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Cd, Zn, Cu, Pb, Co, Ni PHYTOTOXICITY ASSESSMENT

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Abstract. In this paper, we investigate using of probit analysis for heavy metals (Pb, Cd, Cu, Zn, Co, Ni) toxicity assessment for spring barley (*Hordeum vulgare* L.) in sod podzolic sandy loam and chernozem soils. Estimation of the heavy metals phytotoxicity by means of $PhLD_{50}$ value was suggested. The $PhLD_{50}$ value is a doze of metal in soil that causes 50% reduction of plant biomass ($mg \cdot kg^{-1}$). According to $PhLD_{50}$ value, metals can be ranked by the effect on biomass reduction as: Cd>Cu>Ni>Co>Pb>Zn (sod podzolic soil) and Cd>Cu>Ni>Co>Zn>Pb (chernozem soil). Results of the study could be useful indicators of Cu, Ni, Co, Cd, Pb and Zn phytotoxicity assessment at the growing of *Hordeum vulgare* (L.) in heavy metals contaminated areas. The $PhLD_{50}$ value demonstrates the comparative toxicity of metals. Tight correlation between studied metals phytotoxicity for plants of spring barley and polarity shift caused by adding to organic matrix – diphenilthiocarbazone (ditizone) for studied metals was observed. This approach may be prominent for metals risk assessment. This work is an attempt to extend our investigations on correlation and methods of polarity assessment and ecotoxicological risk of different groups of contaminants.

Keywords: heavy metals, phytotoxicity, probit analysis, ecotoxicological assessment, $PhLD_{50}$, pollution, dipole moment, barley plants

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INTRODUCTION

Environmental degradation is a widely recognized global challenge. Some of the problems affecting the world now are acid rain, global warming, hazardous wastes, over population, ozone depletion, smog and environmental pollution (Kaonga *et al*. 2010). Environmental pollution is one of the major causes of environmental degradation worldwide. Heavy metal pollution not only affects the production and quality of crops, but also influences the quality of the atmosphere and water bodies, threatens health and life of animals and human beings by inclusion into food chains. Most severe is that this kind of pollution is covert, long term and non-reversible (Zhang *et al.* 2008). Heavy metals are also one of the major contaminating agents in our food supply (Kaonga *et al*. 2010, Zhang *et al*. 2008, Gill 2014, Kabata-Pendias and Mukherjee 2007, Valavanidis and Vlachogianni 2010, Ryzhenko 2012, Ryzhenko and Kavetsky 2015, Alloway 2010). Major causes of environmental heavy metals pollution are: use of sewage sludge in agriculture, metal mining, extensive use of pesticides and chemical fertilizers (Jakubus 2012, Wu *et al*. 2012, Campaña *et al*. 2014. Wang *et al*. 2003, Liu *et al*. 2007). Specifically, metals are non-degradable and, therefore, they can persist for long periods in aquatic and terrestrial environments (Kaonga *et al*. 2010, Zhang *et al*. 2008, Liu *et al*. 2007, Römbke and Moltmann 1996). Investigation of heavy metals phytotoxicity in polluted soil is important because pollutants concentration in crops determines the quality of agricultural products.

Phytotoxic effects of different heavy metals concentration on seed germination and seedling growth in various crops: *Daucus carrota* (L.), *Raphanus sativus* (L.), *Beta vulgaris* (L.), *Lycopersium esculentum* (L.) and *Solanum melongena* (L.), *Vigna radiata* (L.), *Vigna angularis* (L.), *Lablab purpureus* (L.), *Lathyrus ordoratus* (L.), *Triticum aestivum* (L.), etc. were reported (Valerio *et al*. 2007, Azimi *et al*. 2006, Ilyin and Syso 2001, Mantorova 2010). There are many indexes of phytotoxicity: Vigor Index, Seedling vigor index, Tolerance index, Relative root elongation, etc (Wang *et al*. 2008, DeLgado *et al*. 2010, Amin *et al*. 2013). LD_{50} index is often used in toxicology assessment as "doze-effect" correlation, showing the dose causes death of 50% of animals in experiment. However, plant death caused phytotoxicity, in our opinion, is not reliable index of toxic process in plants. Most of the phytotoxicity indexes mentioned above estimate reduction of physiological process or morphological characteristics (e.g. plants weight, height, size of the root, etc.). Due to natural variability, most of the plant observation methods (notations) use, as standard approach, observation of 10%, 50%, and 90% of the whole population of plants changes (Kabata-Pendias and Mukherjee 2007, Ryzhenko and Kavetsky 2015, Ilyin and Syso 2001, Mantorova 2010, Wang *et al*. 2008, DeLgado *et al*. 2010, Amin *et al*. 2013).

Similarly, for estimation of phyto-toxicological effect of impact pollution we propose to use phytotoxic dose 50% ($PhLD_{50}$) caused reduction of 50% of initial weight (height, length of root, etc.). $PhLD_{50}$, therefore, could assess the phytotoxicity effect very well because the index implies the dose of pollutant reducing 50% of plant weight. The higher is value of $PhLD_{50}$ index – the less HM phytotoxicity.

Usually LD_{50} index is used in toxicology as "doze-effect" correlation (Fig. 1). LD_{50} index calculated with application of *probit analysis* allows comparing toxicity of each toxicant for clear assessment (Bliss 1934, Dospekhov 1985). We applied the same approach – *probit analysis* for estimation of dose effecting 50% reduction of initial weight of plants to assess the HM phytotoxicity in soils for spring barley (*Hordeum vulgare* L.) in condition of impact polluted sod podzolic sandy loam and chernozem soils.

