

RESPONSE OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.) TO SOIL CONTAMINATION WITH ZINC

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Abstract

Phytoremediation is one of the ways of removing toxic metals from soil. Phytoremediation relies on using plants which are highly capable of absorbing heavy metals to remove them from soil. In order to determine the effect of the degree of soil contamination with zinc on the production of biomass and concentration of zinc in ryegrass, a pot experiment was carried out, consisting of 6 treatments in which gradually increasing doses of zinc were used: control treatment (without zinc), 25, 50, 100, 200 and 400 mg Zn kg⁻¹ of soil. The experiment was conducted on two types of soil, different in the cation exchange capacity: sand and sandy loam. The tested plant was perennial ryegrass cv. Nira.

The toxic effect of zinc on the growth of ryegrass was much more pronounced in the case of plants grown on sand than on sandy loam. When ryegrass was grown on sand, the toxic effect of zinc was observable even when the lowest rate of zinc had been introduced to soil: the biomass of the first cut was considerably depressed. On sandy loam, the toxic effect of zinc was not manifested until the highest rate of the contaminant had been introduced to soil (400 mg Zn kg⁻¹ of soil).

The experiment has demonstrated the presence of a strong, statistically verified correlation between the content of zinc in soil extracted in 1 M HCl and the concentration and uptake of this metal in the following cuts of ryegrass. The concentration of zinc in grasses from the first cut was very high: 1660 mg Zn kg⁻¹ d.m. on sand and 1200 mg Zn kg⁻¹ d.m. on sandy loam. A one-year cultivation of perennial ryegrass only slightly lowered the content of zinc in soil. Although the concentration of zinc in harvested grass was very high, the total uptake was just a small percentage (1-2%) of the quantity of this metal introduced to soil. Perennial ryegrass cv. Nira is capable of accumulating very high amounts of zinc and is well tolerant to a high content of Zn in soil, which is why it can be used for sowing on land polluted with this heavy metal.

Key words: zinc, phytotoxicity, phytoremediation, perennial ryegrass.

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REAKCJA ŻYCICY TRWAŁEJ (*LOLIUM PERENNE* L.) NA ZANIECZYSZCZENIE GLEBY CYNKIEM

Abstrakt

Jednym ze sposobów oczyszczania gleb z toksycznych metali jest fitoremediacja, polegająca na wykorzystaniu roślin o dużej zdolności do pobierania znacznych ilości metali ciężkich do usuwania ich z gleby. W celu określenia wpływu stopnia zanieczyszczenia gleby cynkiem na produkcję biomasy i koncentrację cynku w życicy trwałej wykazano doświadczenie wazonowe, składające się z 6 obiektów, na których zastosowano wzrastające ilości cynku: obiekt kontrolny (bez Zn), 25, 50, 100, 200 i 400 mg Zn kg⁻¹ gleby. Doświadczenie prowadzono na dwóch glebach, różniących się pod względem pojemności sorpcyjnej: piasku i glinie piaszczystej. Rośliną testowaną była życica trwała, odmiany Nira.

Toksyczne działanie cynku na wzrost życicy ujawniło się w znacznie większym stopniu w przypadku uprawy na piasku niż na glinie piaszczystej. W doświadczeniu na piasku już po zastosowaniu najmniejszej dawki cynku (25 mg Zn kg⁻¹ gleby) nastąpiło istotne zmniejszenie masy pierwszego odrostu traw, natomiast na glinie piaszczystej toksyczne działanie cynku ujawniło się dopiero po największym zanieczyszczeniu gleby tym metalem (400 mg Zn kg⁻¹ gleby).

Badania wykazały istnienie silnej, statystycznie udowodnionej korelacji między zawartością cynku w glebie, ekstrahowanego 1 M HCl, a koncentracją oraz pobraniem Zn z plonem kolejnych odrostów życicy. Wraz ze wzrostem zanieczyszczenia gleby cynkiem istotnie zwiększała się koncentracja oraz pobranie tego metalu przez trawy. Stężenie cynku w życicy pierwszego odrostu, rosnącej na glebie o największym zanieczyszczeniu tym metalem, było bardzo wysokie i wynosiło 1660 mg Zn kg⁻¹ s.m. na piasku oraz 1200 mg Zn kg⁻¹ s.m. na glinie piaszczystej. Jednoroczna uprawa życicy trwałej tylko nieznacznie wpłynęła na zmniejszenie zawartości cynku w glebie. Pomimo bardzo wysokiej koncentracji cynku w trawach całkowite pobranie stanowiło tylko niewielką część (1-2,5%) ilości tego metalu wprowadzonego do gleby. Życica trwała odmiany Nira charakteryzuje się zdolnością do akumulacji bardzo dużych ilości cynku oraz dobrze znosi wysoką koncentrację Zn w glebie, dlatego może być wykorzystywana do zasiedlania terenów zanieczyszczonych tym metalem ciężkim.

