

PROBIT ANALYSIS FOR Cd, Pb, Cu, Zn PHYTOTOXICITY ASSESSMENT

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The aim of investigation was to develop a new approach in Cd, Zn, Cu, Pb phytotoxicity assessing. This approach provided the probit analysis using. Methods included probit analysis, thin layer chromatography, statistical methods (calculation of the least significant difference, correlation analysis). We applied «doze-effect» model to obtain the ranking of the metals according to their phytotoxicity in spring barley field. We offered to estimate the phytotoxicity by $PhLD_{50}$ index. Research results were: graphic formalization of “dose-effect” dependence and calculation of phytotoxic doses ($PhLD_{50}$ and $PhLD_{95}$) for Cd, Cu, Pb, Zn in polluted soil. According to $PhLD_{50}$ value we conducted a comparative assessment of Cd, Cu, Pb, Zn phytotoxicity relatively to spring barley. According to $PhLD_{50}$ value metals could be ranked: Cd>Cu>Pb>Zn. The most toxic metal was Cd. $PhLD$ values, on which the estimation of metals phytotoxicity, were: Cd — 50, Cu — 129, Pb — 537, Zn — 603 mg/kg mobile forms in turf-podzol sandy loam soil. Our findings are relevant to estimating the metal hazard and controlling the condition of the crop growth.

Key words: heavy metals, phytotoxicity, probit analysis.

Investigation of damaging effect factors (for ex. pollutants toxic effects) on biological objects usually requires probit analysis. The idea of probit analysis was first published by Bliss in 1934 in an article devoted to the impact of pesticides on the dead pests percentage [1]. Bliss suggested to calculate the percent of dead pests using block probability— *probability unit* (or *probit*), which got the complete definition by Finney [2]. It is known that measuring of the lethal dose for an exact individual is almost impossible, because the death of the plant organism often occur not immediately. If the dose is not sufficient to cause the death of the plant organism, it also turns out over time. Moreover, the accurately determination of the dose at which 100% of individuals die is not possible and not acquitted. That's why usually there is the determination of the dose at which 50% and 95% of individuals are dying (or biomass decreasing). These doses are taken as averages characteristics of lethal effect of damaging factors and indicate respectively LD_{50} and LD_{95} [3]. Methods of probit analysis using, and calculation of plant or animal organisms death resulted by the toxic factor in agrocenoses in field are described in [3].

Transformation of dead plants percent to *probit*, and “dose-effect” curve are shown in Table 1 and in Figure 1.

Probit analysis is widely used in Toxicology because LD_{50} and LD_{95} are among the most important indexes in assessing the toxicity of the substance. The more LD_{50} and LD_{95} , the less toxic to certain populations. Usually LD_{50} index is used in toxicology as “doze-effect” correlation for assessment of toxicity substances for human's life with following methodology of extrapolating data [4, 5]. However, there are not ecotoxicological studies which should to determine the LD_{50} and LD_{95} for living components of the ecosystem. Although, the biological productivity of ecosystems is an important parameter which characterized the condition of ecosystems and comfortable human existence in it as the last link in the food chain [6, 7].

Modern ecotoxicologic assessment of pollutants danger includes several indexes bypassing attention of calculation lethal doses for biological objects in ecosystem [8–12]. In multimetallic chronic pollution, which are typical for natural conditions, it is impossible to establish a clear “doze-effect” dependence

Table 1. Table of transformed percentage killed plant into probit [1–3]

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

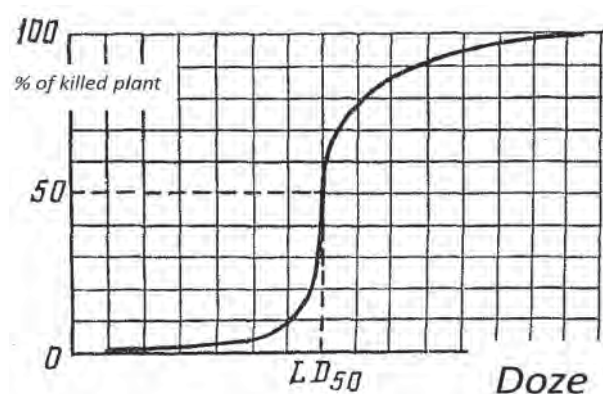


Fig. 1. Curve of dose-effect correlation

for each metal. But, in practice, single heavy metals pollution occurs rarely. Therefore, the simulation conditions of soil contamination impact allow to define the “dose-effect” with various concentrations of metal in the soil and to establish lethal dose in plant (LD_{50} and LD_{95}). Such an experiment will be assessed phytotoxicity each of the pollutants that can be used in calculating the risk of contamination of toxic metals in the environment. The need for such research is of particular relevance in terms of pollutant contamination of agro-ecosystems, quantity and quality crop production which is very important for humans as consumers of it [6–12]. In addition, the establishment of indicators lethal doses will enable comparative evaluation of the toxicity of pollutants in relation to biological objects.

