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Proposal of new convenient extractant for assessing phytoavailability of heavy metals in contaminated sandy soil

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Abstract The aim of the study was to compare the usefulness of 1 M HCl with aqua regia, EDTA, and $CaCl_2$ for the extraction of phytoavailable forms of Cu, Ni, and Zn on coarse-textured soils contaminated with these metals. Two microplot experiments were used for the studies. Reed canary grass (*Phalaris arundinacea*), maize (*Zea mays*), willow (*Salix viminalis*), spartina (*Spartina pectinata*), and miscanthus (*Miscanthus × giganteus*) were used as test plants. They were grown on soil artificially spiked with Cu, Ni, and Zn. The experimental design included a control and three increasing doses of metals. Microplots ($1 \text{ m}^2 \times 1$ m deep) were filled with sandy soil (clay—6%, pH 5.5, Corg—0.8%). Metals in the form of sulfates were dissolved in water and applied to the plot using a hand liquid sprayer. During the harvest, samples were collected from aboveground parts, roots, and the soil and then tested for their Cu, Zn, and Ni contents. The metal content of the soil was determined using four tested extractants. It was found that Cu and Ni were accumulated in roots in bigger amounts than Zn. The usefulness of the extractants was evaluated based on the correlation between the content of metals in the soil and the plant ($n = 32$). This study demonstrated that 1 M HCl, aqua regia, and EDTA were more efficient or equally useful for the assessment of the phytoavailability of Cu, Ni, and Zn as $CaCl_2$. Due to the ease of performing

determinations and their low cost, 1 M HCl can be recommended to assess the excess of Cu, Ni, and Zn in the coarse-textured soils.

Introduction

Soil contamination with heavy metals is a major problem in Europe. A study conducted in 2011–2012 by the European Soil Data Centre of the European Commission indicates that heavy metals are one of the main causes of soil contamination in the EU (34.8%) (Panagos et al. 2013).

Accumulation of heavy metals in the soil may become a potential threat to the entire ecosystem. Soil contamination with these elements may negatively affect the plants and, if they enter the food chain, they may pose a threat to human health (Baraud and Leleyter 2012; Fent 2004; Stanisławska-Glubiak et al. 2012).

The availability of metals to plants depends on a number of chemical and biological factors such as pH, organic matter content, or soil texture (Kabala et al. 2011; Kabata-Pendias 2004; McLaughlin et al. 2000; Schramel et al. 2000; Van Raij 1998; Yu et al. 2004). The evaluation of soil contamination involves determining the concentration of heavy metals and comparing the results against the standard criteria. In many countries, this evaluation is carried out on the basis of the so-called total concentration determined by using strong acids such as aqua regia, $HClO_4$, or HNO_3 (ESdat 2015). These procedures, however, are complicated, time consuming, and quite costly (McBride et al. 2011). Moreover, many authors have reported that the total concentration does not reflect the real risk of contamination (McLaughlin et al. 2000;

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McBride et al. 2009; Menzies et al. 2007; Rao et al. 2008). Measuring the availability of heavy metals for plants is essential for the evaluation of the environmental risk associated with their excess in the soil. Many studies conducted worldwide have indicated that weak extraction solutions such as $Ca(NO_3)_2$ (Black et al. 2011), NH_4NO_3 (Pinto et al. 2015), or $CaCl_2$ (McBride et al. 2003; Menzies et al. 2007; Meers et al. 2007; Zhu et al. 2012) are suitable for the assessment of phytoavailability.

In some European countries (Poland, Latvia, Estonia), 1 M HCl is used for the assessment of micronutrient deficiency in the soil for fertilization purposes. Although it is a strong extractant, our preliminary studies have shown that it may also reflect an actual threat of heavy metal pollution on contaminated sites (Stanislawska-Glubiak and Korzeniowska 2010; Stanisławska-Glubiak and Korzeniowska 2014). It concerns especially coarse-textured soils, which constitute a majority of soils in Poland. The procedure of extraction with the use of 1 M HCl is much simpler and more cost-effective compared to the determination of the total concentration. The works of other authors have confirmed its usefulness for the evaluation of soil contamination with heavy metals (Allen 1993; Chowdhury et al. 2010).

The aim of this study was to compare the usefulness of 1 M HCl with aqua regia, EDTA, and $CaCl_2$ for the extraction of phytoavailable forms of Cu, Ni, and Zn from coarse-textured soil spiked with these heavy metals.

Material and methods

Microplot experiments

Testing the usefulness of extractants involved using two microplot experiments with energy crops. The experiment I was conducted in 2007–2008, with three plant species: reed canary grass (*Phalaris arundinacea*), maize (*Zea mays*), and willow (*Salix viminalis*). The experiment II was carried out in 2009, with two species: spartina (*Spartina pectinata*) and miscanthus (*Miscanthus × giganteus*). Both experiments ware performed in four replicates at the Experimental Station Baborowko near Poznan (middle-west Poland).

