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ESTIMATION OF THE OPTIMAL Cu CONTENT IN DIFFERENT SOIL TYPES BASED OF THE DYNAMIC MODEL FOR COPPER ACCUMULATION IN ABOVE GROUND PARTS AND ROOTS (ON THE EXAMPLE OF BARLEY *Hordeum vulgare* L. PLANTS)

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Abstract

Copper is one of the essential microelements for both animals and plants and plays an important role in a number of physiological processes. However, it becomes toxic to plants when entering high concentrations. The urgency of the work to determine the optimum and critical levels of copper content in soils, especially in the agricultural production, is caused by permanent technogenic pollution of soils with heavy metals. An increase in the content of copper in soils can cause changes in biochemical processes in plants, their morphology, and, ultimately, reduce productivity. The construction of complex dynamic models of heavy metals entering plants is not always justified, since most of the coefficients can be obtained only in laboratory experiments under conditions which are very different from natural ones. In our experiment, it was shown that it is possible to determine optimal and critical levels of soil contamination by heavy metals on the basis of an analysis of the dynamics of their accumulation in different parts of plants. Optimal and critical levels of contamination of two types of soils (sod-podzolic and chernozem) with copper were determined based on the analysis of the dynamics of Cu accumulation in the above-ground and root parts of barley plants (*Hordeum vulgare* L.) in vegetation trials. The concentration of copper in barley plants remains relatively constant throughout the IV-IX stages of organogenesis (20-60 days from the date of emergence). The Cu accumulation in the roots of barley plants linearly followed its content in the soil, while the accumulation rate in the shoots decreases with increasing copper content in the soil. The double excess of Cu accumulation in barley roots on sod-podzolic soil as compared to chernozem is probably due to agrochemical characteristics of soils. A function is proposed that reflects the dependence of the copper content in the shoots on its concentration in plant roots, which has the form of the sum of the exponential accumulation function and the linear elimination function due to the operation of the active molecular transport system Cu in plants: $Y = c \times X^a + b \times X \times (a - 1)^{-1}$. Approximation of the experimental data by this function made it possible to determine its coefficients: $a = 0.430 \pm 0.014$; $b = 0.020 \pm 0.005$; $c = 3.31 \pm 0.81$. Analysis of the dynamics of copper accumulation in the shoot and root parts of plants made it possible to determine the concentration at which, according to Becker's theory, a change takes place from the accumulative to the barrier type of metal accumulation, that is, the transition from increased accumulation of copper by a plant to protective mechanisms limiting the supply of metal. Optimum copper accumulation in barley plant was 7.6 mg/kg, with a total soil content of 3.5 and 6.9 mg kg for sod-podzolic soils and for chernozem, respectively. The calculated value of the «critical» concentration of copper in plant roots, at which its entry into the shoot due to passive transport and excretion due to active molecular transport mechanisms become equal, for barley is 650 mg/kg, and at this level the copper content in the shoot is 31 mg/kg. This level can be achieved with total soil Cu of 300 and 590 mg/kg for sod-podzolic soil and for chernozem, respectively.

Keywords: *Hordeum vulgare* L., barley, copper, sod-podzolic soil, chernozem, optimal level, critical level, dynamic model

Copper is one of the essential minor-nutrient elements. It plays an important role in a number of physiological processes [1] but becomes toxic to plants at high concentrations [2, 3]. In different soils, the total content of copper is from 20 to 110 mg/kg. However, Cu concentration is much lower in soil solutions and varies from 30 to 241 μg/l [4-6]. Emissions of industrial enterprises, the constant use of various products based on copper, in particular, pesticides and fertilizers, can lead to the accumulation of copper in the soils of agriculturally used areas. Significant accumulation of Cu affects not only the soil microbiocenosis but also the physical properties of the soil [7]. Therefore, the study of the mechanism of Cu entry from soil into plants remains relevant, especially for agricultural plants.

