International Journal of Bioorganic Chemistry
2017; 2(4): 163-173
http://www.sciencepublishinggroup.com/j/ijbc
doi: 10.11648/j.ijbc.20170204.12

Cd, Zn, Cu, Pb, Co, Ni Phytotoxicity Assessment as Function of Its Substance Polarity Shift

Nataliia O. Ryzhenko[1], **Sergiy V. Kavetsky**[2], **Volodymyr M. Kavetsky**[3]

[1]Department of Ecological Safety, State Ecological Academy of Post-Graduate Education and Management, Kyiv, Ukraine

[2]Department of Plant Nutrition and Soil Fertility, Faculty of Agricultural Chemistry and Soil Science, National University of Life and Environment Science of Ukraine, Kyiv, Ukraine

[3]Ecology Department, Faculty of Natural Sciences, National University of Kyiv-Mohyla Academy, Kyiv, Ukraine

Email address:

alsko2011@ukr.net (N. O. Ryzhenko), kavetskiy@yahoo.com (S. V. Kavetsky), Kavetskyv@ukr.net (V. M. Kavetsky)

To cite this article:

Nataliia O. Ryzhenko, Sergiy V. Kavetsky, Volodymyr M. Kavetsky. Cd, Zn, Cu, Pb, Co, Ni Phytotoxicity Assessment as Function of Its Substance Polarity Shift. *International Journal of Bioorganic Chemistry*. Vol. 2, No. 4, 2017, pp. 163-173. doi: 10.11648/j.ijbc.20170204.12

Received: February 11, 2017; **Accepted**: April 25, 2017; **Published**: June 20, 2017

Abstract: In this paper we investigate the use of probit analysis for heavy metals (Pb, Cd, Cu, Zn, Co, Ni) toxicity for spring barley (Hordeum vulgare L.) in sod -podzolic sandy loam soil and chernozem soil. Toxicity of studied reduced in the following order: Cd>Cu>Ni>Co>Zn>Pb (chernozem soil). Tight correlation between studied metals phytotoxicity for plants of spring barley and polarity shift caused by adding to organic matrix - diphenilthiocarbazone (Ditizone) the metals under study was observed. It approach may be prominent for metals risk assessment.

Keywords: Metals, Phytotoxicity, Probit Analysis, Assessment, Pollution, Dipole Moment

1. Introduction

Environmental degradation is a widely recognized global challenge. Some of the problems affecting the world currently are acid rain, global warming, hazardous wastes, over population, ozone depletion, smog and environmental pollution [1]. 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 [2]. Heavy metals are also one of the major contaminating agents in our food supply [1, 2-8]. Major causes of environmental heavy metals pollution are: use of sewage sludge in agriculture, metal mining, extensive use of pesticides and chemical fertilizers [9-13]. Specifically metals are non-degradable and therefore can persist for long periods in aquatic and terrestrial environments [1, 2, 13, 14]. 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 vegetable 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 [15-18]. There are many indexes of phytotoxicity: Vigor Index, Seedling vigor index, Tolerance index, Relative root elongation, *etc* [19, 20, 21]. 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 a reliable index of toxic process in plants. Most of the phytotoxicity indices 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 [4, 6, 17, 18, 19, 20, 21].

Similarly, for estimation of phyto-toxicological effect of impact pollution we propose to use phytotoxic dose 50% (PTD_{50}) caused reduction of 50% of initial weight (height, length of root *etc*). PTD_{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 PTD_{50} index – the less HM phytotoxicity.

Usually LD_{50} index is used in toxicology as "doze-effect" correlation (pic. 1). LD_{50} index calculated with application of *probit analysis* allows comparing toxicity of each toxicant for clear assessment [22, 23]. We applied 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 [4, 5, 6, 7]. Phytotoxicity of heavy metals is considered inhibitory for plant growth [4, 5, 6, 7, 19, 20, 21]. The presence of heavy metals in soil disrupts the pattern of nutrient uptake in plant because of nutrient metal interaction [4, 5, 7].

Tight correlation between pesticides behavior in environment and their polarity was demonstrated in papers of Kavetsky V. [24, 25]. 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). Ground on this Kavetsky V. and Bublik L. 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) [24, 25, 26]. But similar ideas for heavy metals (HM) have not been suggested or applied by any authors yet.

Although ions don't have a dipole moment but they can influence on polarity of substances containing these ions. We assume that all metals may influence on polarity of compounds in which they include 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*). *Ditizone* forms complexes with Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+} compounds. Dipole moments of metals ditizonates were defined using thin layer chromatography. Dipole moments were determined as a correlation between *Rf* value of metal ditizonate and *dialectical permeability of mobile phase*. Defining the polarity of metals ditizonate we tried to detect correlation between ditizonates polarity shift, caused by adding of metals, and phytotoxicity.

2. Method

Spring barley (*Hordeum vulgare L.*) was selected as a model plant. Spring barley (*Hordeum vulgare* L.) is one of the important cereals crop 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 g. Chernozem soil has the following: pH_{salt} 6.2, organic matter by Turin 2.89%, CEC 27.1 mg eqv/100 g.