In the year preceding the planting/seeding plants, concrete-framed microplots (1 m^2 × 1 m deep without bottom) were filled with Haplic Luvisols soil—the most common type of soil in Poland. It was a coarse-textured soil with a low content of clay, low pH, and low content of organic matter (Table 1). In both experiments, I and II, the same soil was used. The soil in microplots was artificially contaminated with Cu, Ni, and Zn in the early autumn in the year before the introduction of tested plants. As soon as the metals were introduced into the soil, the white mustard (*Sinapis alba*) was sown as a transitional plant to obtain a level of contamination as stable as possible. Mustard was harvested in the end of autumn, just before winter. Plant cultivation accelerates the establishment of equilibrium between the soil solution and the solid phase.

In the experiment I, the following doses of metals were applied: 0-the control (no metals), Cu1–50, Cu2–100, Cu3–200, Ni1–40, Ni2–80, Ni3–160, Zn1–200, Zn2–400, and Zn3–800 mg kg^{-1}. In the experiment II, metal doses were increased to evoke a stronger plant reaction: 0, Cu1–100, Cu2–200, Cu3–400, Ni1–60, Ni2–100, Ni3–240, Zn1–300, Zn2–600, and Zn3–1200 mg kg^{-1}. In total, 120 microplots were used in experiment I (3 plants × 10 treatments × 4 replicates) and 80 in experiment II (2 plants × 10 treatments × 4 replicates).

Metals in the form of sulfates were dissolved in water and applied to the microplots using a hand liquid spreader. To thoroughly mix the metals with the soil, they were first introduced into the 15–30-cm soil layer, mixed, and then into the 0–15-cm layer, where they were carefully mixed again.

In both experiments, the plants were planted in the spring, 1 year after the introduction of metals into the soil. In the experiment I, willow was planted on 13 April 2007, reed canary grass on 02 May 2007, and maize on 02 May 2007 and 29 April 2008. In experiment II, spartina and miscanthus were planted on 14 May 2009. All the test plants, apart from reed canary grass, were initially planted in higher density, and after 2 months, plant thinning was performed, leaving 5 plants of willow, 12 of maize, 5 of spartina, and 2 of miscanthus on the microplot. In the case of reed canary grass, 6 g of seeds was sown per plot. In all 3 years of the studies, basic NPK fertilization was applied in the spring at 10: 2: 8 g per plot, respectively. The plants on microplots were hand weeded and watered during the periods of insufficient rainfall.

Table 1 Characteristics of the experimental soil (0–30 cm)

Soil fraction (mm) %			C org %	pH KCl	Concentration mg kg^{-1}					
2.0–0.05	0.05–0.002	< 0.002			P[a]	K[a]	Mg[b]	Cu[c]	Ni[c]	Zn[c]
80	14	6	0.8	5.5	85	116	51	11.7	7.6	39.3

[a] Egner

[b] Schachtschabel

[c] Aqua regia

Table 2　Some parameters of the extraction/digestion methods of metals from soil

Extractant	Soil/solution ratio	Shaking/heating time	Temperature in °C	Approximate cost in %[a]
1 mol HCl	1:10	1 h	20	30
0.02 M EDTA in an ammonium acetate buffer at pH 4.65	1:5	30 min	20	60
0.01 M CaCl$_2$	1:10	2 h	20	20
Aqua regia (HCl/HNO$_3$ = 1/3)	1:10	±2 h	120–200	100

[a] Aqua regia cost was 100%

The yield of the aboveground parts of the test plants was determined during the harvest. In experiment I, willow was collected on 25 October 2007 and 01 October 2008 (branches with leaves) and maize on 3 September 2008 and 6 September 2007 (stems with leaves and cobs). Reed canary grass was collected at the stage of heading, providing only one cut of biomass in the first year and three cuts in the second year of growing, on 25 September 2007 and 23 July, 8 August, and 23 August 2008. In experiment II, single cuts of spartina and miscanthus were collected during the heading phase on 20 October 2009 and 27 October 2009, respectively.

The samples of aboveground parts of plants, including leaves of willow, stems with leaves of maize, and whole

Table 3　Copper concentration in the soil determined by tested methods in mg kg^{-1}