It was established [8] that the interspecific differences in terms of copper accumulation in the plant-soil system can reach 9-fold values. According to Baker's heavy metals absorption model, the accumulative, barrier or indicative type of protective reactions can be formed in plants depending on the content of metals [8]. The nature of protective reactions is determined by the processes controlling the entry and distribution of Cu in the organs and tissues of plants. The entry of various metals ions from the soil solution and their distribution in the cells of roots, xylem, in the apoplast and cytosol of the above-ground parts of plants are carried out both passively (due to osmosis) and through active transport with the involvement transporters encoded by different genes [9]. In recent years, the greater focus has been placed on the molecular mechanisms of these processes. For *Arabidopsis thaliana*, the participation of ferric reductase oxidases of the FRO family in changing the form of copper oxidation (Cu/Cu^{2+}), affecting the absorption of copper by plant roots from the soil, has been described [10]. Also, *Arabidopsis* shows the important role of genes of the COPT family (copper transporter), products of which are localized in the plasmatic membrane of the root cells, in the entry of Cu from the rhizosphere [11]. It is assumed that members of the zinc-regulated transporters (ZRT) family and iron-regulated transport proteins (IRT) can indirectly participate in the absorption and transfer of Cu to *Arabidopsis thaliana* and *Medicago truncatula* [12-13].

Various Cu-transporting adenosine triphosphatases of the HMA family are involved in the transfer of both mono- and divalent copper cations from the root symplast to the plant xylem, and also from the cytoplasm to the vacuole of the cell. In the opposite direction, the specific transport of only copper (II) ions through the plasmalemma is probably carried out by proteins of the COPT subfamily of the CTR family, which are also responsible for the absorption of Cu in leaves and other above-ground parts of the plant [11, 12, 17, 18]. For *Arabidopsis* and rice, seven proteins of the COPT type, the genes of which were expressed in virtually all tissues of the root and shoot, have been identified [18, 19]. In *Brassica napus*, the balance of intensity of *HMA5* and *ZIP4* genes expression was established; the protein products of this genes are localized in the plasmalemma and provide, respectively, the transport of Cu ions from the cytosol to the apoplast and the entry of Cu into the cytosol [20]. It has been shown that in response to an increase in the concentration of Cu in the medium, the intensity of *HMA5* gene expression sharply increases, while *ZIP4* gene expression is completely blocked, which suggests that these genes may participate in the regulation of intracellular homeostasis of Cu in order to limit its accumulation up to lethal concentrations.

Usually, the Cu content is from 2 to 50 mg/kg dry weight, depending on the plant species. The value of 5-20 mg/kg is optimal for most plants; the symptoms of toxicity appear above this value, and the symptoms of deficiency appear below this value [21, 22]. Both the deficit and excess of Cu affect the physiological processes in plants and, ultimately, the productivity [23, 24].

Construction of complex dynamic patterns of heavy metals entering plants is not always justified, since most of the coefficients can be obtained only during model experiments, the conditions of which are very different from the natural ones. Our experiment shows that it is possible to determine the optimal and critical levels of soil contamination by heavy metals on the basis of analysis of the accumulation dynamics in different parts of plants.

The purpose of the paper was to study the dynamics of copper accumulation in barley plants depending on its amount in the soil to assess the optimal and critical values of the content of this trace element.

Techniques. Vegetation experiments were performed on barley plants (*Hordeum vulgare* L.) of the Zazersky 85 variety. Plants were grown in vessels containing 4.5 kg of sod-podzol soil or leached heavy loamy chernozem. Agrochemical soil indicators, determined using the conventional methods [25], were pH_{KCl} 5.47 and 5.53, respectively; humus 1.7% and 4.8% (by Tyurin); exchange K_2O 64.7 and 134.3 mg/kg (by Maslova); mobile P_2O_5 805 and 214 mg/kg (by Kirsanov); hydrolytic acidity 2.7 and 3.0 mg-equ/100 g; the sum of the exchange bases 7.6 and 31.7 mg-equ/100 g (by Kappen). The total content of copper in soils was 3.8 and 9.1 mg/kg, which was used as a control. Prior to sowing, nutrients were added to the soil at the rate of $N_{200}P_{100}K_{100}$ mg/kg soil according to the active ingredient, Cu was added in the form of aqueous solutions of the nitric acid salt $Cu(NO_3)_2 \cdot 3H_2O$ up to 50, 100, 150 and 200 mg/kg for sod-podzol soil, 100, 150, 300 and 400 mg/kg – for heavy loamy chernozem. Barley was grown for 60 days from the date of the seedling to the milky stage. The sowing density is 13 plants on a vegetation vessel of 22 cm in diameter, 5 biological replications of the experiment.

