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ZINC UPTAKE FROM SOILS AT VARIOUS TIMES POLLUTED WITH HEAVY METALS

POBIERANIE CYNKU W RÓŻNYM CZASIE Z GLEB ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

Abstract: The interest in the subject of trace element concentrations and their behaviour in the environment is increasing, therefore investigations focused at determining abilities for harmful metals accumulation in plants growing on soil permanently or freshly polluted with heavy metals, contribute to development of this area of research. The paper aimed at an assessment of yielding and accumulation of zinc in buckwheat (*Fagopyrum esculentum* Moench) and sunflower (*Helianthus annuus* L.) cultivated on soils at various times polluted with this metal.

Much lower yields of buckwheat and sunflower were obtained on light soils, irrespective of supplementary mineral fertilization or the level and date of soil pollution with heavy metals. A toxic effect of heavy metals "freshly" applied to the soil was visible only on light soil, additionally fertilized with NPK. Plants cultivated on soils with higher content of these metals usually accumulated at least several times higher amounts of zinc, both in their shoots and roots. Additional NPK fertilization often increased Zn content. The largest amounts of zinc were absorbed by plants from light soils freshly and permanently polluted with heavy metals, on which additional mineral (NPK) fertilization was applied, and next from medium soil, freshly polluted with heavy metals and fertilized with NPK.

Keywords: zinc, pot experiment, heavy metals, sunflower, buckwheat

Introduction

Zinc is the element widely common in the environment, which constitutes about 0.007 % of the earth's crust. It is a component of almost 100 enzymes and fulfills numerous important functions in plant metabolism [1]. Among others it influences plant resistance to drought and diseases, regulates cell membrane permeability and pro-

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portions of the key component content in cell [2]. Considering the microelements crucial for living organisms, zinc deficiencies are the cause of the greatest losses in crop yields worldwide [3].

Zinc presence in plants is connected not only with its content in soil, but also with soil ability to bind this element, which may result in either deficiency or sometimes excess, *ie* its toxic effect upon plants [4].

Plants show deficiency of this element at its contents from 15 to 20 mg $\cdot$ kg^{-1} d.m. through reduction of their biomass, leaf chlorosis, poor flower setting and early ageing of older leaves [5, 6]. Zinc deficiency in plan tissues causes production of reactive oxygen species, mainly immediately in result of disturbed ratio of this element concentration to Cu. The outcome of this process is inhibition of protein synthesis and increase in Fe accumulation leading to damage of cell membranes, chlorophyll and enzymes participating in the photosynthesis process, which is visible as chlorotic lesions on plant leaves [7].

Human activity, such as ore extracting and processing, generation of municipal and industrial sewage or agriculture, contribute to pollution with zinc of considerable areas of agricultural lands [8–12]. Beside anthropogenic pollution, in some regions so called fault soils, which normally contain considerable amounts of this element [13]. However, bioaccumulation of Zn from the soil in these regions is not the major hazard to plant or animal organisms. On the other hand, their correct development is threatened by cadmium and lead simultaneously present in the rock, which have a toxic effect both on plants and animals [14, 15]. Therefore, in order to reduce, among others, Zn accumulation in the food chain in these areas, a recultivation by means of phytoremediation has been suggested [12]. The process is effective at properly selected plant species and the best are so called hyperaccumulators which have the ability of taking up about 1 % of the available amount of metal available from the soil during one vegetation period [16, 17].

The paper aimed at an assessment of yielding and zinc accumulation in buckwheat (*Fagopyrum* Mill.) and sunflower (*Helianthus annus* L.) cultivated on soil at different times polluted with this metal.

Materials and methods

The pot experiment was conducted in the vegetation hall of the Faculty of Agriculture and Economics, University of Agriculture in Krakow, at Mydlniki near Krakow. Plastic pots with 7 kg of soil d.m. were used for the experiment. Light and medium soil with heavy metal content close to natural (collected in Mydlniki area) and light and medium soils polluted with heavy metals (collected in Bukowno region) were used for the experiment. Detailed data concerning physicochemical properties of the soil materials used for the experiment were presented in Table 1.

Zn concentrations in the soils originating from Bukowno area were generally several times higher in comparison with the soils from Mydlniki. The soils from Mydlniki were characterized by a neutral reaction, cation exchange capacity (assessed using Kappen's method) on the level of 105.5 mmol(+) $\cdot$ kg^{-1} (light soil – ML) and 504.2

mmol(+) · kg^{-1} (medium soil – MM) and organic carbon content (assessed by Tiurin's method) 12.1 (ML) and 25.2 g · kg^{-1} soil d.m. (MM), respectively.