During the evolution of plants, only a few heavy metals were incorporated in metabolic process. Phytotoxicity of plants to various heavy metals occurs by surpassing critical levels. It depends on the capability of species, cultivars and genotypes to handle appropriately uptake, translocation, incorporation into organic compounds, and cellular compartmentation of these metals. Phytotoxicity of heavy metals is the result of the imbalance between the uptake of an element and incapability of the metabolism to cope with its cellular, especially cytosolic concentration (Kabata-Pendias and Mukherjee 2007, Valavanidis and Vlachogianni 2010, Ryzhenko 2012, Ryzhenko and Kavetsky 2015). Phytotoxicity of heavy metals is considered inhibitory for plant growth (Kabata-Pendias and Mukherjee 2007, Valavanidis and Vlachogianni 2010, Ryzhenko 2012, Ryzhenko and Kavetsky 2015, Wang *et al*. 2008, DeLgado *et al*. 2010, Amin *et al*. 2013). The presence of heavy metals in soil disrupts the pattern of nutrient uptake in plant because of nutrient metal interaction (Kabata-Pendias and Mukherjee 2007, Valavanidis and Vlachogianni 2010, Ryzhenko 2012).

Tight correlation between pesticides behavior in environment and their polarity was demonstrated in papers of Kavetsky and Ryzhenko (2008), and Kruk and Kavetsky (1999). Pesticides polarities were determined by dipole moments. Each of pesticides properties such as persistent in plant and soil, solubility in different solutions, volatility, etc. had high correlation with pesticides dipole moment (μ). Based on this, Kavetsky and Bublik (1989) worked out the aLgorithm of extraction and chromatographing of pesticides with different dipole moment and work out the scale of pesticides phytotoxicity according to their dipole moment (μ) (Kavetsky and Ryzhenko 2008, Kruk and Kavetsky 1999). But similar ideas for heavy metals (HM) have not been suggested or applied by any authors yet.

Although ions do not have a dipole moment, they can influence the polarity of substances containing these ions. We assume that all metals may influence the polarity of compounds in which they are included in the same way. Based on that assumption, we added different metals to model matter. As a model matter we used the *dyphenilditiokarbazone* (short name – *ditizone*). Defining the polarity of metals ditizonate we tried to detect correlation between ditizonates polarity shift, caused by adding of metals, and phytotoxicity.

MATERIALS AND METHODS

Spring barley (*Hordeum vulgare* L.) was selected as a model plant. It is one of the major cereal crops in Ukraine. Mean standard deviations, variance, and minimum, maximum, standard errors were calculated from at least three replicates. The experimental results were interpreted using standard statistical methods.

The soils of experimental pots were: sod podzolic sandy loam on layered glacial sands (sod podzolic) and calcareous deep chernozem on loamy loess (cernozem). Sod podzolic soil has the following physic chemical characteristics: pH_{salt} – 5.5; organic matter by Turin – 0.87%, CEC – 6.3 mg eqv·100^{-1} g. Chernozem soil has the following features: pH_{salt} – 6.2, organic matter by Turin – 2.89%, CEC – 27.1 mg eqv·100^{-1} g.

Studied trace elements: Cd, Pb, Zn, Cu, Co, Ni were applied separately in the amount equal to the following concentration in the soils (Table 1):

TABLE 1. SCHEME OF ADDING TOXIC DOSES OF METALS INTO THE SOIL

Control (no HM application)	
Cu^{2+}:	Zn^{2+}:
100 mg·kg^{-1} of the soils	600 mg·kg^{-1} of the soils
150 mg·kg^{-1} of the soils	900 mg·kg^{-1} of the soils
200 mg·kg^{-1} of the soils	1,200 mg·kg^{-1} of the soils
300 mg·kg^{-1} of the soils	1,500 mg·kg^{-1} of the soils
Co^{2+}:	Ni^{2+}:
60 mg·kg^{-1} of the soils	70 mg·kg^{-1} of the soils
300 mg·kg^{-1} of the soils	210 mg·kg^{-1} of the soils
480 mg·kg^{-1} of the soils	350 mg·kg^{-1} of the soils
540 mg·kg^{-1} of the soils	420 mg·kg^{-1} of the soils
600 mg·kg^{-1} of the soils	700 mg·kg^{-1} of the soils
Cd^{2+}:	Pb^{2+}:
15 mg·kg^{-1} of the soils	150 mg·kg^{-1} of the soils
30 mg·kg^{-1} of the soils	300 mg·kg^{-1} of the soils
60 mg·kg^{-1} of the soils	450 mg·kg^{-1} of the soils
90 mg·kg^{-1} of the soils	900 mg·kg^{-1} of the soils
150 mg·kg^{-1} of the soils	1,200 mg·kg^{-1} of the soils
300 mg·kg^{-1} of the soils	1,500 mg·kg^{-1} of the soils

That amount corresponds with adopted in Ukraine Maximum Allowed Concentration (MAC) in soil (Medvedev *et al.* 1998). The following metals salts: $Pb(NO_3)_2$, $ZnSO_4{\cdot}H_2O$, $CuSO_4{\cdot}7H_2O$, $CdSO_4$, $NiSO_4{\cdot}6H_2O$, $CoSO_4{\cdot}7H_2O$ were used for the trace elements application. The investigation was conducted in green house conditions. Plants grew in plastic Mitcherlikh's pots. Soil preparation, pots filling, and trials were carried out in accordance with standard methodic (Dospekhov 1985, Medvedev *et al*. 1998). The metals were added to soil during soil preparation before filling the pots. Then, spring barley germinated seeds were planted into the pots and, in the stage of 3 leaves, the recommended population was established.

The studied elements were extracted by 1 M HCl from the soils. The method of HM determination was thin layer chromatography (TLC). Method widely was used in our previous investigation and officially recognized in Ukraine (Kavetsky *et al*. 2001).

The method of polarity determination was based on the correlation between *Rf* of a substance and the value of *dialectical permeability (*ε*)* of mobile phase during separation of the substance in the thin layer (TLC) (Kavetsky and Bublik 1989). *Ditizone* forms compounds with Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}. Dipole moments of metals ditizonates were defined by using of thin layer chromatography. Dipole moments were determined as a correlation between *Rf* value of metal ditizonate and *dialectical permeability of mobile phase*.

Ditizonates of Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+} were successively separated on chromatographic plate "Silufol" in mobile phases with different ration of hexane: acetone. Mobile phases with different ratio of hexane: acetone has different *dielectric constant* (ε), and, therefore, *Rf* of same substances were different.