The content of Cu in the above-ground biomass and plant roots was determined after 20, 30, 45 and 60 days from the date of seedlings (respectively, in the IV, V, VII and IX stages of organogenesis) [26]. During selection, plant roots were washed from the soil in distilled water. The experiments were performed in 3-fold biological and 2-fold analytical replications. The mass of the above-ground and root parts of the plants (for the air-dry state) was evaluated using the gravimetric method. The Cu content was determined by the atomic absorption method using the SpectrAA 250 Plus spectrometer (Varian, Inc., USA) as described [27]. Plant samples were mineralized with dry ashing according to RF State Standard GOST 26657-85.

The table and figures show the mean values (*M*) and their standard errors (*M*±SEM). The significance of differences with the control plants was established for the mean values using Student's *t*-test, for the variances using *F*-test at the significance level $p < 0.05$. For statistical data processing, we used Microsoft Office Excel 2003 and STATISTICA v.6 software package (StatSoft, Inc., USA, http://www.statsoft.com).

Results. The dynamics of copper accumulation in plant organs, depending on the metal content in the soils and the age of barley, is presented in the table. During the growth of plants, the amount of metal in the root and above-ground biomass changed insignificantly.

Accumulation of copper in barley (*Hordeum vulgare* L.) plants of the Zazersky 85 variety at different stages of organogenesis depending on the element content in soils (M ± SEM, greenhouse test)

Indicator	Introduced Cu, mg/kg	Organogenesis stage according to Kuperman			
		IV	V	VII	IX
	Sod-podzol sandy loam soil				
Cu content in the above-ground biomass, mg/kg	0	8.8±0.9	9.3±0.9	6.2±0.8	6.2±0.8
	50	18.6±1.2	23.0±1.2	18.8±1.0	21.6±1.1
	100	27.6±1.1	27.0±1.1	23.3±0.9	25.6±0.9
	150	29.1±1.9	31.0±1.9	23.7±1.6	25.6±1.7
	200	33.6±2.3	35.0±2.3	26.8±1.9	27.7±2.0
Cu content in roots, mg/kg	0	11.1±1.2	12.9±1.1	11.0±1.0	7.7±1.1
	50	94.2±5.7	84.2±5.3	109±5	92.6±5.3
	100	236±14	176±13	221±12	210±13

Table continued

	150	323±14	272±13	288±12	324±13
	200	517±18	489±17	439±15	474±17
	Leached heavy loam chernozem				
Cu content in the above-ground biomass, mg/kg	0	11.3±1.1	13.6±1.2	8.9±1.3	8.6±1.2
	100	19.5±1.6	23.6±1.8	16.9±1.8	16.3±1.7
	150	23.8±1.5	26.6±1.6	21.3±1.7	19.5±1.6
	300	25.2±1.3	29.5±1.5	24.7±1.5	23.0±1.5
	400	27.7±1.1	32.1±1.2	27.5±1.2	27.3±1.2
Cu content in roots, mg/kg	0	17.3±0.5	17.0±0.4	17.3±0.4	15.3±0.5
	100	67.0±8.2	81.2±6.4	104±7	81.0±7.6
	150	97.0±10	126±8	143±8	118±9
	300	223±8	224±6	252±7	246±7
	400	372±18	296±14	310±15	343±17

The analysis of the variance of data on the content of copper in the above-ground and root parts of the plants showed the dependence of the Cu amount in the above-ground parts on the physiological phase of development (for sod-podzol soil $F = 9.5$, for chernozem $F = 60.9$, $F_{0.05} = 3.49$) and did not identify such dependence for the roots (for sod-podzol soil $F = 1.98$, for chernozem $F = 0.58$, $F_{0.05} = 3.49$). The observed dependence is due to the increased accumulation of copper at the V stage of organogenesis and its decrease at later stages of plant development (see Table). However, in general, these changes do not exceed 20%.

