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ULTRASONIC CLEANING OF PLANT ROOTS IN THEIR PREPARATION FOR ANALYSIS ON HEAVY METALS

A b s t r a c t

The paper focuses on ultrasonic method used for cleaning of root material collected from a pot experiment. In the experiment, red fescue was grown on differently contaminated soils amended with organic additions. Determined were total concentrations of Cu and Zn in root material prepared for analysis by hand washing and the use of ultrasonic bath. The results indicate significant differences between the two methods.

Key words: ultrasonic, contaminated soils, heavy metals, pot experiment, roots

INTRODUCTION

The pot experiments using soils contaminated with heavy metals are widely used in the agricultural and earth sciences. This type of experiment makes possible to determine the effect of defined soil pollution on plants in strictly controlled conditions. Pot experiments, in contrast to field experiments, are easy for carrying out and enable thorough sampling of plant material in a laboratory. Pot experiments, compared to the field ones, facilitate statistical analysis of results and are much easier for drawing conclusions. Changing conditions of the experiments, such as soil moisture, sun exposure and temperature, are the most important factors that in the field experiment can cause uncontrolled variability in results [Petersen 1994]. Therefore, in contrast to the field ones, pot experiments may be performed in one growing season and do not need to be repeated in the next seasons. Additional advantages of pot experiments are: low cost of experiment the ability to analyze the various factors affecting the vegetation, the possibility of utilization of soil used in the experiment [Żurbicki 1975].

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In spite of the fact that plant material produced in the pot experiments may be easily acquired, there are still some problems with collecting the underground parts of plants for analysis without their contamination by soil particles. The problem becomes particularly aggravating in the case of obtaining fine roots (eg. fine roots of grasses) wherein soil particles are bound to the surface of epidermis. Manual cleaning of root material in this case is not a sufficient method to obtain uncontaminated analytical material for further analysis. Therefore, authors of this study examined the effects of ultrasonic methods used for plant roots cleaning.

Ultrasonic cleaning methods are commonly used in dental and technical sciences. Many studies indicate that the use of ultrasonics does not destroy or alter the properties of materials treated to cleaning (eg. sensitive membranes, metallic surfaces or ceramic materials). Additional advantage of ultrasonic cleaning is possibility to remove contaminants consist of fine materials [Lamminen et al. 2004, Muthukumarana et al. 2004, Weller et al. 1980].

This article presents a comparison of heavy metal concentrations in plant roots collected from the pot experiment, and cleaned by using the traditional method (hand washing) and ultrasonic method (in ultrasonic bath).

MATERIAL AND METHODS

The pot experiment was carried out in 2014. Three various kinds of soils, differently contaminated with heavy metals, were put into the pots (1 kg per pot) and amended with additives modifying solubility of heavy metals, i.e. ground lignite ("brown coal": 50 g per pot), compost (50 g in pot), and beech litter (40 g in pot). Tab. 1 presents the basic properties of soils and additives.

Tab. 1. Soil and additives basic properties

Symbol	Description	Texture	Fraction < 0,002 mm	pH in KCl	Organic carbon content	Cu concentration	Zn concentration
			%		%	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$
Soil No. 1	Forest soils	SiL		7.2	0.9	301	79
Soil No. 2	Agricultural soils			7.3	1.0	281	85
Soil No. 3				7.5	0.8	275	110
BC	Brown coal	-		4.5		-	
CO	Compost			6.7			
BL	Beech liter			5.4			

Soil material was collected from the area adjacent to the Copper Smelter Legnica, from the surface soil layer (0-30 cm). Soil material No 1 was collected

from forested stand, and two other soil materials (No 2 and 3) - from agricultural areas (two sites). The soil was sieved and then dried. Dried soil material was used in the pot experiment. Soils were amended with organic additives modifying metals solubility. Soil material was supplemented with water to reach water field capacity. After two weeks red fescue was sown (rate of seeding – 30 g per 1 m^2). Pot experiment was carried out 20 weeks. At this time the grass was mown three times. After 20 weeks the roots were collected. The experiment was conducted in triplicate. The experiment lasted six weeks.

