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Original Research

Assessment of *Zea mays* Sensitivity to Toxic Content of Zinc in Soil

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Abstract

Our work aims to assess *Zea mays* sensitivity to soil pollution with zinc. The aim was realized on the basis of studies on zinc's effect on the germination and initial growth of *Zea mays*, determining toxicity thresholds (PT) and estimating the toxic zinc content in zinc, at which a significant inhibition of germination and growth of roots occurs and a significant decline in this plant yields. On the basis of estimated values of EC10, EC20, and EC50 toxicity indicators and zinc phytotoxicity thresholds (PT), it was demonstrated that *Zea mays* is a plant little sensitive to over-the-norm zinc concentrations in soils, and should not be used as an indicator plant.

Keywords: zinc, toxicity thresholds, toxicity criteria, maize, phytotoxkit

Introduction

Zinc belongs to the most mobile and bioavailable elements in soil, therefore its high concentrations in soil solution may have a phytotoxic effect limiting crop yield and quality [1-3]. The phytotoxic effect of zinc may depend on soil properties, plant species, and the plant development stage, but also on metal properties and their bioavailability [4]. The state of the soil environment pollution with zinc is usually determined on the basis of the element total content in soil. However, this criterion is too general for the assessment of the toxic zinc level, because only a small part of the element total content in soil is available to plants. The degree of zinc availability to plants is diverse and depends on soil properties such as particle size composition, organic matter content, or pH [5]. Total content of zinc in soils may rather inform about its potential, not actual toxicity [6]. An equally important factor modifying zinc phytotoxicity is the plant itself, its species, and cultivar features. Among crops, cereals respond to zinc excess in soil to the greatest extent by showing a decline in yields: alfalfa, peas, lettuce, and spinach are moderately sensitive; and the most resistant are potatoes, beans, and clover [2, 6, 7]. In world literature maize is broadly described as a crop sensitive to zinc deficiency [8, 9]. On the other hand, there are few publications discussing its sensitivity to zinc excess in soil.

Our work aims to assess *Zea mays* sensitivity to soil pollution by zinc. The aim was realized on the basis of studies on zinc effect on germination and initial growth of *Zea mays* (Phytotoxkit), determining toxicity thresholds (PT10, PT20, and PT50) and estimating the toxic zinc content in zinc, at which a significant inhibition of germination and growth of roots occurs and a significant decline in this plant yields.

Materials and Methods

Studies on zinc toxicity to *Zea mays* were conducted under conditions of laboratory and pot experiments. Both experiments were carried out simultaneously on two soils, i.e. light and heavy. Light soil, with particle size composition of weakly loamy sand, was characterized by a slightly acid pH (pH = 6.20), whereas the heavy soil with particle size composition of silt clay revealed acid reaction (pH = 4.10). Organic matter content in the soils was 16 g (light soil) and 22 $g \cdot kg^{-1}$ (heavy soil). Total zinc content in the

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light soil was 62 mg and in the heavy soil 158.3 mg·kg^{-1}. According to limit heavy metal contents, the soil used for the experiment revealed an elevated content of Zn (degree I) [10]. Zinc soluble in 1 mol HCl·dm^{-3} constituted 36% of its total content in light and 16% in heavy soil.

Both in the laboratory and pot experiment the test plant was maize, Bora cv. Three levels of zinc: Zn_0 – 0 mg (control), Zn_1 – 50 mg, Zn_2 – 250 mg, and Zn_3 – 750 mg·kg^{-1} d.m. of soil were applied in both experiments. Zinc was applied as $ZnSO_4 \cdot 7H_2O$ (c.p.a, manufactured by POCH).

Laboratory test – Phytotoxkit™

The effect of soil pollution with zinc on germination and initial growth of *Zea mays* was studied in the laboratory experiment. For this purpose the Phytotoxkit™ test was conducted [11]. The studied soils were placed on test plates (21 × 15.5 × 0.8 cm) and moistened with distilled water with added zinc-containing medium (to maximum water capacity). The maximum water capacity of light soil was 27.4% and 33.2% heavy soils. The test plates with properly moistened soil were covered by a paper filter and maize seeds were sown at a rate of 8 pieces per plate. The plates prepared in this way were incubated in a horizontal position at 25ºC in darkness for 72 hours. Afterward, the image was registered by digital camera and the root length was measured using Image Tools software for image analyses. The whole experiment was conducted in three replications for each analyzed combination (zinc dose – plant – soil).