Table 1

Physicochemical properties of soils used for the experiment

Parameter		Unit	Bukowno		Krakow-Mydlniki	
			Light soil	Medium soil	Light soil	Medium soil
Use		—	Coppice	Meadow	Wasteland	Meadow
Granulometric group		—	Loose sand	Sandy loam	Light loamy sand	Medium loam
pH_{H2O}		—	5.73	7.40	6.96	6.81
Hydrolytic acidity (Hh)		[mmol(+) · kg^{-1}]	29.8	8.40	10.50	5.70
Cation exchange capacity (CEC)			168.0	483.6	105.5	504.2
Organic C		[g · kg^{-1} d.m.]	16.4	29.3	12.1	25.2
Cd	Total form	[mg · kg^{-1} d.m.]	1.22	5.53	0.78	2.50
Pb			29.1	311.6	21.9	66.1
Zn			188.6	440.6	66.4	101.9

The soil materials from Bukowno area somewhat differed by the determined physicochemical properties. Light soil from Bukowno (BL) revealed pH = 5.73, cation exchange capacity 16.0 mmol(+) · kg^{-1} and organic C content 16.4 g · kg^{-1} soil d.m., whereas in case of medium soil (BM) pH was 7.40, cation exchange capacity 483.6 mmol(+) · kg^{-1} soil d.m. and organic C content 29.3 g · kg^{-1} d.m.

Water solutions of $CdSO_4 \cdot 7H_2O$, $(CH_3COO)_2Pb \cdot 3H_2O$ and $ZnSO_3 \cdot 7H_2O$ were added to light soil from Mydlniki (objects 9–12) to compare the possibilities of heavy metal uptake by plants from the soils "freshly" polluted and for a long time polluted with zinc. In this way cadmium, lead and zinc contents in these soils were equaled to the values assessed in the soils from Bukowno. In case of zinc, 122.2 mg Zn was added to the light soil and 338.7 mg Zn · kg^{-1} to medium soil (Table 2). The second factor was additional mineral fertilization applied in the objects 5–8 and 11–12.

Fertilizer components were applied under the forecrop (buckwheat) as NH_4NO_3, KH_2PO_4 and KCl in the following amounts:1.4 gN, 0.4 gP_2O_5 and 1.3 gK_2O per pot. Solutions of fertilizer NPK components and heavy metals were mixed with the whole soil mass in a pot one week prior to the first plant sowing.

The test plant, buckwheat (*Fagopyrum esculentum* Moench), "Panda" cv., was sown on 26 May 2011 and after its harvesting, an aftercrop – sunflower (*Helianthus annuus* L.) was sown on 25.07.2011 in the amount of 12 seeds per pot. During the plant vegetation the soil moisture was maintained on the level of 50 % (at the initial period) and then 60 % of the maximum water capacity. Water losses were supplemented with distilled water.

Table 2

Pot experiment design

No.	Object description	Object symbol
1	Bukowno light soil	BL
2	Bukowno medium soil	BM
3	Mydlniki light soil	ML
4	Mydlniki medium soil	MM
5	Bukowno light soil + NPK	BL + NPK
6	Bukowno medium soil + NPK	BM + NPK
7	Mydlniki light soil + NPK	ML + NPK
8	Mydlniki medium soil + NPK	MM + NPK
9	Mydlniki light soil + heavy metals	ML + h.m.
10	Mydlniki medium soil + heavy metals	MM + h.m.
11	Mydlniki light soil + heavy metals + NPK	ML + h.m. + NPK
12	Mydlniki medium soil + heavy metals + NPK	MM + h.m. + NPK

Zinc content was assessed in the harvested plant material (tops and roots) after previous mineralization in a muffle furnace and digestion in nitric(V) and hydrochloric acids, using ICP-OES Optima 7300 DV, atomic emission spectrometer (PerkinElmer).

Results and discussion

The yield of buckwheat and sunflower and zinc content in the yields were statistically verified using analysis of variance, while a multiple comparison procedure was conducted basing on Tukey's test, at $\alpha \leq 0.05$. Columns marked with the same letter do not differ significantly at significance level $\alpha \leq 0.05$. Yielding and zinc concentrations were presented separately for the tops and roots, whereas Zn uptake as a sum of this element amount absorbed from a pot with tops and roots yield of buckwheat and sunflower.