Dipole moment index (μ) was calculated as (Kavetsky and Bublik 1987):

$$\mu = \frac{Rf_2^2\ x\varepsilon_1 - Rf_1^2\ x\varepsilon_2}{Rf_2^2 - Rf_1^2} \qquad (1)$$

Where: *Rf* – ratio between distance passed the spot of ditizonate of a metal to distance passed the mobile phase with certain *dialectical permeability* (ε).

Probit analysis was applied according to Dospekhov (1985). Probit values were found in "Bliss table" which transformed the percentage of killed plant into probit (Bliss 1934). The idea of the probit function was published by C.I. Bliss (1899–1979) in *Science* in 1934 on how to treat data such as the percentage of a pest killed by pesticide (Table 2) (Bliss 1934). Next, the method introduced by Bliss was transformed into *Probit Analysis* for toxicological applications by D.J. Finney (1917–). The mathematical interpretation of experimental data by using *S*-curve of doze-effect correlation is difficult (Fig. 1). Usually, according to *Probit Analysis*, the linear correlation between lg D and *probit* value is used.

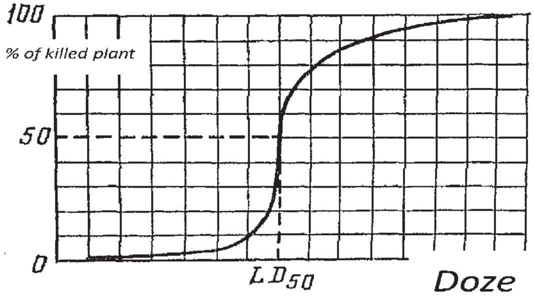

Fig. 1. Curve of doze-effect correlation (Bliss 1934, Dospekhov 1985)

TABLE 2. TRANSFORMED PERCENTAGE OF KILLED PLANT INTO PROBIT (BLISS 1934, DOSPEKHOV 1985)

Killed plant (%)	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	5.05	7.33

Experimental data are shown in Table. Except experimental data, Table 3 includes the values of lg **D** (where **D** is a 1 M HCl extracted forms in soil, $mg \cdot kg^{-1}$) and probit values.

TABLE 3. HEAVY METALS POLLUTION IMPACT ON CONCENTRATION OF ITS AVAILABLE FORM IN SOIL AND REDUCTION OF SPRING BARLEY BIOMASS

Sod podzolic						
Heavy metal	D 1 M HCl extracted forms in soil, $mg \cdot kg^{-1}$	Plants' weight, g	Plants weight compared to control, %	Reduction of spring barley biomass, %	lg D	Probit values
Cd	22.9±0.3	25.3±0.20	80.70	19.3	1.36	4.12
	46.4±0.5	18.2±0.10	57.80	42.2	1.67	4.80
	77.1±0.6	12.3±0.10	39.30	60.7	1.89	5.28
	101.2±0.8	7.3±0.10	23.55	76.5	2.00	5.74
	153.1±1.2	1.4±0.05	4.40	95.6	2.18	6.75

Heavy metal	D 1 M HCl extracted forms in soil, mg·kg^{-1}	Plants' weight, g	Plants weight compared to control, %	Reduction of spring barley biomass, %	lg D	Probit values
Sod podzolic						
Pb	231.9±2.6	27.2±0.2	86.50	13.5	2.37	3.92
	347.7±3.8	24.6±0.2	78.30	21.7	2.54	4.23
	695.1±4.3	15.2±1.5	48.30	51.7	2.84	5.05
	930.0±5.0	7.5±0.5	24.19	75.8	2.97	5.71
	1158.3±5.6	1.7±0.1	5.50	94.5	3.06	6.64
Cu	67.2±0.9	28.2±0.3	89.70	10.3	1.83	3.72
	102.9±1.6	25.1±0.3	80.00	20.0	2.01	4.16
	135.5±1.9	15.0±0.1	48.40	51.6	2.13	5.05
	173.8±1.8	5.5±0.1	17.60	82.4	2.24	5.92
Zn	427.4±4.2	26.8±0.3	85.40	14.6	2.63	3.96
	550.3±4.9	24.8±0.3	79.10	20.9	2.74	4.19
	685.7±5.2	11.5±0.2	37.1	62.9	2.84	5.33
	743.0±6.0	3.5±0.1	11.20	88.8	2.87	6.23
Co	36.5±0.4	30.8±0.4	98.0	2.0	1.56	2.95
	125.0±1.2	29.2±0.2	93.0	7.0	2.10	3.52
	159.6±1.7	16.64±0.1	53.0	47.0	2.20	4.92
	191.0±2.0	8.80±0.5	28.0	72.0	2.28	5.58
	219.6±2.0	3.2±0.1	10.2	89.8	2.34	6.28
Ni	39.0±0.4	30.9±0.5	98.5	1.5	1.59	2.95
	91.4±1.0	29.3±0.5	93.2	6.8	1.96	3.52
	148.9±1.5	17.0±0.2	54.0	46.0	2.17	4.90
	178.9±1.8	8.0±0.1	25.5	74.5	2.25	5.67
	210.0±2.2	2.8±0.1	9.0	91.0	2.32	6.34
Chernozem						
Cd	20.8±0.2	30.2±0.4	94.30	6.0	1.32	3.45
	41.7±0.4	23.4±0.3	73.10	26.9	1.62	4.39
	68.2±0.5	15.8±0.2	49.30	50.7	1.83	5.03
	92.5±0.7	10.5±0.2	33.9	66.1	1.97	5.41
	138.9±1.5	5.6±0.1	17.50	82.5	2.14	5.95
Pb	212.6±2.4	29.4±0.3	91.73	8.3	2.33	3.59
	319.7±4.0	31.5±0.3	98.41	1.6	2.50	2.95
	653.8±5.7	18.7±0.2	58.50	41.5	2.82	4.8
	902.5±7.8	10.0±0.2	32.3	67.7	2.95	5.47
	1062.0±9.8	3.3±0.1	10.20	89.8	3.03	6.23
Cu	59.5±0.6	30.8±0.3	96.10	3.9	1.77	3.25
	87.6±1.0	28.9±0.3	90.30	9.7	1.94	3.72
	111.0±1.4	20.0±0.2	64.52	35.5	2.05	4.64
	144.3±1.2	15.4±0.2	48.10	51.9	2.16	5.05
Zn	382.3±3.5	29.5±0.1	92.20	7.8	2.58	3.59
	483.5±3.8	27.7±0.3	86.70	13.3	2.68	3.87
	640.5±5.8	16.3±0.2	52.58	47.4	2.81	4.92
	656.5±7.0	9.8±0.2	30.50	69.5	2.82	5.52

