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Letter to Editor

Effects of Thiuram on Uptake of Copper, Zinc and Manganese by *Valeriana Officinalis* L.

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Abstract

This work is a continuation of previous studies on the effects of fungicides on the uptake of trace elements by herbs [1, 2]. Copper, zinc and manganese content was determined in *Valeriana officinalis* grown in the soil free from additives and in the soil sprayed with thiuram. The content of bioavailable forms of these elements was established in pure soil and in the soil with fungicide addition. The introductory part of the work involves the analysis of the soil used for study (determination of organic matter content, pH, mechanical analysis, total content of Cu, Zn and Mn). The analysis showed that the soil used for the study was not contaminated with any of the examined elements.

Keywords: thiuram, *Valeriana officinalis*, herbs, Cu, Zn and Mn

Introduction

The evaluation of heavy metals content in different medicinal plants are important because their excessive accumulation in plant material may weaken their therapeutic effect or even become a source of additional chronic exposure of a patient's organism to heavy metals. A turn to natural medicine is observed nowadays. *Valeriana officinalis* L. is a commonly used medicinal plant. The herbal component of the medicines is dried roots and rhizomes containing volatile oil (0.5–2%), valepotriates (iridoid compounds) organic acids, alkaloids, tannins and mineral salts. *Valeriana officinalis* root extract has a tranquilizing effect on whole nervous system, is a relaxing agent and decreases the tension of smooth muscles of the digestive tract, urinary tract, bile ducts and peripheral blood vessels [3, 4]. Now it is a component of many pharmaceuticals and herbal blends available in pharmacies. Valerian is a common herb in Europe, as well as in Asia and North America. Since the supply of wild plants does not fully meet the demand for this product, cultivation of *Valeriana officinalis* has started. One of the pesticides used in the cultivation of medicinal herbs is thiuram (tetramethyl thiuram disulfide) – a contact fungicide. It is used to prevent blade diseases and as seed dressing (dose 0.75-1kg/ha) [5].

Some pesticides tend to form complexes with metals [6], so the purpose of our study was to examine the effects of thiuram on the uptake of copper, zinc and manganese by the above-ground parts and roots of *Valeriana officinalis*.

Zinc is one of the most mobile metals in the soil, which is caused by both its exchangeable forms and compounds with organic matter. Maximum zinc content in arable land soils was established at 250-300 ppm [7]. All forms of readily soluble zinc are also bioavailable but the uptake level differs considerably depending on the genus, species and variety of the plant. Mean content of zinc in the above-ground plants parts, free from pollution, is 10-70 ppm [7].

Only a small proportion of copper occurs in soil in mobile (readily soluble and exchangeable) forms. Organic matter plays a key role in binding copper in soils. The range of total content of copper in soils is from 1 to 140 ppm, and its mean amount indicates a strict connection with the kind and type of soil [7]. The amount of copper taken up by plants is proportional to its content in the ground.

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Plant roots accumulate considerable amounts of this element. Compared with other trace elements, copper shows low mobility in plants. According to Kabata, its average content in the above-ground parts of plants is 5-20 ppm, while Ruszkowska et al. consider 3.3-6.6 ppm as optimum content in crop blades [7].

In comparison with other metals, manganese is weakly bound by organic matter, which makes it relatively mobile in organic soils. Mean content of manganese for different kinds and types of soils varies from 100 to 1300 ppm [7]. The uptake of manganese by plants is controlled by metabolism. Mn mobility in plant tissues increases as its supply becomes richer. Its mean content in clover and grass varies from 25 to 160 ppm [7]. The above-ground parts of green plants have the highest content of the element, which is related to its concentration in chloroplasts.

