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USEFULNESS OF DIFFERENT EXTRACTION SOLUTIONS FOR DETERMINATION OF PLANT AVAILABILITY OF HEAVY METALS

PRZYDATNOŚĆ RÓŻNYCH ROZTWORÓW EKSTRAKCYJNYCH DO OZNACZANIA FITOPRZYSWAJALNOŚCI METALI CIĘŻKICH

Abstract: the objective of the study was to evaluate usefulness of different extraction solutions for determination of plant availability of heavy metals. The results obtained indicated relationships between the amounts of Cd, Cu, Pb and Zn extracted, soil physical-chemical properties and extraction solution used. In general, lesser amounts of examined metals were extracted from light soil than from medium soil. The amounts of heavy metals extracted with the use of all examined methods increased with an increase of soil acidity and a level of soil pollution with heavy metals, however it generally decreased with an increase of organic carbon in soil. A strong positive correlation between the contents of Cd, Cu, Pb and Zn in plants and the amounts of heavy metals extracted from soil was observed. The higher values of correlation coefficients were obtained when soft extraction solutions (soil solution and 0.01 mol · dm^{-3} $CaCl_2$) were used when compared with strong extraction solutions (1 mol · dm^{-3} HCl and *aqua regia*).

Keywords: heavy metals, extraction solutions, soil properties, plant availability

Increasing pollution of natural environment with heavy metals as a result of civilization development often leads to excessive accumulation of these elements in the food chain: soil-plant-animal-man. The properties of soil, which is the first link in this system, significantly affect the extent and speed of allocation of trace elements in the food chain.

Determination of availability of trace elements for plants is essential for evaluation of their toxicity for potential consumers. This is performed by chemical tests – soil extractions. A level of soil pollution with heavy metals has so far been determined

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based on their total contents [1]. However, this method is not accurate enough as the total content of heavy metals in soil includes a variety of their forms with different solubility and mobility. Furthermore, there exist a large number of soil factors of great changeability that can significantly influence biological availability of heavy metals [2]. Therefore, evaluation of the content of soluble forms of heavy metals, ie biologically available, would be more suitable for agricultural needs and precise assessment of contamination of cultivated plants with heavy metals [3].

The objective of this study was to examine usefulness of selected extraction solutions for determination of plant availability of heavy metals.

Materials and methods

The study was carried out in the years 2006–2007 in natural climatic conditions. Microplot experiments were conducted at the Experimental Station of the Agriculture and Biology Faculty of Warsaw University of Life Sciences located close to the city of Skierniewice. Ground vases dug in soil in open air were treated as microplots. Soil in the ground vases was differentiated by 3 pH levels (4.0, 5.0 and 6.0), 3 levels of organic C (0.6, 0.9 and 1.2 %), 2 contents of fraction below 0.02 mm (15 and 30 %) and 4 levels of the content of heavy metals: equivalent to the content in natural conditions (pollution of 0^o), increased content (pollution of I^o), weak pollution (II^o), and medium pollution (III^o), according to Kabata-Pendias et al [1]. The experiments embraced 216 microplots that altogether formed 72 combinations. The radish (*Raphanus sativus* L.) and the Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*) were used as experimental plant.

The content of Cu, Cd, Pb and Zn was determined in soil samples with the use of the following methods: the analysis of heavy metals in soil solution and extraction with the use of:

– *aqua regia*,
– HCl at a concentration 1 mol $\cdot$ dm^{-3},
– CH_3COONH_4 at a concentration of 1 mol $\cdot$ dm^{-3},
– $CaCl_2$ at a concentration of 0.01 mol $\cdot$ dm^{-3}.

Soil solutions were obtained with the use of the vacuum displacement method [4]. The contents of heavy metals in soil extracts and solutions were determined with the use of the AAS method. After mineralization of plant material samples in a mixture of concentrated acids HNO_3, $HClO_4$ and H_2SO_4 the total contents of zinc, cadmium, copper and lead were determined with the use of AAS.

Statistical analysis of obtained results concerned the multifactorial variance analysis and simple regression. The analysis were made for all 216 observations and two tested plants.

Results and discussion

The amount of elements extracted from soil was significantly linked with physical-chemical properties of soil and a degree of heavy metal pollution. Of all examined soil properties, a degree of heavy metal pollution and soil reaction had the strongest effects on amounts of heavy metals extracted with the use of examined solutions (Tables 1–4).

