

Metals Phytotoxicity Assessment and Classification

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Keywords: Trace metals, Plants, Phytotoxicological Classification, Assessment, Pollution, Phytotoxicity.

Abstract. In this paper, the influence of trace metals (Cd, Pb, Cu, Co, Ni, Zn) on plants of spring barley (*Hordeum vulgare* L.) was investigated in polluted sod podzolic sandy loam on layered glacial sands and calcareous deep chernozem on loamy loess soils. We propose to estimate the phytotoxicity with help of phytotoxicological classification. The phytotoxicological classification of trace metals gives the possibility to assess their hazard for plants. On the base of indicators such as plant up-taking index (UI), phytolethal dose ($PhLD_{50}$), Dipole moment (μ), Phyto Maximum Allowable Concentration (PMAC) a phytotoxicological classification of hazardous trace metals was suggested. The four classes of danger in phytotoxicological classification of hazardous trace metals were offered. According to phytotoxicological classification, Cd, Co, Ni belong to the first class of hazard, Cu – to the second class of hazard, Zn – to the third class of hazard, Pb – to the fourth class of hazard. Phytotoxicological classification of hazardous trace metals gives the possibility to comprehensively estimate the danger of trace metals for plants as a biological object that plays a very important role in the life of ecosystem. This approach may be applied for another trace metals risk assessment for other plants.

Introduction

One of the main harmful and widespread in the territorial and nomenclature aspects of ecosystem contaminants is trace metals, the assessment of which hazards among different groups of toxicants is ambiguous [1, 4, 29]. After all, trace metals-trace elements, on the one hand, provide normal livelihoods of organisms, and on the other hand, in the high concentration they are toxic to biota [4, 15, 28]. Anthropogenic trace metals contamination of ecosystems as a result of the application of industrial, transport, agrarian and other technologies causes a damage of the functioning of plants as an important component in ecosystem [2, 3, 9, 11]. Often plants are the main accumulator of trace metals in polluted ecosystem. In the same time, plants play an important role in ecosystem as biomass producers and as biodiversity creators [11, 12, 17, 19, 21]. That's why it would be reasonable to get the tools of objective assessment of trace metals influence on plants in polluted ecosystem. Complex approach to trace metals hazard assessment for plants was suggested. It relies on using the phytotoxicologic classification which includes several criteria: $PhLD_{50}$ value, $PhLD_5$ value, *Phyto Maximum Allowable Concentration*, polarity of metal compounds, Bioaccumulation. Obtaining the $PhLD_{50}$ value, $PhLD_5$ value, Polarity (μ) of metal compounds and assessment of trace metals by these values was represented in previous papers [20, 23].

Materials and Methods

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 (chernozem). Sod podzolic soil has the following physic chemical characteristics: pH_{salt} 5.5; organic matter by Turin, Walkley-Black 0.87%, CEC 6.3 Cmol/kg. Chernozem soil has the following: pH_{salt} 6.2, organic matter by Turin, Walkley-

Black 2.89%, CEC 27.1 Cmol/kg. Control concentration of trace metals in soil (1 M HCl, mg kg⁻¹) was: Cd – 0.1; Pb - 0.3; Cu = 0.92; Zn - 2.4; Ni - 1.1; Co-1.5 (sod podzolic); Cd = 0.11; Pb - 0.32; Cu - 2.6; Zn - 5.3; Ni - 2.3; Co – 2.5 (chernozem). The following trace metals salts: Pb(NO₃)₂, ZnSO₄·H₂O, CuSO₄·7H₂O, CdSO₄, NiSO₄·6H₂O, CoSO₄·7H₂O were used for the trace elements application.

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

1. Cu²⁺: 100 mg kg⁻¹ of the soils, 150 mg kg⁻¹ of the soils, 200 mg kg⁻¹ of the soils, 300 mg kg⁻¹ of the soils.

2. Zn²⁺: 600 mg kg⁻¹ of the soils, 900 mg kg⁻¹ of the soils, 1200 mg kg⁻¹ of the soils, 1500 mg kg⁻¹ of the soils.

3. Co²⁺: 60 mg kg⁻¹ of the soils, 300 mg kg⁻¹ of the soils, 480 mg kg⁻¹ of the soils, 540 mg kg⁻¹ of the soils, 600 mg kg⁻¹ of the soils.