Experiment/year	Plant	Treatment[a]	pH	Aqua regia	HCl	EDTA	CaCl$_2$
I/2007	Reed canary grass	0	5.2	8.9	5.2	2.8	0.1
		Cu1	5.2	78.9	73.1	57.7	0.7
		Cu2	5.2	168.0	146.0	122.0	2.0
		Cu3	4.9	224.0	212.0	177.0	4.6
	Maize	0	5.3	8.1	5.4	2.8	0.1
		Cu1	5.3	93.4	85.1	68.2	0.7
		Cu2	5.2	124.0	115.0	93.6	1.3
		Cu3	5.0	265.0	239.0	201.0	4.7
	Willow	0	5.3	9.2	5.9	3.1	0.1
		Cu1	5.2	60.4	54.3	44.7	0.7
		Cu2	5.2	191.0	172.0	148.0	2.3
		Cu3	5.0	300.0	280.0	238.0	8.8
I/2008	Reed canary grass	0	5.1	8.4	4.6	2.9	0.1
		Cu1	5.0	106.0	94.9	76.2	1.0
		Cu2	4.9	183.0	166.0	139.0	1.5
		Cu3	4.7	238.0	213.0	174.0	1.6
	Maize	0	5.1	8.7	4.9	3.3	0.1
		Cu1	5.1	70.3	61.9	49.9	0.4
		Cu2	5.1	108.0	103.0	82.3	0.7
		Cu3	4.8	248.0	243.0	191.0	5.6
	Willow	0	5.1	6.8	3.6	2.7	0.2
		Cu1	5.1	69.7	61.1	50.2	0.8
		Cu2	5.0	134.0	118.0	111.0	1.6
		Cu3	4.8	207.0	176.0	156.0	5.7
II/2009	Spartina	0	5.5	10.5	7.2	3.8	0.1
		Cu1	5.5	256.0	239.0	204.0	2.5
		Cu2	5.3	447.0	419.0	368.0	11.0
		Cu3	5.1	668.0	621.0	540.0	37.4
	Miscanthus	0	5.4	9.6	5.7	3.6	0.1
		Cu1	5.2	219.0	231.0	181.0	5.6
		Cu2	5.0	385.0	369.0	324.0	11.6
		Cu3	4.9	599.0	532.0	483.0	26.3

[a] Experiment I: Cu1–50, Cu2–100, Cu3–200 mg kg^{-1}. Experiment II: Cu1–100, Cu2–200, Cu3–400 mg kg^{-1}

aboveground parts of grasses, were collected always during harvest. The samples of maize roots were also collected during the harvest. Due to the lack of the possibility of obtaining samples of the roots of perennial crops in the first year of growing, the roots of the plants which had been removed during the thinning were used (2007 and 2009). Reed canary grass was the exception, as its roots were collected in the first growing season by removing two rows of plants per plot during the harvest. In the second growing season (2008), root samples of this plant were collected during the final harvest. All plant samples (especially roots) were carefully washed, dried at 60 °C, and finely ground. Soil samples were collected every year in the autumn after the harvest. Air-dry samples were passed through a sieve with the mesh size less than 2 mm.

Chemical\analyses

All chemical analyses were performed in the Central Laboratory of the Institute of Soil Science and Plant Cultivation in Pulawy, certified by the Polish Centre of Accreditation (certificate no. AB 339) according to PN-EN ISO/IEC 175 17025.

Total organic carbon in soil (TOC) was determined by Tiurin method using potassium dichromate (PN-ISO14235: 2003), pH was established potentiometrically in 1 mol KCl dm^{-3} (ISO10390: 2005), P and K were determined using Enger-Riehm method (Polish standards no. PN-R-04023:1996 and PN-R-04022:1996 adequately), Mg by Schachtschabel method (PN-R-04020:1994), and texture was evaluated by the aerometric method (PN-R-04033: 1998).

Table 4 Nickel concentration in the soil determined by tested methods in mg kg^{-1}

Experiment/year	Plant	Treatment[a]	pH	Aqua regia	HCl	EDTA	CaCl$_2$
I/2007	Reed canary grass	0	5.2	7.1	1.3	0.9	0.1
		Ni1	5.3	45.5	37.1	30.0	7.0
		Ni2	5.2	86.6	74.7	61.5	20.3
		Ni3	5.2	181.0	161.0	138.0	54.5
	Maize	0	5.3	8.3	1.6	1.1	0.2
		Ni1	5.2	67.5	56.0	43.4	11.7
		Ni2	5.1	74.9	62.8	55.2	20.6
		Ni3	5.1	187.0	164.0	142.0	58.0
	Willow	0	5.3	7.5	1.9	1.2	0.2
		Ni1	5.2	50.1	41.8	33.3	10.9
		Ni2	5.1	100.0	87.0	78.8	27.1
		Ni3	5.1	151.0	139.0	124.0	60.4
I/2008	Reed canary grass	0	5.1	6.0	1.3	0.8	0.1
		Ni1	5.1	61.1	49.4	39.9	9.5
		Ni2	5.0	136.0	110.0	91.2	24.2
		Ni3	5.0	192.0	160.0	134.0	41.5
	Maize	0	5.1	6.5	2.0	1.2	0.1
		Ni1	5.0	48.9	35.7	28.8	5.3
		Ni2	4.9	104.0	79.7	70.3	25.1
		Ni3	4.9	180.0	151.0	134.0	56.4
	Willow	0	5.1	6.1	2.1	1.6	0.4
		Ni1	5.1	49.6	39.6	36.5	15.2
		Ni2	5.0	80.5	65.5	58.4	23.0
		Ni3	5.0	149.0	129.0	112.0	59.3
II/2009	Spartina	0	5.5	7.4	2.5	1.4	0.2
		Ni1	5.5	100.0	84.3	71.1	20.5
		Ni2	5.4	163.0	148.0	130.0	50.6
		Ni3	5.1	256.0	245.0	221.0	111.0
	Miscanthus	0	5.4	7.0	3.0	1.9	0.4
		Ni1	5.3	106.0	91.8	78.5	30.4
		Ni2	5.3	147.0	131.0	114.0	43.1
		Ni3	5.2	291.0	258.0	224.0	111.0