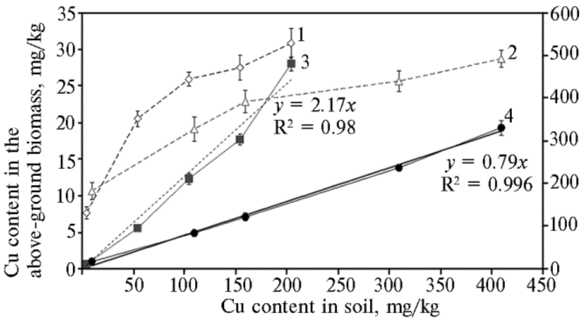

Fig. 1. Accumulation of Cu in barley (*Hordeum vulgare* L.) plants of the Zazersky 85 variety in the above-ground biomass on sod-podzol soil (1) **and chernozem** (2) **and in the roots on sod-podzol soil** (3) **and chernozem** (4) **depending on the element content in soils.** The average experimental values with an error are presented, dashed lines are equations and significance of linear approximation (greenhouse test).

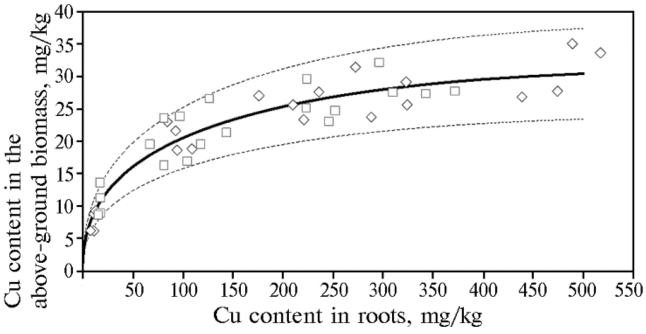

Fig. 2. Accumulation of Cu in barley plants (*Hordeum vulgare* L.) of the Zazersky 85 variety in the above-ground biomass, depending on the element content in the roots on sod-podzol soil (◇) and chernozem (□). The solid line is the approximation by the function (2), dashed lines – 95% confidence interval (greenhouse test).

Figure 1 shows the dynamics of copper accumulation in the above-ground and root parts of plants, depending on its content in soils of two types. The copper content in the roots had a definite linear dependence on the content of trace elements in the soil (the dashed lines in the figure represent the equations and the significance of linear approximation), while the accumulation rate in the above-ground biomass decreased with the increase in copper amount in the soil. A two-fold excess in the accumulation of Cu in barley roots on sod-podzol soil, compared with chernozem, is probably due to the agrochemical characteristics of soils and more solid fixation of copper in chernozem compared to sod-podzol soil. After the experiment was completed, the availability of copper from soils was determined

[25]. On average, in all variants with the introduction of copper into the substrate for sod-podzolic soil, the proportion of acid-soluble fraction (1 M HCl extraction) of the total copper content was 81±2%, of the total chernozem content 73±3%. The proportion of the mobile fraction (extraction with acetate-ammonium buffer, pH 4.8) was 43±4% and 7±2% for sod-podzol soil and chernozem, respectively.

Figure 2 shows Cu content in the above-ground biomass, depending on its content in the plant roots. It is obvious that the entry of Cu from the roots into the above-ground biomass is not due to the type of soil on which the plants were grown.

The entry of copper into the above-ground biomass from the roots of plants is regulated by the mechanisms of both passive (due to osmosis) and active transport with feedback, that is, the rate of Cu entry into the above-ground biomass from the plant roots should be proportional to the concentrations difference and inversely proportional to the copper content in the roots of plants:

$$\frac{\partial Y}{\partial X} = \frac{a \times Y - b \times X}{X}. \tag{1}$$

The solution of this equation is the following function:

$$Y = c \times X^{a} + \frac{b \times X}{a - 1}, \tag{2}$$

where Y is copper content in the above-ground biomass; X is the content of copper in the roots of plants; a, b, c are the coefficients.

The solid line (see Fig. 2) represents the calculated accumulation of copper in barley biomass at a = 0.430 ± 0.014; b = 0.020 ± 0.005; c = 3.31 ± 0.81. Dashed lines show 95% confidence interval of the approximation.