4. Ni²⁺: 70 mg kg⁻¹ of the soils, 210 mg kg⁻¹ of the soils, 350 mg kg⁻¹ of the soils, 420 mg kg⁻¹ of the soils, 700 mg kg⁻¹ of the soils.

5. Cd²⁺: 15 mg kg⁻¹ of the soils, 30 mg kg⁻¹ of the soils, 60 mg kg⁻¹ of the soils, 90 mg kg⁻¹ of the soils, 150 mg kg⁻¹ of the soils, 300 mg kg⁻¹ of the soils.

6. Pb²⁺: 150 mg kg⁻¹ of the soils, 300 mg kg⁻¹ of the soils, 450 mg kg⁻¹ of the soils, 900 mg kg⁻¹ of the soils, 1200 mg kg⁻¹ of the soils, 1500 mg kg⁻¹ of the soils.

The metals were added to the soil in such quantities before sowing the spring barley. That amount corresponds with adopted in Ukraine Maximum Allowed Concentration (MAC) in soil [27]. 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 [5, 27]. The trace 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 trace metals determination was thin layer chromatography (TLC). The method is based on the extraction of metal ions from solutions by *diphenyldithiocarbazone* (*ditizon*). Complex compounds of metals (*dithizonates*) are formed in a certain range of pH. Further, the colored *dithizonates* of metals are identified by chromatography in a thin layer of the adsorbent with qualitative and quantitative determination. Method officially recognized in Ukraine [13].

Trace element determination in the plants was carried out after wet digestion by mixture of H₂SO₄ and HClO₄ [8]. The method of the trace metals determination was the thin layer chromatography (TLC). The plant up-take index (*UI*) was calculated in equation 1 [21]:

$$UI = \frac{C_{plant}}{C_{soil}} \quad (1)$$

where *C_{plant}* – metal's concentration in plant (or a part of plant), mg kg⁻¹; *C_{soil}* – metal's concentration (available form) in 0-20 cm soil layer, mg kg⁻¹.

Now a methodology that would determine the safe concentration of trace metals directly for plants in the soil is absent. After all, the existing standards for the content of trace metals in environmental objects are sanitary-hygienic and focused just on human health [14, 16, 25]. Determination of the trace metals safe level in the soil for plants can help to objectively assess state of the ecosystem and prevent the trace metals dangerous influence on plant [24]. In this studies, the algorithm of calculation of Phyto Maximum Allowable Concentration (*PMAC*) was proposed similar to the existing approach of calculation of Maximum Allowable Toxic Concentration (*MATC*) (equation 2) [18]. In the toxicology practice, the scheme to substance toxicity assessment using the *LOEC* and *NOEC* is quite effective and widely used [6, 16, 25]. These indicators are used also to

calculate the Maximum Allowable Toxic Concentration (*MATC*) for assessing the toxicity of substances in the aquatic environment. *MATC* is calculated by the formula [18]:

$$MATC = \sqrt{(NOEC) * (LOEC)} \quad (2)$$

where *NOEC* is No Observed Effect Concentration; *LOEC* is Lowest Observed Effect Concentration.

We propose to determine the Phyto Maximum Allowable Concentration by the formula:

$$PMAC = \sqrt{C_{contr} * PhLD_5} \quad (3)$$

where C_{contr} – background concentration (on the control variant of experiment – without additional metal input);

The $PhLD_5$ is phytotoxic dose 5% ($PhLD_5$) caused reduction of 5% of initial weight (height, length of root etc.).

In our opinion, 5% reduction of initial weight (height, length of root etc.) is the minimal effect, which is similar to the *LOEC* shows the preliminary changes in the productivity of the plant population. Moreover, the level of significance of deviations, which are considered sufficient for ecological and biological research at the level of 5% ($p < 0.05$) was chosen.

Bioaccumulation capacity and the metal toxic effects are characterized by the plant up-take index [7]. In our investigation, the plant up-take indexes at 10% and 50% reduction of initial weight of plants were proposed for the phytotoxicologic classification.

For each indicator ($PhLD_5$, $PMAC$, $PhLD_{50}$, μ , UI_{10} , UI_{50}), the number of classes in phytotoxicological classification is determined by the Sturges (1926) equation [26]:

$$k = 1 + 3.32 \lg(n), \quad (4)$$

where n - the number of options.

The number of options is 12 because in two soils we investigated 6 trace metals. That's why the number of classes was calculated by equation (4): $k = 1 + 3.32 \lg(12) \approx 4$.