When the test was completed the soil material from each plate was dried at room temperature, thoroughly mixed, and subjected to chemical analysis. In the soil material, zinc content in 1 mol HCl·dm^{-3} was assessed by means of atomic emission spectrometry (ICP-EAS) based on inductively coupled plasma by JY 238 ULTRACE apparatus (Jobin Yvon). Conducted measurements allowed us to estimate EC10, EC20, and EC50 toxicity indices, i.e. zinc content in soil causing 10%, 20%, or 50% inhibition of seed germination, and test plant root growth and lowest observable effect concentrations (LOEC), which caused a significant inhibition of root growth. EC10, EC20, and EC50 toxicity indices were determined on the basis of linear regression equations. Inhibition of germination or root growth was assumed as the dependent variable, whereas the independent variable was zinc content in soil (mg·kg^{-1}). Taking into consideration a 10, 20, or 50% inhibition of germination and root growth process, values 90, 80, or 50 were substituted for variable y. After an appropriate transformation of the equation due to variable x, Zn mg·kg^{-1} d.m. EC 10, EC20, and EC50 of zinc were computed for the test plants under conditions of light and heavy soils. Determined value EC10 denoted the highest zinc concentrations in soil, which still does not cause a harmful effect for the test plants. LOEC was determined on the basis of ANOVA and Tukey's test.

Pot Experiment

The aim of the pot experiment was to determine the thresholds of toxicity (PT10, PT20, and PT50) that represent zinc content in maize shoots or roots causing a decline in its yield by 10, 20, or 50% and estimating toxic content of zinc in soil at which a significant decrease in this plant yield occurs. A two-year pot experiment was conducted in polyethylene pots with a volume of 5 kg of air-dried soil in four replications. Maize was harvested at the 7-9 leaves stage, after 40 days of vegetation. Equal mineral fertilization: 0.225 g N, 0.14 g P, and 0.275 g K·kg^{-1} d.m.of soil was applied on all treatments. Mineral salts such as ammonium nitrate(V), potassium dihydrogen phosphate(V), and potassium chloride were supplied before plant sowing.

After harvest the plant material was dried in a dryer with forced air flow at 70ºC and the amount of yield dry mass was determined [12]. Zinc concentrations in the shoots and roots were determined after dry combustion and dissolving the ash in 1:3 H_2O:HNO_3 v/v. Zinc concentrations in soil were assessed after sample extraction with 1 mol HCl·dm^{-3}. Zinc content in the obtained solutions was determined by means of atomic emission spectrometry with inductively coupled plasma (ICP-AES) on a JY 238 ULTRACE apparatus (Jobin Yvon). Zinc toxicity thresholds PT10, PT20, and PT50 were determined using a simple regression method [13]. Also, the dependence between maize yield and the content of zinc form soluble in 1 mol HCl·dm^{-3} in soils was determined. On this basis limit the numbers of zinc content toxic for maize in soil were determined [5]. Obtained results were verified statistically using one factor ANOVA and Tukey's Test. The test was applied when no equality was revealed between means. ANOVA was conducted at the significance level α=0.05. The significance of simple regressions was verified at the significance level α=0.05. All results were elaborated upon using Statistica 8.1.

Results

Seed germination was the first parameter used to assess *Zea mays* sensitivity to zinc. The degree germination inhibition for maize seeds was between 0 to 25% (light soil) and from 0 to 17% (heavy soil) (Fig. 1), depending on the soil and zinc level. The second parameter used for an assessment of plant response to zinc was growth of test plant roots (Table 1, Fig. 2). The degree of root growth inhibition depending on zinc dose was between 0 and 33% in light soil and from 0 to 29% in heavy soil. In heavy soil a significant reduction of maize root length was observed at 250 mg Zn·kg^{-1} d.m. (LOEC) (Table 1). Considering the soils, a weaker response (as germination inhibition) was observed in the heavy rather than the light soil. The relationship was confirmed by the determined toxicity indices EC10, EC20, and EC50, which assumed higher values for seeds in heavy soil than for seeds in light soil (Table 2).

Table 1. Roots growth depending on zinc dose in light and heavy soils.

