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METALS PHYTOTOXICITY ASSESSMENT AND PHYTO MAXIMUM ALLOWABLE CONCENTRATION

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Abstract. In this paper, the influence of 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 highlight the metals phytotoxicity with help of the phyto maximum allowable concentration. The phyto maximum allowable concentration is a permissible level of metals for plants in the polluted soil and represents the safe degree for plants in contaminated ecosystem. The phyto maximum allowable concentration gives the possibility to estimate and to forecast the danger of 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 metals phytotoxicity assessment for other plants.

Keywords: trace metals (Cd, Pb, Cu, Co, Ni, Zn), phytotoxicity assessment, spring barley, phyto maximum allowable concentration

INTRODUCTION

Metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons (Nagajyoti *et al*. 2010, Tangahu *et al*. 2011, Mamatha *et al*. 2014). Metals, such as cadmium, copper, lead, nickel, cobalt and mercury are

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major environmental pollutants, particularly in areas with high anthropogenic pressure. Metal accumulation in soils and plants is of concern in agricultural production due to the adverse effects on food safety and marketability, crop growth due to phytotoxicity, and environmental health of soil organisms. The influence of plants and their metabolic activities affects the geological and biological redistribution of heavy metals through pollution of the air, water and soil (Nagajyoti *et al.* 2010, Gyuricza *et al.* 2010).

Anthropogenic 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 the ecosystem (Bradl 2005, Alloway 2010, Kabata-Pendias and Mukherjee 2007). Often, plants are the main accumulator of metals in the polluted ecosystem (Ryzhenko and Kavetsky 2017). At the same time, plants play an important role in the ecosystem as biomass producers and as biodiversity creators (Rombke and Moltmann 1996, Kabata-Pendias and Mukherjee 2007, Sardar *et al.* 2013). Usually, phytotoxicity is considered as a harmful influence of metal on plant growth and development (Kabata-Pendias and Mukherjee 2007, Nagajyoti *et al*. 2010, Satpathy *et al*. 2014, Gill 2014, Ryzhenko 2017). However, the setting of a safe level of toxicant for the plant is also very important, because it can help to prevent and to control the negative effects of metals in the ecosystem (Ryzhenko *et al*. 2017a).

Today a methodology that would determine the safe concentration of metals directly for plants in the soil is absent. After all, the existing standards for the content of metals in environmental objects are sanitary-hygienic and focused just on human health (Lewis 1998, Smirnov *et al*. 2002, Warne and van Dam 2008). Determination of the metals safe level in the soil for plants can help to objectively assess state of the ecosystem and prevent the metals dangerous influence on plant (Ryzhenko *et al*. 2017b). The phyto maximum allowable concentration (PMAC) was suggested as a safe level of metal in the soil for plants.

MATERIALS AND METHODS

Spring barley (*Hordeum vulgare* L.) was selected as a model plant. Spring barley is one of the important cereals crop in Ukraine. Mean standard deviations, variance, and minimum, maximum, standard errors were calculated from four replicates. The experimental results were interpreted using standard statistical methods.

The soils of experimental pots were: sod podzolic sandy loam on layered glacial sands (Podzol soil) and calcareous deep chernozem on loamy loess (Chernozem soil). 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 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 diphenyldiithiocarbazone (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. The method is officially recognized in Ukraine (Kavetsky *et al*. 2001). The thin layer chromatography for the determination of trace metals in soils and plants is not only very sensitive, but also a highly productive and inexpensive method that allows to effectively determine the six metals in the sample simultaneously. 1 M HCl was used as an extractor in the method of metal determination (TLC) for not only available, but also mobile and potentially mobile forms of metals in the soil. After all, it is known that the total form of the metal in the soil does not give a complete picture of the behavior of metals in the "soil-plant" system. 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 experiment

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 metal 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 methodology (Dospekhov 1985, Medvedev *et al*. 1998, Gorodniy 2008). The metals were added to soil during soil preparation before filling the pots. Then, spring barley germinated seeds were planted into the pots and, at the stage of 3 leaves, the recommended population was established.