We offer to define the lethal indexes for plant (phytotoxicity) using known probit analysis analogously to LD_{50} and LD_{95} .

Phytotoxic lethal dose ($PhLD_{50}$ and $PhLD_{95}$) is the amount of pollutants in the soil (or plant) ($mg\ kg^{-1}$) which results 50% (or 95%) death of plants.

Our research has been devoted to defining indexes $PhLD_{50}$ and $PhLD_{95}$ of Cd, Zn, Cu, Pb for spring barley in polluted turf-podzol sandy loam soil.

Impact pollution is a one-time (or non-systematic) contamination of soil pollutants leading to disruption of biotic component of ecosystems, i.e. it is formed the artificially created biogeochemical endemic [13–15].

Impact and chronic pollution of toxic metals in agro-ecosystems generally occurs near proximity anthropogenic (industrial) landscapes. In order to predict the harmful effect of metals behavior in polluted conditions in such landscapes, it is necessary to have ecotoxic preliminary data about these pollutants, for example, their kinetics, soil profile migration, effects on micro biota, plant up-taking and others properties. For this purpose, the model experiment included the artificial adding of different doses of Cd^{2+} , Pb^{2+} , Cu^{2+} , Zn^{2+} salts in sod podzolic sandy loam soil.

Materials and Methods

The soil of experiment was sod podzolic sandy loam on layered glacial sands (sod podzolic). 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. Crop was spring barley. Research conducted at the Institute of Chernigov APP UNAAS. Experimental studies conducted over the years 1999–2012.

Scheme of experiment (adding of toxic metals salts to soil)

Control (no HM application)	
Cu ²⁺ :	Zn ²⁺ :
100 mg kg ⁻¹ of the soils (1 MAC)	600 mg kg ⁻¹ of the soils (2 MAC)
150 mg kg ⁻¹ of the soils (1,5 MAC)	900 mg kg ⁻¹ of the soils (3 MAC)
200 mg kg ⁻¹ of the soils (2 MAC)	1200 mg kg ⁻¹ of the soils (4 MAC)
300 mg kg ⁻¹ of the soils (3 MAC)	1500 mg kg ⁻¹ of the soils (5 MAC)
Cd ²⁺ :	Pb ²⁺ :
15 mg kg ⁻¹ of the soils (5 MAC)	150 mg kg ⁻¹ of the soils (5 MAC)
30 mg kg ⁻¹ of the soils (10 MAC)	300 mg kg ⁻¹ of the soils (10 MAC)
60 mg kg ⁻¹ of the soils (20 MAC)	450 mg kg ⁻¹ of the soils (15 MAC)
90 mg kg ⁻¹ of the soils (30 MAC)	900 mg kg ⁻¹ of the soils (30 MAC)
150 mg kg ⁻¹ of the soils (50 MAC)	1200 mg kg ⁻¹ of the soils (40 MAC)
300 mg kg ⁻¹ of the soils (300 MAC)	1500 mg kg ⁻¹ of the soils (50 MAC)

That amount corresponds with those adopted in Ukraine Maximum Allowed Concentration (MAC) in soil.

The following metals salts: Pb(NO₃)₂, ZnSO₄·H₂O, CuSO₄·7H₂O, CdSO₄, were used for the trace elements application. Spring barley (*Hordeum vulgare* L.) was selected as a model plant. Soil preparation, pots filling, and trials were carried out in accordance with standard methods [3]. 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 (№ 50-97, 19.06.1997) [16].

For studying the total biomass we used the average value of biomass within agrodempopulation, which, in our case, consists of a set of spring barley individuals in per unit area with a certain concentration of mobile and potentially mobile forms of metals in the soil. 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. Probit analysis was applied according to Dospekhov V. [3]. This method applies to the generalized system modifications probit. It allows only estimating of *PhLD*₅₀ and *PhLD*₉₅ approximately, because it cannot allow calculating the confidence intervals of these values. Thus it may be possible some errors in values of “doza-effect”, and as a result in *PhLD*₅₀ and *PhLD*₉₅. However, on the accuracy of *PhLD*₅₀ it affects very slightly. To avoid these shortcomings and

draw the line that best fits the experimental set points, it is necessary to use more complex system modifications *probit*. However, the uses of sophisticated methods are not always beneficial, because in most cases this accuracy is not required.

