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DEGRADATION, REHABILITATION, AND CONSERVATION OF SOILS

Triad Method for Assessing the Remediation Effect of Humic Preparations on Urbanozems

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Abstract—The data on the pollutant content, ecological toxicity, and structural and functional specifics of soil microbial communities in urbanozem sampled in the city of Kirov were used to describe the remediation effect of humic substances (lignohumate and nanomagnetitohumate). The integral index of environmental risk on contaminated and background soil sites was calculated using the triad method. Based on varying Chemical Risk Index, Ecotoxicological Risk Index, and Ecological Risk Index, this method proved that humic substances are able to reduce ecological toxicity and transform the ecophysiological indices of biota in urban soils. The most vivid effect of humic products has been revealed on introduction of 0.0025 and 0.01% mass. The biological activity of nanomagnetitohumate and lignohumate, rather than their ability to bind toxicants, is apparently the principal factor controlling their remediating effect.

Keywords: humic substances, lignohumate, nanocomposites, biotesting, bioindication, environmental risk index

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INTRODUCTION

The soil cover in urban areas is subject to a high anthropogenic load. Urban soils differ from background soils in their physical, chemical, and biological properties [4, 13, 16]. It is noted that elevated concentrations of contaminants and the loss in bioorganic potential, i.e., the sum of living and humified organic soil substances, plays a leading role in the decreasing capacity of urban soils to perform their ecological functions [9, 11, 17, 20, 24]. Humic substances efficiently contribute to improving physicochemical properties of soils, activation of microflora, nutrient migration, and finally, the rehabilitation of soil and vegetation cover [10, 12].

Under certain conditions, application the industrial analogues of natural humic substances (humic preparations) to urban soils may exert a favorable impact on the ecological status of soils [3, 15, 32]. A high interest in humic preparations promotes improving the routine technologies of their production, broadening the raw materials base by using new ranks of coals, peat, shale, and peloids, as well as implementing innovations in this field. In particular, preparations are known that are produced on the basis of accelerated humification of lignin-containing raw material, i.e., lignohumate [1, 27], as well as those obtained from increasing the number of reaction centers by including nano-size particles of metals or their oxides to a humic matrix [5, 21, 28, 29]. Acuteness and appropriateness of humate application requires biotic control over soils and checking of the remediation activity and the ecological safety of humic preparations [33].

Remediation activity of humic preparations should be evidently estimated in line with the biotic concepts by considering the set of data on chemical and biological analyses of soils before and after soil treatment with humates. Similar to many other cases, the main problems in biotic concept implementation for assessing the humic preparations imply the choice of mode for integrating information into a single index and interpretation of quantitative index values as qualitative criterion of soil condition. There is a history of applying detoxication coefficient as an integral index for assessing the remediation activity of humates [6, 7]. However, we consider the methodology applied at the interdisciplinary level, i.e., the triad method, to be the most objective and versatile, as it integrates a triad of ecological data, i.e., chemical, biological and toxicological indices [26, 31].

This study is aimed at estimating the remediation effect of two humic preparations (lignohumate and nanomagnetitohumate) upon the urbanozem samples on the basis of triad-based approach.

MATERIALS AND METHODS

The research was based on the pot experiment with two urbanozems sampled from two sites in the city of Kirov, i.e., the conventionally uncontaminated background area and the test plot in the industrial district, where the comprehensive survey in 2010–2011 revealed the elevated concentrations of a number of heavy metals, and both phyto- and ecotoxic properties of soils [14]. A mixed sample of urbanozem was taken from a 0 to 20 cm horizon at the test plot of 10 m^2 in the area situated 5 m away from a highway with heavy traffic. The soil showed the following total content of heavy metals (mg/kg): Cd—4.61; Ni—97.23; Pb—249.11; and Cr—296.21. The initial soils had a loamy texture. The soil of natural moisture was cleaned from artifacts and passed through a 2–4 mm sieve.

The following humic substances produced on the basis of modern synthesis techniques were the objects of study:

Humic preparation 1 (HP 1)—*lignohumate K* (Scientific and Production Assosiation RET, Russia) obtained from the artificial humification of lignosulfonate. It contains an insignificant amount (mass %) of N (0.37); S (~ 4.0); C (35.0); K (3.0); and P (0.1). Its composition is specified by the prevalence of fulvic acids and acid-soluble fractions over the humic acids: 90 and 10%, respectively.