The ranges of classes of each indicator (UI_{10} , UI_{50} , $PhLD_{50}$, μ , C_{contr} , $PMAC$) were obtained by following equations [10]:

1. The lower limit of first class was calculated by formula:

$$\text{Lower limit 1 class} = x_{\min} - dx/2, \quad (5)$$

where

$$dx = \text{Lim} / k, \quad (6)$$

$$\text{Lim} = x_{\max} - x_{\min}, \quad (7)$$

2. The upper limit of first class was calculated by formula:

$$\text{Upper limit 1 class} = x_{\min} + dx/2 - \sigma, \quad (8)$$

where σ is measurement uncertainty.

3. The upper and lower limits of second and other classes were calculated by formula 9 and 10:

$$\text{Lower limit 2 class} = \text{Upper limit 1 class} + \sigma, \quad (9)$$

$$\text{Upper limit 2 class} = \text{Lower limit 2 class} + dx - \sigma. \quad (10)$$

Results and Discussion

Table 1 shows the values of $PhLD_{5,50}$ and $PMAC$ for all investigated trace metals, as well as the background concentration in soil (0-20 centimeters). The polarity for each trace metals ditizonates were: Zn(H Dz) 2 – 8.24; Pb(H Dz) 2 – 8.33; Co(H Dz) 2 – 8.54; Ni(H Dz) 2 – 8.91; Cd(H Dz) 2 – 8.95; Cu(H Dz) 2 – 7.60 [22]. The $PMAC$ could be used as an environmental standard that regulate the safe level of pollutants in the soil for plant. Whereas the Phyto Maximum Allowable Concentration is the harmless level of trace metals in the soil for plant, this value was suggested for developing of phytotoxicity classification.

Table 1. $PhLD_{5,50}$, $PMAC$, and background concentration in soil (0-20 centimeters, 1 N HCl, mg kg⁻¹)

Metal	$PhLD_5$	$PhLD_{50}$	C_{contr} (Background concentration in soil, 0-20 cm)	$PMAC$
Sod podzolic (1 M HCl, mg kg ⁻¹)				
Cd	14.72	50	0.10±0.02	1.21
Pb	186.64	537	0.30±0.05	7.48
Zn	394.46	603	2.40±0.30	30.77
Cu	62.91	129	0.92±0.10	7.60
Co	57.94	155	1.50±0.15	9.77
Ni	50.12	135	1.10±0.10	7.40
Chernozem (1 M HCl, mg kg ⁻¹)				
Cd	19.25	68	0.11±0.02	1.46
Pb	264.24	661	0.32±0.05	9.20
Zn	380.19	616	5.30±0.50	44.90
Cu	65.61	141	2.60±0.20	13.10
Co	74.13	162	2.50±0.20	13.61
Ni	69.98	150	2.30±0.30	12.69
$F, 0.05$	0.999	0.816	0.108	0.426

The plants up-taking (bioaccumulation) is one of the most important indices in the study of the harmful influence of the pollutants on plant. In the case of trace metals, plant up-taking availability is especially important, because trace metals, on the one hand, are nutrient elements, and on the other hand, in high concentrations, are toxic to plants. Besides, the plant up-taking index (UI) gives an opportunity to compare the different bioavailability of the trace metals for plant. In addition, investigation of trace metals up-take by plant in polluted soil is important because pollutants concentration in crops determines the quality of agricultural products. Thereby, index of plant up-taking helps to assess the danger of trace metals and to predict the harmful effects both for human and plants. The plant up-taking indexes of trace metals were obtained in the toxic diapason of concentration in the soil. Reduction of phytomass depending on cobalt's concentration in plant and sod podsolc soil is shown in Fig. 1 as an example. Reduction of phytomass depending on other trace metals in plant and both soils was obtained similar to cobalt.