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

Table 1. *Scheme of experiment.*

Control (no HM application)	
Cu^{2+}:	Zn^{2+}:
100 mg kg^{-1} ^{o}f the soils,	600 mg kg^{-1} ^{o}f the soils,
150 mg kg^{-1} ^{o}f the soils,	900 mg kg^{-1} ^{o}f the soils,
200 mg kg^{-1} ^{o}f the soils,	1200 mg kg^{-1} ^{o}f the soils),
300 mg kg^{-1} ^{o}f the soils	1500 mg kg^{-1} ^{o}f the soils
Co^{2+}:	Ni^{2+}:
60 mg kg^{-1} ^{o}f the soils	70 mg kg^{-1} ^{o}f the soils
300 mg kg^{-1} ^{o}f the soils	210 mg kg^{-1} ^{o}f the soils,
480 mg kg^{-1} ^{o}f the soils $^{+}$	350 mg kg^{-1} ^{o}f the soils,
540 mg kg^{-1} ^{o}f the soils	420 mg kg^{-1} ^{o}f the soils,
600 mg kg^{-1} ^{o}f the soils	700 mg kg^{-1} ^{o}f the soils
Cd^{2+}:	Pb^{2+}:
15 mg kg^{-1} ^{o}f the soils,	150 mg kg^{-1} ^{o}f the soils,
30 mg kg^{-1} ^{o}f the soils,	300 mg kg^{-1} ^{o}f the soils,
60 mg kg^{-1} ^{o}f the soils,	450 mg kg^{-1} ^{o}f the soils,
90 mg kg^{-1} ^{o}f the soils,	900 mg kg^{-1} ^{o}f the soils,
150 mg kg^{-1} ^{o}f the soils,	1200 mg kg^{-1} ^{o}f the soils,
300 mg kg^{-1} ^{o}f the soils	1500 mg kg^{-1} ^{o}f the soils

That amount corresponds with those adopted in Ukraine Maximum Allowed Concentration (MAC) in soil [27]. The following metals salts: $Pb(NO_3)_2$, $ZnSO_4$. H_2O, $CuSO_4$. $7H_2O$, $CdSO_4$, $NiSO_4 \cdot 6H_2O$, $CoSO_4 \cdot 7H_2O$ were used for the trace elements application. The investigation was conducted under greenhouse conditions. Plants grew in plastic Mitcherlikh's pots. Soil preparation, pots filling, and trials were carried out in accordance with standard methods [23, 27]. The metals were added to soil during soil preparation before filling the pots. Barley germinated seeds were planted in 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 used in our previous investigation and officially recognized in Ukraine [28].

The method of polarity determination based on correlation between *Rf* of a substance and value of *dialectical permeability (ε)* of mobile phase during separation of the substance in thin layer (TLC) [24, 26]. 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 ratios 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 reported elsewhere in literature [24, 26, 29]:

$$\mu = \frac{Rf_2^2\ x\varepsilon_1 - Rf_1^2\ x\varepsilon_2}{Rf_2^2 - Rf_1^2}, \tag{1}$$

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 V.[23]. Probit values was found in "Bliss table" which transformed percentage killed plant into probit [22]. The idea of the probit function was published by Chester Ittner Bliss (1899–1979) in *Science* in 1934 on how to treat data such as the percentage of a pest killed by pesticide (table 2) [22]. The method introduced by Bliss was further evolved in *Probit Analysis*, for toxicological applications by D. J. Finney. The mathematical interpretation of experimental data by using *S*-curve of doze-effect correlation is difficult (figure 1).

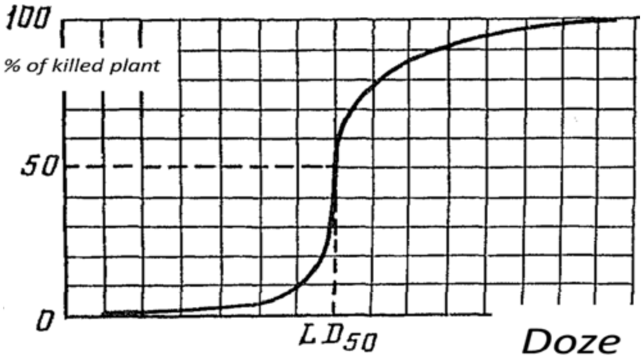

Figure 1. *Curve of doze-effect correlation [22, 23].*

Table 2. *Table of transformed percentage killed plant into probit [22, 23].*

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

Usually, according to *Probit Analysis* the linear correlation between LgD and *probit* value is used.

3. Result

Experimental data are shown in Table 3. Except experimental data table 3 includes the values of lg D (where D is a 1 M HCl extracted forms in soil, mg 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 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
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

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	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
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 showed that all the heavy metals individually affected 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 [1, 3, 4, 12, 17]. 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 [1, 2, 4, 5]. 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 [4, 17, 18]. For example, Zn, Fe, Cu, Cr, and Co are critical nutrients but may become toxic elements at greater amounts.