[a] Experiment I: Ni1–40, Ni2–80, Ni3–160 mg kg^{-1}. Experiment II: Ni1–60, Ni2–100, Ni3–240 mg kg^{-1}

The concentrations of Cu, Ni, and Zn in the soil were determined using four different methods: (1) aqua regia—hydrochloric acid + nitric acid as 3 + 1 (PN-ISO 11466:2002), (2) 1 M HCl (Gembarzewski and Korzeniowska 1990), (3) EDTA (Lakanen and Ervio 1971), and (4) CaCl$_2$ (Houba et al. 2000) (Table 2). After the extraction, metals were determined using FAAS method.

Heavy metals in plant tissue were determined by the FAAS method, having first dry ashed the material in a muffle furnace and digested it with 20% nitric acid (PN-R-04014: 1991). A standard reference material IPE 952 (International Plant-Analytical Exchange) from Wageningen (Netherlands) was used for quality control purposes.

The concentration of metals in plant tissues was determined in the samples from each replication and in the soil from the samples obtained by combining all four replications together.

Statistical analyses

For metal concentration in plant tissue, one-way ANOVAs were conducted, followed by the Tukey test ($P < 0.05$). Linear correlation and regression analyses were calculated for 3 years and five plants together ($n = 32$) using the Statgraphics Plus 5.1 software (Statistical Graphics Corporation). Regression analyses were performed to relate the concentration of each metal in the plant tissues to the metal concentration extracted with each tested method from the soil.

Table 5 Zinc concentration in the soil determined by tested methods in mg kg^{-1}

Experiment/year	Plant	Treatment[a]	pH	Aqua regia	HCl	EDTA	CaCl$_2$
I/2007	Reed canary grass	0	5.2	40.4	15.1	9.2	1.2
		Zn1	5.2	272.0	232.0	205.0	59.4
		Zn2	5.2	541.0	411.0	353.0	135.0
		Zn3	4.8	705.0	559.0	485.0	232.0
	Maize	0	5.3	36.0	15.1	9.4	1.3
		Zn1	5.3	283.0	247.0	212.0	65.2
		Zn2	5.1	604.0	481.0	427.0	175.0
		Zn3	5.0	571.0	459.0	404.0	171.0
	Willow	0	5.3	38.0	15.2	9.2	1.4
		Zn1	5.2	279.0	241.0	222.0	87.5
		Zn2	5.1	485.0	382.0	345.0	165.0
		Zn3	4.9	664.0	539.0	485.0	234.0
I/2008	Reed canary grass	0	5.1	35.1	13.80	10.0	0.9
		Zn1	5.0	254.0	194.0	174.00	42.8
		Zn2	4.9	462.0	348.0	349.00	120.0
		Zn3	4.7	520.0	401.0	427.00	168.0
	Maize	0	5.1	37.3	14.8	10.0	0.7
		Zn1	5.0	304.0	238.0	219.0	62.7
		Zn2	4.7	376.0	303.0	293.0	118.0
		Zn3	4.6	512.0	420.0	399.0	168.0
	Willow	0	5.1	29.3	9.5	8.2	1.7
		Zn1	5.0	272.0	208.0	196.0	79.6
		Zn2	4.8	362.0	271.0	255.0	145.0
		Zn3	4.6	461.0	377.0	365.0	204.0
II/2009	Spartina	0	5.5	44.2	20.3	12.7	1.3
		Zn1	5.4	472.0	437.0	378.0	139.0
		Zn2	5.4	734.0	652.0	597.0	221.0
		Zn3	5.1	732.0	682.0	590.0	274.0
	Miscanthus	0	5.4	33.5	13.8	7.3	1.7
		Zn1	5.3	417.0	386.0	331.0	134.0
		Zn2	4.9	567.0	527.0	496.0	242.0
		Zn3	5.0	648.0	582.0	529.0	268.0

