  $\text{CaCl}_2$ , in Germany  $\text{NH}_4\text{NO}_3$ , in Switzerland  $\text{NaNO}_3$ , while the DTPA and EDTA have been both separately adopted in French legislation [12].

Ideally, the best suitable single extractant is the one which could extract metals from soil under the conditions similar to those between plant roots and soil solution [13]. Chelating agents DTPA and EDTA extract metals from mobile and potentially mobile forms, while the neutral salts such as

$\text{NH}_4\text{NO}_3$  and  $\text{CaCl}_2$  extract only the directly available metals [14]. According to some studies [15,16], the DTPA-extractable metals are not plant-available metals and therefore neutral salt solutions provide better relationship between soil-extractable metals and plant tissue accumulation. On the other side, numerous investigations showed that the selection of the best suitable extractant for the prediction of the plant uptake depends mainly on the environmental conditions [17,18].

Thus, the first step in researching the Cu-distribution and mobility in the soil in one region is the selection of the best suitable single extraction procedure. Testing the different extractants includes not only providing extractions, but also determination of the total Cu in the soil and main chemical properties (pH, organic matter content, CEC) that influence the mobility and extractability of this element. All this and the lack of before-done similar researches in the arable soils in the north-western part of the Republic of Srpska define the main objectives of our work: 1. Determination of the copper mobility and availability by comparing the result of the three most commonly applied single extraction procedures (single extractions with DTPA,  $\text{CaCl}_2$  and  $\text{NH}_4\text{NO}_3$ ), 2. Assessment of the efficiency of the applied single reagents for the Cu extraction from the soils 3. Estimation of the degree of the soil pollution with Cu by comparing the determined available and total contents with the maximum allowed, 4. Estimation of the sufficiency of the Cu in the investigated soils by comparing the determined content of the available Cu with the reference values.

## 2. MATERIAL AND METHODS

This research was carried out in the arable soils in the north-western part of the Republic of Srpska and included 48 soil samples. The investigated region is located between the river Sava in the north and the inner Dinarides slopes in the south, while the rivers Una and Bosna are its borders in the west and east (Figure 1). The region includes areas with different intensity of agricultural production. In the first line, the plains and fields situated in the fertile river valleys of Sava, Vrbas, Una, Bosna and their tributaries are primarily used for crop and farming production. On the other side, the mountainous part which includes plateaus on the medium-high mountains Cemernica, Manjaca and Uzlocmac, and initial zone of Dinarides are predominately rich in pastures and extensive crop production. Between these two areas are hilly

terrains which are dominating by fruit growing. The soils in the region are formed on the parent material with various geological origins producing the variety of soil types and therefore their heterogeneity [19].

The samples of the arable soils were taken from 48 locations, from top-soil layer, depth 0-25 cm. Sampling was carried out with the agrochemical probe by forming the average sample which was then analyzed. During the process of sampling, the information relevant for the description of each location was also recorded: usage of the agricultural land, cultivated crops, distance from the industrial facilities, major roads and water streams, GPS coordinates etc.

Prior to the analyses, the average soil samples were air dried at room temperature, then crushed in a mortar and sieved through a 2 mm sieve in order to remove stones and pebbles. The fraction of the soil with size <2 mm was used for all soil analyses. Main chemical properties of the soils were determined according to standard agrochemical methods: 1.

Acidity (pH) in KCl,  $c=1$  mol/L (soil: solution ratio 1:2,5), 2. Content of carbonates ( $\text{CaCO}_3$ ) with Scheibler calcium-metre, 3. Organic matter content applied method by Tjurin [20] 4. Cations exchange capacity by saturation with NaOAc,  $\text{pH}=7$  [21].

Single extractions of the copper from the soil were done with three extractants: DTPA,  $\text{CaCl}_2$  and  $\text{NH}_4\text{NO}_3$  (Table 1) DTPA-extractable Cu was determined with the solution, which includes DTPA  $c=0,005$ mol/L, triethanol-amine (TEA)  $c=0,1$  mol/L and  $\text{CaCl}_2$   $c=0,01$ mol/L, buffered at  $\text{pH}=7,3$ . During the extraction, 20 g of the soil was shaken with 40 mL of the DTPA solution on the rotational shaker. For  $\text{CaCl}_2$  extraction, 10 g soil were shaken with 100 mL of  $\text{CaCl}_2$  solution. In the case of the  $\text{NH}_4\text{NO}_3$  extraction, ratio of the soil and extraction solution was 10 g: 50 mL. After shaking the extractants with soil on the rotational shaker during the time of extraction, the extracts obtained for every of the three single extraction procedure, were filtrated through a Whatman No. 44 filter paper into pure flasks.