Słowa kluczowe: cynk, fitotoksyczność, fitoremediacja, życica trwała.

INTRODUCTION

The emission of zinc from industrial sources, compared to that of other trace elements, is relatively high. Moreover, zinc in soil is a highly mobile element, which makes it a serious threat to the natural environment. In Poland, the percentage of soils to a greater or lesser degree contaminated with zinc is estimated to equal *ca* 11.3% (TERELAK et al. 1995).

Soil contamination with zinc causes retardation of plant growth, and its accumulation in crops may reach values dangerous to animals and people (BARAN, JASIEWICZ 2009). The phytotoxic effect of zinc depends mainly on soil properties, such as soil reaction, organic matter content or soil absorbing complex, as well as the species of a crop and its current developmental phase (ROSZYK et al. 1988, RUSZKOWSKA 1991, ŁYSZCZ, SPIAK et al. 2000, KABATA-PENDIAS 2002, BARAN et al. 2008, GAMBUŚ et al. 2004).

Reclamation of soils burdened with high levels of heavy metals is a difficult and expensive process, which is why it is not conducted on a large scale. Phytoremediation is one of the ways in which excess heavy metals are removed from soil. Phytoremediation means growing plants which are highly capable of absorbing heavy metals from soil, thus removing them from the soil environment. Hyperaccumulators are the plant species which can contain up to 1-2% of a toxic element. In theory, such plants could remove as much as 200-1000 kg of metals per 1 ha. However, as there are no detailed agronomic recommendations concerning the cultivation of such plants, these species usually yield too little biomass. Phytoremediation can also involve typical crops. Although they do not accumulate as much of trace metals as hyperaccumulators, these plants produce much more biomass and can therefore improve the effects of soil remediation. The advantages of phytoremediation techniques are their low cost and easy application. The disadvantages include low efficacy and the problem of handling the biomass which contains large amounts of toxic elements (SAS-NOWOSIELSKA et al. 2004, KARCZEWSKA 2005, MARECIK et al. 2006).

The aim of this study was to evaluate the usefulness of perennial ryegrass, cv. Nira for phytoremediation of soils contaminated with zinc.

MATERIAL AND METHODS

This study has been performed as a strict pot experiment set up in 2007 in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment consisted of 6 treatments in which increasing quantities of zinc in the form of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ were added to soil prior to sowing ryegrass: control treatment (no Zn added), 25, 50, 100, 200 and 400 mg Zn kg^{-1} of air dry soil.

The experiment was carried out on two soils, sand and sandy loam, different in the sorption capacity. The content of organic carbon was 5.67 g kg^{-1} in sand and 10.05 g kg^{-1} in sandy loam. Some of the physicochemical properties of the soils are presented in Table 1.

The pots were filled with 8.0 kg of air dry soil mass. The tested plant was perennial ryegrass (*Lolium perenne* L.) cv. Nira. The grass was cut three times in the early phase of heading. Each treatment comprised four replications.

The nitrogen, phosphorus, potassium and magnesium fertilization was identical in all the fertilized treatments. When setting up the experiment, 0.4 g N, 0.2 g P, 0.4 g K and 0.08 g Mg per pot were applied. After the first cut, additional 0.5 g N, 0.4 g K and 0.1 g Mg were introduced to soil, and after the second cut the soil was amended with 0.25 g N, 0.2 g K and 0.05 g Mg per pot. Nitrogen was applied as NH_4NO_3 ; phosphorus was used in the form of KH_2PO_4 ; potassium was introduced as KH_2PO_4 and KCl, whereas magnesium was taken as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