[a] Experiment I: Zn1–200, Zn2–400, Zn3–800 mg kg^{-1}. Experiment II: Zn1–300, Zn2–600, Zn3–1200 mg kg^{-1}
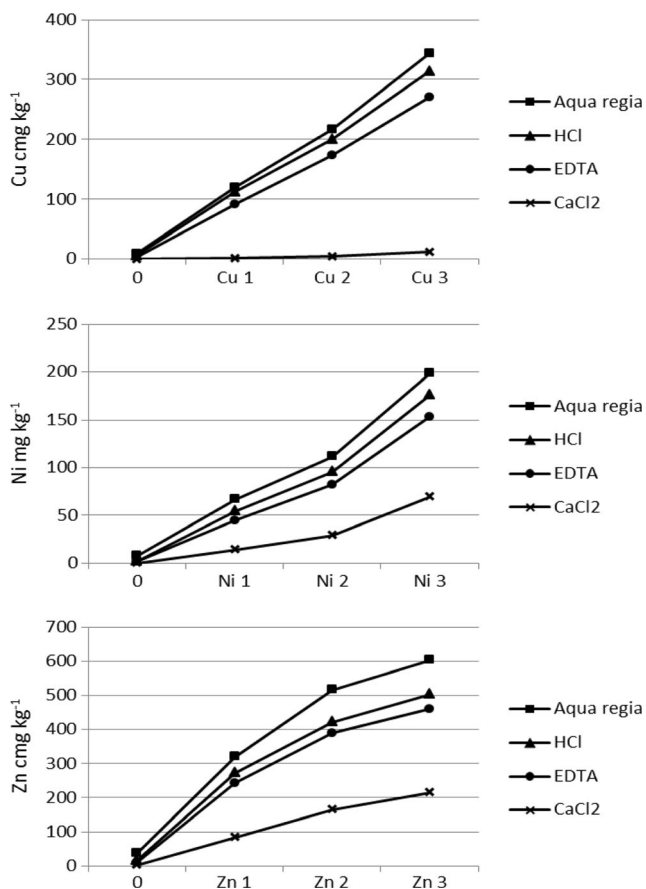
Fig. 1 Metal concentration in the soil depending on the dose and extractant—means for 3 years and five plants. Description of treatments 1, 2, and 3—see notes under Tables 3, 4, and 5

Table 7 Correlation (r values) between metal levels extracted by tested extractants (calculated for 3 years and 5 plants, $n = 32$)

	HCl	EDTA	CaCl$_2$
Cu			
Aqua regia	0.999***	0.992***	0.905***
HCl	–	0.999***	0.901***
EDTA	–	–	0.908***
Ni			
Aqua regia	0.998***	0.995***	0.961***
HCl	–	0.999***	0.969***
EDTA	–	–	0.975***
Zn			
Aqua regia	0.992***	0.991***	0.963***
HCl	–	0.996***	0.968***
EDTA	–	–	0.971***

***P value ≤ 0.0001

(Table 3). The concentration of Ni for aqua regia ranged between 6.0–291.0, for 1 M HCl 1.3–258.0, for EDTA 0.8–224.0, and for CaCl$_2$ 0.1–111.0 mg kg^{-1} (Table 4). Analogically, the concentrations of Zn were 29.3–732.0, 9.5–682.0, 7.3–590.0, and 0.7–274.0 mg kg^{-1} (Table 5).

The soil in experiment II (2009) showed higher metal contamination than the soil in experiment I (2007–2008). In the experiment I, the total metal concentrations in the soil in the treatments with the highest metal doses exceeded the Polish standards 1.5–2.5 times, while in the experiment II, by approximately 3.0–4.5 times (Regulation of the Minister of the Environment 2002).

1 M HCl had the highest, and CaCl$_2$ the lowest extraction strength among the three tested extractants (Fig. 1). 1 M HCl solution extracted 82–92%, while EDTA 74–78% of metals from their total pool determined in aqua regia. CaCl$_2$ extracted 29–32% of Ni and Zn, and only 3% of Cu compared with aqua regia (Table 6).

The studies of other authors have confirmed a high extraction strength of 1 M HCl (Bakircioglu et al. 2011, Gediga et al. 2015; Kantek and Korzeniowska 2013; Korzeniowska and Stanisławska-Glubiak 2015a; Stanislawska-Glubiak and Korzeniowska 2010).

Although the four tested methods extracted different amounts of Ni and Zn from the soil, the correlation between all of them for Ni and Zn was very high and exceeded 0.96

The r^2 values of the regression were used to assess these methods.