On the control objects, *ie* on the soils from both Bukowno and Mydlniki, without an additional NPK fertilization and without heavy metal supplement, a similar yielding of the test plant shoots and roots was registered, however the yields of tops gathered particularly from light soils were visibly smaller (Fig. 1).

Applied mineral fertilization of the analyzed soils, both those strongly polluted with heavy metals and with natural content of these elements, markedly increased the cultivated crops yielding. It was more noticeable for buckwheat, under which NPK fertilization was applied and light soil from which about 4-fold bigger total dry mass yields were harvested jointly from both cultivated crops on the objects receiving NPK treatment. Application of heavy metal salts solutions to the soils from Mydlniki (ML and MM), equalizing their level to the content registered in the soils from Bukowno (BL and BM), did not decrease the yield of buckwheat tops and roots or sunflower cultivated as an aftercrop.

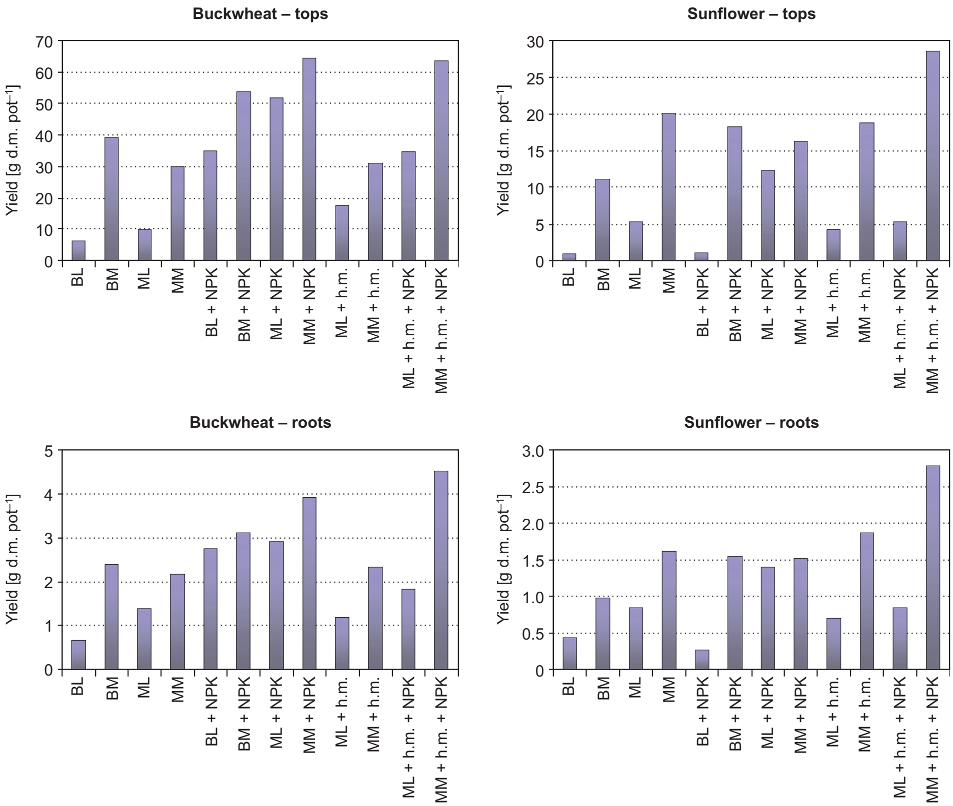

Fig. 1. Yields of buckwheat and sunflower tops and roots

A comparison of crop yielding on the artificially polluted objects (ML and MM) with plant biomass harvested from “soils naturally polluted with heavy metals”(BL and BM), did not reveal a decline in yield in result of introducing soluble forms of the analyzed metals to the soil from Mydlniki. On the other hand, a toxic effect of heavy metals “freshly” applied to the soil became evident in the object with light soil additionally fertilized with NPK (ML+h.m.+NPK) where significantly smaller yield of both buckwheat and sunflower was gathered. In case of medium soil, not such effect was noted and even a larger yield of sunflower aboveground parts was harvested from the soil fertilized with NPK with heavy metal supplement.

As might have been expected, zinc accumulation in plants cultivated on the soil from the lead-zinc ore extraction region (BL and BM) was much higher than in the plants cultivated on soil from the vicinity of Krakow (ML and MM) (Table 3).