Sod podzolic						
Heavy metal	D 1 M HCl extracted forms in soil, mg·kg^{-1}	Plants' weight, g	Plants weight compared to control, %	Reduction of spring barley biomass, %	lg D	Probit values
Co	41.5±0.4	31.1±0.4	99.0	1.0	1.62	2.67
	132.7±1.5	30.0±0.4	95.6	4.4	2.12	3.25
	164.0±1.7	18.5±0.3	58.9	41.1	2.21	4.73
	215.8±2.5	9.8±0.2	31.2	68.8	2.33	5.5
	245.5±2.5	0.6±0.1	1.8	98.2	2.39	7.05
Ni	43.0±0.3	31.1±0.3	99.0	1.0	1.63	2.67
	97.0±0.7	29.6±0.3	94.3	5.7	1.99	3.45
	154.8±1.1	18.2±0.2	58.1	41.9	2.19	4.80
	186.5±2.0	8.6±0.2	27.4	72.6	2.27	5.61
	222.5±2.4	3.5±0.1	11.1	88.9	2.35	6.23

RESULTS AND DISCUSSION

Results showed that all the heavy metals individually affected the weight of barley as compared to control (Table 3). There could be many and varied causes of weight reduction. However, factors affecting cell division and cell expansion depending on HM properties might have played a key role from the ecotoxicological view point (Kaonga *et al*. 2010, Gill 2014, Kabata-Pendias and Mukherjee 2007, Wang *et al.* 2003, Ilyin and Syso 2001). Applied amounts of Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} reduced the total weight of the whole plant.

Phytotoxicity effect of HM

Heavy metal poisonousness is the product of multifaceted interaction of chief noxious ions with other vital or non-essential ions. The metals can be a source of decrease in the hydrolysis products viz., α-amylase, Phosphatase, RNAs and proteins. They disrupt enzymes activities by substituting metal ions from the metalo-enzymes and prevent various physiological developments of plants (Kaonga *et al.* 2010, Zhang *et al*. 2008, Gill 2014, Kabata-Pendias and Mukherjee 2007). Different rare metals are crucial for plants, showing main roles in plant anabolism, catabolism and biosynthesis, together as cofactors for enzymes and as metabolic yields (Kabata-Pendias and Mukherjee 2007, Ilyin and Syso 2001, Mantorova 2010). For example, Zn, Fe, Cu, Cr, and Co are critical nutrients but turn into toxic elements at greater amounts.

Relationship between lg **D** of Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and probit on the studied soils are shown in Figures 2 and 3. $PhLD_{50}$ and $PhLD_{95}$ for each investigated metals in two studied soils were calculated. $PhLD_{50}$ is the doze of a metal in soil that causes 50% reduction of plant biomass (mg·kg^{-1}). $PhLD_{95}$ is a doze of a metal in soil that causes 95% reduction of plant biomass (mg·kg^{-1}). Only the $PhLD_{50}$ was used in our studies for HM phytotoxicity assessment.

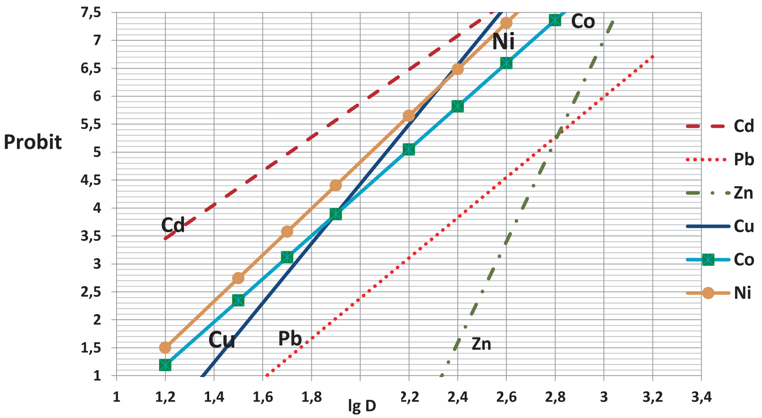

Fig. 2. Correlation between lg **D** of heavy metals and probit in the condition of sod podzolic sandy loam on layered glacial sands

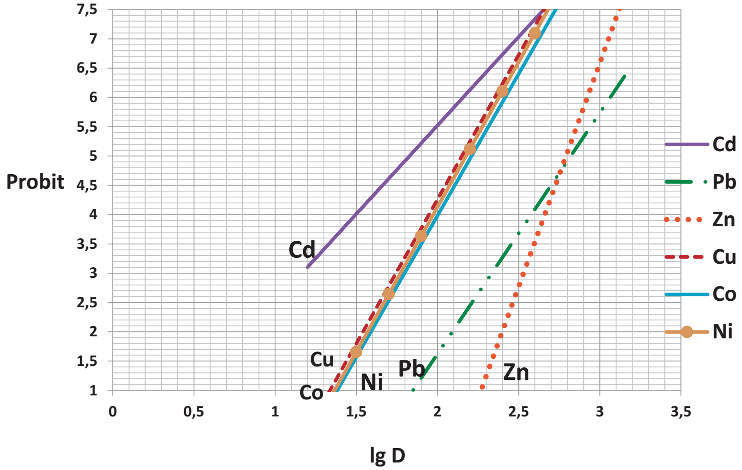

Fig. 3. Correlation between lg **D** of heavy metals and probit in the condition of calcareous deep chernozem on loamy loess

The correlation between lg **D** of Cd^{2+} and a probit for sod podzolic sandy loam on layered glacial sands was:

$$y = 3.0274x - 0.1749 \qquad (2)$$

If probit equals 5 ($PhLD_{50}$ calculation):

$$5 = 3.0274x - 0.1749, \text{ and } x=1.7 \qquad (3)$$

The antilogarithm (1.7)=50-$PhLD_{50.}$

Equations (Table 4), $PhLD_{50}$ values, and $PhLD_{95}$ (Table 5) values were obtained for all heavy metals in both studied soils.