Results and discussion

The concentration of metals in the soil

The concentrations of metals in the soil increased with the increase of their doses. The so-called total concentration of Cu in the soil determined in aqua regia ranged from 6.8 to 668.0 mg kg^{-1}, extracted with 1 M HCl from 3.6 to 621.0, EDTA from 2.7 to 540.0, and CaCl$_2$ from 0.1 to 37.4 mg kg^{-1}

Table 6 Metal concentration in soil in mg kg^{-1} (mean for all the plants and doses)

Metal	Aqua regia (Ar)	HCl	HCl/Ar	EDTA	EDTA/Ar	CaCl$_2$	CaCl$_2$/Ar
Cu	172.3	158.3	0.92	134.5	0.78	4.4	0.03
Ni	95.7	81.8	0.85	70.6	0.74	28.1	0.29
Zn	368.5	303.0	0.82	275.4	0.75	116.3	0.32

($P \leq 0.0001$) (Table 7). It was also valid for $CaCl_2$ which extracted much less Ni and Zn than the other solutions. However, in the case of Cu, the correlation between $CaCl_2$ and the other extractants was much lower and did not exceed 0.92 ($P \leq 0.0001$).

It should be noted that the increase of the extracted metals from the soil was due not only to their increasing dose but also to the decreasing soil pH due to the use of sulfates (max. 0.4 unit) (Tables 3, 4, and 5). However, it did not interfere to assess the usefulness of the tested extractants. A good extractant should provide a reliable assessment of the phytoavailability of metals irrespective of soil acidification.

The concentration of metals in plants

The concentration of Cu, Ni, and Zn in the test plants increased systematically with increasing the doses of these metals (Table 8). The concentration of metals in the aboveground parts of the test plants ranged between 2.6–11.4 for Cu, 0.4–5.1 for Ni, and 3–1086 mg kg^{-1} for Zn. The concentration in roots was higher than in the aboveground parts and varied between 3.9–70.7, 1.3–181.0, and 25–1611 mg kg^{-1}, respectively.

The analysis of the data from Table 8 showed that miscanthus had a higher metal concentration in both

Table 8 Metal concentration in plants in mg kg^{-1}

Experiment/year	Plant	Treatment[a]	Cu		Ni		Zn	
			Aboveground parts	Roots	Aboveground parts	Roots	Aboveground parts	Roots
I/2007	Reed canary grass	0	3.3 b	8.8 d	1.1 d	8.2 d	70 d	58 d
		1	5.0 a	24.1 c	10.1 c	42.2 c	383 c	354 c
		2	4.1 ab	43.7 b	14.0 b	67.5 b	758 b	660 b
		3	5.3 a	57.9 a	24.9 a	133.0 a	987 a	1494 a
	Maize	0	3.1 c	6.9 c	1.7 d	1.6 d	13 d	25 d
		1	5.1 b	32.4 b	2.7 c	60.4 c	294 c	406 c
		2	7.0 a	39.1 b	5.4 b	91.4 b	419 b	789 b
		3	6.3 ab	70.7 a	10.4 a	169.0 a	622 a	1502 a
	Willow	0	2.8 b	7.5 d	0.7 d	2.6 c	52 d	62 d
		1	3.5 ab	20.0 c	3.5 c	4.9 c	376 c	120 c
		2	3.5 ab	24.7 b	5.7 b	15.0 b	429 b	178 b
		3	4.0 a	38.7 a	9.3 a	46.6 a	591 a	279 a
I/2008	Reed canary grass	0	4.8 b	7.3 d	0.9 d	1.6 c	43 d	151 d
		1	5.7 ab	19.0 c	13.7 c	50.9 b	237 c	682 c
		2	6.1 a	33.9 b	21.2 b	65.5 b	366 b	882 b
		3	5.6 ab	47.5 a	31.6 a	133.0 a	776 a	1611 a
	Maize	0	3.8 c	5.7 c	0.9 b	2.1 d	27 d	53 d
		1	4.2 c	17.2 b	1.1 b	29.5 c	314 c	310 c
		2	6.1 b	18.7 b	2.6 ab	80.4 b	409 b	457 b
		3	7.8 a	49.5 a	10.7 a	132.0 a	588 a	807 a
	Willow	0	2.6 ab	3.9 c	1.1 d	1.3 c	84 b	64 b
		1	2.5 b	6.0 b	11.2 c	10.1 b	456 a	256 a
		2	3.1 ab	18.1 a	14.8 b	11.6 b	459 a	203 a
		3	3.5 a	18.1 a	20.8 a	26.6 a	458 a	280 a
II/2009	Spartina	0	3.5 c	5.2 d	0.4 d	5.7 d	20 d	51 d
		1	4.8 b	21.1 c	5.5 c	60.9 c	172 c	378 c
		2	5.0 b	26.2 b	11.2 b	92.0 b	338 b	501 b
		3	6.1 a	65.4 a	39.4 a	181.0 a	911 a	1031 a
	Miscanthus	0	2.8 d	4.8 c	0.5 d	3.9 d	32 d	89 d
		1	5.2 c	34.4 b	7.6 c	68.6 c	366 c	783 c
		2	7.6 b	50.9 a	9.4 b	139.0 b	787 b	1230 b
		3	11.4 a	48.3 a	54.1 a	165.0 a	1086 a	1441 a

[a] The dose of Cu or Ni or Zn, respectively (see Tables 3, 4, and 5). The same letters for each plant-metal combination indicate the lack of significant differences according to Tukey's test ($P < 0.05$)

aboveground parts and roots than the other plants, while willow had a significantly lower metal concentration in the roots. It should be noted, however, that in the experiment II, soil contamination with metals was higher compared to the experiment I.