The shoots of buckwheat and sunflower gathered from light soil from Bukowno (BL) accumulated over 12-fold more of zinc that assessed in the analogous soil materials from Mydlniki (ML). In case of medium soil, the differences were about 2.5-fold. Additionally NPK fertilization of the analyzed soils, despite markedly increased yielding, did not lead to so called “dilution effect” of the element in a larger yield [5, 18].

Table 3

Zinc (Zn) content in tops and roots of buckwheat and sunflower [mg · kg^{-1} d.m.]

Objects	Buckwheat		Sunflower	
	tops	roots	shoots	roots
BL	574.1^{c}	239.0^{ef}	911.5^{bc}	1573.6^{c}
BM	121.9^{a}	52.4^{abc}	89.1^{a}	81.2^{a}
ML	47.4^{a}	88.9^{abc}	72.1^{a}	116.6^{a}
MM	50.9^{a}	22.4^{a}	36.4^{a}	40.5^{a}
BL + NPK	538.4^{c}	247.4^{g}	1424.9^{c}	1990.8^{d}
BM + NPK	99.9^{a}	65.7^{abc}	95.2^{a}	70.7^{a}
ML + NPK	95.5^{a}	55.8^{abc}	150.6^{a}	97.2^{a}
MM + NPK	67.3^{a}	47.5^{ab}	48.6^{a}	48.3^{a}
ML + h.m.	225.3^{b}	94.8^{bc}	551.4^{bc}	747.9^{b}
MM + h.m.	229.6^{b}	121.8^{cd}	217.2^{a}	222.5^{a}
ML + h.m. + NPK	588.0^{c}	172.3^{de}	542.5^{b}	635.6^{b}
MM + h.m. + NPK	220.9^{b}	112.3^{bc}	197.9^{a}	166.8^{a}

Means marked with the same letter in columns do not differ significantly according to Tukey's test at $\alpha \leq 0.05$.

In case of sunflower cultivated on light soil polluted with heavy metals (BL + NPK), some synergic effect was observed, *ie* increased uptake and accumulation of zinc, both in the tops and roots.

A similar phenomenon occurred for buckwheat cultivated on soils freshly polluted with heavy metals. A supplement of these metals to both soils with initial natural heavy metal contents (ML + h.m and MM + h.m.) generally increased several times zinc accumulation in the test plants. It was also observed that applied supplementary mineral (NPK) fertilization increased zinc content in the tops and roots of buckwheat growing on light soil (ML + h.m. + NPK) and in these plants' tops when grown on medium soil (MM + h.m. + NPK).

Element uptake by plants and its removal from soil is a ratio of the yield of cultivated plants and the elements content in the yield [4]. In conditions of the presented experiment, the biggest amounts of zinc were absorbed by buckwheat yield from light soils: permanently and freshly polluted with heavy metals and additionally fertilized (BL + NPK and ML + h.m. + NPK), but also from medium soil freshly polluted and additionally fertilized (MM + h.m. + NPK) (Table 4). These amounts were several times bigger than absorbed even from the soils permanently polluted with heavy metals but without any supplementary fertilization. In case of sunflower cultivated as an aftercrop, the highest quantities of zinc were taken up by the plants cultivated on the soil in MM + h.m. + NPK object and from the MM + h.m. object, *ie* with the same freshly polluted soil, but without supplementary fertilization.

Table 4

Total uptake of zinc with yield of buckwheat and sunflower tops and roots [μg · pot^{-1}]

Object	Buckwheat	Sunflower	Total
BL	3 725	1 806	5 531
BM	4 889	1 079	5 968
ML	585	479	1 064
MM	1 573	794	2 367
BL + NPK	19 664	2 138	21 802
BM + NPK	5 580	1 863	7 443
ML + NPK	5 121	2 057	7 178
MM + NPK	4 479	859	5 338
ML + h.m.	4 082	2 759	6 841
MM + h.m.	7 245	4 478	11 723
ML + h.m. + NPK	20 717	3 394	24 111
MM + h.m. + NPK	14 549	6 114	20 663

Analyzed plant material: shoots of buckwheat and sunflower were assessed for their forage usefulness. Considering assumed [18] standards of zinc content, it should be stated that the harvested material of sunflower plants growing both on medium and light soils from Mydlniki without supplements and additionally fertilized with NPK, fulfilled the requirements for forage use which for Zn range from 50 to 100 mg · kg^{-1} d.m. of plant material [18]. In case of buckwheat growing on soils polluted for a long and short time, the forage criterion of the analyzed material was fulfilled only in case of plants growing on medium soil from Bukowno additionally fertilized with NPK. Shoots of both buckwheat and sunflower gathered from the other objects did not fulfill the standards of forage usefulness and therefore should be destined for industrial processing.