TABLE 4. CORRELATION BETWEEN LG D AND PROBIT

Metal	Equations
	Sod podzolic
Cd	$y = 3.0274x - 0.1749$ (R^2=0.94)
Pb	$y = 3.6038x - 4.8227$ (R^2=0.92)
Zn	$y = 9.036x - 20.099$ (R^2=0.85)
Cu	$y = 5.3198x - 6.2087$ (R^2=0.93)
Co	$y = 3.8571x - 3.4384$ (R^2=0.80)
Ni	$y = 4.1516x - 3.4822$ (R^2=0.88)
	Chernozem
Cd	$y = 3.0225x - 0.5224$ (R^2=0.99)
Pb	$y = 4.113x - 6.6035$ (R^2=0.84)
Zn	$y = 7.6369x - 16.317$ (R^2=0.89)
Cu	$y = 4.9278x - 5.594$ (R^2=0.95)
Co	$y = 4.8313x - 5.6795$ (R^2=0.71)
Ni	$y = 4.944x - 5.7593$ (R^2=0.92)

Cadmium was the most toxic for spring barley in our investigation. Cadmium has a bad reputation for being highly toxic and threatening to plant growth (Kabata-Pendias and Mukherjee 2007, Valavanidis and Vlachogianni 2010, Ryzhenko 2012, Alloway *et al.* 2010, Azimi *et al.* 2006). This metal had least $PhLD_{50}$ and $PHLD_{95}$ in two studied soils (Table 5). With the increase of cadmium application, the plants total weight per pot was reduced, beginning with applied concentration of 22.9 $mg \cdot kg^{-1}$ of the sod podzolic soils and 20.8 $mg \cdot kg^{-1}$ of chernozem. Cadmium concentration in sod podzolic soil 153 $mg \cdot kg^{-1}$ caused the 95.6% biomass reduction. Decreasing of 50.7% of barley weight was resulted by 68.2 $mg \cdot kg^{-1}$ in chernozem. Concentration of Cd^{2+} 138.9 $mg \cdot kg^{-1}$ in chernozem leads to decreasing of 82.5% of barley weight.

In plants, heavy metals such as cadmium (Cd) and nickel (Ni) are greatly toxic in relatively low amount (Ilyin and Syso 2001, Wang *et al.* 2008). Cd is one of the most highly dispersed metals in terms of anthropogenic activities (Azimi *et al.* 2006). The agricultural soils are contaminated by fertilizer impurities (Cd^{2+}), use of refuge derived compost and sewage sludge (Cd^{2+}). Cadmium is easily taken up by plants because, geochemically, it is quite a mobile element in water and soil ecosystems (Ryzhenko and Kavetsky 2015, Azimi *et al.* 2006, Ilyin and Syso 2001). Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and, finally, death (Kabata-Pendias and Mukherjee 2007, Kaonga *et al.* 2010, Ilyin and Syso 2001, Gill 2014). Cadmium has no recognized favorable effects in plants and is solely lethal (Kabata-Pendias and Mukherjee 2007, Kaonga *et al.* 2010, Azimi *et al.* 2006). The inhibition of root Fe(III) reductase induced by Cd led to Fe(II) deficiency, and it seriously affected photosynthesis (Kabata-Pendias and Mukherjee 2007, Kaonga *et al.* 2010, Ilyin and Syso 2001). In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants (Kabata-Pendias and Mukherjee 2007, Kaonga *et al.* 2010, Ryzhenko 2012). Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots (Azimi *et al.* 2006, Mantorova 2010).

Copper (Cu) is known to be important and poisonous for numerous biological systems. It is considered as a micronutrient for plants and plays an important role in CO_2 assimilation and ATP synthesis (Kabata-Pendias and Mukherjee 2007, Kaonga *et al.* 2010, Sardar *et al.* 2013). Cu is also an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain (Kabata-Pendias and Mukherjee 2007, Kaonga *et al.* 2010, Wang *et al.* 2008, Mantorova 2010). However, plants grown in the Cu-polluted soils store abundant portion of metals in roots (Jakubus 2012; Ilyin and Syso 2001). Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Kabata-Pendias and Mukherjee 2007, Ducic and Polle 2005). Exposure of plants to excess Cu generates oxidative stress and ROS (Kabata-Pendias and Mukherjee 2007, Ilyin and Syso 2001, Wang *et al.* 2008, Ducic and Polle 2005). Oxidative stress causes the disturbance of metabolic pathways and damage to macromolecules (Jakubus 2012, Wang *et al.* 2008, Ducic and Polle 2005). In the present research, copper concentration in soil (67.2 $mg \cdot kg^{-1}$) caused the 10.3% reduction of biomass (Sod podzolic soil) and 59.5 $mg \cdot kg^{-1}$ caused the 3.9% reduction of biomass (chernozem soil) (Table 3). Only 102.9 $mg \cdot kg^{-1}$ of copper in Sod podzolic soil resulted in 20% of weight reduction. And finally, 82.4% of weight reduction was obtained by copper concentration of 173.8 $mg \cdot kg^{-1}$ in sod podzolic soil. In our studies the phytotoxicity of copper was in the second place after cadmium in two soils (Table 3). The $PHLD_{50}$ of copper was 129 $mg \cdot kg^{-1}$ (sod podzolic soil) and 141 $mg \cdot kg^{-1}$ (chernozem soil).