Humic preparation 2 (HP 2)—*nanomagnetitohumate* (*Biokhimmash*, Russia) was produced by a mechanic-chemical synthesis from humic acids of oxidized brown coal and highly-active magnetite nanoparticles. The content of humic acids bound to magnetite does not exceed 10% mass of the total amount [23]. The elemental composition (mass %) of this preparation was the following: N (2.8); C (33.8); H (2.6); Fe (15.3); and K (14.0). The elevated amount of nitrogen is related to the residual NH_4Cl used in magnetite synthesis. A high content of potassium in this substance results from the use of potassium in humate synthesis (the potassium content in the initial humic preparation is equal to 23%).

Heavy metals (Pb, Cd, Ni, and Cr) in both HPs are found in trace amounts.

Dry HP were added to soil samples in three concentrations: 0.0025; 0.01; and 1% mass (equivalent to 0.025; 0.1 and 10 g/kg). The mixtures were mixed up thoroughly and were placed into vegetative vessels (plastic containers enclosing 500 g of the air-dry soil). Each experiment was performed in three replicates. The urbanozem sample not treated with any humic preparation was taken as a control soil sample (hereinafter, control). The background soils (hereinafter, background) sampled from the test plot in the southwestern part of the city in the recreational forest (where the soil occurs in the most favorable condition according to the study [14]) were taken as the natural reference samples.

Immediately after filling the vessels with the soil material, 2 g of lawn grass mixture *Universal* (Russia) was planted in each vessel. The grass mixture consisted of *Festuca pratensis* (30%), *F. rubra* (35%), *Lolium perenne* (15%), and fescue–ryegrass hybrid (20%). Watering corresponded to the mean monthly rainfall rate for Kirov in June and July. Illumination was artificial with alternating 12 hours of light and 12 hours of darkness regime.

After the vegetation (56 days) grass was harvested. Herbs were cut in the end of the experiment. The soil toxicity and the effect of humic preparations were estimated by the phytomass weight. The soil samples were removed from vessels, released from roots and were analyzed for a number of chemical, toxicological, and bioindicative indices.

Chemical studies included determination of pH in water extracrs and content of mobile heavy metal species with atomic–adsorption spectroscopy after their extraction with acetate–ammonium buffere solution (pH 4.8) according to the standard procedures. The principal agrochemical parameters (NPK and C_{org}) were measured by routine methods.

Toxicological analyses used three testing systems on reacting organisms of different taxonomic and trophic levels (*Scenedesmus quadricauda* algae, *Daphnia magna* crustacea, and *Escherichia coli* gene modified bacteria) in line with the standard procedures (FR.1.39.2007.03223 similar to ISO 8692-1; FR.1.39.20070322, ISO 7346-1); (PND F T 14.1:2:3:4.11-04; PND F T 16.1:2.3:3.8-04 similar to ISO-11348-2).

Bioindication studies comprised the determination of the following indices:

—intensity of soil respiration by the value of substrate-induced respiration of soil enriched in glucose and the microbial respiration intensity without adding glucose. Proceeding from the obtained data, the microbial biomass value and the microbial metabolic coefficient were calculated according to [24, 25];

—structure of soil micromycetes by inoculation of soil suspension in agarized Czapek's medium; synecological analysis based on the total amount of colony-forming units and the share of dark-pigmented fungi species stable to unfavorable factors;

—urease enzyme activity by colorimetry method based on measuring the amount of ammonia formed from urea hydrolysis using the Nessler reagent;

—catalase fermentation activity using gasometry based on measuring the rate of hydrogen peroxide decomposition according to the extracted hydrogen volume;

—the increment in the lawn grass aboveground biomass after cutting plants at the 56th day of experiment. Plants were dried at 105°C, and the phytomass was determined by weighing.

In calculations, the background value of each index was taken as 100%, and the corresponding values in other test options were expressed in percent as related to the background ones (in treated soil and in urbanozem untreated with humic preparations). The data obtained were statistically processed with MS Excel 2003 and Statistica 6.0 software. The difference between each treatment and the control as well as treatment were evalueted using a one-way analysis of variance (ANOVA) followed by LCD test.

To assess quantitatively the remediation effect of HP in contaminated soils, we used the triad method, which permits revealing of the variation in toxicity, soil biota status, and the content of contaminants in the soils treated with HP as compared to the control and the background in a relative dimensionless value scale (ranging from a 0 to 1) [26]. The complexity of this integral Environmental Risk Index (EnvRI) provides the advantages of triad approach upon assessment of the effect of humic preparations as compared, for example, to the calculation of binding constants or detoxication coefficients [7]. The values of environmental risk indices obtained by the triad method provide evidence on the influence of humic preparations at various biological levels, from organism to population or community. The Chemical Risk Index, Ecotoxicological Risk Index, and Ecological Risk Index (ChemRI, EtoxRI, and EcoRI) are calculated in three stages, i.e., the comparison of data obtained with the background data for every index; the choice of transitional function depending on the deviation from the background values for each index; calculation of integral indices (ChemRI, EtoxRI, and EcoRI)) [26, 31].