Results and Discussion

Experimental data are shown in Table 2. Except experimental data Table 2 includes the values of lg D (where D is a 1 M HCl extracted forms in soil, mg kg⁻¹) and *probit* values.

Probit value 5 corresponds to the 50% of biomass reduction, *probit* value 6,64 corresponds to the 95% of biomass reduction in plant population (Table 1) [1–3]. For example, inhibition 19,3% of biomass corresponds to the *probit* 4.12 in the polluted by Cd soil (tables 1, 2); [1–3].

Relationship between LgD of Cd²⁺, Pb²⁺, Zn²⁺, Cu²⁺, Co²⁺, Ni²⁺ and *probit* on the studied soils are shown in figures 2, 3, 4. Lg of dose marked on the *ox*, and values of *probit* marked on the *oy*.

The correlation between LgD of Cd²⁺ and a *probit* for sod podzolic sandy loam on layered glacial sands was:

$$y = 3.0274x - 0.1749 \quad (1)$$

If *probit* equals 5 (*PhLD*₅₀ calculation), from here:

$$5 = 3.0274x - 0.1749, \text{ and } x = 1.7 \quad (2)$$

The antilogarithm (1.7) = 50 – *PhLD*₅₀.

If *probit* equals 6.64 (*PhLD*₉₅ calculation), from here:

Table 2. Heavy metals pollution impact on concentration of its available form in sod podzolic soil and reduction of spring barley biomass

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

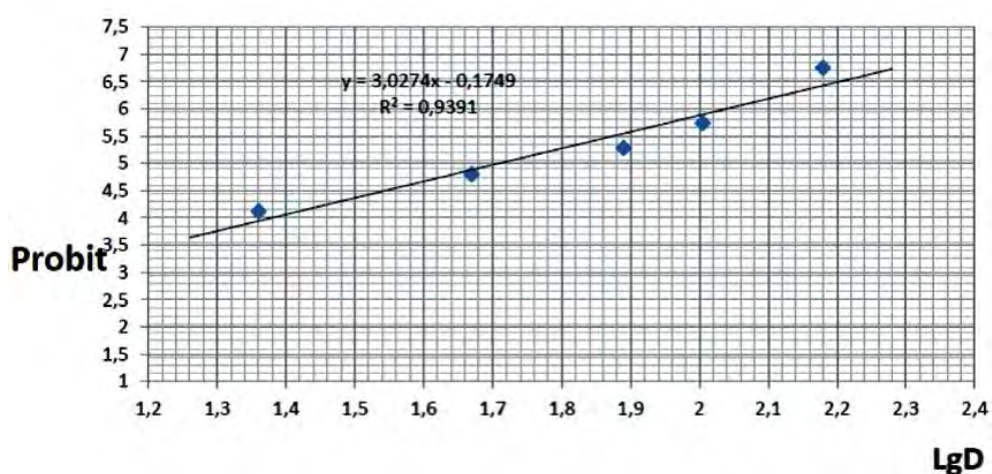


Fig. 2. Correlation between LgD of Cd and *probit* in the condition of sod podzolic sandy loam on layered glacial sands

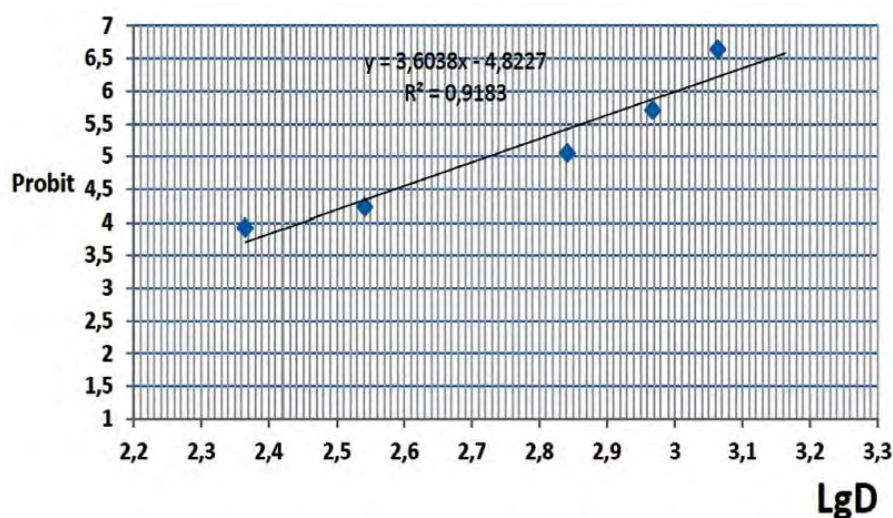


Fig. 3. Correlation between LgD of Pb and *probit* in the condition of sod podzolic sandy loam on layered glacial sands