As a rule, lower amounts of Pb, Cd, Cu and Zn were extracted from light soil when compared with medium soil (Table 1). The content of organic carbon had no statistically proven significant effect on amounts of heavy metals extracted with the use of examined solutions (Table 1). Nevertheless, with an increase of organic carbon in soil there was observed dropping off content of heavy metals extracted with examined solutions (Table 2). On the other hand, the amounts of extracted heavy metals enlarged with an increase of soil pollution with these elements (Table 3).

Table 1

The average content of heavy metals [mg · kg^{-1} d.m. of soil] in relation to the soil category and the extraction method used

Soil category	Cd					Cu				
	Extraction method									
	1	2	3	4	5	1	2	3	4	5
Light	0.88	0.71	0.19	0.07	0.0004	43.72	37.60	0.54	0.12	0.03
Medium	1.08	0.93	0.28	0.11	0.0005	48.86	40.65	0.64	0.15	0.03
$LSD_{0.05}$	0.101	0.097	0.036	0.023	0.0001	5.070	5.070	3.986	0.131	0.001
	Pb					Zn				
Light	80.07	75.76	3.79	0.04	0.002	98.03	57.47	9.25	4.77	0.07
Medium	89.84	83.99	4.60	0.06	0.008	135.6	77.85	11.95	8.50	0.11
$LSD_{0.05}$	5.508	4.815	1.289	0.035	0.001	12.88	6.601	2.722	3.436	0.009

Explanation for Tables 1–4: Extraction method: 1 – *aqua regia*; 2 – HCl 1 mol · dm^{-3}; 3 – CH_3COONH_4 1 mol · dm^{-3}; 4 – $CaCl_2$ 0.01 mol · dm^{-3}; 5 – soil solution.

Table 2

The average content of heavy metals [mg · kg^{-1} d.m. of soil] in relation to the content of organic carbon in soil and the extraction method used

Organic C [%]	Cd					Cu				
	Extraction method									
	1	2	3	4	5	1	2	3	4	5
0.6	1.02	0.85	0.24	0.12	0.0005	48.03	40.62	0.89	0.14	0.03
0.9	0.97	0.77	0.23	0.09	0.0004	47.99	40.95	0.47	0.13	0.03
1.2	0.95	0.83	0.22	0.06	0.0003	42.85	35.82	0.41	0.12	0.02
$LSD_{0.05}$	0.124	0.118	0.052	0.028	0.0001	6.207	5.865	0.192	0.032	0.001
	Pb					Zn				
0.6	90.59	86.26	4.36	0.10	0.003	121.9	70.04	11.37	7.99	0.13
0.9	85.77	79.70	4.29	0.03	0.002	117.9	67.93	11.22	6.93	0.09
1.2	78.50	73.68	3.93	0.02	0.001	110.7	65.00	9.21	4.98	0.04
$LSD_{0.05}$	8.121	7.083	1.578	0.044	0.0008	18.95	9.711	4.005	2.055	0.01

Table 3

The average content of heavy metals [mg · kg^{-1} d.m. of soil] in relation to a level of soil pollution and extraction method used

Pollution degree	Cd					Cu				
	Extraction method									
	1	2	3	4	5	1	2	3	4	5
0	0.26	0.11	0.15	0.07	0.0001	7.77	3.65	0.14	0.05	0.01
I	0.62	0.49	0.21	0.08	0.0003	28.04	21.66	0.27	0.10	0.02
II	1.03	0.87	0.26	0.10	0.0004	54.23	45.22	0.64	0.16	0.03
III	2.01	1.80	0.31	0.11	0.0007	95.11	85.98	1.30	0.21	0.04
$LSD_{0.05}$	0.189	0.180	0.050	0.045	0.00008	9.465	7.445	0.243	0.041	0.006
	Pb					Zn				
0	14.05	12.26	0.28	0.02	0.0006	47.45	16.96	3.02	1.46	0.04
I	52.38	48.35	1.37	0.04	0.0010	85.74	48.36	5.03	3.02	0.08
II	92.80	88.11	3.17	0.06	0.0020	125.3	83.36	9.49	6.11	0.09
III	180.6	170.8	11.97	0.08	0.0030	208.8	121.9	24.85	15.95	0.14
$LSD_{0.05}$	10.64	10.28	1.822	0.051	0.0002	24.05	12.32	3.850	1.010	0.008

The content of microelements in soil including heavy metals is associated with the soil agronomic type and content of soluble forms of these elements increases in soil together with an increase of the content of leachates [3, 5].

Solubility of heavy metals decreases with an increase of the content of organic carbon in soil [3]. Then again, as found by Diatta and Grzebisz [6], the content of organic carbon in soil is not a reliable and steady factor that has an effect on the content of Zn, Pb and Cd extracted from soil with the use of different solutions.