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

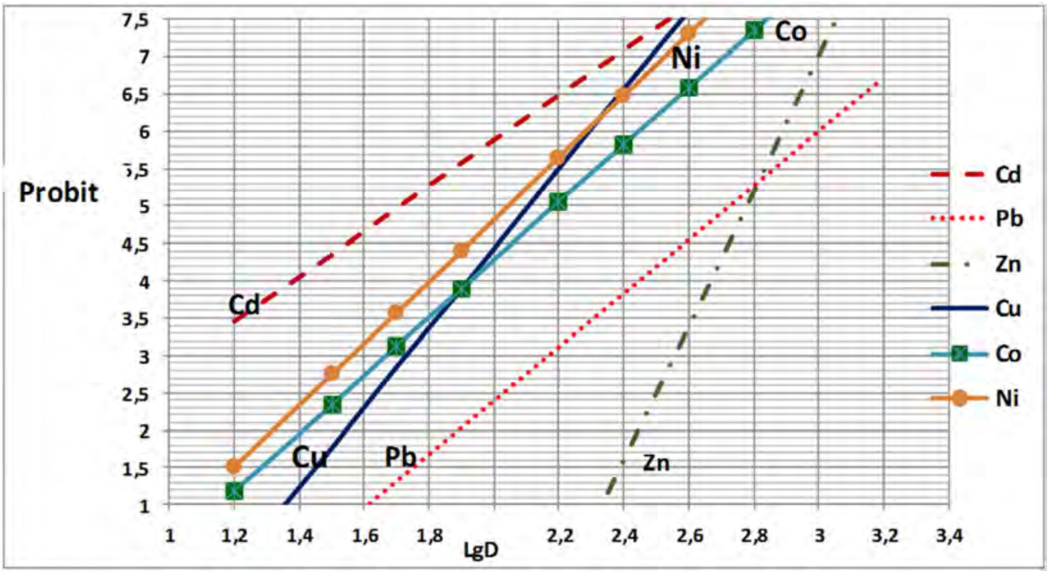

Figure 2. *Correlation between LgD of Trace Metals and probit in the condition of sod podzolic sandy loam on layered glacial sands.*

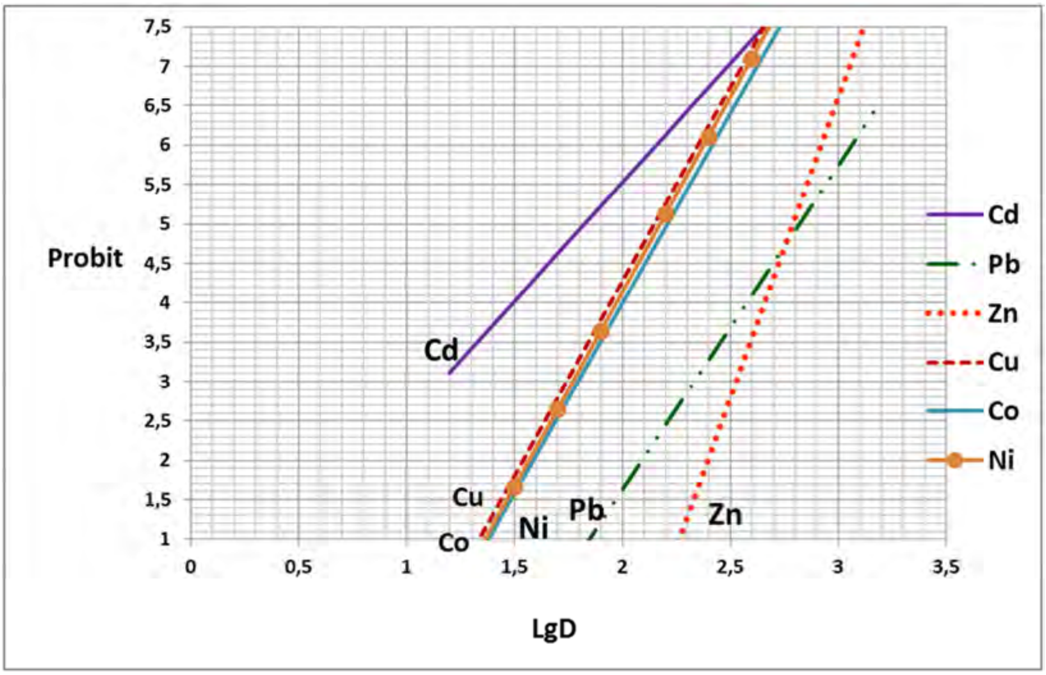

Figure 3. *Correlation between LgD of Trace Metals and probit in the condition of calcareous deep chernozem on loamy loess.*

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

$$y = 3.0274x - 0.1749 \tag{2}$$

If probit equals 5 (PTD_{50} *calculation*), from here:

$$5=3.0274x - 0.1749, \text{ and } x=1.7 \tag{3}$$

The antilogarithm (1.7)=50 - PTD_{50}.

Equations (table 4), PTD_{50} values*,* and PTD_{95} (table 5) values were obtained for all heavy metals in both studied soils.

Table 4. *Correlation between LgD and probit.*

Metal	Regression 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 [4, 5, 7, 8, 16]. This metal had least PTD_{50} and PTD_{95} in two studied soils (table 4). With increase of cadmium application the plants total weight per pot reduced beginning with applied concentration of 22.9 mg kg^{-1} of the sod podzolic soils and 20.8 mg kg^{-1} of the chernozem. Cadmium concentration in sod podzolic soil 153 mg kg^{-1} caused the 95.6% biomass reduction. Decreasing of 50.7% barley weight was resulted by 68.2 mg kg^{-1} in chernozem. Concentration of Cd^{2+} 138.9 mg kg^{-1} in chernozem leads to decreasing of 82.5% of barley weight.