Calculation of environmental risk index by the chemical indices. The results obtained for tested samples were compared to the background values. For transition to the scale normalized from a 0 to 1, we applied the functions of type (1):

$$\mathrm{ChemRI}_i = \begin{cases} \dfrac{C_i}{C_{\mathrm{background}i}} \times 0.50, & \text{if} \quad C_i \le C_{\mathrm{background}i} \\ 0.50 + \left(\dfrac{C_i - C_{\mathrm{background}i}}{10C_{\mathrm{background}i} - C_{\mathrm{background}i}} \right) \times 0.50, & \text{if} \quad C_{\mathrm{background}i} < C_i \le 10C_{\mathrm{background}i} \\ 1, & \text{if} \quad 10C_{\mathrm{background}i} < C_i \end{cases} \quad (1)$$

where ChemRI_i is the transformed value, the index of soil status according to the concentration of *i*th chemical index; C_i is the concentration of the ith chemical index in the sample; $C_{\mathrm{background}i}$ is the concentration of the *i*th chemical index in the background.

The data obtained on all investigated sample components were generalized as the arithmetic mean $\mathrm{IS}_{\mathrm{ch}i}$:

$$\mathrm{ChemRI_c} = \frac{\sum_{i=1}^{n} \mathrm{ChemRI}_{\mathrm{ch}i}}{n}, \quad (2)$$

where ChemRI is the soil status index by chemical data; and n is the number of analyzed indices.

Calculation of environmental risk index by the toxicological indices. The values of testing functions of the testing systems (bioassay) were compared to the values obtained for the background sample from equation (3):

$$\mathrm{P}_i = \frac{\left|\mathrm{T}_i - \mathrm{T}_{\mathrm{background}i}\right|}{\mathrm{T}_{\mathrm{background}i}}, \quad (3)$$

where P_i is the deviation of the testing function in the *i*th bioassay in the sample from the background; T_i is the testing function value in the sample; and $\mathrm{T}_{\mathrm{background}i}$ is the testing function value in the background sample.

To convert to the scale standardized from 0 to 1, we applied the functions of type (4):

$$\mathrm{ChemRI}_i = \begin{cases} 0, & \text{if} \quad \mathrm{P}_i \le 0.20 \\ \dfrac{\mathrm{P}_i - 0.20}{0.80 - 0.20}, & \text{if} \quad 0.20 < \mathrm{P}_i \le 0.80. \\ 1, & \text{if} \quad \mathrm{P}_i > 0.80 \end{cases} \quad (4)$$

Bioindication indices were assessed in a similar way using equation (3) and functions (4). The status index by toxicological and bioindication parameters (EtoxRI, and EcoRI) were calculated from the arithmetic mean of EtoxRI, and EcolRI, respectively (similar to equation (2)).

Upon calculating the integral status index by the triad of indices, i.e., chemical, toxicological, and bioindicative (ChemRI, EtoxRI, and EcoRI), we used the *weight coefficients* equal to 1.5 and 2.0:

$$\mathrm{EnvRI} = \frac{\mathrm{ChemRI} + 1{,}5\mathrm{EtoxRI} + 2{,}0\mathrm{Ecol}}{1.0 + 1.5 + 2.0}. \quad (5)$$

The attribution, suggested by Dagnino et al. [26], of *weight coefficients* seems to be justified in this case, since these are biotic (toxicological and bioindication) indices that are most informative in terms of sustaining stable status of ecosystems and performing ecological functions by soils, in particular, such function as a habitat for living beings [30].

Thus, the integral index of soils status calculated by the triad method shows the variation in the content of contaminants, the integral toxicity and bioindication parameters of soils in the presence of HP as compared to those indices in the same samples without HPs. A decreasing EnvRI value points to the improving ecological status of soils with introduced HP, while increasing EnvRI value attests to its degradation. The

Table 1. Influence of humic preparations on mobile species of heavy metals in soils. Mean +/– standart deviation.