Since the coefficient is $a < 1$, so the function of copper content in the above-ground biomass of plants (2) summarizes both the entry of Cu from the roots and the reverse metal transport from the above-ground part to the root of the plants. In this case, the first term of the function ($c \times X^{a}$), characterizing the accumulation, completely coincides with the description of the regularity of copper entering barley plants established by R.D. Davis and P.H.T. Beckett [2, 3]. They showed that before reaching the copper concentration, which is critical for plants, the dependence of its accumulation in young barley plants is described by a linear function of the logarithms $\mathrm{Log}(y) = a + b \times \mathrm{Log}(x)$, where y is Cu content in plants, and x is the concentration in the nutrient solution.

The second part of the function (2) is provided by the active reverse transport system and corresponds to a direct linear dependence on the copper concentration. The linear dependence of gene expression activity, which provides transport of Cu in the plant, on its content was shown for *Brassica napus* [20]: in response to an increase in the concentration of Cu in the medium, the intensity of *HMA5* gene expression sharply increases, while *ZIP4* gene expression is completely blocked.

Mechanisms of heavy metals content regulation in plant organs and tissues form the corresponding type of protective reaction according to Becker's theory: in case of shortage of a trace element in the soil, a storage type of accumulation is observed, in case of excess – the barrier type of accumulation is observed. The indicator of the transition from one type of accumulation to another is the ratio of the metal content in the above-ground and root parts of the plant: if it is > 1, then the type is accumulative, if < 1, then the type is barrier [8]. Consequently, according to the dynamics of Cu accumulation in the above-ground and root parts of plants, it is possible to determine the concentration of Cu in the soil at which a change in the type of metal accumulation from the accumulative to the barrier takes place. This point will correspond to the copper content in the soil, which is optimal for plant nutrition:

$$\frac{Y}{X}=c\times X^{a-1}+\frac{b}{a-1}=1\,, \tag{3}$$

$$X=\sqrt[a-1]{\frac{a-b-1}{c\times(a-1)}}\;. \tag{4}$$

The content of Cu in the roots and above-ground biomass of the plant, which is optimal for barley (7.6 mg/kg) is determined using the approximation coefficients obtained for the function (2). Based on the linear dynamics of copper accumulation in the roots (see Fig. 1), the total content of Cu in the soil, which is optimal for barley, is 3.5 and 6.9 mg/kg for sod-podzol soil and for chernozem, respectively.

The experiment used the quantities of Cu in soils that do not cause obvious toxic effects in barley. It can be assumed that when the critical Cu content in the roots is reached, its entry into the above-ground biomass due to passive transport and removal by means of active molecular mechanisms of transport will be equal:

$$\frac{\partial Y}{\partial X}=c\times a\times X^{a-1}+\frac{b}{a-1}=0\,. \tag{5}$$

Based on this assumption, it is possible to calculate the critical accumulation of Cu in plant roots:

$$X=\sqrt[a-1]{\frac{b}{c\times a\times(1-a)}}\;. \tag{4}$$

The Cu content in the roots, which is critical for barley, 650 mg/kg, can be determined using the approximation coefficients obtained for the function (2). At this value, the content of copper in the above-ground biomass will reach 31 mg/kg. Accordingly, the total content of Cu, which is critical for barley, in sod-podzolic soil and chernozem is 300 and 590 mg/kg, respectively.

Thus, the intensity of copper accumulation in barley plants does not depend on the physiological phase of plant development and remains relatively constant throughout the IV-IX stages of organogenesis. The accumulation of Cu in the roots of barley plants is linear and determined by the content of copper in the soil. A twofold excess in the accumulation of Cu in barley roots on the sod-podzol soil in comparison with chernozem is probably due to the characteristics and buffer capacity of soils. The dependence of the copper content in the above-ground biomass on its amount in the roots is the sum of accumulation exponential function and removal linear function, which is due to the operation of the system of active molecular transport of Cu in plants. Based on the Becker theory (1981) and the analysis of the dynamics of copper accumulation in the above-ground and root parts of plants, the calculated metal content in biomass, which is optimal for barley, is 7.6 mg/kg. In the experiment, it corresponded to a total Cu content in the sod-podzol soil 3.5 mg/kg, in chernozem 6.9 mg/kg. The calculated value of the conditionally critical copper accumulation in the roots of plants, at which its entry into the above-ground biomass due to passive transport and removal by means of active molecular mechanisms of transport are equal, for barley is 650 mg/kg, while the amount of Cu in the above-ground part will be 31 mg/kg.

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