In plants, trace metals such as cadmium (Cd) and nickel (Ni) are significantly toxic in relatively low amounts [17, 19]. Cd is one of the most highly dispersed metals by anthropogenic activities [16]. 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 mobile element in water and soil ecosystems [6, 7, 16, 17]. 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 [1, 3, 4, 17]. Cadmium has no recognized favorable effects in plants but solely lethal [1, 4, 16]. The inhibition of root Fe(III) reductase induced by Cd led to Fe(II) deficiency, and it seriously affected photosynthesis [1, 3, 4, 16, 17]. 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 [1, 4, 6]. Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots [16, 18].

Copper is known to be important and poisonous for numerous biological systems. Copper (Cu) is considered as a micronutrient for plants and plays important role in CO_2 assimilation and ATP synthesis [1, 4, 5, 17, 30]. Cu is also an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain [1, 4, 17, 18, 19]. However, plants grown in the Cu-polluted soils store abundant portion of metals in roots [1, 3, 4, 5, 7, 9]. 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 [4, 31]. Exposure of plants to excess Cu generates oxidative stress and ROS [4, 17, 19, 31]. Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules [9, 19, 31]. In the present research, the cooper concentration in soil 67.2 mg kg^{-1} caused the 10.3% reduction of biomass (Sod podzolic soil) and 59.5 mg kg^{-1} caused the 3.9% reduction of biomass (Chernozem soil) (table 2). Already 102.9 mg kg^{-1} of cooper in Sod podzolic soil resulted to 20% of weight reduction. And finally, 82.4% of weight reduction was obtained by copper concentration of 173.8 mg kg^{-1} in sod podzolic soil. In our studies the phytotoxicity of copper was in second place after cadmium in two soils (table 4). The PTD_{50} of copper was 129 mg kg^{-1} (Sod podzolic soil) and 141 mg kg^{-1} (Chernozem soil).

Nickel had less phytotoxicity effect for spring barley than copper and cadmium in our investigation. PTLD_{50} of Ni was 135 mg kg^{-1} (Sod podzolic soil) and 150 mg kg^{-1} (Chernozem soil). Some authors noted that there is no evidence of an essential role of Ni in plant metabolism [32]. In our studies with increase of nickel application the plants total weight per pot reduced beginning with applied concentration of 39 mg kg^{-1} of the Sod podzolic soil and of 43 mg kg^{-1} of the Chernozem soil (table 1). Phytotoxicity also depends on nickel availability in the soil solution [4, 9, 17, 32, 33]. Nickel concentration 178.9 mg kg^{-1} in Sod podzolic soil reduced barley biomass on 74.5% while 222.5 mg 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 [4, 5, 33, 34, 35]. Phytotoxic nickel concentrations vary widely among plant species and cultivars and have been reported in the range 40 to 246 mg kg^{-1} DW plant tissue [4]. Excess of Ni^{2+} in soil causes various physiological alterations and diverse toxicity symptoms such as chlorosis and necrosis in different plant species [1, 4, 9, 33, 34, 35]. 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 [4, 17, 33, 34].

Cobalt PTD_{50} were 155 mg kg^{-1} (Sod podzolic soil) and 162 mg kg^{-1} (Chernozem soil) (table 4). 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 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 [1, 4, 17, 30]. Very little information is available regarding the phytotoxic effect of excess Co. Excess of Co 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 excess Cu or Cr, Co significantly decreased water potential and transpiration rate. While diffusive resistance and relative water content increased in leaves of cauliflower upon exposure to excess Co [3, 4, 32]. In our investigations, high level cobalt concentration in two soils resulted to significant reduction of barley biomass. For example, 219.6 mg kg^{-1} of Co^{2+} in Sod podzolic soil caused the 89.8% of weight decreasing, and 245.5 mg kg^{-1} of Co^{2+} in Chernozem soil caused the 98.2% of weight decreasing. Concentration of Co^{2+} from 36.5 mg kg^{-1} to 125.0 mg kg^{-1} in Sod podzolic soil didn't result to significant reduction of barley biomass. Within this range of cobalt concentration in soil barley weight inhibition was from 5 to 7%. However, the 159.6 mg kg^{-1} of cobalt in Sod podzolic soil leads already to 47% reduction of biomass (table 3). 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 existence of protective barrier in plant. This barrier could play a permissive role for up-taking trace elements in low level concentration in soil. In the case of certain high concentration of Co in soil the metal begins to destroy that barrier and plant sharply react by reduction of biomass.

Lead and *zinc* had the highest PTD_{50} value in two studied soils (table 5). PTD_{50} of lead was 537 mg kg^{-1} in Sod podzolic and 661 mg kg^{-1} in chernozem. PTD_{50} of lead are significantly higher than the one of Cd^{2+}, Ni^{2+}, Co^{2+}. Concentrations of lead in soil resulted to reduction of 80-90% of barley biomass were several times higher, than other metals. Reduction of 94.5 and 89.8% of barley biomass on two studied soils accordingly (table 3) resulted by very high concentration of Pb^{2+} (1158.3 mg kg^{-1} in sod podzolic and 1062.0 mg kg^{-1} in chernozem soil). Lead concentration of 319.7 mg kg^{-1} in chernozem soil resulted to only 1.6% of barley biomass reduction, while less lead concentration of 212.6 mg kg^{-1} in chernozem soil resulted to 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 [33]. 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 [4, 34]. On the other hand, lead biological role, mode of action at low concentrations in plants studied very poorly [17, 35, 36, 37]. 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 leads properties. There are many research confirmed that leaf up-take in plant more intensive and ponderable for lead [4, 17, 35, 36, 37]. All in all, our study shows that phytotoxicity effect of lead and zinc is significantly less compare to the other metals.