Many authors have reported that soil reaction is the most important factor to determine solubility of heavy metals in soil [3, 7–9]. The results of this study indicated a significant negative effect of soil reaction on amounts of heavy metals extracted from soil with the use of CH_3COOH at a concentration 1 mol · dm^{-3}, $CaCl_2$ at a concentration 0.01 mol · dm^{-3} as well as with soil solution. On the other hand, amounts of metals extracted with the use of *aqua regia* and HCl at a concentration 1 mol · dm^{-3} did not depend on soil reaction (Table 4). Gambus and Rak [8] showed that together with an increase of soil pH, strong extraction solutions release bigger amounts of cadmium. However, this relation was reversed when soft extraction solutions were applied. Similar relations were observed by Gediga [9] in the case of manganese.

The biggest amounts of examined heavy metals were extracted from soil with the use of *aqua regia* when compared with the effects of other examined extraction methods. The amounts of heavy metals extracted with the use of HCl at a concentration 1 mol · dm^{-3} were: for Pb from 73 to 99 %, for Cd from 25 to 99 %, for Cu from 35 to

99 % and for Zn from 24 to 90 % of the total content of these elements in soil (ie of the amounts extracted with the use of *aqua regia*).

Table 4

The average content of heavy metals [mg · kg^{-1} d.m. of soil] in relation to soil reaction and the extraction method used

pH	Cd					Cu				
	Extraction method									
	1	2	3	4	5	1	2	3	4	5
6.0	0.80	0.66	0.22	0.06	0.0002	43.63	36.59	0.46	0.11	0.02
5.0	1.04	0.86	0.23	0.10	0.0003	45.56	38.57	0.62	0.12	0.03
4.0	1.10	0.93	0.24	0.12	0.0006	49.68	42.22	0.68	0.17	0.04
$LSD_{0.05}$	0.349	0.246	0.044	0.033	0.0001	7.456	6.882	0.159	0.027	0.007
	Pb					Zn				
6.0	84.10	79.19	2.87	0.03	0.0006	110.2	60.30	7.09	2.49	0.04
5.0	85.69	79.56	3.50	0.06	0.0010	117.6	67.53	8.96	5.25	0.08
4.0	85.07	80.89	6.21	0.07	0.0040	125.3	71.15	15.75	12.17	0.15
$LSD_{0.05}$	8.283	7.998	1.869	0.022	0.0002	15.77	8.085	3.334	2.208	0.009

When compared with their total contents (ie the amounts extracted with the use of *aqua regia*), the quantities of heavy metals extracted from soil with the use of CH_3COONH_4 solution were: 10–70 % of the total content of Cd, 1–15 % of Pb, 1–4 % of Cu and from 2 do 25 % of the total content of Zn in soil. When $CaCl_2$ at a concentration 0.01 mol · dm^{-3} was used the amounts Cd, Pb and Cu extracted were below 1 % of their total content in soil. In contrast, the amount of Zn extracted with the use of calcium chloride ranged from 1 to 18 % of the total content of this element in soil. The amounts of examined metals determined with the use of soil solution constituted just about fractions of the percent when compared with their total contents in soil. Gambus et al [10] showed that strong extraction solutions, such as HCl at a concentration 1 mol · dm^{-3} released into the filtrate a big fraction of soil metals, such as Cu, Ni and Zn. On the other hand, unbuffered solutions, such as $CaCl_2$ at a concentration 0.01 mol · dm^{-3} or NH_4NO_3 at a concentration 1 mol · dm^{-3} extracted at most 1.1 % of examined metals. Similar relations were observed for manganese by Gediga [9] as well as by Gambus and Rak [8] in the case of cadmium.

The results obtained showed a significant decrease of yield in both tested plant species with an increase of soil pollution with heavy metals. At the same time, the content of heavy metals in plants significantly increased with an increase of soil pollution with heavy metals (Table 5).

The results of the analysis of correlation coefficients between the amounts of heavy metals in plants and soil indicated that the amounts of heavy metals in plants were positively correlated with the amounts of these elements extracted from soil with the use of different extraction methods (Table 6).