Nickel had less phytotoxicity effect for spring barley than copper and cadmium in our investigation. $PHLD_{50}$ of Ni was 135 $mg \cdot kg^{-1}$ (sod podzolic soil) and 150 $mg \cdot kg^{-1}$ (chernozem soil). Some authors noted that there is no evidence of an essential role of Ni in plant metabolism (Eid *et al.* 2012). In our studies, with increase of nickel application, the plants total weight per pot was reduced, beginning with applied concentration of 39 $mg \cdot kg^{-1}$ of sod podzolic soil and of 43 $mg \cdot kg^{-1}$ of chernozem soil (Table 3). Phytotoxicity also depends on nickel availability in the soil solution (*Soil Guideline Values…* 2009, Kukier and Chaney 2004). Nickel concentration 178.9 $mg \cdot kg^{-1}$ in sod podzolic soil reduced barley biomass to 74.5%, while 222.5 $mg \cdot kg^{-1}$ resulted in 88.9% of weight reduction in chernozem soil. Nickel phytotoxicity has been frequently studied with commonly reported systems including chlorosis followed by yellowing and necrosis of leaves, restricted growth, and tissue injury (Kabata-Pendias and Mukherjee 2007, Kukier and Chaney 2004). Phytotoxic nickel concentrations vary widely among plant species and cultivars, and have been reported in the range from 40 to 246 mg·kg^{-1} DW plant tissue (Kabata-Pendias and Mukherjee 2007). Excess of Ni^{2+} in soil causes various physiological alterations and diverse toxicity symptoms such as chlorosis and necrosis in different plant species (Kukier and Chaney 2004, Eid *et al.* 2012). Plants grown in high Ni^{2+}-containing soil showed impairment of nutrient balance and resulted in disorder of cell membrane functions. Thus, Ni^{2+} affected the lipid composition and H-ATPase activity of the plasma membrane (Kukier and Chaney 2004, Ilyin and Syso 2001).

Cobalt $PHLD_{50}$ were 155 mg·kg^{-1} (sod podzolic soil) and 162 mg·kg^{-1} (chernozem soil) (Table 5). Zn, Cu and Co are essential to plant growth and needed in small (micro) quantities, however, their excessive concentration in plant tissues may cause toxic symptoms. These nutrients are vital physiologically and are important constituents of enzymes, thus, critical for a number of plant functions. In plants, Co complex is found in the form of vitamin B12. Plants can accumulate small amount of Co from the soil. Uptake and distribution of Co in plants is species-dependent and controlled by different mechanisms (Sardar *et al.* 2013, Ilyin and Syso 2001). Very little information is available regarding the phytotoxic effect of Co excess. Its excess restricted the concentration of Fe, chlorophyll, protein and catalase activity in leaves. High level of Co also affected the translocation of P, S, Mn, Zn and Cu from roots to tops in plant. In contrast to an excess of Cu or Cr, Co significantly decreased water potential and transpiration rate, whereas diffusive resistance and relative water content increased in leaves of cauliflower upon exposure to an excess of Co (Gill 2014, Kabata-Pendias and Mukherjee 2007, Eid *et al.* 2012). In our investigations, high level of cobalt concentration in two soils resulted in significant reduction of barley biomass. For example, 219.6 mg·kg^{-1} of Co^{2+} in sod podzolic soil caused 89.8% of weight decreasing, and 245.5 mg·kg^{-1} of Co^{2+} in chernozem soil caused 98.2% of weight reduction. Concentration of Co^{2+} ranging from 36.5 mg·kg^{-1} to 125.0 mg·kg^{-1} in sod podzolic soil did not result in significant reduction of barley biomass. Within this range of cobalt concentration in soil barley weight inhibition was from 5 to 7%. However, 159.6 mg·kg^{-1} of cobalt in sod podzolic soil leads already to 47% reduction of biomass (Table 2). Such dramatic increasing of phytotoxicity effect at the excess of Co concentration in soil from 125.0 to 159.6 mg·kg^{-1} could be explained by the existence of protective barrier in a plant. This barrier could play a permissive role for up-taking trace elements in low level concentration in soil. In the case of high concentration of Co in soil, the metal begins to destroy that barrier and plant sharply reacts by the reduction of biomass.

Lead and **zinc** had the highest $PhLD_{50}$ value in two studied soils (Table 5). $PhLD_{50}$ of lead was 537 mg·kg^{-1} in sod podzolic and 661 mg·kg^{-1} in chernozem. $PhLD_{50}$ of lead are significantly higher than the one of Cd^{2+}, Ni^{2+}, Co^{2+}. Concentrations of lead in soil resulted in the reduction of 80–90% of barley biomass were several times higher than other metals. Reduction of 94.5 and 89.8% of barley biomass in two studied soils was the (Table 2) result of very high concentration of Pb^{2+} (1158.3 $mg \cdot kg^{-1}$ in sod podzolic and 1062.0 $mg \cdot kg^{-1}$ in chernozem soil). Lead concentration of 319.7 $mg \cdot kg^{-1}$ in chernozem soil contributed to only 1.6% of barley biomass reduction, while less lead concentration of 212.6 $mg \cdot kg^{-1}$ in chernozem soil resulted in higher phytotoxical effect (8.3% biomass reduction). Lead toxic effect for plants is more controversial. Many nonessential and toxic for plant growth trace elements (e.g. Cd or Pb) are absorbed by plants rapidly when present in growing medium (Kukier and Chaney 2004). Some investigation showed that nonessential doses of Pb do not inhibit biomass production, but stimulate plant growth as well as micronutrients. Lead presents in all living organisms and its toxicity and vital necessity for plants is well-proven (Soldatova and Khryanin 2008). On the other hand, its biological role, mode of action at low concentrations in plants are studied very poorly (Mamatha *et al.* 2014, Satpathy *et al.* 2014, Egoshina and Shikhova 2008). We may assume that toxic or stimulation effects also depend on other environmental factors (e.g. ratio of nutrients in soil solution, organic matter, pH, etc.) and lead's properties. There are many research which confirmed that lead uptake in plants is more intensive (Mamatha *et al.* 2014, Satpathy *et al.* 2014, Egoshina and Shikhova 2008). All in all, our study shows that phytotoxicity effect of lead and zinc is significantly less comparable to the other metals.