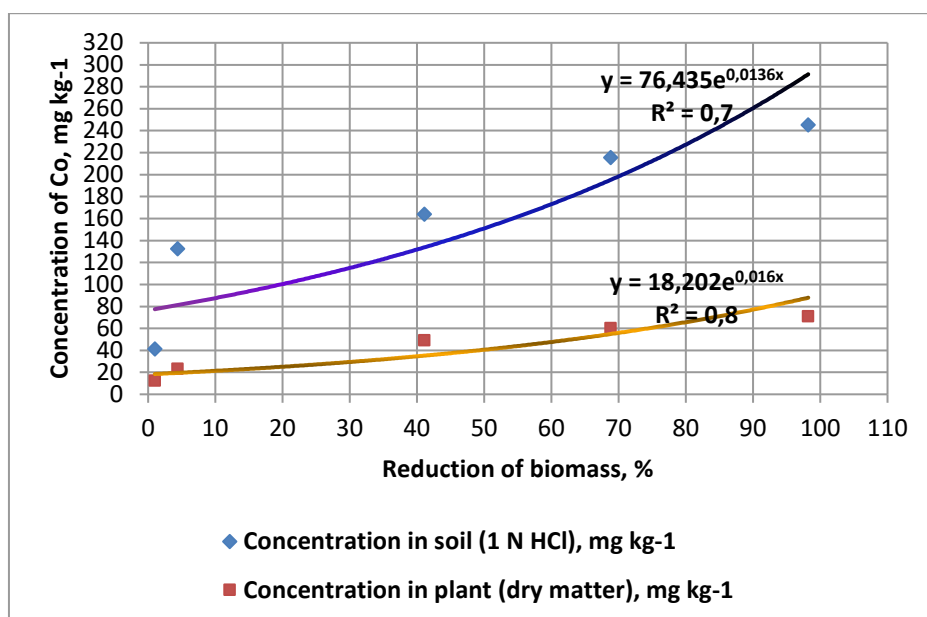


Figure 1. The barley phytomass reduction depending on concentration of Co in plant and soil (Chernozem) mg kg^{-1}

Results of plant up-taking indexes are shown in Table 2. Plant up-taking indexes were found at 5, 10, 50, 80 % reduction of biomass of spring barley. Cadmium plant up-taking indexes were the highest among all investigated trace metals. Lead and cobalt had lowest plant up-taking indexes.

There were obtained the coefficients of variation (*CV*) and coefficients of oscillation (*Kr*) of plant up-taking indexes in both soils (Table 2). The coefficients of variation allowed revealing insignificant variability of plant up-taking in contaminated soil by trace metals. This made it possible to conclude that in conditions of contamination, each metal has a rather narrow range of bioaccumulation ability.

Table 2. Plant up-taking indexes (*UI*) at 5, 10, 50, 80% reduction of phytomass of spring barley

Biomass reduction, %	Cd	Pb	Cu	Zn	Co	Ni
Sod podzolic						
5	0.600	0.220	0.540	0.280	0.120	0.580
10	0.603	0.194	0.515	0.277	0.263	0.596
50	0.645	0.184	0.535	0.291	0.289	0.573
80	0.611	0.163	0.542	0.271	0.300	0.584
<i>Kr</i> , % ($p < 0.5\%$)	7.32	29.96	5.07	7.15	74.07	3.94
<i>CV</i> , % ($p < 0.5\%$)	2.92	10.77	2.01	2.59	29.74	1.43
Chernozem						
5	0.620	0.120	0.460	0.290	0.250	0.540
10	0.537	0.122	0.520	0.298	0.222	0.500
50	0.572	0.189	0.458	0.279	0.267	0.560
80	0.533	0.158	0.440	0.249	0.296	0.570
<i>Kr</i> , % ($p < 0.5\%$)	15.38	46.86	17.04	17.56	28.60	12.90
<i>CV</i> , % ($p < 0.5\%$)	6.18	19.32	6.43	6.66	10.38	4.94

It has been proved that under the conditions of ecosystem pollution the most intense bioaccumulation is characterized by Cd, Cu, Ni, moderate – Zn, Co, and the smallest – Pb. There were obtained the plant up-taking indexes (*UI*) at 5, 10, 50, 80% reduction of phytomass of *Hordeum vulgare* L. The highest plant up-taking indexes were recorded for Cd (0.533-0.645) in polluted ecosystem. Plant up-taking availability allows predicting the amount of metal present in plant and helps to determine the quality of agricultural products. There was obtained the following ranking of the trace metals Cd> Ni> Cu> Zn> Co> Pb according the value of their up-taking index in polluted ecosystem.

Phytotoxicological classification. The phytotoxicological classification of hazardous trace metals has been developed on the basis of such indicators as: C_{contr} , $PhLD_5$, $PhLD_{50}$, $PMAC$, μ , UI_{10} , UI_{50} . There were obtained the coefficient of correlation (r , $p < 5\%$) between values among themselves ($PhLD_5$, C_{contr} , $PMAC$, $PhLD_{50}$, μ , UI_5 , UI_{10}) for Cd, Pb, Zn, Cu, Ni, Co.