HP concentration, mass %	Pb	Cd	Ni	Cr
	mg/kg			
Background				
0	3.67 ± 1.28*a*	0.32 ± 0.08*a*	0.63 ± 0.11*a*	4.30 ± 0.21*a*
Urbanozem (control)				
0	11.69 ± 2.81*b*	0.58 ± 0.10*b*	1.00 ± 0.22*b*	5.32 ± 0.58*b*
Urbanozem treated with nanomagnetitohumate				
0.0025	8.42 ± 2.02*c*	0.54 ± 0.12*b*	0.79 ± 0.17*ab*	5.35 ± 0.59*b*
0.01	5.54 ± 1.31*ac*	0.45 ± 0.09*ab*	0.80 ± 0.17*ab*	5.34 ± 0.58*b*
1	4.78 ± 1.14*a*	0.46 ± 0.09*ab*	0.94 ± 0.21*b*	5.40 ± 0.60*b*
Urbanozem treated with lignohumate				
0.0025	10.32 ± 2.47*bc*	0.43 ± 0.09*ab*	0.75 ± 0.10*ab*	5.29 ± 0.57*b*
0.01	5.01 ± 1.20*a*	0.43 ± 0.08*ab*	0.82 ± 0.18*ab*	5.26 ± 0.56*b*
1	4.45 ± 1.07*a*	0.44 ± 0.08*ab*	0.97 ± 0.21*b*	5.40 ± 0.60*b*

Hereinafter, significant differences ($p < 0.05$) among treatments, within the same sampling interval, are indicated with different letter.

value close to 0 (the background soil status) is the most favorable for the functioning of biota.

RESULTS AND DISCUSSION

Effect of humic preparations on the content of heavy metals and agrochemical indices of soils. The humic preparations are known to exert a protective effect against a wide range of contaminants. The mode of protective effect manifestation in respect to certain contaminants depends on many factors, above all, on the application rates and the composition of humic preparations. The experimental results proved that the addition of 0.01 and 1% of lignohumate and nanomagnetitohumate affected the concentration of the mobile species of lead and cadmium, but did not affect the content of mobile species of nickel and chromium in urbanozem (Table 1).

The effect of humic preparations on the toxicological indices of soils. The soil capacity to provide the plant growth is a good indicator of soil quality. The increment of the above-ground biomass of lawn grass was assessed in the vegetation experiment. The control sample (urbanozem untreated with HP) showed lower values than the background soils (the increment was 28% lower). The application of humic preparations exerted an ambiguous effect on the vegetation parameters of the lawn grass mixture in the experiment (Fig. 1).

The treatment with application of lignohumate showed the highest increment of the above-ground plant biomass. The trend to slowing down of plant growth was revealed for urbanozems treated by nanomagnetitohumate. The plant growth decreased markedly with the increasing concentration of nanomagnetitohumate in the soil. In treatments with 0.01 and 1% introduced nanomagnetitohumate, the dry biomass decreased more than by 50% in respect to control and background soils. The morphological changes in the leaves and their necrosis were observed.

In addition to lawn grass, the standard laboratory bioassays were used for the study of the effect of humic preparations on toxicological soil parameters. Three species of various trophic levels were used for bioassays, i.e., *S. quadricauda, D. magna,* and *E. coli*. The control sample (urbanozem untreated by HP) was toxic for all three test systems, and the test-functions of bioassays were inhibited by 48–70% as related to the background sample. The treatment with humic preparations exerted a significant effect on ecotoxic properties manifested by soils (Fig. 2).

The toxic effect disappeared completely upon introducing HP. The pattern of bioassay response was almost independent on HP concentrations and the bioassay species. The results obtained for bioassays theoretically may be true for a wider range of biological objects. Introduction of HP may supposedly exert a stimulating effect on soil invertebrates, soil algae, and micromycetes.

Influence of humic preparations on soil biota. HP are known to exert both stimulating and inhibiting