It was found that Cu and Ni accumulated much more in the roots than in the aboveground parts. It was indicated by roots/aboveground parts ratio, which ranged from 5.1 to 6.1 (mean, 5.6) for Cu and 1.8–16.0 (mean, 6.7) for Ni. In the case of Zn, the ratio was significantly lower and ranged from 0.5 to1.6 (mean, 1.3), which means that this metal was accumulated to a similar degree in roots and aboveground parts (Table 9). It is widely known that plants accumulate Zn mainly in their aboveground parts, while Cu in roots (Menzies et al. 2007, Stanislawska-Glubiak and Korzeniowska 2010; Yoon et al. 2006). In the case of Ni, it is not so clear, and different authors have presented different results. Menzies et al. (2007) argued that Ni, similarly to Cu, was mainly accumulated in roots, while Korzeniowska and Stanisławska-Glubiak (2015b) have shown that, being mainly accumulated in aboveground parts, it was more similar to Zn.

Relationship between soil and plant tissue metal levels

The comparison of the tested extractants was carried out on the basis of linear regression describing the relationship between the metal concentration in plant tissues and in the soil. The analysis of the correlation coefficients (r^2) allowed for identifying the most useful extractants. This method has been used by many authors to assess the effectiveness of different soil tests (Baraud and Leleyter 2012; Chojnacka et al. 2005; Matula 2009; McBride et al. 2011; Meers et al. 2007; Pinto et al. 2015).

In our study, the concentrations of metals in the aboveground parts and roots were correlated with the concentrations of metals in the soil determined using aqua regia, 1 M HCl, EDTA, and CaCl$_2$ (Table 10). The calculations were performed using both aboveground parts and roots due to differences among tested metals in their transfer from belowground to aboveground parts of plants.

Table 9 Roots/aboveground parts ratio (mean for 2 years and all doses ± SE)

Plant	Cu	Ni	Zn
Reed canary grass	6.1 ± 1.2	4.3 ± 0.6	1.6 ± 0.3
Maize	5.5 ± 1.1	16.0 ± 3.8	1.6 ± 0.2
Willow	5.4 ± 0.9	1.8 ± 0.5	0.5 ± 0.1
Spartina[a]	6.1 ± 1.9	6.0 ± 1.9	1.4 ± 0.3
Miscanthus[a]	5.1 ± 1.1	5.3 ± 2.4	1.6 ± 0.3
Mean	5.6 ± 0.2	6.7 ± 2.4	1.3 ± 0.2

[a] Mean only for 1 year and all doses

Table 10 Linear correlation coefficients (r^2 values) for metal concentrations in plants regressed against soil metal concentrations determined by various extractants (calculated for 3 years and 5 plants, $n = 32$)

Part of plant	Total	HCl	EDTA	CaCl$_2$
Cu				
Cu-aboveground parts	44.77***	44.62***	44.10***	31.26**
Cu-roots	55.89***	56.70***	53.95***	34.48**
Ni				
Ni-aboveground parts	70.49***	70.49***	69.52***	69.37***
Ni-roots	78.40***	77.95***	77.02***	66.05***
Zn				
Zn-aboveground parts	71.05***	69.02***	69.91***	75.65***
Zn-roots	51.75***	50.23***	52.22***	48.25***

≤0.001; *≤0.0001

It is commonly known that there is a strong barrier of the Cu transfer from the roots to the shoots, much stronger than for other metals (McBride 2001; McBride et al. 2009). Therefore, the assessment of Cu phytoavailability requires testing the roots, not the shoots. On the contrary, in the case of Zn, it is necessary to use aboveground parts (Menzies et al. 2007). This has been confirmed by our study. In the case of Cu, higher correlation coefficients were obtained for the roots, while in the case of Zn—for aboveground parts. For Ni and Cu, higher coefficients were obtained for roots. Therefore, a further evaluation of extractants for Cu and Ni was carried out on the basis of the correlation coefficients for the roots, while for Zn—for aboveground parts.

Generally, the tested extractants estimated the phytoavailability of Ni and Zn more efficiently than the phytoavailability of Cu. Correlation coefficients r^2 reached the level from 66.05 to 77.95 for Ni, 69.02–75.65 for Zn, and only 34.08–56.70 for Cu (Table 10).