Table 5

Yield of examined plant species [g d.m.] and their content of heavy metals [$mg \cdot kg^{-1}$ d.m.] in relation to soil pollution with heavy metals

Pollution degree	Chinese cabbage					Red radish				
	yield	Cd	Cu	Pb	Zn	yield	Cd	Cu	Pb	Zn
0°	1023.0	0.23	9.50	2.63	59.1	710.2	0.09	0.18	1.24	6.81
I°	730.0	0.52	12.18	3.58	108.7	543.0	0.29	0.43	4.22	17.62
II°	507.1	0.88	15.10	6.27	150.6	431.7	0.30	0.57	4.23	22.44
III°	132.3	1.34	19.03	8.40	241.8	175.2	0.37	0.70	5.53	23.27
$LSD_{0.05}$	44.43	0.112	0.336	1.290	11.56	113.10	0.031	0.076	0.910	1.269

Table 6

Correlation coefficients (r) between the content of heavy metals in soil and amounts of these metals in plants

Extraction method	Cd	Cu	Pb	Zn
Aqua regia	0.64	0.78	0.75	0.71
HCl 1 $mol \cdot dm^{-3}$	0.70	0.77	0.74	0.74
CH_3COONH_4 1 $mol \cdot dm^{-3}$	0.73	0.70	0.81	0.72
$CaCl_2$ 0.01 $mol \cdot dm^{-3}$	0.87	0.80	0.85	0.74
Soil solution	0.91	0.81	0.87	0.79

The higher values of correlation coefficients were obtained for soft extraction solutions (soil solution and $CaCl_2$) than for stronger ones (HCl and *aqua regia*). On the other hand, Gambus et al [10] showed that strong extraction solutions that released big amounts of copper were also very good indicators of plant availability of this metal, contradictory to soft extraction solutions. The results of Gambus and Rak [8] indicated that soft extraction solutions (eg $CaCl_2$ at a concentration 0.01 $mol \cdot dm^{-3}$) were most useful for evaluation of plant available forms of cadmium. Furthermore, HCl solution at a concentration 1 $mol \cdot dm^{-3}$ was completely ineffectual in determination of plant availability of cadmium. Burzynska and Sapek [11] showed greater usefulness of $CaCl_2$ at a concentration 0.01 mol $mol \cdot dm^{-3}$ for evaluation of plant available forms of zinc than that of HCl at a concentration 1 $mol \cdot dm^{-3}$.

Conclusions

1. The amounts of heavy metals (Cd, Cu, Pb and Zn) extracted from soil depended on extraction solution used and soil physical-chemical properties. In general, lesser amounts of examined metals were extracted from light soil than from medium soil. A decrease of the amounts of heavy metals extracted with the use of examined extraction solutions was observed with an increase of organic carbon in soil.

2. The amounts of heavy metals extracted from soil decreased with a reduction of extraction power of solutions used as follows: *aqua regia* > HCl > CH_3COONH_4 > > $CaCl_2$ > soil solution.

3. The content of examined heavy metals in plants was strongly positively correlated with the amounts of heavy metals obtained with examined extraction methods. At the same time, the higher values of correlation coefficients were obtained when soft extraction solutions (soil solution and 0.01 mol · dm^{-3} $CaCl_2$) were used when compared with strong extraction solutions (1 mol · dm^{-3} HCl and *aqua regia*).

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PRZYDATNOŚĆ RÓŻNYCH ROZTWORÓW EKSTRAKCYJNYCH DO OZNACZANIA FITOPRZYSWAJALNOŚCI METALI CIĘŻKICH

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Abstrakt: Celem pracy było określenie przydatności różnych roztworów ekstrakcyjnych do oceny fitoprzyswajalności metali ciężkich.

Przeprowadzone badania wykazały zależność pomiędzy ilością Cd, Cu, Pb i Zn ekstrahowanych z gleby a jej właściwościami fizyczno-chemicznymi oraz zastosowanym roztworem ekstrakcyjnym. Z gleby lekkiej ekstrahowano zazwyczaj mniejsze ilości badanych metali niż z gleby średniej. Ilość badanych pierwiastków ekstrahowanych z gleby wszystkimi analizowanymi metodami zwiększała się wraz ze wzrostem zakwaszenia gleby oraz wraz ze wzrostem stopnia zanieczyszczenia gleby metalami, a zazwyczaj zmniejszała się wraz ze wzrostem zawartości węgla organicznego w glebie. Zaobserwowano silną dodatnią korelację pomiędzy zawartością Cd, Cu, Pb i Zn w roślinie a ilością metali ciężkich ekstrahowanych z gleby. Większe wartości współczynników korelacji otrzymano dla ekstrakcji przy użyciu słabych ekstrahentów (roztwór glebowy i 0,01 mol · dm^{-3} $CaCl_2$) niż w przypadku mocnych roztworów ekstrakcyjnych (1 mol · dm^{-3} HCl i woda królewska).

Słowa kluczowe: metale ciężkie, roztwory ekstrakcyjne, właściwości gleby, dostępność dla roślin