$PhLD_{50}$ of **zinc** was 603 $mg \cdot kg^{-1}$ (sod podzolic soil) and 616 $mg \cdot kg^{-1}$ (chernozem soil). In the present research, zinc had higher toxic effect when compared to lead in chernozem soil, and vice versa in sod podzolic soil. It proves the idea that soil properties influence substance toxicity for plants. Zinc and cooper are microelements often added to podzolic soils as a fertilizer. In our experiment, zinc concentration of 427.4 $mg \cdot kg^{-1}$ resulted in 14.6% of biomass reduction in sod podzolic soil and 382.3 $mg \cdot kg^{-1}$ contributed to 7.8% of biomass reduction in chernozem soil. Zn^{2+} concentration of 743.0 $mg \cdot kg^{-1}$ led to 88.8% of biomass reduction in sod podzolic soil (Table 3). In some previous studies, zinc salts, such as zinc chloride, are proved to be less harmful to the germination of seeds. These results were confirmed by Somova and Pechurkin who showed a high tolerance of plants to zinc salts (Somova and Pechurkin 2009). In non-tolerant plants, Zn toxicity is apparent in soils with high Zn content which could affect inhibition of root elongation and chlorosis of young leaves (Nicholson *et al*. 1997, González and Lobo 2013, Naz *et al*. 2013). Though Zn was once not considered to be highly toxic, phytotoxicity of zinc is usually reported in acid and heavily sludged soils. In our investigation, zinc and lead were least phytotoxic for spring barley among all metals in the two soils.

$PhLD_{50}$ and $PhLD_{95}$ indexes on sod podzolic soil are slightly lower than on chernozem. It could be explained by higher buffer capacity of chernozem than the one of sod podzolic soils. The toxic effect of heavy metal on plant growth depends not only on the amount of toxic metal taken up from the soil. The toxicity of heavy metal in soil also depends on the bioavailable fraction which may be modified by rhizosphere processes, or content of phosphate, lime, organic matter or other soil properties (Nicholson *et al*. 1997, González and Lobo 2013). The uptake of metals from soil into plants and their phytotoxicity effect are affected by soil chemistry, metal speciation (i.e. inorganic and organic complexation depending on HM properties), and molecular transport and storage processes in plants (Kabata-Pendias and Mukherjee 2007). These processes can be summarized in terms of metal bioavailability, which reflects the fraction of a metal in soil that is available for plant uptake.

TABLE 5. THE $PhLD_{50}$ AND $PhLD_{95}$ OF Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} IN SOD PODZOLIC AND CHERNOZEM SOILS
(1 M HCl EXTRACTED FORMS IN SOIL, $mg \cdot kg^{-1}$)

Metal	$PhLD_{50}$	$PhLD_{95}$
	Sod podzolic	
Cd	50	200
Pb	537	1514
Zn	603	913
Cu	129	263
Co	155	398
Ni	135	311
	Chernozem	
Cd	68	234
Pb	661	1660
Zn	616	1000
Cu	141	302
Co	162	363
Ni	150	324

Summarizing the results, the metals can be ranked by descending phytotoxic order for two studied soils as follow:

Sod podzolic soil: Cd>Cu>Ni>Co>Pb>Zn.

Chernozem soil: Cd>Cu>Ni>Co>Zn>Pb.

HM polarity, correlation between HM polarity and HM phytotoxicity effect

The aim of our studies included also considering the correlation between chemical and physical properties of HM substances in soil and phytotoxicity. One of the most prominent and integral factors that determines the HM behavior in the environment may be the polarity of metals substances (Kavetsky and Ryzhenko 2008, Forrest *et al*. 2014). The dipole moment induced by nonhomogeneous charge distribution in a molecule can be a useful parameter for prediction of toxic potency (Forrest *et al*. 2014, Kruk and Kavetsky 1999).

Our approach to correlation between toxicity and polarity based on assumption that studied metals may influence the polarity of the substances, to which they are included, in the same tendency. So, the higher the polarity of the organic substances with a metal, the higher toxicity of the metal for similarity with pesticides when it was proven that the higher polarity, the more toxic pesticide.

On the other hand, heavy metals in soils may form many unpredictable compounds with components of liquid and solid phase of soil. Recently, it has become very popular to determine trace elements compounds in soil with the application of different physicochemical methods (chromatography, voltammetry, etc.) with further estimation of biological properties of these substances. However, trace elements substances composition of soils varies very much with soil characteristics.

Determination of the value of dipole moment of a substance depends on different factors such as dielectric permeability of mobile phase, aggregative consistence of substance, etc. Therefore, the main challenge is creation of equal conditions for determination of the trace metals substances dipole moment. Once we are able to create equal conditions for determination of each metal substances dipole moment, we may compare the influence of a metal on its substance polarity and, thereby, toxicity of each metal.

To tie up the studied trace element in compound with the same organic matrix, we use ditizone with further determination of metals compounds dipole moment by TLC as it was explained in the "Materials and Methods" part.

The values of the metals ditizonates dipole moments are shown in Table 6. The highest value of dipole moment (μ) had Cu^{2+} ditizonate (HDz) (9.13 Debye). The dipole moment of Cd^{2+} HDz was 8.95 Debye. The lowest value of dipole moment had ditizonate of zinc. According to the value of the heavy metals dipole moments (μ), the heavy metals can be ranked in the following descending order: Cu>Cd >Ni>Co>Pb>Zn.