Some physicochemical properties and texture class of soils used in experiment

Texture-classes USDA classifi- cation	Soilfraction			C org. g kg ⁻¹ of soil	pH _{KCl}	Hh mmol H ⁺ kg ⁻¹ of soil	Base saturation mmol (+) kg ⁻¹ of soil	CEC mmol (+) kg ⁻¹ of soil	mg Zn kg ⁻¹ of soil
	diameter (mm)								
	2.0-0.05	0.05-0.002	< 0.002						
Sandy loam	72.3	25.3	2.4	10.05	6.16	14.1	57.0	71.1	14.03
Sand	86.7	12.1	1.2	5.67	5.72	8.3	18.3	26.6	7.19

Grass seeds were sown after three weeks of the incubation of soil with the applied salts. After emergence, 20 plants of ryegrass were left in each pot. The soil moisture was maintained as the level of 70% of the maximum water capacity.

In order to determine the initial concentration of Zn in soil (extraction in 1 M HCl) in all the treatments, an additional trial was set up in which soil was incubated for three weeks with the same quantities of zinc per pot as used in the plant growing experiment.

Dried and ground plant samples were mineralized (separately for each pot) in a mixture of concentrated nitric (V), chloric (VII) and sulphuric (VI) acids, in a volumetric ratio between the acids equal 40:10:1. Zinc was determined in the mineralized samples with the atomic absorption spectrometric method.

Prior to the experiment, the soils were analyzed to determine the following parameters: pH in 1 M KCl by potentiometry, content of organic carbon by Kurmies method (WALINGA et al. 1992), base saturation and hydrolytic acidity (Hh) by Kappen method, Zn concentrations by flame AAS after soil extraction in 1 M HCl and soil particle size distribution using the laser diffraction method (BUURMAN et al. 1997).

After the third cut, soil samples were taken from each pot. They were dried and passed through a 1 mm mesh sieve. Afterwards, zinc in soil samples was determined with the AAS method, following soil extraction in 1 M HCl.

Statistical processing of the data involved analysis of variance for a one-factor pot experiment in a completely random, orthogonal arrangement. Significance of differences between the treatment means was verified using *t*-Student test. Correlations between selected traits were determined with the analysis of correlation and regression.

RESULTS AND DISCUSSION

In the treatments set up on sand, as the soil contamination with zinc increased (treatments with 25, 50, 100, 200 and 400 mg Zn kg⁻¹ soil), grass yields from all harvested cuts tended to decrease (Table 2). A strong negative correlation appeared for pots filled with zinc contaminated soil between the content of zinc in soil and the harvested mass of the three cuts (Figure 1). In the first cut, a significant decrease in the yield was already noticeable in response to the lowest zinc rate (25 mg Zn kg⁻¹ of soil); in the second cut, it was the second smallest rate, i.e. 50 mg Zn kg⁻¹ of soil, that led to

Table 2

Effect of soil contamination with zinc on the yielding of perennial ryegrass

Zn rate (mg kg ⁻¹ of soil)	Yields of perennial ryegrass (g of d.m. pot ⁻¹)							
	sand				sandy loam			
	1 st cut	2 nd cut	3 rd cut	total yield	1 st cut	2 nd cut	3 rd cut	total yield
0	18.40	22.73	16.03	57.16	27.97	19.90	21.37	69.24
25	15.97	21.13	15.93	53.03	27.60	21.73	20.80	70.13
50	14.97	20.57	15.70	51.24	29.67	21.03	20.73	71.43
100	14.33	19.50	14.23	48.06	28.63	21.03	20.17	69.83
200	12.73	19.47	14.27	46.47	29.70	22.03	20.00	71.73
400	8.83	17.13	13.40	39.36	24.37	20.73	20.50	65.60
LSD _{0.01}	1.81	2.00	1.41		2.50	3.11	2.94	
LSD _{0.001}	2.55	2.83	1.99		3.53	4.40	4.15	