PTD_{50} of *zinc* was 603 mg kg^{-1} (Sod podzolic soil) and 616 mg kg^{-1} (Chernozem soil). In the present research, zinc had higher toxic effect compare to lead in chernozem soil, and vice versa in sod podzolic soil. It proves idea that soils properties influence substance toxicity for plants. Zink and cooper are microelements often added to podzolic soils as a fertilizer. In our experiment zinc concentration of 427.4 mg kg^{-1} resulted the 14.6% of biomass reduction in Sod podzolic soil and 382.3 mg kg^{-1} resulted the 7.8% of biomass reduction in Chernozem soil. Zn^{2+} concentration of 743.0 mg kg^{-1} leads to 88.8% of biomass reduction in Sod podzolic soil (table 3). In some previous studies, zinc salts such as zinc chloride is 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 [38]. 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 [33, 39, 40]. 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 two soils.

PTD_{50} and PTD_{95} indexes on sod podzolic soil are slightly lower than on chernozem. It could be explained by higher buffer capacity of chernozem than 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 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 [17, 18, 39, 40]. 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 [1, 2, 4]. These processes can be summarized in terms of metal bioavailability, which reflects the fraction of a metal in soil that is available for uptake into a plant.

Table 5. *The PTD_{50} and PTD_{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 kg^{-1}).*

Metal	PTD_{50}	PTD_{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.

4. Discussion

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 determines the HM behavior in environment may be polarity of metals substances [6, 24, 25, 41]. The dipole moment induced by nonhomogeneous charge distribution in a molecule can be a useful parameter for prediction of toxic potency [6, 24, 25, 41].

Our approach to correlation between toxicity and polarity based on assumption that studied metals may influence on the polarity of the substances, to which they are included, in the same tendency. So, the higher is 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 is.

On the other hand, heavy metals in soils may form unpredictable many compounds with components of liquid and solid phase of soil. Recently, has become very popular to determine of trace elements compounds in soil with application of different physicochemical methods (chromatography, voltammetry *etc.*) with farther 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 dialectical 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 furthermore toxicity of each metal.

To tie up the studied trace element in compound with same organic matrix we use ditizone with farther determination of metals compounds dipole moment by TLC as it was explained in chapter "material and methods".

The values of the metals ditizonates dipole moments are in the 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 PTD_{50} values.*

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

Due to the polarity change caused adding of the different metals we suggest estimate the metals toxicity properties in ecosystem. Thus, we attempted to find correlation between 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.

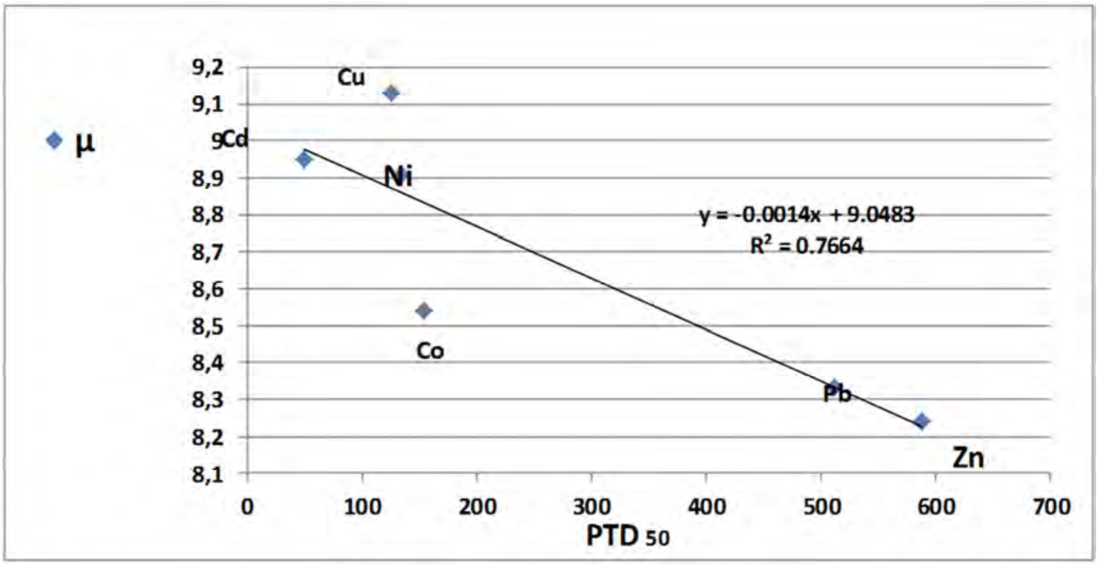

Figure 4. *Correlation between HM PTD_{50} value and HM HDz^- dipole moment (μ) for sod podzolic soil.*