Method of collecting root material

A lump of soil with grass roots was pulled out of the pot. The protruding above-ground parts of plants were cut at soil level. Then, the block of soil was gently hand-patted, leaving the roots. Root mass was carefully triturated a mortar with pestle, to get rid of the remnants of dry soil. Material prepared in this way was thereafter divided into two parts and prepared to further cleaning in two ways. Photos 1-4 represent the stages of roots preparation for the chemical analysis.

Phot. 1. Block of soil with roots

Phot. 2. Roots mixed with soil clods ground with pestle in a mortar

Phot. 3. Roots separated with pestle in mortar

Phot. 4. Roots prepared for further cleaning

Methods of roots cleaning

Two methods were used for cleaning the roots, i.e. 1) hand washing and shaking with distilled water, and 2) ultrasonic cleaning. Table 2 presents the stages of roots cleaning in each of two methods.

Tab. 1. Methods of roots cleaning and preparation for chemical analysis

Traditional method (1)	Method with ultrasonic cleaning (2)
- Hand washing in water; - Shaking with distilled water (1 h) - Shaking with distilled water (1h) - Hand washing in distilled water - **Drying at 105 deg., and wet digestion**	- Hand washing in water; - Shaking with distilled water (1 h) - Shaking with distilled water (1h) - Hand washing in distilled water - Cleaning in ultrasonic bath (5 min, 50 mHz) - Hand washing in distilled water - Cleaning in ultrasonic bath (5 min, 50 mHz) - **Drying at 105 deg., and wet digestion**

Roots material after cleaning was dried in 105 deg. and wet digested in concentrated perchloric acid. Copper and zinc concentrations were determined in samples by FAAS method [Ostrowska et al. 1991].

RESULTS AND DISCUSSION

The concentration of heavy metals in shoots oscillated at the level de-scribed widely in literature [Chen 2004, Beesly 2014, Takács 2001]. The concentrations of copper and zinc, in most cases were lower in the samples subjected to ultrasonic cleaning. These differences ranged from 1% to 57% (in the case of copper) and from 1% to 21% (in the case of zinc). Only a few samples cleaned in ultrasonic bath indicated higher concentrations of metals than the samples without ultrasonic cleaning (and the differences ranged from 1% to 12%). Fig. 1 and 2 present copper and zinc concentrations in root samples.

Higher concentrations of copper in the samples after ultrasonic cleaning were found in the case of soils No 1, i.e. that with higher content of clay fraction. It is worth noticing that this soil contained lower concentrations of Cu and Zn than the concentrations of those metals in the samples of grass roots. Probably insufficient cleaning of roots without ultrasonic assistance allowed the fine soil particles stick to the surface on the roots. The remnants of fine soil particles caused a “dilution effect” to the metal concentrations in root samples which

resulted in lowered concentrations of heavy metals measured after root digestion.