Experimental Procedures

Soil samples (0-20 cm) were taken from a recreational plot in Jedlicze situated about 20 km from Łódź, according to ISO standards [8]. The following general properties of the agricultural soil used for the study were determined: pH, organic matter content and mechanical analysis. Air-dried samples were analyzed. Soil pH was determined in both H_2O and $1 mol\cdot L^{-1}$ KCl solutions. 25 mL of suitable extractant were added to 10 g of soil and left for 24 hours. Next, soil pH was measured using a pH-meter Delta 350 (Mettler). For organic matter determination the dry soil was placed in a muffle furnace to follow the decrease in mass at 550°C [9]. Mechanical analysis was carried out using stainless steel sieves (0.02; 0.1; 1.0 mm). The results of the analyses are given in Table 1.

Table 1. Results of soil analyses.

Analysis	Results
Soil pH (H_2O) Soil pH (KCl)	6.81 - neutral 5.82 – slightly acid
Organic matter determination	5.43%
Mechanical analysis Fractions Φ 1 – 0.1 mm Φ 0.1 – 0.02 mm Φ < 0.02 mm	 89.5% 9.91% 0.64% Category of soil: loamy sand
Content of copper Content of zinc Content of manganese	Total forms: 19.6 ppm Total forms: 88.7 ppm Total forms: 84.4 ppm

Total amount of the metals in pure soil was determined in soil mineralizates by the FAAS method. A mixture of concentrated acids (21 mL HCl - 36% and 7 mL HNO_3 - 65%) was poured on 0.4500 g weighed amounts of soil, with granularity below 0.1 mm and left for 16 hours at room temperature to allow slow oxidation of organic matter. Next the samples were mineralized using a "Plazmatronika" mineralizer. The results of total amounts of the metals in soil mineralizates are presented in Table 1. The method accuracy was confirmed by the analysis of certified reference material Light sand Soil with normal analyte levels 7001, certificate No. 0217-CM-7001-04. Recovery was 96% for Cu, 99% for Zn, and 92% for Mn.

Next, the content of bioavailable forms of Cu, Zn and Mn was determined in pure soil used for the cultivation of *Valeriana officinalis* and the soil with the addition of thiuram in doses corresponding with those used during plant cultivation (3mg per pot). Thiuram used for the examination was produced in "Organika-Azot" S.A. Chemical Plant in Jaworzno. The content of the metals was determined after three; six; nine and twelve weeks of contact of thiuram with the soil. For determination of bioavailable metal forms, air-dried soils were passed through a 2mm sieve and then extracted with 1 M HCl for 1 h [10]. Zinc, copper and manganese in the soil were determined by the FAAS method using AAS spectrometer GBC 932 plus, in the reducing oxy-acetylene flame with the use of an appropriate lamp and wavelength Zn-213.9 nm; Cu-324.7 nm; Mn-279.5 nm. The results of the analyses are given in Table 2.

Table 2. Statistical analysis for bioavailable forms of copper, zinc and manganese determination in soil*.

No.	Soil	Contents of bioavailable forms of metals in soil [ppm]		
		Cu	**Zn**	**Mn**
1.	Without thiuram	1.99 ± 0.08	7.48 ± 0.58	10.8 ± 0.6
2.	3 weeks with thiuram	2.79 ± 0.18	12.1 ± 0.7	19.6 ± 0.8
3.	6 weeks with thiuram	3.30 ± 0.21	10.5 ± 0.5	18.4 ± 0.8
4.	9 weeks with thiuram	3.34 ± 0.26	11.0 ± 0.7	19.3 ± 0.9
5.	12 weeks with thiuram	3.71 ± 0.24	11.5 ± 0.6	18.4 ± 0.7

*n = 5; p = 95%, n – number of sample, p – confidence level.

In order to study the effects of thiuram on the uptake of zinc, copper and manganese by plants, *Valeriana officinalis* were grown under the pots method in laboratory conditions. Five series of *Valeriana officinalis* were grown, one without spraying with the fungicide and four others in which plants were treated with thiuram. Each series consisted of three samples of plants cultivated under the same conditions. The plants were sprayed with the fungicide (3 mg thiuram /pot) four months after they were sown. The plants were cut after three, six, nine and twelve weeks from the moment they had been sprayed with thiuram.