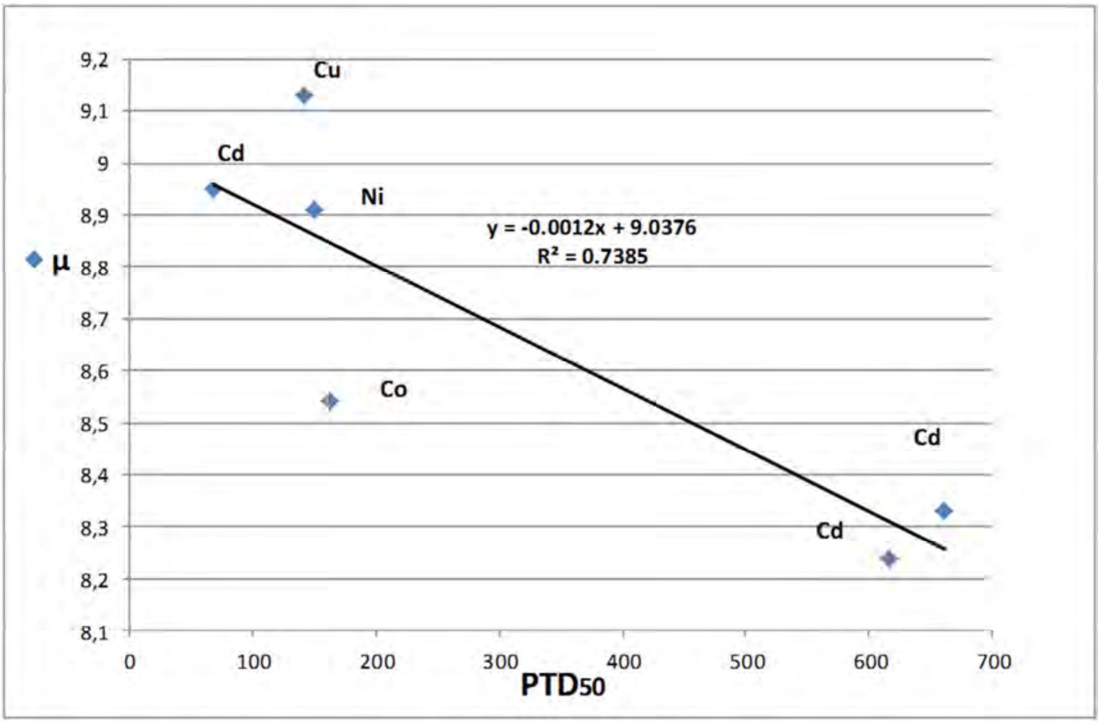

Figure 5. *Correlation between HM PTD_{50} value and HM HDz^{-}dipole moment (μ) for chernozem.*

Correlation between two indices 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 soil (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 PTD_{50} and dipole moment in two studied soils was sufficiently near. Such closeness of approximation in two soils also confirms occurrence of tight correlation between PTD_{50} and dipole moment (μ). It also witness that soil properties does not change heavy metals toxicity for the plant.

5. Conclusions

The results helps 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 influenced differently on the 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 resulted in 80-90% reduction of of barley biomass were several times higher, than other metals. The high concentration of Pb^{2+} (1158.3 mg kg^{-1} in Sod podzolic and 1062.0 mg kg^{-1} in Chernozem soil) resulted to reduction of 94.5 and 89.8% of barley biomass accordingly.

We suggested to estimate the heavy metals phytotoxicity by means of PTD_{50} value. The PTD_{50} value is a doze of metal in soil that causes 50% reduction of plant biomass (mg kg^{-1}). The phytotoxic effects of selected metals can be ranked by 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 at the growing of *Hordeum vulgare L.* in heavy metals contaminated areas.

This study shows the tight correlation between HM PTD_{50} value (phytotyoxicity) and shift of substance dipole moment (μ) caused by addition of studied metals to *dyphenilditiokarbazone* (*ditizone*). The results extend possibility to assess risk of phytotoxicity (as well as another ecotoxicological risks) by dipole moment shift of the metals substance. Therefore, further investigation of influence substances polarity on their toxicity, bioavailability, and mobility can be prominent.

References

[1] C. C. Kaonga, J. Kumwenda, H. T. Mapoma (2010) Accumulation of lead, cadmium, manganese, copper and zinc by sludge worms; Tubifex tubifex in sewage sludge \\Int. J. Environ. Sci. Tech., 7 (1), pp. 119-126

[2] X. Zhang, A. J. Lin, F. J. Zhao, G. Z. Xu, G. L. Duan, and Y. G. Zhu (2008) Arsenic accumulation by the aquatic fern Azolla: comparison of arsenate uptake, speciation and efflux by A. caroliniana and A. filiculoides\\ Environmental Pollution, vol. 156, no. 3, pp. 1149–1155.

[3] Mukti Gill (2014) Heavy metal stress in plants: a review\\ International Journal of Advanced Research, Volume 2, Issue 6, pp. 1043-1055.

[4] Kabata-Pendias, A., Mukherjee, A. B. (2007) trace Elements from Soil to Human. Berlin: Springer-Verlag.

[5] Valavanidis A., Vlachogiani Th. (2010) Metal Pollution in ecosystems. Ecotoxicology Studies and Risk Assessment/ A. Valavanidis, Th. Vlachogiani //Science advances on Environment, Toxicology and Ecotoxicology issues: www.chem.-tox-ecotox.org/wp/wp-content/uploads/2010/01/02-Metals-17_01_2010.