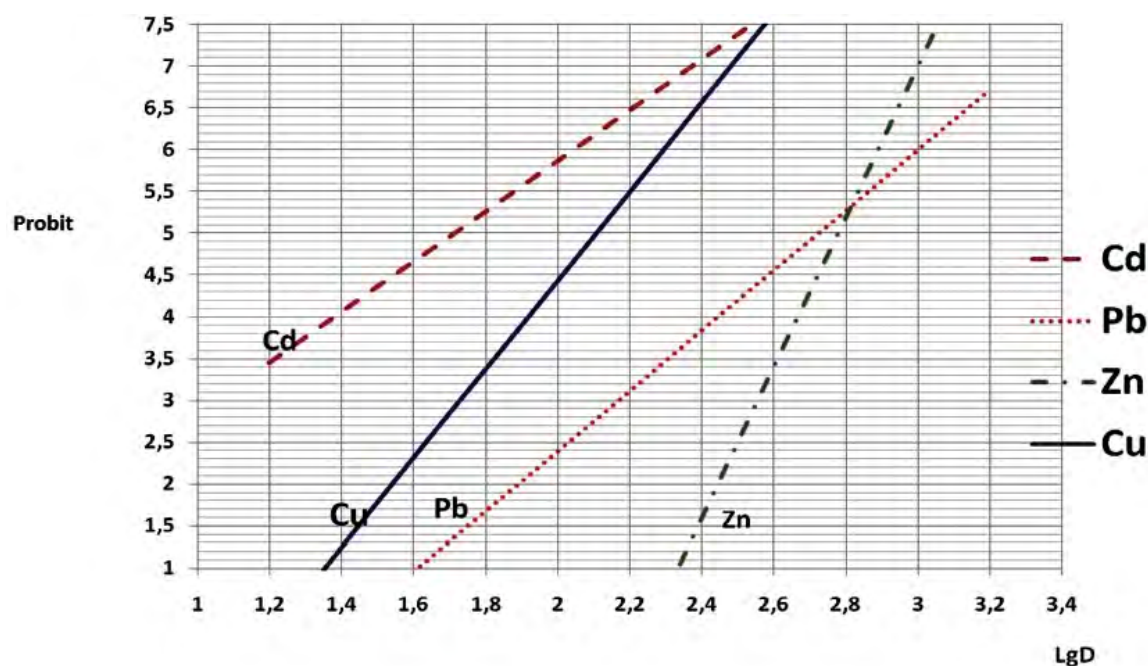


Fig. 4. Correlation between LgD of Trace Metals and *probit* in the condition of sod podzolic sandy loam on layered glacial sands

$$6.64 = 3.0274x - 0.1749, \text{ and } x = 2.3 \quad (3)$$

The antilogarithm $(2.3) = 199.5 - PhLD_{95}$.

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

$$y = 3.6038x - 4.8227 \quad (4)$$

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

$$5 = 3.6038x - 4.8227, \text{ and } x = 2.73 \quad (5)$$

The antilogarithm $(2.73) = 537 - PhLD_{50}$.

If *probit* equals 6.64 ($PhLD_{95}$ calculation), from here:

$$6.64 = 3.6038x - 4.8227, \text{ and } x = 3.18 \quad (6)$$

The antilogarithm $(3.18) = 1513.6 - PhLD_{95}$.

The correlation between between LgD and *probit* is presented in table 3. Values of $PhLD_{50}$

Table 3. 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	$y = 4.1516x - 3.4822 (R^2 = 0.88)$

Table 4. The $PhLD_{50}$ and $PhLD_{95}$ of Cd^{2+} , Pb^{2+} , Zn^{2+} , Cu^{2+} in Sod podzolic soil (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

and $PhLD_{95}$ for each metal are presented in Table 4.

So $PhLD_{50}$ and $PhLD_{95}$ indicators characterize phytotoxicity of metals. For assessing the phytotoxicity of these toxicants used $PhLD_{50}$. According to $PhLD_{50}$ summarizing the results the metals can be ranked by descending phytotoxic order as follow: Cd>Cu>Pb>Zn.

According to $PhLD_{95}$ the metals can be ranked by descending phytotoxic order as follow: Cd>Cu>Zn>Pb. This is because the large doses of lead and other metals causes the defense mechanisms of plant organisms that are responsible for the normal up-taking. Often, in such cases, the break down of defense mechanisms results to freely up-taking toxicants instead nutrients. Such phenomenon describes in many environment axioms, in particular in Shelford's regularity. Diapason of resistance (tolerance) is between organism limit factors (pessimisms). That's why commonly known toxicological practice of lethal dose establishing operates 50% changing of population. Furthermore, it is known that plants root up-taking of lead has less intensity than other ways of plant up-taking.