Table 1. The single extractions applied in this work

Extractant	Concentration (mol/L)	Soil: extractant ratio (weight:volume)	Extraction time (h)	Temperature	Reference
DTPA	0,005	1:2	2	room temp.	[22]
$\text{NH}_4\text{NO}_3$	1	1:5	2	room temp.	[23]
$\text{CaCl}_2$	0,1	1:10	2	room temp.	[24]

For the total contents of Cu, the following acid digestion [10,25] of the soils was carried out:  $2.00 \pm 0.01$  g of the soil was transferred to a 100 mL Erlenmeyer flasks, 20 mL of concentrated  $\text{HNO}_3$  was added and the flasks were covered with the funnels. The content was boiled at  $150^\circ\text{C}$  for two hours. After cooling, 3 mL of 33% solution of  $\text{H}_2\text{O}_2$  was added and the content was additionally boiled for 15 min. to ensure complete dissolution (oxidation) of soil organic matter. The procedure with  $\text{H}_2\text{O}_2$  was repeated once again after cooling. The content was then transferred into a 100 mL volumetric flask with deionized water and filtered through a Whatman No. 44 filter paper into a flask.

In the extracts obtained after single extractions and the acid digestion, the contents of Cu was determined by method of the atomic absorption spectrophotometry, on the instrument Perkin Elmer AAnalyst 400. The measurements were carried on the wave length of 324.75 nm, using the air/acetylene flame. Calibration standard solutions with the concentrations of 0.2, 0.4, 0.8 and 1.0 mg Cu/L were prepared by dilution of the standard stock solution (1000 mg/L, Perkin Elmer) with the used extractants.

All laboratory vessels used for determination of the total metal contents were prewashed in the 10%  $\text{HNO}_3$  and then washed with deionized water. Standard solutions for AAS quantification of metals were made using commercial standards for AAS (Perkin Elmer, USA) and the proper extractant for dilution. In all cases, standard solutions and blank were treated in the same way as the real samples to minimize the interferences during analysis.

All analyses and measurements were carried out in three replicates and all chemicals used in the experiments were analytically pure (p.a. grade). Internal control soil (soil sample from the interlaboratory proficiency testing) was analyzed to assess reliability of the results. The recovery for total Cu was 93,6%.

The statistical analyses included determination of the mean, median, standard deviation ( $\sigma$ ), Pearson's correlation coefficient ( $r$ ), coefficient of variation etc. and were performed using the Microsoft software package (Excel) and Statistica 10 software. The Origin 7.5 was applied for graphical presentation of the extraction efficiency for applied single extractants.

### 3. RESULTS AND DISCUSSION

Total content of Cu in all investigated soil samples was lower than the maximum allowed for unpolluted soils (60 mg Cu/kg, [4]). Beside this, the determined mean for the total Cu (21,77 mg/kg) is lower than the average value for worldwide soils (30

mg Cu/kg, [4]). The determined available contents of Cu with all three used extractants (Table 2) showed low level of Cu mobility and availability. This is probably consequence of the lower total Cu and its higher contribution in the chemically less mobile forms in the investigated soils.

Table 2. The contents of Cu and the main chemical properties of the investigated soils

	unit	mean	st.deviation	minimum	maximum
Cu, HNO <sub>3</sub>	mg/kg	21,77	9,44	7,70	42,90
Cu, DTPA	mg/kg	2,50	1,54	0,38	6,64
Cu, NH <sub>4</sub> NO <sub>3</sub>	mg/kg	0,10	0,06	0,02	0,27
Cu, CaCl <sub>2</sub>	mg/kg	0,07	0,04	0,01	0,15
pH		5,73	1,12	3,75	7,37
Organic matter	%	1,84	1,01	0,70	5,40
CEC	meq/100g	21,48	11,07	8,75	49,75
CaCO <sub>3</sub>	%	1,85	6,43	0,00	33,10