Table 3. Statistical analysis for the content of metals in *Valeriana officinalis**.

No.	Plants	Content of metals in plants [ppm]					
		Cu		**Zn**		**Mn**	
		Above – ground parts	Roots	Above – ground parts	Roots	Above – ground parts	Roots
1.	Without thiuram	6.93 ± 0.30	13.1 ± 0.8	28.1 ± 1.4	75.1 ± 3.1	30.4 ± 1.6	16.8 ± 0.8
2.	3 weeks with thiuram	8.66 ± 0.55	10.4 ± 0.6	33.8 ± 1.2	41.8 ± 2.7	28.9 ± 1.9	18.2 ± 0.9
3.	6 weeks with thiuram	9.57 ± 0.58	16.9 ± 1.1	40.4 ± 1.9	102 ± 3	39.2 ± 1.2	38.5 ± 2.6
4.	9 weeks with thiuram	6.55 ± 0.27	12.2 ± 0.9	30.4 ± 1.4	112 ± 5	36.3 ± 1.3	26.6 ± 1.4
5.	12 weeks with thiuram	7.11 ± 0.42	11.7 ± 0.7	24.9 ± 1.3	129 ± 6	23.0 ± 1.9	11.2 ± 0.6

*n = 5; p = 95%, n – number of sample, p – confidence level

The over-ground parts were separated from the roots, washed in distilled water and dried at 45°C. Then microwave mineralization with a mixture of nitric acid (V) (6 mL) and hydrogen peroxide (2mL) was applied. Copper, zinc and manganese in the resulting solution were determined by FAAS. The results of copper, zinc and manganese determination in *Valeriana officinalis* are given in Table 3. Accuracy of the method was confirmed by the analysis of certified reference materials INCT-MPH-2 Mixed Polish Herbs. Recovery was 102% for Cu, 91% for Zn and 91% for Mn.

Results and Discussion

The soil is soft acidic and belongs to the granulometric group of loamy sand (Table 1). Due to the low pH and content of organic matter (5.43%) the metals present in the soil are readily available to plants. The bioavailable form content of copper in pure soil (Table 2) accounts for about 10% of its total amount (Table 1). In the case of manganese the level of bioavailable forms (Table 2) accounts for about 12% of total manganese in the soil (Table 1). In the case of zinc bioavailable forms (Table 2) accounts for 9% of total zinc (Table 1). Analysis shows that the soil used for the study was not contaminated with copper, zinc or manganese (Tables 1, 2). According to the Directive of the Minister of the Environment passed in 2002, the content of zinc in built-up and urbanized areas should not exceed 300 ppm in the surface layer of the soil, copper – 150 ppm [11]. The addition of thiuram to the soil caused an increase of bioavailable forms of all of the metals (Cu about 40%; Zn about 70%; Mn about 90%) after the first three weeks (Table 2) in comparison to values without thiuram. Further contact of thiuram with the soil did not have a significant effect on the content of manganese. The contents of Cu increase by about 18% up to the 6^{th} week and in the next period it is almost constant. In the case of Zn, a slight decrease in its contents by about 12% is observed in the 6^{th} week.

The analysis of V. officinalis grown in the soil without additives shows that the contents of Cu, Zn and Mn in the examined parts of plants (above-ground parts and roots) differ between the elements (Table 3). The results of Cu and Zn determination in the plants indicate that roots accumulate considerable amounts (about 65% of total Cu and 73% of total Zn). In contrast, in the case of manganese the above-ground parts contain higher amounts of the metal - 64% of total Mn). The addition of thiuram to the soil caused a decrease of the content of copper and zinc in the roots by about 21% – Cu and 44% – Zn (observed after only 3 weeks of the contact of the plant with the fungicide), and a slight increase of the content of the metals in the above-ground parts (by about 25% for Cu and 20% for Zn). A gradual increase of the content of the metals continued until the sixth week. According to the literature data [12-15] a sparingly soluble complex of copper and thiuram is probably formed, so copper becomes less available to the roots of the plants. After the about 6 weeks the content of Cu in the roots increases, which may be caused by the decomposition of thiuram and release of Cu in the roots zone. A similar observation was made also in the case of zinc.