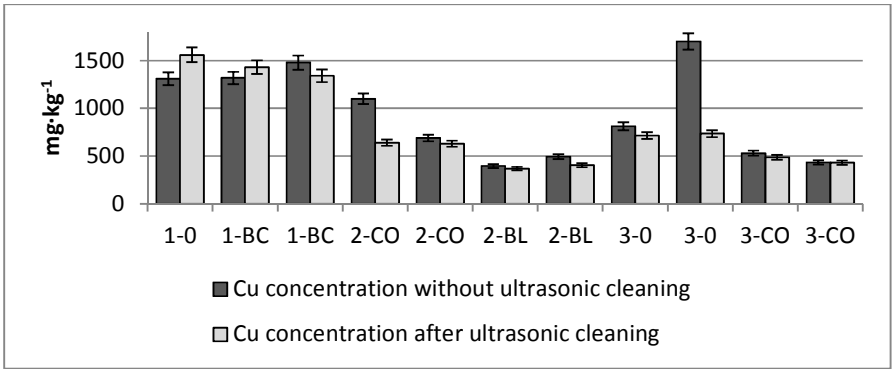

Fig. 1. Copper concentrations in variously cleaned root samples

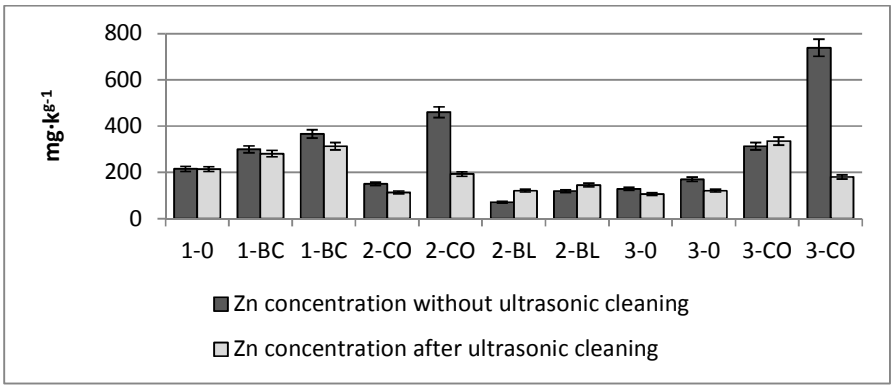

Fig. 2. Zinc concentrations in variously cleaned root samples

Statistical correlation analysis was performed to check whether the differences between metal concentrations in variously cleaned plant roots depend on total concentrations of metals in the samples. Fig. 3 and 4 present related scatterplots. Pearson's correlation coefficient for Cu is -0,428 and for Zn is -0,025.

Statistical analysis indicated significant correlation between the concentrations of copper in the root samples and the differences between analytical results obtained for variously cleaned material (the higher content of copper in the roots, the lower difference between the results obtained with two cleaning methods). In the case of zinc no such correlation was observed. However the

results indicate a significant influence of the ultrasonic cleaning method on final results of the analysis. It is also worth noticing that the differences between replicates of the same samples are lower after ultrasonic cleaning method compare to the first method.

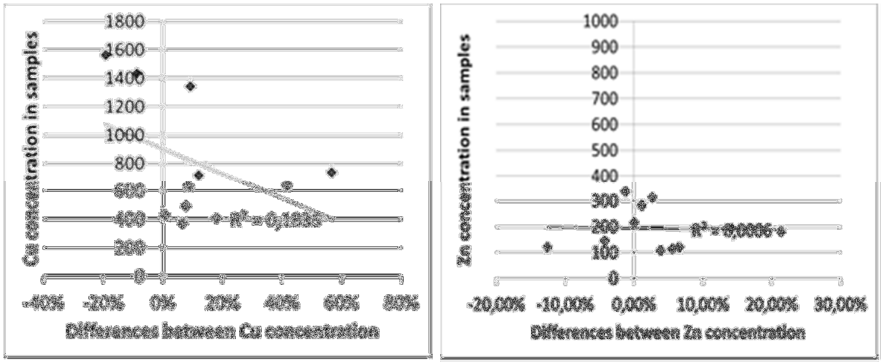

Fig 3 and 4. Scatterplot for heavy metals concentrations and differences between concentrations in samples

CONCLUSIONS

- The use of ultrasonic method for cleaning the root material of plants grown in contaminated soils significantly affects the results of Cu and Zn analyses. In most cases the use of ultrasonic method caused the reduction in the concentration of metal in roots.
- Differences between replicates of the same samples are lower after ultrasonic cleaning method compare to the method without using of ultrasonic cleaning.
- The results confirm the suitability of ultrasonic method for cleaning plant roots and clearly indicate more efficient removal of soil particles from the surface of root epidermis.

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ZASTOSOWANIE METODY OCZYSZCZANIA ULTRADŹWIĘKAMI W ANALIZIE KONCENTRACJI METALI CIĘŻKICH W MATERIALE KORZENIOWEM POBRANYM Z DOŚWIADCZENIA WAZONOWEGO

S t r e s z c z e n i e

Przedstawiono wyniki zastosowania metody ultradźwiękowego czyszczenia materiału korzeniowego pobranego z doświadczenia wazonowego. W doświadczeniu wykorzystano różnie zanieczyszczone gleby z dodatkami organicznymi a następnie wysiano kostrzewę czerwoną. Oznaczono całkowite zawartości Cu i Zn w wybranych próbkach korzeni kostrzewy po

ich oczyszczaniu ręcznym oraz po zastosowaniu oczyszczania metodą ultradźwiękową. Wyniki wskazują na znaczne różnice pomiędzy zastosowanymi metodami.

Słowa kluczowe: ultradźwięki, gleby zanieczyszczone, metale ciężkie, doświadczenie wazonowe, korzenie