Dose Zn mg·kg^{-1}	Light soil		Heavy soil	
	mm	SD[1]	mm	SD
0	38.45	4.90	77.75[b2]	7.30
50	33.55	8.89	63.10[ab]	1.26
250	32.01	13.34	58.06[a]	6.10
750	25.71	4.79	54.98[a]	5.32
$LSD_{0.05}$	s.i.[3]		11.56	
LOEC[4]	s.i.		250 mg	

[1]SD – standard deviation,
[2]homogenous groups according to Tukey test, α = 0.05,
[3]s.i. – statistically insignificant,
[4]LOEC – the lowest zinc dose causing a significantly inhibition growth roots of the test plant

Zinc detox ability was overcome by maize (EC50 reduction of root length by 50%) from zinc content 1194 mg Zn in light soil, and 1320.6 mg Zn·kg^{-1} d.m. in heavy soil (Table 2). The obtained results testify to a relatively small reduction of root growth in the analyzed soils. Moreover, in the case of soil comparison it is difficult to demonstrate unanimously in which of them zinc effect on root growth was less inhibitory. However, it may be stated that lower zinc doses and its lower contents in soil acted for the benefit of light soil in comparison with heavy soil. With growing zinc dose and its content in soil (a dose of 750 mg·kg^{-1}, parameters EC20 and EC50) less inhibitory conditions for root growth were stated for heavy soil.

Toxicity thresholds (PT) of zinc were estimated for maize using the linear regression method (Table 3). A decrease in total yield was assumed as an dependent variable, whereas the independent variable was zinc contents in shoots and roots (mg·kg^{-1} d.m.). Four regression equations were computed describing the relationships between a decrease in maize yield and zinc content in shoots and zinc concentration in roots. Three toxicity thresholds (PT10, PT20, and PT50) were considered, which represent zinc content in shoots or roots causing a decline in yields by 10, 20, or 50% [14]. This results from the computed data that the values of toxicity thresholds depended in the first place on zinc content in the individual parts of maize and the type of soil. Three times lower values of PT10, PT20, and PT50 for shoots than for roots were revealed, which evidences a stronger effect on a decline in maize yields of elevated zinc content in this plant part than in roots. On the other hand, considering the influence of soil type, between 3 and 36% lower values of toxicity thresholds for shoots were revealed in heavy than in light soil, whereas an opposite relationship was stated for toxicity thresholds PT20 and PT50 for roots.

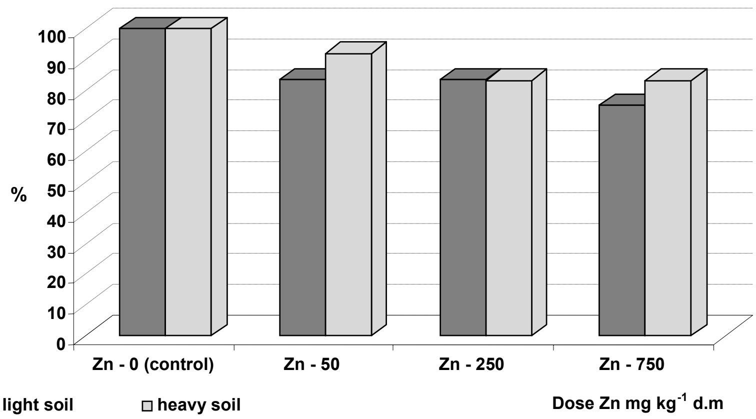

Fig. 1. Germination of maize seeds [%] depending on zinc dose in light and heavy soils.

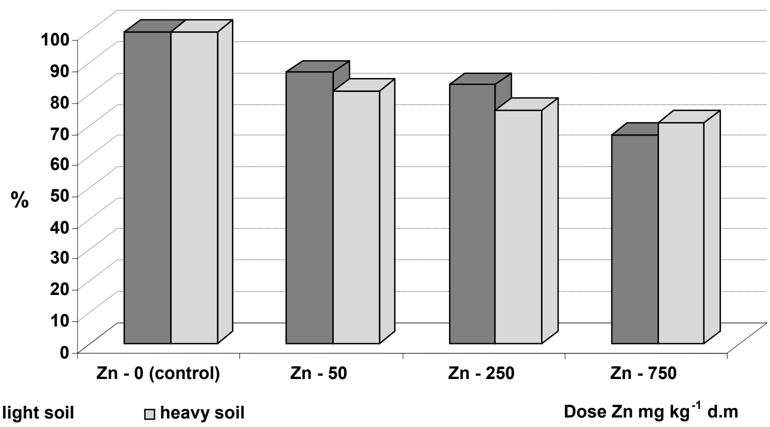

Fig. 2. Inhibition of root growth [%] depending on zinc dose in light and heavy soils.