[6] Ryzhenko N., Kavetsky V.(2015) Assessment of heavy metal's (Cd, Zn, Cu, Pb, Co, Ni) toxicity for plants by polarity of their dytizonates and LD_{50} \\ Agroecol. J., Kyiv, №3, pp. 52-60.

[7] Ryzhenko N. O. (2012). Bioaccumulation of Pb, Cd, Zn, Cu in the condition of impact pollution – ecotoxicology criteria of environment quality, Kyiv, J. of ecological sciences, №1, 46-55, (10 pages), http://ecoj.dea.gov.ua/wp-content/uploads/2013/02

[8] Brian J. Alloway (Editor) (2010) Heavy metals in soils. Trace elements and Metalloids in Soils and their Bioavailability. Third edition, Alloway Brian J., UK, Springer, (235 pages).

[9] Monika Jakubus (2012) Phytotoxicity and speciation of copper and nickel in composted sewage sludge \\ J. Elem. (DOI: 10.5601/jelem.2012.17.1.04): pp. 43–56.

[10] Stephen G. Wu, Li Huang, Jennifer Head, Da-Ren Chen, In-Chul Kongand Yinjie J. Tang (2012) Phytotoxicity of Metal Oxide Nanoparticles is Related to Both Dissolved Metals Ions and Adsorption of Particles on Seed Surfaces \\ J Pet Environ Biotechnol, 3: 4, http://dx.doi.org/10.4172/2157-7463.1000126

[11] Domingo Horacio Campaña, Milena Esther Uribe Echevarría, Ariel Oscar Airasca and María Luisa Andrade Couce (2014) Physicochemical and Phytotoxic Characterisation of Residual Sludge from the Malting of Barley \\ J. Pollut. Eff. Cont., 2: 2, http://dx.doi.org/10.4172/2375-4397.1000115

[12] Wang QR, Cui YS, Liu XM, Dong YT, Christie P.(2003) Soil contamination and plant uptake of heavy metals at polluted sites in China \\ J Environ Sci Health A Tox Hazard Subst Environ Eng.; 38 (5): pp. 823-838.

[13] J. Liu, C.-Q. Duan, Y.-N. Zhu, X.-H. Zhang, and C.-X. Wang (2007) "Effect of chemical fertilizers on the fractionation of Cu, Cr and Ni in contaminated soil," Environmental Geology, vol. 52, no. 8, pp. 1601–1606.

[14] Rombke, Jorg, Moltmann, Johann F. (1996). Applied Ecotoxicology, Lewis Publishers: Boca Raton, 38-45.

[15] Marlon Escoto Valerio, Juan Fernández García, Francisco Martín Peinado (2007) Determination of phytotoxicity of soluble elements in soils, based on a bioassay with lettuce (Lactuca sativa L.)\\ Science of the Total Environment, 378: 63–66.

[16] A. A. Azimi, T. Navab Daneshmand and A. Pardakhti (2006) Cadmium absorption and accumulation in different parts of kidney beans, radishes and pumpkins \\ Int. J. Environ. Sacli.. Tech., Vol. 3, No. 2, pp. 177-184.

[17] Ilyin V. B., Syso A. I. (2001) Microelements and Heavy Metals in soils and plants at Novosibirsk region \ V. B. Ilyin, A. I. Syso, Novosibirsk, RAS, pp. 30-31.

[18] G. F. Mantorova (2010) Heavy metals in soil and plant production under conditions of anthropogenic pollution\ *Agro XXI*, № 1–3, pp. 52-54.

[19] Wang Xuejiang chen Ling, Xia Siqing, Zzhao Jianfu (2008) Changes of Cu, Zn, and Ni chemical speciation in sewage sludge co-composted with sodium sulfide and lime \ Journal of Environmental Sciences 20 (2008), pp. 156–160.

[20] M. Mar Delgado, José V. Martin, Rosario Miralles De Imperial, Cristina León-Cófreces, M. Cruz. García (2010) Phytotoxicity of uncomposted and composted poultry manure \ African Journal of Plant Science Vol. 4 (5), pp. 154-162.

[21] Hira Amin, Basir Ahmed Arain, Farah Amin, Muhammad Ali Surhio (2013) Phytotoxicity of Chromium on Germination, Growth and Biochemical Attributes of Hibiscus esculentus L.\ American Journal of Plant Sciences, 4, pp. 2431-2439, http://dx.doi.org/10.4236/ajps.2013.412302

[22] Bliss C. I. (1934) The method of probits/ C. I. Bliss //. Science.- vol. 79 (2037): 38–39.

[23] Dospekhov B. A. (1985) Methodology of field experiment \ B. A. Dospekhov, Moscow: Agropromizdat, pp. 313-316.

[24] Kavetsky V. M., Ryzhenko N. O. (2008) Physical and Chemical Criteria for Pesticides Determination and Risk Assessment in Ecosystem/V. M. Kavetsky, N. O. Ryzhenko//Polish J. Chem. Vol. 82. – P. 361-369.

[25] Kruk L. S., Kavetsky V. M. (1999) Harvest Pesticides Pollution assessment depend on its polarity \ Agroecology and Biotechnology J., - Kyiv, p. 357.