The strongest relation was between $PhLD_5$ - $PMAC$, $PhLD_5$ - $PhLD_{50}$, C_{contr} - $PMAC$, $PhLD_5$ - μ , $PhLD_{50}$ - μ , UI_{10} - $PhLD_5$, UI_{50} - $PhLD_{50}$. High correlation (r ($p < 5\%$) $> 0.6-0.88$) had almost all the indices with μ . This indicates the correlation between toxicity and physical and chemical properties of the pollutants. Between μ and UI_{10} as well as UI_{50} , the coefficient of correlation ($p < 5\%$) was 0.89-0.9, which proves the high correlation between metal's bioaccumulation and polarity of trace metals substances.

The example of calculation of classes ranges for $PhLD_5$ (data from Table 1):

$$Lim = x_{max} - x_{min} = 399.46 - 14.72 = 379.74, \quad (11)$$

$k=4$,

$$dx = Lim / k = 379.74 / 4 = 94.94 \approx 95, \quad (12)$$

$$Lower\ limit\ 1\ class = x_{min} - dx/2 = 14.72 - 95/2 = -32.78, \quad (13)$$

$$Upper\ limit\ 1\ class = x_{min} + dx/2 - \sigma = 14.72 + 95/2 - 1.0 \approx 61, \quad (14)$$

$$Lower\ limit\ 2\ class = Upper\ limit\ 1\ class + \sigma = 61 + 1.0 = 62, \quad (15)$$

$$Upper\ limit\ 2\ class = Lower\ limit\ 2\ class + dx - \sigma = 62 + 95 - 1.0 = 156, \quad (16)$$

$$Lower\ limit\ 3\ class = Upper\ limit\ 2\ class + \sigma = 156 + 1.0 = 157, \quad (17)$$

$$Upper\ limit\ 3\ class = Lower\ limit\ 3\ class + dx - \sigma = 157 + 95 - 1.0 = 251, \quad (18)$$

$$Lower\ limit\ 4\ class = Upper\ limit\ 3\ class + \sigma = 251 + 1.0 = 252, \quad (19)$$

$$Upper\ limit\ 4\ class = Lower\ limit\ 4\ class + dx - \sigma = 252 + 95 - 1.0 = 346, \quad (20)$$

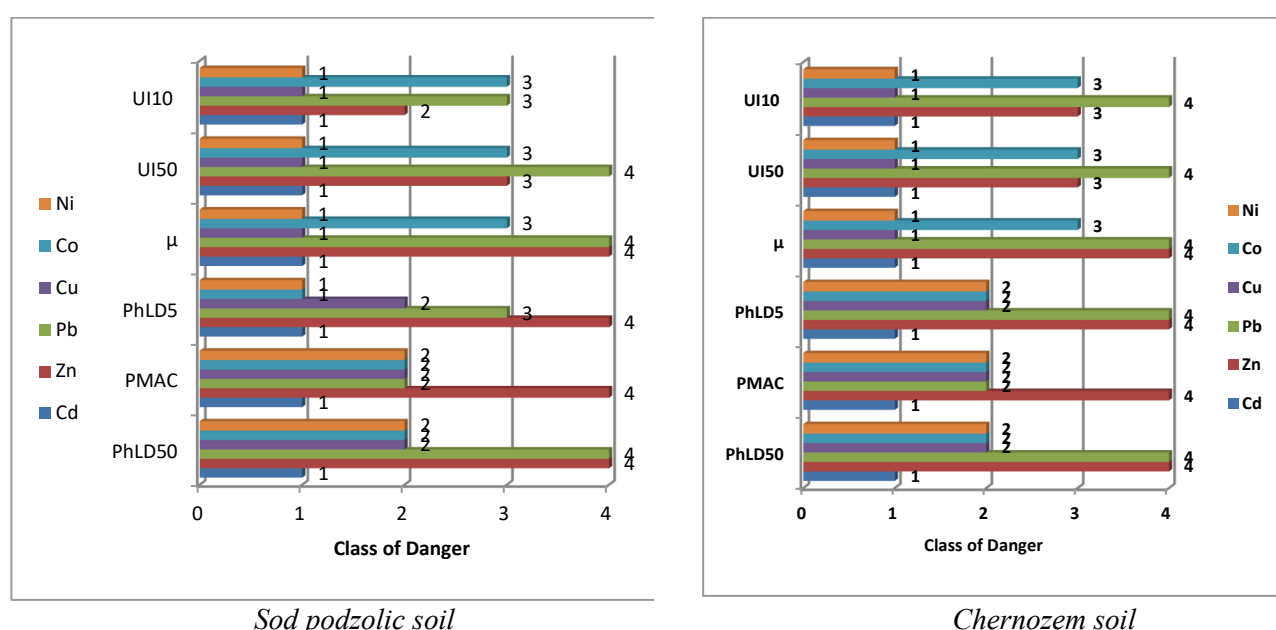
There were obtained the ranges of other values ($PhLD_{50}$, $PMAC$, μ , UI_{50} , UI_{10}) similar to $PhLD_5$.