Some studies indicate that low doses of lead are often not only inhibit the production

of biomass crops, but also cause a stimulating effect which is observed when feeding micronutrient [15–20]. It is established that small amounts of lead needed to plant organisms. Although lead is present in all living organisms, it is proven its vital necessity. But on the other hand, toxicity or biological role or mechanisms of lead's action are studied very poorly [19–21]. Therefore, setting up of experiments for studying the "dose-effect" dependence should consider not only the effect of depression, but stimulating effect of metals in small quantities. Indeed, all metals in small quantities play role as ultra-trace elements [15–21].

We offer to use the probit analysis for assessing the phytotoxicity metals.

Graphic formalization of "dose-effect" dependence for Cd, Cu, Pb, Zn relative to spring barley was built. The $PhLD_{50}$ and $PhLD_{95}$ for each metal were calculated.

We suggested to estimate the heavy metals phytotoxicity by means of $PhLD_{50}$ value. The results helps to compare phytotoxicity of studied metals Cd, Cu, Zn, Pb for plants of Spring barley (*Hordeum vulgare* L.) on sod podzolic sandy loam on layered glacial sands (sod podzolic). The $PhLD_{50}$ value is indicates not only 50% reduction of biomass for spring

ПРОБІТ-АНАЛІЗ ЗА ОЦІНЮВАННЯ ФІТОТОКСИЧНОСТІ Cd, Pb, Cu, Zn

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Мета роботи полягала у розробленні нового підходу при оцінюванні фітотоксичності важких металів (Cd, Zn, Cu, Pb), використовуючи пробіт-аналіз. Методами досліджень були пробіт-аналіз, хроматографія у тонкому шарі сорбенту, статистичні методи (розрахунок найменшої суттєвої різниці, кореляційний аналіз). Результатами досліджень були: графічні формалізації залежності «доза-ефект» з розрахунком фітотоксичних доз ($PhLD_{50}$ та $PhLD_{95}$) для Cd, Cu, Pb, Zn в умовах дерново-середньопідзолистого ґрунту за імпаکتного забруднення. За значенням $PhLD_{50}$ проведено порівняльну оцінку фітотоксичності Cd, Cu, Pb, Zn стосовно ячменю ярого. Найбільш токсичним виявився Cd. Таким чином, ряд фітотоксичності важких металів за величиною $PhLD_{50}$ має вигляд: Cd>Cu>Pb>Zn. Величини $PhLD_{50}$, за якими проведено оцінку фітотоксичності металів, становили для Cd — 50, Cu — 129, Pb — 537, Zn — 603 мг/кг рухомих форм на дерново-середньопідзолистому ґрунті. Результати дослідження стосуються оцінки небезпеки металу і контролю за станом росту сільськогосподарських культур.

Ключові слова: важкі метали, фітотоксичність, пробіт-аналіз.

ПРОБИТ-АНАЛИЗ ПРИ ОЦЕНКЕ ФИТОТОКСИЧНОСТИ Cd, Pb, Cu, Zn

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Цель работы заключалась в разработке нового подхода при оценке фитотоксичности тяжелых металлов (Cd, Zn, Cu, Pb), используя пробит-анализ. Методы исследований включали пробит-анализ, хроматографию в тонком слое сорбента, статистические методы (расчет наименьшей существенной разницы, корреляционный анализ). Результатами исследований были: графические формализации зависимости «доза-эффект» с расчетом фитотоксичных доз ($PhLD_{50}$ и $PhLD_{95}$) для Cd, Cu, Pb, Zn в условиях дерново-среднеподзолистости почвы с импаکتного загрязнения. По значению $PhLD_{50}$ проведена сравнительная оценка фитотоксичности Cd, Cu, Pb, Zn по отношению к ячменю яровому. Наиболее токсичным оказался Cd. Таким образом, ряд фитотоксичности тяжелых металлов по величине $PhLD_{50}$ имеет вид: Cd>Cu>Pb>Zn. Размер $PhLD_{50}$, по которой проведена оценка фитотоксичности металлов, составляла Cd — 50, Cu — 129, Pb — 537, Zn — 603 мг/кг подвижных форм на дерново-среднеподзолистой почве. Результаты исследования имеют отношение к оценке опасности металла и контролю за состоянием роста сельскохозяйственных культур.

Ключевые слова: тяжелые металлы, фитотоксичность, пробит-анализ.