In this study, the algorithm of calculation of PMAC was proposed similar to the existing approach of calculation of maximum allowable toxic concentration (MATC) (equation 1) (Rand 1994). In the toxicology practice, the scheme to substance toxicity assessment using the lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) is quite effective and widely used (Smirnov *et al*. 2002, Warne and van Dam 2008, *Guidance Document on…* 2005, *Globally Harmonized System…* 2011). These indicators are also used to calculate the MATC on behalf of assessing the toxicity of substances in the aquatic environment. MATC is calculated by the following formula (Rand 1994):

$$\mathrm{MATC} = \sqrt{(\mathrm{NOEC}) * (\mathrm{LOEC})} \tag{1}$$

Where:
NOEC – no observed effect concentration
LOEC – lowest observed effect concentration

We propose to determine the PMAC by the following formula:

$$\mathrm{PMAC} = \sqrt{\mathrm{C_{contr}} * \mathrm{PhLD_5}} \tag{2}$$

Where:
C_{contr} – background concentration (on the control variant of experiment – without additional metal input)
$PhLD_5$ – phytotoxic 5% dose ($PhLD_5$) which caused the 5% reduction of initial weight (height, length of root, etc.)

In my opinion, the 5% reduction of initial weight is the minimal effect, which is similar to the LOEC and 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 5% level ($p < 0.05$) was chosen. The algorithm of obtaining the $PhLD_5$ was represented in previous papers (Ryzhenko *et al*. 2017b).

RESULTS AND DISCUSSION

Table 2 presents the values of $PhLD_5$ and PMAC for all investigated metals, as well as the background concentration in the soil (0–20 cm). PMAC was obtained with the help of equation 2. The PMAC for Cd in sod podzolic soil was calculated in this way:

$$PMAC_{Cd} = \sqrt{0.1 * 14.72} = 1.21 \text{ mg·kg}^{-1} \quad (3)$$

The PMAC for Ni, Pb, Cu, Co, Zn in the two soils were calculated similarly.

Table 2. $PhLD_5$, PMAC, and the background concentration in the soil (0–20 cm, 1 N HCl, mg·kg^{-1})

Metal	$PhLD_5$	C_{contr} (background concentration in soil, 0–20 cm)	PMAC
		Sod podzolic (1 M HCl, mg·kg^{-1})	
Cd	14.72	0.10±0.02	1.21
Pb	186.64	0.30±0.05	7.48
Zn	394.46	2.40±0.30	30.77
Cu	62.91	0.92±0.10	7.60
Co	57.94	1.50±0.15	9.77
Ni	50.12	1.10±0.10	7.40
		Chernozem (1 M HCl, mg·kg^{-1})	
Cd	19.25	0.11±0.02	1.46
Pb	264.24	0.32±0.05	9.20
Zn	380.19	5.30±0.50	44.90
Cu	65.61	2.60±0.20	13.10
Co	74.13	2.50±0.20	13.61
Ni	69.98	2.30±0.30	12.69

The lowest value of the PMAC had Cd, the highest value of the PMAC had Zn in the two studied soils. The chernozem soil had higher values of the PMAC than Sod podzolic soil. It could be explained by higher content of organic matter, granulometric composition of soil and other properties of chernozem soil.

According to the value of PMAC, the metals can be ranked in the following descending order: Zn > Co > Cu > Ni > Pb > Cd. The PMAC could be used as an environmental standard that regulates the safe level of pollutants in the soil for plant.

CONCLUSIONS

As a result of this investigation, it was proposed to use the PMAC as a permissible level for plants in the soil in the polluted ecosystem. The algorithm of calculation of PMAC was based on the approach of the existing calculation of MATC.

The PMAC was obtained for *Hordeum vulgare* L. for all researched metals in the 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 PMAC gives the possibility to set the permissible level of metal in the soil for plant as a biological organism, but not from the point of view of hygienic regulation. The using of concept of PMAC may be suitable for receiving the permissible level of metals in different soils for other plants in polluted ecosystems. Phyto maximum allowable concentration gives the possibility to estimate the danger caused by metals directly for plants as a biological object that play a very important role in the ecosystem.

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