TABLE 6. DIPOLE MOMENT (μ) of DITIZONATES of Zn^{2+}, Pb^{+2}, Co^{2+}, Ni^{2+}, Cd^{+2}, Cu^{2+} AND THEIR $PhLD_{50}$ VALUES

HM ditizonates	μ, Debye	HM $PhLD_{50,}$ $mg \cdot kg^{-1}$ (1 M HCl extracted forms in soil)	
		Sod podzolic	Chernozem
Zn(HDz)2	8.24	603	616
Pb(HDz)2	8.33	537	661
Co(HDz)2	8.54	155	162
Ni(HDz)2	8.91	135	150
Cd(HDz)2	8.95	50	68
Cu(HDz)2	9.13	129	141

Because of the polarity change caused adding of the different metals, we suggest to estimate the metals toxicity properties in ecosystem. Thus, we attempted to find correlation between the polarity of metals ditizonates and its phytotoxicity. It was hypothesized that the toxic potency would be greater when the dipole moment is higher. The graphic image of correlation between dipole moment and phytotoxicity effect is shown in Figures 4 and 5.

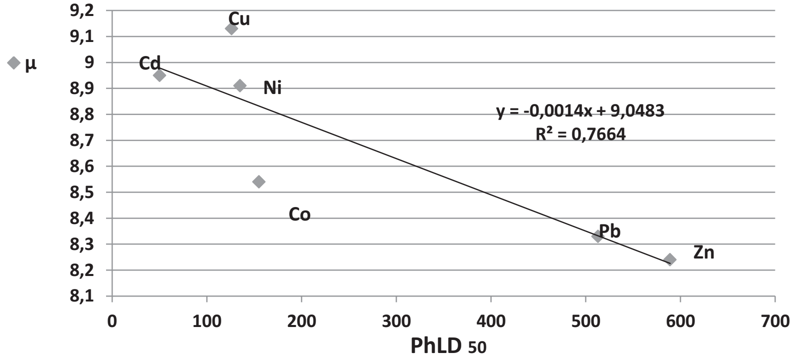

Fig. 4. Correlation between HM $PhLD_{50}$ value and HM HDz dipole moment (μ) for sod podzolic

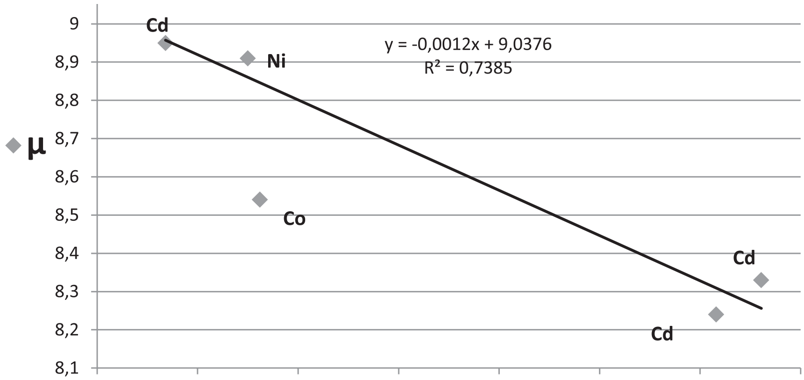

Fig. 5. Correlation between HM $PhLD_{50}$ value and HM HDz dipole moment (μ) for chernozem

Correlation between the two indexes can be described with linear regression on chernozem – y = -0.0012x + 9.0376 and on sod podzolic – y = -0.0014x + 9.0483; (sod podzolic) soils are tight and very much alike. The validity of the approximations were sufficiently high in sod podzolic (R^2=0.77) as well as in chernozem (R^2=0.74).

In spite of differences in soils characteristics, the values of the correlation between $PhLD_{50}$ and dipole moment in two studied soils were sufficiently near. Such closeness of approximation in two soils also confirms the occurrence of tight correlation between $PhLD_{50}$ and dipole moment (μ). It is also confirmation that soil properties do not change heavy metals toxicity for the plant.

CONCLUSIONS

The results help to compare phytotoxicity of studied metals Cd, Cu, Ni, Co, Zn, Pb for plants of spring barley (*Hordeum vulgare* L.) on sod podzolic sandy loam on layered glacial sands (sod podzolic) and calcareous deep chernozem on loamy loess (chernozem). Each metal differently influenced whole plant weight reduction. The most phytotoxic metal in our studies was cadmium. Zinc had more phytotoxicity effect as compared to lead in chernozem soil than one in sod podzolic soil. The concentrations of lead in soil, which resulted in 80–90% reduction of barley biomass, were several times higher than other metals. The high concentration of Pb^{2+} (1158.3 $mg \cdot kg^{-1}$ in sod podzolic and 1062.0 $mg \cdot kg^{-1}$ in chernozem soil) resulted to reduction of 94.5 and 89.8% of barley biomass, respectively.

We suggest to estimate the heavy metals phytotoxicity by means of $PhLD_{50}$ value. The $PhLD_{50}$ value is a doze of metal in soil that causes 50% reduction of plant biomass ($mg \cdot kg^{-1}$). According to $PhLD_{50}$ value, metals can be ranked by the effect on biomass reduction as: Cd>Cu>Ni>Co>Pb>Zn (sod podzolic soil) and Cd>Cu>Ni>Co>Zn>Pb (chernozem soil). Results of the study may be useful indicators of Cu, Ni, Co, Cd, Pb and Zn phytotoxicity assessment for the growing of *Hordeum vulgare* (L.) in heavy metals contaminated areas.

$PhLD_{50}$ value demonstrates the comparative toxicity of metals for a plant. The $PhLD_{50}$ value could be a useful approach for toxicity assessment of any pollutant. This value gives the possibility to predict the behavior of metal in the ecosystem. Estimation phytotoxicity by means of $PhLD_{50}$ value could be applied for another contaminates and plants.

This study shows the tight correlation between HM $PhLD_{50}$ value (phytotyoxicity) and shift of substance dipole moment (μ) caused by an addition of studied metals to *dyphenilditiokarbazone* (*ditizone*).

The results extend possibilities to assess the risk of phytotoxicity (as well as other ecotoxicological risks) by evaluation of the metals substance dipole moment shift. Highlighting of the correlation between a substance polarity and phytotoxicity helps to provide the systematical approaches of another pollutants (e.g. pesticides residue, organic pollutant) toxicity assessment. Therefore, further investigation on substances polarity impact on its ecotoxicological characteristics (toxicity, bioavailability, mobility, etc.) could be prominent.

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