Table 2. The zinc content of soils causes 10%, 20%, and 50% inhibition of seed germination and root growth of maize.

Parametr	Index	Light soil	Heavy soil
		mg·kg^{-1} s.m.	
Seeds germination	EC10	160.2	269.5
	EC20	463.2	686.2
	EC50	1372.3	1936.2
Root growth	EC10	51.1	30.3
	EC20	336.8	352.9
	EC50	1194.0	1320.6

Statistically significant linear regression at α = 0.05

Due to easy uptake and translocation of zinc from roots to shoots and its accumulation in vegetative and generative parts, too high concentration of this element in the soil solution is harmful to plants. Therefore, the studies estimated the threshold value from which the toxic effect of zinc begins, i.e. when a reduction of green mass resulting in a decline in yield may be expected. Using the linear regression method zinc content toxic for plants was determined in both light and heavy soils. The assumed toxicity criteria were 10, 20, and 50% decline in yields in effect of zinc doses in comparison with the control. In numerous investigations their authors suggest that even a 10% decrease in yield is significant and economically important, whereas they treat a 50% decline in yield as a very sharp criterion [5]. Taking into consideration a 10, 20, and 50% decrease in yield as a result of toxic zinc effect on plants, the values of 90, 80 or 50 were substituted for variable *y*.

After an appropriate transformation of the equation due to variable *x* Zn mg·kg^{-1} d.m., zinc content toxic for maize in light and heavy soils was computed (Table 4). Zinc contents in soil, toxic for maize, at which a 10, 20 and 50% decline in yield was noted were, respectively: 238 mg Zn, 470 mg Zn, and 1167.4 mg Zn in light soil; and 217.7 mg Zn, 666.6 mg Zn, and 2013.3 mg Zn·kg^{-1} d.m.in heavy soil. Analysis of the concentrations of zinc soluble in 1 mol HCl·dm^{-3} in light and heavy soil, at which a considerable decrease in maize yield occurred, revealed that in light soil lower zinc contents caused 20 and 50% diminishing of maize yield in comparison with heavy soil. This evidences better availability of this metal in conditions of light soil. The fact was also confirmed by zinc solubility in 1 mol HCl·dm^{-3}, which was better in light than heavy soil (80% of its total content in light soil and 57% in heavy soil). In light soil soluble zinc constituted between 66 and 98%, whereas in heavy soil from 45 to 75% of its total content. It was also stated that with the growing toxic effect of zinc its contents causing a 20% or 50% decline in maize yields in heavy soil were from 1.5 times to twice higher in comparison with light soil.

Discussion

The author's own studies revealed that maize is little sensitive to soil pollution with zinc. Therefore, it may be presumed that maize is not a good marker of zinc excess in the environment. Rybak et al. [15] obtained similar results in their research on phytotoxicity of post-flotation sludge and sewage sludge. In his investigations on an assessment of various plant species' sensitivity to cadmium, An [4] also demonstrated that maize is not a proper marker of soil pollution with this metal. Values EC50 shoot, which he

Table 3. Zinc phytoxicity thresholds PT10, PT20, and PT50 for maize

Part of plants	Soil	Phytoxicity thresholds mg·kg^{-1} d.m.		The regression equation	R^2	p
Shoots	Light	PT10	276	[1]y = 95.39-0.022x	0.81	<0.0006
		PT20	745			
		PT50	2151			
	Heavy	PT10	203	y = 94.31-0.021x	0.82	<0.0005
		PT20	674			
		PT50	2085			
Roots	Light	PT10	794	y = 99.97-0.013x	0.83	<0.0004
		PT20	1590			
		PT50	3978			
	Heavy	PT10	604	y = 94.58-0.008x	0.80	<0.0007
		PT20	1921			
		PT50	5873			

[1]y – yield decrease in %, x – zinc contents in shoots and roots (mg·kg^{-1} d.m.)

Table 4. Content of zinc soluble in 1 mol HCl in soils, resulting in 10, 20, and 50% reduction in yield of maize (shoot + roots).