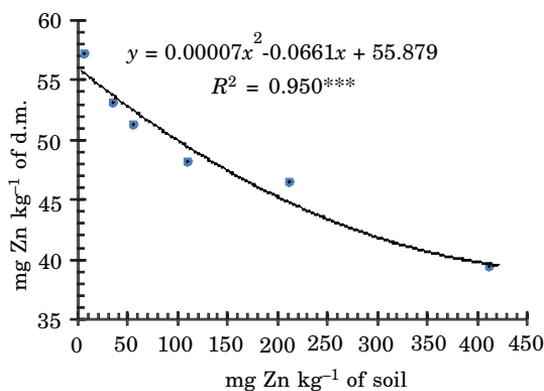


Fig. 1. Total yield of perennial ryegrass as affected by zinc content in sand (extracted in 1 M HCl)

a significantly lower grass yield; with respect to the third cut, it was not until the rate of 100 mg Zn kg⁻¹ had been introduced to soil that the grass yield declined significantly. The largest and significantly high decrease in the grass biomass occurred on the soil with the highest rate of the contaminant, i.e. 400 mg Zn kg⁻¹ of soil. In that treatment, the biomass of ryegrass was lower by 52% in the first, 25% in the second and 16% in the third cut than in the control treatment. In a study conducted by BARAN (2011) on sand, the rates of 250 and 750 mg Zn kg⁻¹ of soil depressed the mass of aerial parts of maize by 10% and 25%, respectively.

Excess zinc is accumulated mainly in the below-ground parts of plants, impairing their function. WERYSZKO-CHEMLEWSKA et al. (2000) demonstrated that a high content of zinc in the substrate caused damage to root cells of horse bean and led to a decreased leaf area and mass. SAGARODY et al. (2009) report that toxic quantities of zinc reduce the uptake of such essential nutrients as nitrogen, magnesium, potassium and iron by plants. They also cause depressed concentrations of chlorophyll in leaves, with an adverse effect on the process of photosynthesis, leading to a retarded growth of plants.

In the present experiment, the emergence of plants growing on sand contaminated with 200 mg or 400 mg Zn kg⁻¹ of soil was evidently worse and the plants developed more slowly. As the supply of mobile forms of Zn in the substrate was being depleted, the toxic effect produced by the metal was considerably weakened. Thus, the negative effect of this element was the strongest in the first cut of ryegrass, but proved much weaker in the last cut.

Contrary results were obtained in the other experiment, set up on sandy loam (Table 2). Statistically verified, the toxic effect of zinc in the soil with a larger CEC was not observable until the highest degree of soil contamination with zinc was tested (400 mg Zn kg⁻¹), but even then it appeared only in the first ryegrass cut. No significant differences were detected between the mass of plants harvested from the treatments which had received zinc in rates less than 400 mg kg⁻¹ (25, 50, 100 and 200 mg Zn kg⁻¹). The results suggest that the adverse effect of zinc is dependent to a great degree on the type of soil, and mainly its CEC. On very light soil, even a small rate of added zinc (25 mg Zn kg⁻¹ of soil) significantly depressed the biomass of ryegrass, whereas on sandy loam a significant decline in the grass yield was observed only under the influence of 400 mg Zn kg⁻¹ of soil. It is obvious that a higher percentage of organic and mineral colloids in the soil with the texture of sandy loam had a protective action and reduced the concentration of active zinc forms in soil. This assumption is supported by the research carried out by SPIAK (1996), in which the value of the zinc migration coefficient, which reflects the ratio of the concentration of this metal in plants to its content in soil, largely depended on the quantity of the soil fraction less than 0.02 mm in diameter. On light soil, following the application of 500 mg Zn kg⁻¹, the value of this index was 36.1, whereas on medium and heavy soil, it fell to 12.3 and 5.9, respectively. GAMBUS et al.

(2004), KABATA-PENDIAS (2002) and MERCIK et al. (2004) also report that solubility and mobility of zinc in soil are depressed when the content of clay fractions, humus and aluminium, iron and manganese oxides increases. This finding explains a much weaker toxic effect of zinc in sandy loam than in sand.

Perennial ryegrass can be regarded as a species well tolerant to high content of zinc in soil. The decrease in the mass of cut grass caused by the highest rate of zinc contamination was relatively small, especially on sandy loam. Relatively good tolerance of grasses to high zinc concentrations in the substrate has also been reported by PASCHKE et al. (2000).