In conclusion it should be stated that the ability to absorb the analyzed element by the test plants of buckwheat and sunflower depended to the greatest extent on the form in which zinc occurred in the substratum. Plants growing on soils from Bukowno region, *ie* polluted for a long time, usually absorbed smaller amounts of Zn in comparison with the quantities taken up from the soils freshly polluted with this element. The relationship is probably due to much bigger content of exchangeable zinc, soluble in the soil from Mydlniki freshly polluted with this metal in comparison with Zn amount in soil from Bukowno. Jiang et al [19] in their research on solubility of various Zn salts, stated that *Sedum alfredii* and maize (*Zea mays* L.), which they cultivated in hydroponics were taking up the greatest quantities of Zn salts which after application to the substratum became a part of exchangeable and soluble fraction of this element. Also the kind of soil caused an increase of Zn uptake, *ie* light soil has a poor ability for metal sorption, in comparison with medium soil. Cation exchange capacity of soil sorption complex depends on many factors, according to Miretzky et al [20] and Galletti et al [21], mainly on: soil pH, organic matter content, content of soil clay fraction and soil moisture. Author's own studies confirmed these relationships: plants harvested from objects growing on light soils, *ie* with lesser abilities for immobilizing this element contained much higher amounts of Zn in comparison with plants gathered from the objects with medium soil, which probably absorbed a considerable part of Zn introduced to the soil.

Conclusions

1. In conditions of conducted experiment much smaller yields of buckwheat and sunflower were obtained on light soil in comparison with medium one, irrespective of supplementary mineral fertilization, as well as the level and date of soil pollution with heavy metals. Toxic effect of heavy metals "freshly" applied to the soil became apparent only on light soil additionally fertilized with NPK.

2. Plants cultivated on soils with bigger content of these metals accumulated usually at least several fold bigger quantities of zinc both in their tops and roots, irrespective of the date of pollution with heavy metals. Additional NPK treatment often increased Zn content.

3. The largest amounts of zinc were taken up by plants from light soil freshly and permanently polluted with heavy metals on which supplementary mineral NPK fertilization was applied and next from medium soil, freshly polluted with heavy metals and fertilized with NPK.

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POBIERANIE CYNKU W RÓŻNYM CZASIE Z GLEB ZANIECZYSZCZONYCH METALAMI CIĘŻKIMI

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Abstrakt: Stale wzrasta zainteresowanie tematyką zawartości pierwiastków śladowych i ich zachowaniem w środowisku, a badania, których celem jest określenie zdolności do akumulowania pierwiastków szkodliwych w roślinach rosnących na glebach trwale oraz świeżo zanieczyszczonych tymi metalami, przyczyniają się do rozwoju tego obszaru badawczego. Celem pracy była ocena plonowania oraz kumulacji cynku w gryce (*Fagopyrum esculentum* Moench) oraz w słoneczniku (*Helianthus annuus* L.) uprawianych w różnym czasie na glebach zanieczyszczonych tym metalem.

Znacznie mniejsze plony gryki i słonecznika uzyskano na glebie lżejszej, niezależnie od dodatkowego nawożenia mineralnego oraz poziomu i terminu zanieczyszczenia gleby metalami ciężkimi. Toksyczny efekt „świeżo" zastosowanych do gleby metali ciężkich ujawnił się jedynie na glebie lekkiej dodatkowo nawożonej NPK. Rośliny uprawiane na glebach z większą zawartość tych metali nagromadzały zwykle co najmniej kilkakrotnie większe ilości cynku zarówno w częściach nadziemnych, jak i korzeniach. Dodatkowe nawożenie NPK często zwiększało tę koncentrację. Największe ilości cynku zostały pobrane przez rośliny z gleby lekkiej świeżo oraz trwale zanieczyszczonej metalami ciężkimi, do której zastosowano dodatkowe nawożenie mineralne (NPK) i z kolei z gleby średniej, świeżo zanieczyszczonej metalami i nawożonej NPK.

Słowa kluczowe: cynk, doświadczenie wazonowe, metale ciężkie, słonecznik, gryka