For Cu and Ni (assessed on the basis of the roots), more aggressive chemically extractants proved to be more useful, while weak CaCl$_2$ was clearly worse. In the case of Zn (assessed on the basis of the aboveground parts), CaCl$_2$ solution turned out to be the most effective. For example, Figs. 2,

Root Cu = 12.7178 + 0.0925109*Soil Cu(HCl), R2=56.70
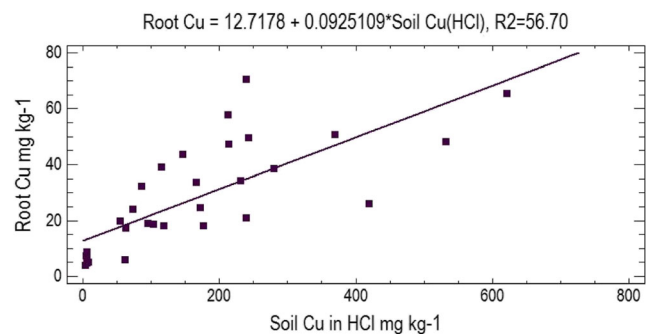
Fig. 2 Linear regression between Cu concentration in the roots and Cu concentration in the soil determined in 1 mol HCl
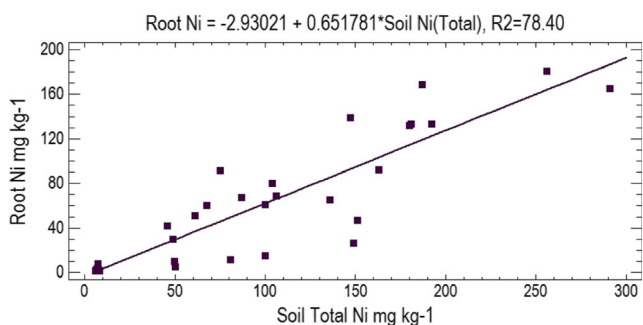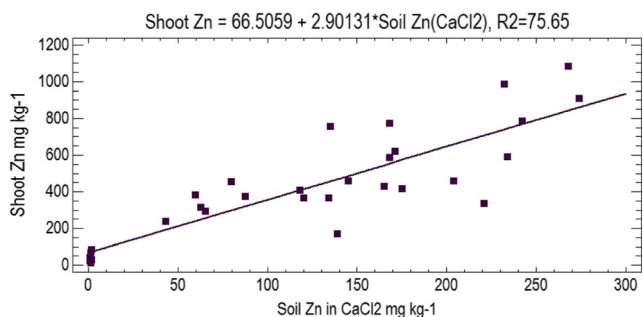
Fig. 3 Linear regression between Ni concentration in the roots and the total Ni concentration in the soil determined in aqua regia

3, and 4 show a graphical presentation of the relationships between plant and soil, for which the highest correlation coefficients were obtained: for Cu-roots and 1 M HCl, for Ni-roots and aqua regia, and for Zn-aboveground parts and CaCl$_2$. It is worth noting that in the case of Zn, the differences between the tested extractants were lower than for Cu and Ni, and the correlation coefficients for all the tested methods were about 70%. It proves the usefulness of CaCl$_2$ and stronger extractants for the assessment of phytoavailable Zn forms in the soil.

The results of the study contradict the results of many other authors who show greater suitability of the weaker extractants than stronger ones to evaluate the contents of phytoavailable metal forms in the soil (Black et al. 2011; McBride et al. 2003; McLaughlin et al. 2000; Menzies et al. 2007; Meers et al. 2007; Zhu et al. 2012). Our studies indicated that aggressive extractants were more effective than CaCl$_2$ solution due to the use of coarse-textured soils with little ability of metal binding (Table 1). The study indicates that on this type of soils, the strength of an extractant is not as important as on the soils with a higher metal binding capacity. McBride et al. (2003) found that on fine-textured soils, with near-natural pH and high contents of clay or organic matter, there was a clear difference between weak and strong extractants, in favor of the weaker ones. The soil used in our studies is characteristic for Poland. Coarse-textured soils with low contents of clay and organic matter, with a relatively low ability of heavy metal binding,

constitute 50% of the Polish soils (Bieganowski et al. 2013; Niewiadomski and Toloczko 2014).

Conclusions

The 1 M HCl had the highest extraction strength of the three tested extractants, followed EDTA and CaCl$_2$. Whereas HCl and EDTA extracted about 70–90% of metals, CaCl$_2$ extracted only 2–30% of their total pool determined in aqua regia. The studies conducted on coarse-textured soils with a low pH and low contents of clay and organic matter have shown that all three stronger extractants were better or equally useful for the evaluation of phytoavailable forms of Cu, Ni, and Zn as CaCl$_2$. In some European countries, 1 M HCl is used to estimate the content of microelements in the soil for the purpose of fertilizer advisory services. In the same time, the assessment of the excess of heavy metals is carried out using aqua regia. It seems that due to the ease of performing determinations and their low cost, 1 M HCl can be recommended to assess the excess of Cu, Ni, and Zn in acid coarse-textured soils.

Fig. 4 Linear regression between Zn concentration in aboveground parts and Zn concentration in the soil determined in CaCl$_2$

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