The gradually increasing levels of zinc contamination of soil caused a very high rise in the concentration of this metal in ryegrass (Figures 2-7). Zinc permeated into plant cells very readily. In the experiment run on sand, following the application of $400 \text{ mg Zn kg}^{-1}$ of soil, the concentration of this metal rose to $1629 \text{ mg Zn kg}^{-1}$ d.m. in the first grass cut (43-fold more

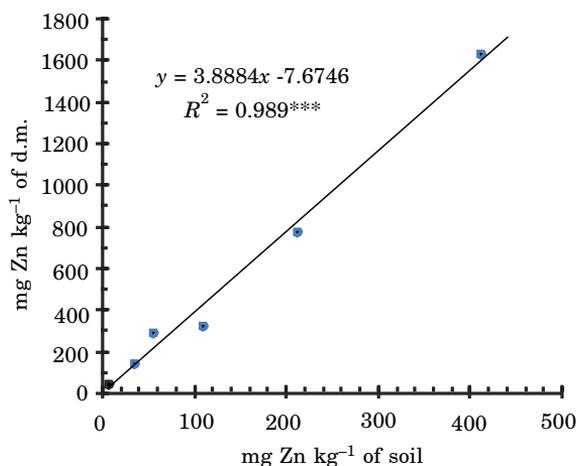


Fig. 2. Zinc concentration in the first cut of perennial ryegrass as affected by zinc content in sand (extracted in 1 M HCl)

than in the treatment with the natural soil content of zinc, Table 3). This element was luxuriously taken up by plants, which confirms very high mobility of zinc in soil. In the trials conducted on sand, a radical increase in the concentration of zinc in the first grass cut was also observed in the pots with a very small amount of this metal introduced to soil (25 and 50 mg Zn kg^{-1} of soil). The concentration of zinc in plants harvested from those treatments increased to 139.8 mg and $287.7 \text{ mg Zn kg}^{-1}$ d.m. of plants, respectively. For comparison, the ryegrass harvested from the control treatment contained $37.8 \text{ mg Zn kg}^{-1}$ d.m. Similar concentrations of this metal in grasses growing on soils unpolluted with zinc have been observed by

Effect of soil contamination with zinc on the concentration of this element in perennial ryegrass

Zn rate (mg kg ⁻¹ of soil)	Zn concentration (mg Zn kg ⁻¹ d.m.)					
	sand			sandy loam		
	1 st cut	2 nd cut	3 rd cut	1 st cut	2 nd cut	3 rd cut
0	37.76	31.69	44.40	54.40	40.28	38.98
25	139.76	53.97	66.73	106.36	53.22	45.85
50	287.67	77.40	93.27	157.22	77.45	59.92
100	317.22	122.58	115.65	307.62	112.96	74.92
200	773.04	300.92	274.83	639.33	208.58	131.78
400	1629.13	1042.55	695.56	1223.41	311.11	187.23

KUCHARCZYK and MORYL (2010). In plants grown for fodder, the threshold level of this microelement is 100 mg Zn kg⁻¹ d.m. The fact that zinc can permeate so easily into plant cells may pose a high risk on farmlands used for growing crops and just very slightly polluted with this metal.

The concentration of zinc in the second and third cut of ryegrass grown on sand also increased significantly following the application of increasing rates of zinc (Figures 3 and 4). When 400 mg Zn kg⁻¹ had been added to soil, the concentration of zinc in the second and third cuts rose 33- and 15-fold, respectively, compared to the control. It should be noticed, however, that the concentrations of zinc in grasses from the second and third cuts were around 2.5-fold smaller than in the ryegrass from the first cut on corresponding treatments. The determination coefficients, which indicate the

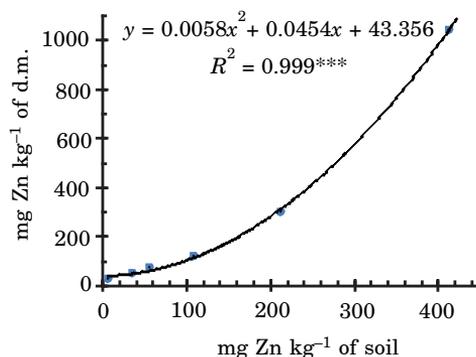


Fig. 3. Zinc concentration in the second cutting of perennial ryegrass as affected by zinc content in sand (extracted in 1 M HCl)