The ranges of each value in phytotoxicological classification of hazardous trace metals are shown in the Table 3.

Table 3. The ranges of each value in phytotoxicological classification of hazardous trace metals

Value	Class of danger of trace metals			
	I Highly dangerous	II Dangerous	III Moderately dangerous	IV Low dangerous
$PhLD_{50}$ ($mg\ kg^{-1}$)	< 126	127–279	278–432	> 433
$PMAC$ ($mg\ kg^{-1}$)	< 6	7–17	18–28	> 29
$PhLD_5$ ($mg\ kg^{-1}$)	< 61	62–156	157–251	> 252
μ (μ)	> 8.79	8.57–8.78	8.35–8.56	< 8.34
UI_{50}	> 0.475	0.474–0.355	0.354–0.235	< 0.234
UI_{10}	> 0.413	0.293–0.412	0.173–0.292	< 0.172

According to the obtained ranges of each of the studied values, all trace metals were classified into one of the four classes of danger (Fig. 2).

**Figure 2.** Distribution of trace metals to 4 classes of danger in sod podzolic and chernozem soils

The phytotoxicological classification of hazardous trace metals is shown in Table 4.

Table 4. The phytotoxicological classification of hazardous trace metals

Metal	The phytotoxicological class of danger of metal (<i>The average value</i>)
Cd	I
Zn	III
Pb	IV
Cu	II
Co	I
Ni	I

According to phytotoxicological classification, Cd, Co, Ni belong to the first class of hazard, Cu – to second class of hazard, Zn – to third class of hazard, Pb – to fourth class of hazard.

Conclusions

As a result of this investigation, it was proposed the phytotoxicological classification of trace metals hazard. The phytotoxicological classification by the following indicators: plant up-taking index (UI_{10} , UI_{50}), phytolethal dose ($PhLD_{50}$), dipole moment of trace metals ditizonate (μ), *Phyto Maximum Allowable Concentration* (PMAC). We offer to use the four classes of danger in phytotoxicological classification of hazardous trace metals.

According to phytotoxicological classification, Cd, Co, Ni belong to the first class of hazard, Cu – to second class of hazard, Zn – to third class of hazard, Pb – to fourth class of hazard. Phytotoxicological classification of hazardous trace metals gives the possibility to estimate the danger of trace metals directly for plants as a biological object that playing a very important role in the life of ecosystem.

We offer to use the *Phyto Maximum Allowable Concentration* as a permissible level for plants in soil in the polluted ecosystem. The algorithm of calculation of *Phyto Maximum Allowable Concentration* based on the approach of the existing calculation of Maximum Allowable Toxic Concentration (MATC) was proposed. The *Phyto Maximum Allowable Concentrations* were obtained for *Hordeum vulgare* L. for all researched trace metals in two soils (mg kg^{-1} ; 1 N HCl): Cd–1.21; Cu – 7.60; Co–9.77; Zn – 30.77; Ni – 7.40; Pb – 7.48 (sod podzolic sandy loam on layered glacial sands), and Cd – 1.46; Cu – 13.10; Co–13.61; Zn– 44.90; Ni – 12.69; Pb – 9.20 calcareous deep chernozem on loamy loess). The *Phyto Maximum Allowable Concentration* gives the possibility to set the permissible level of metal in soil for plant as a biological organism, but not from the point of view of hygienic regulation. The using of concept of *Phyto Maximum Allowable Concentration* may be suitable for receiving a permissible level of trace metals in different soils for other plants in polluted ecosystems.

Conflict of Interest

The author declares that there is no conflict of interest.

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