In previous work [2] the effects of thiuram on the uptake of zinc by Melissa officinalis was studied. The analysis shows that Melissa cultivated without thiuram also accumulated zinc mainly in the roots of the plants. Both the aboveground parts of the plants and the roots accumulated lower amounts of zinc in comparison to plants grown without the addition of the fungicide. It was found that the lowest amount of zinc is present in the plants sprayed with thiuram and cut after 3 weeks. After the following weeks the content of zinc in Melissa gradually increased, but in each case its amount is higher in the roots than in the above-ground parts.

In the case of manganese the addition of thiuram to the soil caused a decrease in the content of the metal in the above-ground plant parts (by approx. 5%) and its increase in the roots (by about 8%). In the literature there are no data on the formation of Mn complexes with thiuram. The uptake of manganese by plants is controlled by metabolism. However, when the metal content in the vicinity of the roots is high the metabolic barrier does not work and passive uptake occurs. Soluble forms are easily available to plants [7]. It is supposed that the addition of thiuram causes a transition of manganese present in the soil into forms which are more readily absorbed by the plants, which manage to increase of its content in the roots. At the same time, a decrease in the content of Mn in the above-ground parts of the plants is observed, which may be explained by increasing sorption ability of the root cell membrane.

References

1. ADAMCZYK D. The effect of thiuram on the uptake of lead and copper by Melissa officinalis, Environ. Eng. Sci., **23**(4), **2006**.
2. ADAMCZYK D. Effect of thiuram on the uptake zinc by Melissa officinalis, Ecol. Chem. Eng., **14**(8), **2007**.
3. SUCHORSKA-TROPIŁO K., OLSZEWSKA -KACZYŃSKA I. Medical botany., SGGW Warsaw **2003** [In Polish].
4. SEIDLER–ŁOŻYKOWSKA K. International Interherba Symposium. Conference materials **2003**.
5. RÓŻAŃSKI L. Vademecum of pesticides. Poznań: Agra-Enviro Lab. **1998**.
6. TUREK A., KOBYŁECKA J., SKIBA E., ADAMCZYK D. Contribution of pesticides in circulation of metals in the environment. Pathways of pollutants and mitigation strategies of their impact on the ecosystems, Monograph, **27**, PAN Lublin **2004**.
7. KABATA–PENDIAS A., PENDIAS H. Biogeochemistry of trace elements., PWN Warsaw **1999** [In Polish].
8. PN-ISO 11259:2001 “Soil quality – Simplified soil description”.
9. OSTROWSKA A., GAWLIŃSKI S., SZCZUBIAŁKA Z. Methods of analyses and estimation of soils and plants properties, Institute of Environment Protection, Warsaw **1991**.
10. Polish Standards. Chemo-agricultural analysis of soil. Determination of bioavailable zinc content PN-92/R-04016. Polish Committee for Standardization, Warsaw, **1992**. [In Polish].
11. Directive of the Minister of the Environment of 09.09.2002. Jour. of Law, No. 165, item 1359 [In Polish].
12. MACIAS B., VILLA M. V., CHICOTE E., MARTIN-VELASCO S., CASTINERAS A. BORRAS J. Copper complexes with dithiocarbamates derived from natural occurring amino acids, Polyhedron **21**, 1899, **2002**.
13. VICTORIANO L., Copper(III) dithiocarbamates, J. Chem Ed. **79**(10), 1252, **2002**.
14. ZHAO Y., ZHENG X., HUANG Z., YANG M. Voltammetric study on the complex of thiram-copper(II) and its application, Anal. Chim. Acta., **482**, 29, **2003**.
15. VICTORIANO L., GRANIFO J., PARRAGUEZ L., GRANIFO J. Copper(III) dithiocarbamates. Biol. Soc. Chil. Quim. **45** (3), 487, **2000**.