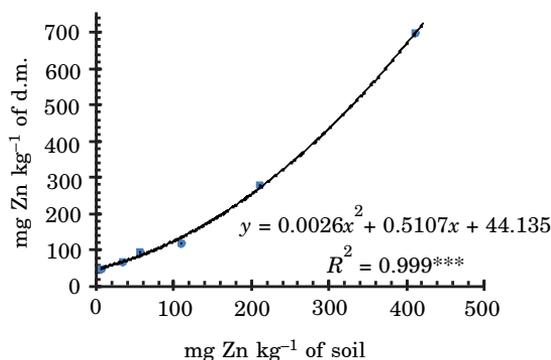


Fig. 4. Zinc concentration in the third cut of perennial ryegrass as affected by zinc content in sand (extracted in 1 M HCl)

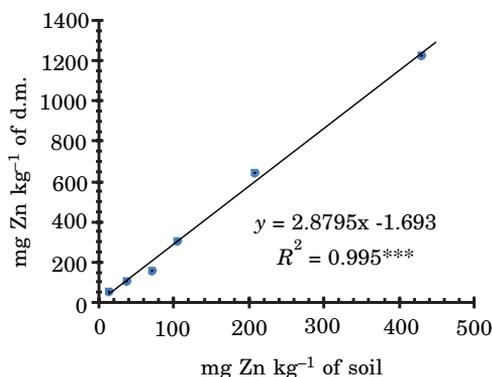


Fig. 5. Zinc concentration in the first cut of perennial ryegrass as affected by zinc content in sandy loam (extracted in 1 M HCl)

relationship between the concentration of Zn in plants and its content in soil (extracted in 1 M HCl) were very high and reached 0.998, 0.999 and 0.999 respectively for the first, second and third cut.

The concentrations of zinc in grasses growing on sand were higher than in plants harvested from the trials on sandy loam (at the corresponding levels of zinc contamination). Particularly big differences in Zn concentration in plants grown on the two different types of soil appeared in the third cut after the application of the two highest rates of zinc, i.e. 200 and 400 mg Zn kg⁻¹ (Table 3).

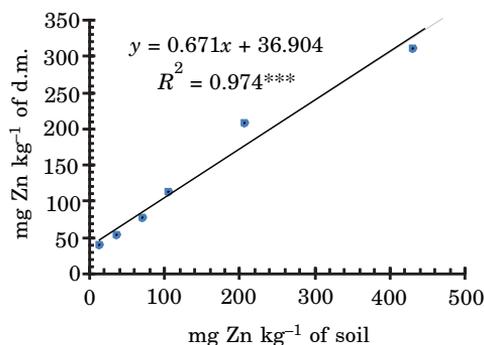


Fig. 6. Zinc concentration in the second cutting of perennial ryegrass as affected by zinc content in sandy loam (extracted in 1 M HCl)

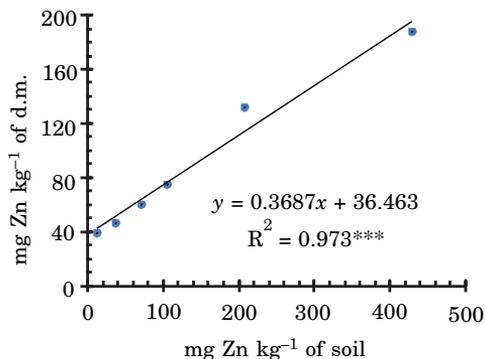


Fig. 7. Zinc concentration in the third cut of perennial ryegrass as affected by zinc content in sandy loam (extracted in 1 M HCl)

Ryegrass does not require large amounts of zinc to satisfy its nutritional demand. Nonetheless, it absorbed the metal in the amounts proportional to its content in soil. According to MISZTAL and LIGEŻA (1996), zinc is one of the most mobile elements and it is its mobility that ensures such an easy uptake of this heavy metal by plants. KICIŃSKA and HELIOS-RYBICKA (1995) reported that the concentration of zinc in plants was largely dependent on the content of this element in soil.

The concentration of zinc ranging between 15 and 50 mg Zn kg⁻¹ of plant dry matter is sufficient to cover the plant's demand for this element. CHANEY and MARSCHNER (after BROADLEY et al. 2007) report that symptoms of a toxic response to zinc appear on leaves most often when the con-