Among the applied extractants, DTPA showed the highest extraction efficiency (Table 2, Figure 2). This is in consonance with the results of other similar researches [15,26,27] in which this reagent is found as an extractant with a higher extraction efficiency than CaCl<sub>2</sub> and NH<sub>4</sub>NO<sub>3</sub>. Higher extraction capacity of DTPA compared to the other two used reagents is mainly caused by different mechanisms of Cu-extraction. Due to a strong complexing ability, DTPA displaces Cu from insoluble organic or organometallic complexes in addition to those absorbed in inorganic soil

components (exchangeable forms). This reagent also extracts copper ions bound to carbonate and Fe- and Mn-oxides. On the other side, unbuffered solutions of the neutral salts (such as NH<sub>4</sub>NO<sub>3</sub> and CaCl<sub>2</sub> used in this research) extract only easily exchangeable forms of Cu from the soil (directly available forms).

In the average DTPA extracted 10,87%, CaCl<sub>2</sub> 0,31% and NH<sub>4</sub>NO<sub>3</sub> 0,44% of the total soil Cu from the investigated soils. The amounts of extracted total Cu for all three single extractions are similar with typically reported for unpolluted soils [18,28,29].

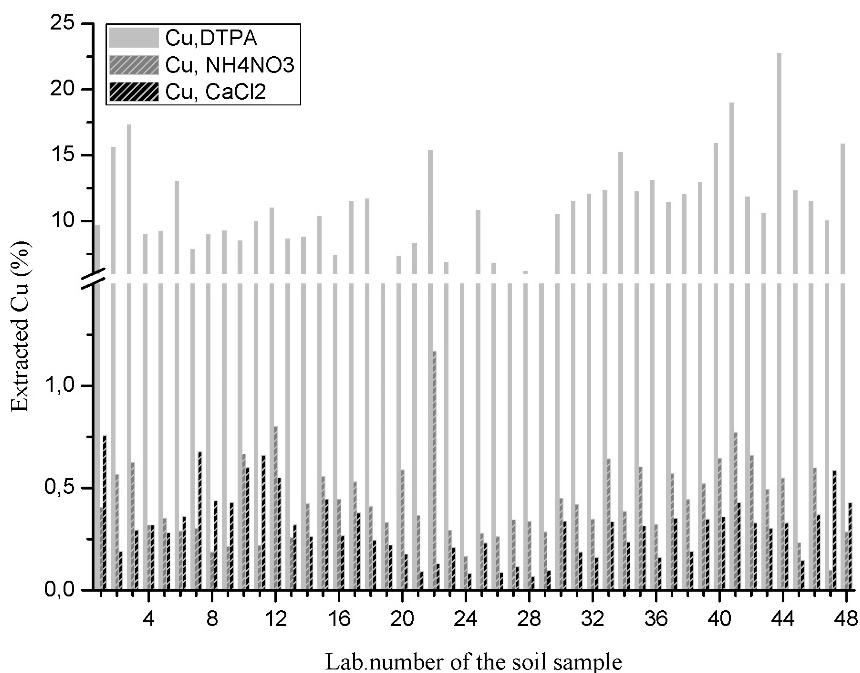


Figure 2. Extraction efficiency of the used single extractants

Figure 2. shows the share of available Cu determined with all used extractants in the total Cu for the singular soil samples. Thanks to this approach, it is possible to detect the locations with higher mobility and availability of Cu in the soil and analyze it in more details by conducting additional researches in order to get more precise information about environmental risk.

Due to the high extraction capacity and fact that it extracts available and potentially available amounts of Cu from the soil, DTPA is used for detection of its deficiency in arable soils [30]. The results of the DTPA extraction in this work showed that in 89,6% of the analyzed soil samples, the

available contents of Cu were high or very high concerning the sufficiency with this element (>0,9 mg/kg). On the other side, the content of Cu extracted with DTPA in all samples was lower than 15 mg/kg, which is the limit value for the toxic effects according to the same classification [30]. Following this norm, the investigated soils are unpolluted. Regarding the determined contents of Cu extracted with NH<sub>4</sub>NO<sub>3</sub> (Table 2), we identified these soils as unpolluted also, because the Cu contents in all analyzed samples were lower than the maximum allowed according the DIN-standard (1 mg/kg, [23]).