[26] Kavetsky V. M., Bublik L. I. (1989) Using Think Layer chromatography for organic substances dipole moment determination // Physical Chemistry J, 13 (4). – p. 1021.

[27] V. V. Medvedev, T. M. Laktionova, S. A. Bal'uk, N. M. Blokhina, V. O. Bililypsky (1998) Method of monitoring of lands which are in the crisis state, Kharkiv, O. N. Sokolovsky' Institute of soil science, (88 pages).

[28] Kavetsky V. N., Makarenko N. A., Buogis A. M. Kavetsky S. V. (2001) Thin Layer Chromatography Methods of the Hg, Zn, Co, Cd, Cu, Ni determination in soil, plant and water, № 50-97, Methodic of determination pesticides residues in food, forage and environment, Kyiv, P. 18-23.

[29] Kavetsky V. M., Bublik L. I. (1987) Determination of organic substances dipole moment. A. P. № 1296930 USSR A1G01N30/9615.11 VNIIGPE. - №3753317/23-25, №10, 15.03.1987.

[30] Kamran Sardar, Shafaqat Ali, Samra Hameed, Sana Afzal, Samar Fatima, Muhammad Bilal Shakoor, Saima Aslam Bharwana, Hafiz Muhammad Tauqeer (2013) Heavy Metals Contamination and what are the Impacts on Living Organisms \\ Greener Journal of Environmental Management and Public Safety, Vol. 2 (4), pp. 172-179.

[31] Ducic T., Polle A. (2005) Transport and detoxification of manganese and copper in plants // Braz. J. Plant Physiol. – vol. 17 no. 1.

[32] Ebrahem M. Eid, Mohamed A. El-Sheikh, Abdulrahman A. Alatar (2012) Uptake of Ag, Co and Ni by the Organs of Typha domingensis (Pers.) Poir. ex Steud. in Lake Burullus and Their Potential Use as Contamination Indicators \\ Open Journal of Modern Hydrology, 2, pp. 21-27; http://dx.doi.org/10.4236/ojmh.2012.21004 Published Online January 2012 (http://www.SciRP.org/journal/ojmh).

[33] Urszula Kukier, Rufus L. Chancy (2004) In Situ Remediation of Nickel Phytotoxicity for Different Plant Species\\ J. OF Plant Nutrition, Vol. 27, No. 3: pp. 465-495.

[34] Soil Guideline Values for nickel in soil. Science Report SC050021 / Nickel SGV (2009) Environment Agency: http://www.environmentagency.gov.uk/clea

[35] Alia Naz, Sardar Khan, Muhammad Qasim, Salma Khalid, Said Muhammad, Muhammad Tariq (2013). Metals toxicity and its bioaccumulation in purslane seedlings grown in controlled environment, Natural Science, Vol. 5 (5), 573-579, (6 pages), http://dx.doi.org/10.4236/ns.2013.55073

[36] Soldatova N. A., Khryanini V. N. (2008). Influence of lead salts on growing processes in plants Cannabis Sativa L, J. News of PGPU, Section of young scientist, № 6 (10) 2008, pp. 215-218.

[37] P. Mamatha, S. Salamma, A. V. N. Swamy, and B. Ravi Prasad Rao (2014) Quantitative and risk analysis of heavy metals in selected leafy vegetables, Der Pharma Chemica, 6 (3), 179-185, (7 pages), http://derpharmachemica.com/archive.html

[38] Deepmala Satpathy, M. Vikram Reddy, and Soumya Prakash Dhal (2014) Risk Assessment of Heavy Metals Contamination in Paddy Soil, Plants, and Grains (Oryza sativa L.) at the East Coast of India, BioMed Research International, Volume 2014, (11 pages), http://dx.doi.org/10.1155/2014/545473

[39] Eghoshina T. L., Shikhova L. N. (2008) Lead in soil and plant at northern west part of European Russia // Announcer of ONU, № 10 (92). – pp. 135-141.

[40] Somova L. A., Pechurkin N. S. (2009) The influence of microbial association on germination on wheat seeds and on the growth of seedlings under impact of Zn salts growth of seedling under impact of of Zn salts \\ Adv.in Space research, №8, pp. 1224-1228.

[41] Nicholson, F. A., Chambers, B. J. and Alloway, B. J. (1997). Effect of soil pH on heavy metal bioavailability. Proceeding of Fourth International Conference on the Bio-geochemistry of Trace Elements, Berkeley, 23-26 June 1997, pp. 499-500.

[42] Águeda González, M. Carmen Lobo (2013). Growth of Four Varieties of Barley (Hordeum vulgare L.) in Soils Contaminated with Heavy Metals and Their Effects on Some Physiological Traits. American Journal of Plant Sciences, 4, 1799-1810, (11 pages), http://dx.doi.org/10.4236/ajps.2013.49221, Published Online September 2013 (http://www.scirp.org/journal/ajps)

[43] Jay Forrest, Paul Bazylewsky, Robert Bauer, Seongjin Hong, ChangYong Kim, John P. Giesy, Jong Seong Khim, and Gap Soo Chang (2014) A comprehensive model for chemical bioavailability and toxicity oforganic chemicals based on first principles \\ Marine science, vol. 1, art. 31, pp. 1-7; http://www.frontiersin.org