centration of the metal exceeds $300 \text{ mg Zn kg}^{-1} \text{ d.m.}$, although more sensitive plants can reveal such symptoms at a content of zinc as low as $100 \text{ mg Zn kg}^{-1} \text{ d.m.}$ of leaves. Other studies also suggest that the response of plants to soil contamination with zinc depends strongly on a plant species (BARAN et al. 2008, BARAN, JASIEWICZ 2009, SPIAK et al. 2000, STANISŁAWSKA-GLUBIAK, KORZENIOWSKA 2005). In the experiment set up on sandy loam, even when the concentration of zinc reached $639 \text{ mg Zn kg}^{-1}$ of dry matter of plants, the yield of ryegrass was high and showed no signs of the toxic influence of zinc. In contrast, when ryegrass grew on sand, the mass of grass decreased significantly at the content of zinc equal $140 \text{ mg Zn kg}^{-1} \text{ d.m.}$ These results demonstrate expressly that the content of organic and mineral colloids in soil is a factor that conditions the toxic influence of zinc on plants, and it seems to be a more important factor than the species of plants or the concentration of zinc in soil.

In the subsequent cuts of ryegrass, the concentration of zinc tended to decline in the treatments established on either of the two types of soil: sand or loam. However, a decrease in the concentration of zinc in the following grass cuts was much bigger in the treatments set up on loam than on sand. In the treatment with the highest zinc contamination dose ($400 \text{ mg Zn kg}^{-1}$ of soil), the third cut of ryegrass grown on loam contained about 6.5-fold less zinc than the first one ($1233 \text{ mg Zn kg}^{-1} \text{ d.m.}$ in the first cut and $187 \text{ mg Zn kg}^{-1} \text{ d.m.}$ in the third one); in ryegrass grown on sand, the analogous difference was about 2.3-fold ($1629 \text{ mg Zn kg}^{-1} \text{ d.m.}$ in the first cut and $696 \text{ mg Zn kg}^{-1} \text{ d.m.}$ in the third one). The results suggest that the solubility, and consequently the bioavailability of zinc and its diffusion to plant roots were diminishing considerably as the plants continued to grow. This process was much more intensive in the soil with a larger sorption complex. The activity of zinc in the experiment carried out on sand was on a constant, high level until the vegetative season terminated. Therefore, perennial ryegrass growing on soil with a low content of soil colloids accumulated high amounts of this heavy metals, regardless of the cut. In turn, zinc in the soil of the texture of sandy loam most probably quickly transformed into a hardly mobile form, which contributed to a big decrease in the concentration of Zn in the tissues of plants harvested in the second and third cut.

The uptake of zinc by ryegrass growing on sand and on sandy loam increased proportionally to the degree of soil contamination with this metal (Table 4). Despite a very high concentration of zinc in grasses growing on heavily polluted soil, the total Zn uptake in the yield from the three cuts was very small compared to the amount of this metal introduced to soil. Consequently, growing ryegrass for one season did not have any considerable effect on the content of zinc in soil after harvest (Table 5). The total uptake of Zn by ryegrass growing on sand was just 1.1-2.2% of the zinc introduced to soil. When ryegrass was grown on loam, the percentage of removed zinc was similar, i.e. 1.3-2.5%. In a study by GAWOREK et al. (2003)

Table 4

Effect of soil contamination with zinc on Zn uptake by perennial ryegrass

Zn rate (mg pot ⁻¹)	Zn uptake (mg Zn pot ⁻¹)							
	sand				sandy loam			
	1 st cut	2 nd cut	3 rd cut	total yield	1 st cut	2 nd cut	3 rd cut	total yield
0	0.69	0.72	0.71	2.12	1.52	0.80	0.83	3.15
200	2.23	1.14	1.06	4.43	2.94	1.16	0.95	5.05
400	4.31	1.59	1.46	7.36	4.66	1.63	1.24	7.53
800	4.55	2.39	1.65	8.59	8.81	2.38	1.51	12.70
1600	9.84	5.86	3.92	19.62	18.99	4.60	2.64	26.23
3200	14.39	17.86	9.32	41.57	29.81	6.45	3.84	40.10

Table 5

Effect of soil contamination with zinc on the content of this metal in the soil (extraction with 1 M HCl) after harvest of perennial ryegrass