Table 3. Correlation coefficients between the Cu contents and main chemical characteristic of the soils

	Cu, HNO <sub>3</sub>	Cu, DTPA	Cu, NH <sub>4</sub> NO <sub>3</sub>	Cu, CaCl <sub>2</sub>
Cu, DTPA	0,88 <sup>a</sup>			
Cu, NH <sub>4</sub> NO <sub>3</sub>	0,81 <sup>a</sup>	0,84 <sup>a</sup>		
Cu, CaCl <sub>2</sub>	0,61 <sup>a</sup>	0,62 <sup>a</sup>	0,69 <sup>a</sup>	
pH	0,59 <sup>a</sup>	0,57 <sup>a</sup>	0,66 <sup>a</sup>	0,54 <sup>a</sup>
CaCO <sub>3</sub>	0,08 <sup>NS</sup>	0,05 <sup>NS</sup>	0,10 <sup>NS</sup>	0,39 <sup>a</sup>
Org.matter	0,23 <sup>NS</sup>	0,30 <sup>b</sup>	0,44 <sup>a</sup>	0,09 <sup>NS</sup>
CEC	0,60 <sup>a</sup>	0,70 <sup>a</sup>	0,69 <sup>a</sup>	0,32 <sup>b</sup>

<sup>a</sup>significant at p<0,01  
<sup>b</sup>significant at p<0,05  
 NS statistically not significant

Correlation analyses show statistically high significant correlation between the total Cu and contents of Cu determined with all used single extractants (Table 3). Beside this, the highest coefficient of correlation is found between the total Cu-content and content extracted with DTPA, which corresponds to the results of the other authors [28,31,32]

Between the contents of copper determined with the use of single extractants, a statistically high

significant correlation is detected, while the highest correlation coefficient was found between Cu contents extracted with DTPA and NH<sub>4</sub>NO<sub>3</sub> (r=0,84). During the study of the efficiency between various single extractants, Etlar and authors [27] found in contaminated soils statistically high significant correlation between the contents of Cu extracted with same extractant with r=0,70.

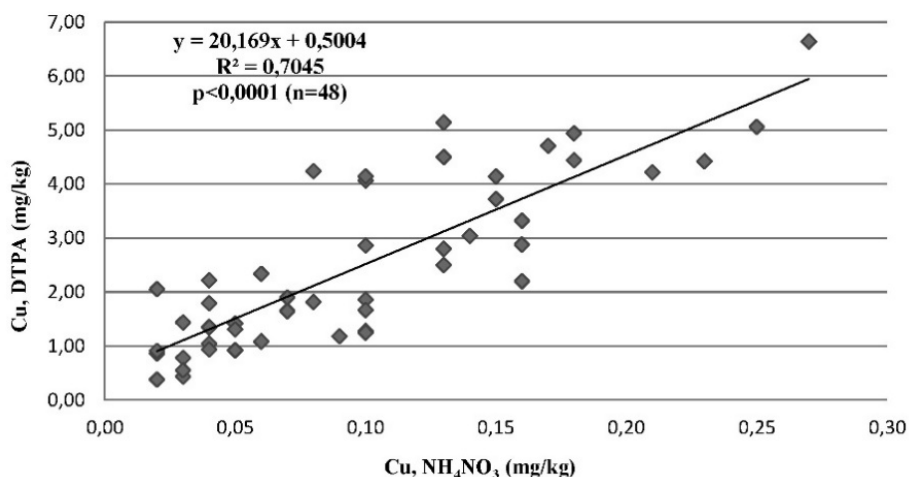


Figure 3. XY-diagram showing the relation between the contents of Cu determined with NH<sub>4</sub>NO<sub>3</sub> and DTPA

The linear regression equation between the available contents of Cu determined with  $\text{NH}_4\text{NO}_3$  and DTPA (Figure 2) confirms these statements and indicates that the DTPA extraction could be used not only for estimation of the Cu-sufficiency, but also for the determination of the limit value for the Cu-content in the unpolluted soils. According to the linear regression equation, the maximum allowed content of Cu extracted with DTPA is 20,67 mg/kg. Therefore, in the soils with the higher Cu-contents, the negative effects for the plants are expected. Since in this work with  $\text{NH}_4\text{NO}_3$ , the contents of Cu similar to or higher than the limit value according to the DIN-standard (1mg/kg, [23]) were not detected, it is necessary to continue with the exploration by including the soil samples with the higher Cu-contents. Further testing will show the significance of the correlation between the contents of Cu determined with these extractants in the broader concentrations range and confirm or deny our assumption.