Soil	Yield decrease	Zn mg·kg^{-1} d.m	The regression equation	R^2	p
Light	10	238.0	y = 100.24-0.04x	0.83	<0.00038
	20	470.3			
	50	1167.4			
Heavy	10	217.7	y = 94.85-0.02x	0.83	<0.00032
	20	666.6			
	50	2013.3			

[1]y – yield decrease in %, x – zinc contents in light and heavy soil (mg·kg^{-1} d.m.).

obtained in his research for sorghum, and cucumber, wheat, and maize, were, respectively 61, 88, 113, 268 mg Cd·kg^{-1} d.m. of soil, which pointed to sorghum as the plant most sensitive to cadmium. Taking into consideration the parameters for toxicity assessment, i.e. seed germination and root growth, it was found that they differed definitely with their sensitivity to zinc effect and the parameter of test plant root growth showed a greater sensitivity than seed germination parameter (Table 2). The relationship was corroborated by observations of other scientists, who reported that seed germination capacity is an indicator which is to a relatively small degree affected by the presence of heavy metals, oil derivatives, and some polymers or lecheates from municipal landfills [16, 17]. It was demonstrated that many plants sensitive to heavy metals germinate in polluted environment but later stop growing, therefore root growth parameter is more sensitive and useful for determining toxicity criteria [4, 17, 18]. Moreover, the authors mentioned above revealed that root growth is a better indicator of plant sensitivity to metals (copper and cadmium) than shoot growth. In the research conducted by Baran et al. [19] on zinc and cadmium harmfulness for *Linum usitatissimum*, *Vicia sativa*, *Pisum sativum*, *Sinapis alba*, *Lepidum sativum*, and *Sorghum saccharatum* also observed weaker inhibition of seed germination as compared with strongly inhibited growth of these plant roots. Moreover, as was demonstrated in the presented research and studies conducted by other authors [20], the properties of soil material considerably influence plant response.

The value of zinc toxicity threshold computed in the presented investigations for maize shoots (PT20) causing a 20% decline in yield corresponded to the results obtained by other authors (Table 5). In the author's own investigations PT20 value for maize shoots was 745 mg Zn·kg^{-1} d.m. for light soil and 674 mg Zn·kg^{-1} d.m. for heavy soil. Estimated toxicity thresholds both in the presented research and determined by other authors pointed to a relatively high maze resistance to elevated zinc concentrations in soil (Table 5).

Plants sensitive to zinc excess comprise wheat, for which PT20 grain was 69 mg and PT20 shoot 169 mg Zn·kg^{-1} d.m. [14]. Also, alfalfa is a crop strongly responding to high zinc concentrations in its shoots with a decline in yield, where PT10 was 200 mg Zn·kg^{-1} d.m. [6]. Grasses belong to plants insensitive to elevated zinc concentrations. According to studies of Paschke et al. [21] a 50% reduction of their yield was observed at zinc concentrations in shoots from 2249 to 5026 mg Zn·kg^{-1} d.m. In the author's own investigations PT50 shoots approximated 2000 mg Zn·kg^{-1} d.m., whereas PT50 roots were almost twice (light soil) and thrice (heavy soil) higher than PT50 shoots. On the other hand, in the research of Spiak et al. [22] the limit to zinc toxicity in plants determined on the basis of tolerance index ranged from 198 (serradella) to 285 (oats) mg Zn in light soils, from 450 (serradella) to 643 (oats) mg Zn in medium soils, and from 500 (mustard) to 2000 (oats) mg Zn·kg^{-1} d.m. in heavy soils. Chaney [23] reported that toxic symptoms in plant tissues are visible at zinc concentrations in leaves > 300 mg·kg^{-1} d.m. However, there are plants that reveal visible symptoms of zinc excess already at zinc content in leaves < 100 mg·kg^{-1} d.m. Limit values for zinc toxicity are different not only for individual species but fluctuate depending on the development stage and plant part. Zinc concentrations in dry mass that in effect lead to depression in yield are generally higher at the earlier than later development stages.

Table 5. Phytotoxicity threshold calculated by different authors.

Plant	[1]Type of thresholds	Threshold mg Zn·kg^{-1} d.m.	Author
Wheat	PT20 shoot	169	Korzeniowska, Stanisławska-Glubiak (2006) [14]
	PT50 shoot	333	
	PT20 grain	69	
	PT50 grain	118	
	PT20 shoot	560	Bowan, Rasmussen (1971) [27]
Maize	PT20 shoot	460	
	PT20 leaf	1000	Chang et al. (1992) [28]
Grass	PT50 shoot	2249-5026	Paschke et al. (2000) [21]
Pea	PT10 shoot	380-500	Stanisławska-Glubiak Korzeniowska (2005) [6]
Alfalfa	PT10 shoot	200	
Lettuce	PT10 leaf	530	