Zn rate (mg kg ⁻¹ of soil)	Sand		Sandy loam	
	Zn content in soil after incubation with ZnSO ₄ ·7H ₂ O	Zn content in soil after harvest	Zn content in soil after incubation with ZnSO ₄ ·7H ₂ O	Zn content in soil after harvest
	mg Zn kg ⁻¹ of soil			
0	7.13	12.82	13.88	15.11
25	35.05	31.23	37.7	37.69
50	56.19	54.76	71.94	67.82
100	109.47	101.44	106.03	105.95
200	211.35	191.90	207.82	207.18
400	411.65	382.52	430.3	429.23

on the efficacy of phytoremediation of soils contaminated with heavy metals, the amount of Zn removed with plant yields did not exceed 2.5% of the content of this metal in the mobile form. A similar level of the uptake of zinc was determined by ANTONKIEWICZ and JASIEWICZ (2003), who investigated maize. Another reason why phytoremediation techniques do not prove to be very effective is the fact that soil samples analyzed after plant harvest contain some quantities of small roots, in which the concentration of heavy metals is much higher than in the aerial parts (BARAN, JASIEWICZ 2009, ZALEWSKA 2010). This is certainly reflected in the results of post-harvest soil chemical analyses.

The uptake of zinc by the yield of the second and third cut of ryegrass, having applied from 200 mg to 1600 mg Zn per pot, as well as the total Zn uptake by the yield of all the three cuts were similar in the trials established on sand and loam in the corresponding treatments. In contrast, a two-fold smaller uptake of Zn in the yield from the first cut of ryegrass growing on the sand most heavily polluted with zinc, compared to the uptake of this metal by plants growing on loam, was due to a strongly depressed mass of plants growing on very light soil with the highest dose of the contaminant.

The determination coefficients for the relationship between the Zn uptake and the content of this metal in soil were very high, for both sand and for sandy loam (Figures 8 and 9).

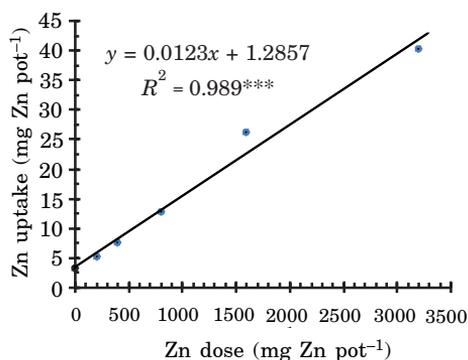


Fig. 8. Zinc uptake by total yield of perennial ryegrass as affected by zinc dose per pot on sand

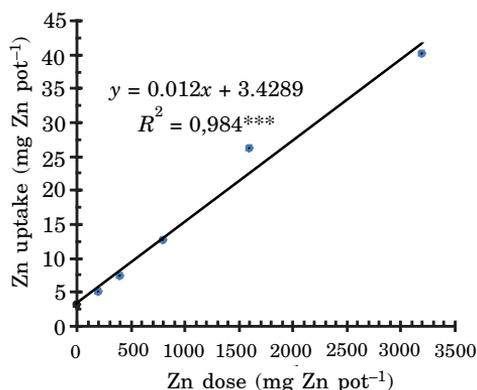


Fig. 9. Zinc uptake by total yield of perennial ryegrass as affected by zinc dose per pot on sandy loam

CONCLUSIONS

1. The toxic effect of zinc on the growth of ryegrass was much more pronounced on sand than on sandy loam. In the trial carried out on sand, even the smallest dose of zinc (25 mg Zn kg⁻¹ of soil) significantly depressed the yield of the first grass cut, whereas on sandy loam, the toxic effect of zinc did not occur until the highest rate of this metal had been introduced to soil (400 Zn kg⁻¹ of soil).

2. As the soil contamination with zinc increased, the concentration and uptake of this metal by ryegrass increased significantly. A very high concentration of Zn in ryegrass growing on soil contaminated with this metal suggests that zinc can easily permeate into plant roots and is very mobile in soil.

3. A one-year cultivation of perennial ryegrass did not have any larger effect on decreasing the content of zinc in soil. Although zinc appeared in very high concentrations in the cut grasses, its total uptake from a pot equalled just 1.1 to 1.5% of the amount of this metal introduced to soil.

4. Perennial ryegrass cv. Nira is characterized by good capability of accumulating very high amounts of zinc and is tolerant to a high content of Zn in soil, which is why it can be grown on areas contaminated with this heavy metal.

5. There is a strong, statistically verified correlation between the content of zinc in soil, extracted in 1 M HCl, and the Zn concentration and uptake by plants. Zinc extraction in 1 M HCl can be used for evaluation of the degree of soil contamination with this metal.

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