The investigated soils show great heterogeneity regarding the main chemical properties, mainly due to the specifics of their pedogenesis (Table 1). Correlation analysis showed that among the determined soil properties, CEC has the highest influence on the total content of Cu as well as on its available contents determined with DTPA,  $\text{NH}_4\text{NO}_3$  and  $\text{CaCl}_2$  (Table 2). The same conclusion was obtained by other authors [33–36]. This is consequence of the high affinity of copper for bounding with organic matter. The content of organic matter, among other factors, significantly determines the adsorptive capacity of the soil. According to the average values for organic matter content and CEC the investigated soils are moderately humous and have moderate adsorptive capacity for the metal cations indicating a lower risk of Cu mobility and transfer from the soils to the plants.

#### 4. CONCLUSIONS

The results of the investigation carried out in this work showed that the level of the Cu mobility and availability from the investigated soils to the plants is low. Overall, the extraction capacity of the tested single extractants for Cu in the investigated soils was following:  $\text{DTPA} > \text{NH}_4\text{NO}_3 > \text{CaCl}_2$ . However, all three methods of single extractions could be successfully applied for the assessment of the availability and mobility of Cu in the investigated soils. In order to assess the most suitable single extraction of the Cu from these soils, it is necessary to

provide further research and to determine the correlation between the content of Cu in cultivated plants and content extracted from the soil with certain single extractant. The statistically high significant correlation between the contents of Cu determined with DTPA and  $\text{NH}_4\text{NO}_3$  ( $r=0,84$ ) in the investigated soils leads us to the conclusion that it would be useful to explore this relation in detail by including the soil samples with higher total and available content of Cu. This could lead us to the answer whether it is possible to determine the maximum allowed level of Cu in the soil according to the DTPA extraction as well as with DIN standard [23].

According to the determined total contents of Cu, as well as the available contents determined with  $\text{NH}_4\text{NO}_3$ , the investigated soils are unpolluted, because the determined contents of Cu was lower than the maximum allowed values for total content (60 mg/kg, [4]) and available content according to DIN-standard (1 mg/kg, [23]). This conclusion is additionally confirmed with the fact that the contents of Cu extracted with DTPA was lower than the allowed maximum according to the Abbreu et al. (15 mg/kg, [29]). Due to the same criteria, the investigated soils are sufficiently supplied with Cu necessary for cultivated plants. All this, together with the determined soil properties, in the first place a moderately adsorptive capacity, indicates a low level of mobility and availability of Cu, and therefore a low environmental risk.

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#### ПРОЦЈЕНА ПРИСТУПАЧНОСТИ БАКРА У ПОЉОРИВРЕДНИМ ЗЕМЉИШТИМА СА РАЗЛИЧИТИМ ЕКСТРАКЦИОНИМ СРЕДСТВИМА

**Сажетак:** У овом раду су представљени резултати процјене приступачности бакра из земљишта примјеном различитих метода појединачних екстракција: ДТРА (0,005 mol/L), CaCl<sub>2</sub> (0,1 mol/L) и NH<sub>4</sub>NO<sub>3</sub> (1 mol/L, DIN стандард). Истраживање је проведено на 48 узорак пољопривредног земљишта узетих у сјевероисточном дијелу Републике Српске. Приликом истраживања су одређени и укупни садржаји Cu киселинском дигестијом земљишта (HNO<sub>3</sub>+H<sub>2</sub>O<sub>2</sub>). У екстрактима добијеним након појединачних екстракција и киселинске дигестије, садржај Cu је одређен методом атомске апсорпционе спектрофотометрије. Укупни садржај Cu био је у интервалу 7,7-42,9 mg/kg што одговара вриједностима за незагађена земљишта. Установљен је низак ниво мобилности и приступачности Cu у испитиваним земљиштима. У просјеку са ДТРА је екстраховано 10,87%, са CaCl<sub>2</sub> 0,31% и са NH<sub>4</sub>NO<sub>3</sub> 0,44% укупног Cu. Такође, установљена је и статистички високо значајна корелација (r=0,84) између садржаја бакра екстрахованог са ДТРА и NH<sub>4</sub>NO<sub>3</sub>, што упућује на могућност да се утврди гранични ниво Cu у незагађеним земљиштима за екстракцију са ДТРА, а што је потребно додатно испитати у новом истраживању којим би били обухваћени и узорци са повишеним укупним садржајем бакра.

**Кључне ријечи:** тешки метали, земљиште, екстракција, загађење.