[1]PT20, 50, 10 – reduction of grain/shoot yield about 20, 50, and 10%

It is worth mentioning that comparing the author's own results for determining zinc toxicity thresholds with results obtained by other researchers is very difficult. On one hand different plant species and their parts are used for analyses, on the other, important issues are different conditions under which investigations were conducted, especially the type of soil. A considerable diversification of toxicity threshold values, even for the same plant species, was also reported by Chaney [23] and Davies [24]. For instance PT50 connected with 50% reduction of radish yield ranges from 36 to 1013 mg $Zn \cdot kg^{-1}$ d.m. On the other hand Paschke et al. [13, 21] demonstrated for various plants PT50 shoot between 1258 and 3214 mg $Zn \cdot kg^{-1}$ d.m. However, determining toxicity thresholds not only for zinc but also for other metals may be useful for identification of plant species, which may be used for reclamation of chemically degraded soils. High values of PT50 computed in the Author's own studies for shoots point to maize as the plant with high potential to be used for reclamation of soil or sludge polluted with zinc. The problem of further utilization of the obtained biomass remains a problem. Biomass that does not fulfil the fodder criteria may be used for energy generating purposes or for compost to be used for turfed areas, not for agricultural production.

Zinc concentrations in the spoil presented above, which cause 10, 20 and 50% declines in maize yield, are high in comparison with other investigations. Stanisławska-Glubiak et al. [5] revealed that a 10% decrease in yield of wheat grain, depending on the soil and its pH, is caused by zinc content of between 16 and 20 mg Zn in very light soil and between 63 and 157 mg $Zn \cdot kg^{-1}$ d.m. in medium soil. On the other hand, in research conducted by Gembarzewski et al. [25] it was calculated that a 10% decline in wheat yield on light, very acid soil is caused by 18 mg Zn, 50% by 34 mg $Zn \cdot kg^{-1}$ d.m., and for clover toxic Zn concentrations in soil were respectively from 7 to 60 mg Zn (10% decrease in yield) and from 30 to 88 mg $Zn \cdot kg^{-1}$ d.m. (50% decline in yield), whereas for maize no decrease in yield was demonstrated under the influence of applied zinc doses. Other investigations have revealed that the zinc limit content in soil at which a decrease in wheat yield occurs is about 100 $mg \cdot kg^{-1}$ of zinc soluble in 1 mol $HCl \cdot dm^{-3}$ [26]. The differences are undoubtedly due to the conditions under which the experiments were conducted, because the values of zinc in soil stated above come from the experiments on soil where various liming levels were applied to diversify their reaction, which may affect the reduction of the amount of zinc-soluble forms in soil. Moreover, the influence might have been modified by diversified species sensitivity to zinc content in soil.

In conclusion it may be said that currently there is no uniform data concerning the method of determining the upper critical zinc content in soils and plants. Some authors state that the plants tolerant to this metal are those that are able to develop and reproduce properly at zinc concentrations other plants are incapable of. Therefore, toxicity criteria that indicate whether a given plant is sensitive to soil pollution with heavy metals should be based on interdependencies between soil and plant, on which the biological and chemical effects of zinc harmfulness is observed.

Conclusions

1. On the basis of estimated values of EC10, EC20, and EC50 toxicity indicators and PT10, PT20, and PT50 zinc phytotoxicity thresholds it was demonstrated that *Zea mays* is a plant little sensitive to over-the-norm zinc concentrations in soils and should not be used as an indicator plant.
2. Zinc contents in soil, toxic for maize, at which a 10, 20, or 50% decline in yield occurred were, respectively: 238 mg Zn, 470 mg Zn, and 1167.4 mg Zn in light soil, and 217.7 mg Zn, 666.6 mg Zn, and 2013.3 mg $Zn \cdot kg^{-1}$ d.m. in heavy soil.
3. Better bioavailability and greater phytotoxicity of zinc were revealed on light than on heavy soil. On light soil lower values of zinc soluble in 1 mol $HCl \cdot dm^{-3}$ caused a 20 and 50% diminishing of maize yield in comparison with heavy soil, which testifies to the better availability of this metal in light soil.
4. Estimated high values of toxicity threshold point to maize as a plant with high potential for use in the reclamation of soils or sludge polluted with zinc.
5. Toxicity criteria that indicate whether a given plant is sensitive to soil pollution with zinc or other heavy metals should be based on the interrelations between soil and plant on which the biological and chemical results of metal harmfulness are observed.

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