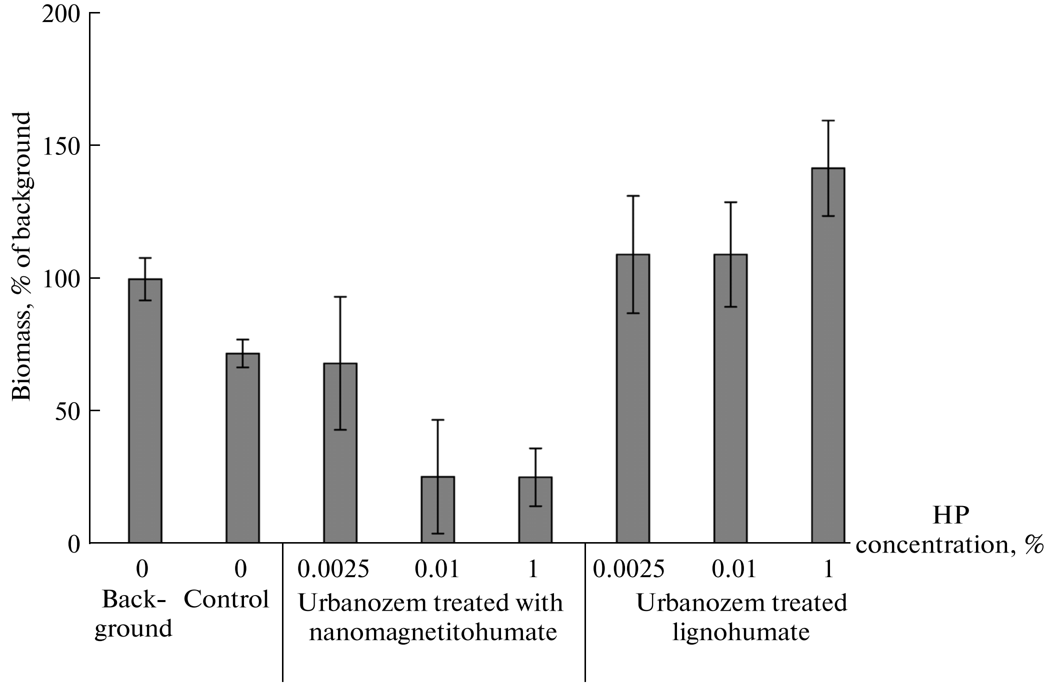

Fig. 1. Influence of humic preparations on plant biomass. Vertical bars indicate standart deviation.

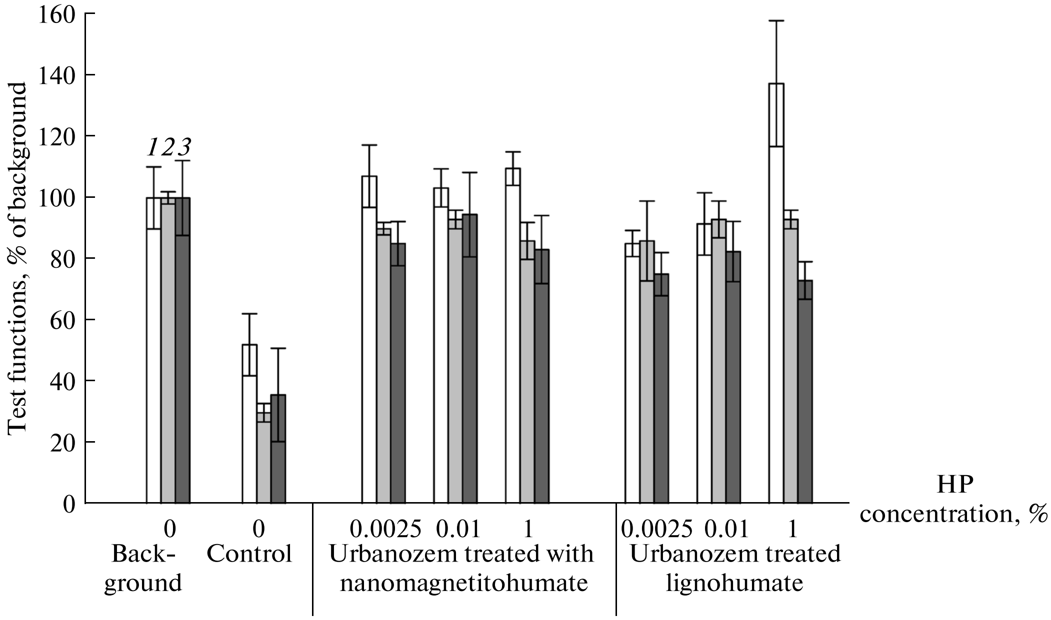

Fig. 2. Influence of humic preparations on responses of biotest systems by the results of pot experiment: *1* increment of *S. quadricauda* cells; *2* survivability of *D. magna* individuals; and *3* bioluminescence of *E. coli*. Vertical bars indicate standart deviation.

effect on soil biota. In the pot experiment, the integral indices of soil biota functioning (CO_2 emission, activity of catalase and urease) as well as individual indices (structural parameters of micromycetal communities) were assessed. Application of humic preparations at small rates (0.025 and 0.01%) stimulated the microbial biomass development and inhibited the microbial respiration parameters; as a result, the microbial metabolic coefficient decreased (Table 2). The decreasing microbial metabolic coefficient may testify to the

Table 2. Influence of humic preparations on the intensity of the substrate-induced respiration (SIR, μmol CO_2/g per hour), microbial respiration (MR, μmol CO_2–C/g per day), content of microbial biomass carbon (C_{mic}, μg C/g soil), and microbial metabolic coefficient (qCO_2, μg CO_2–C/mg C_{mic}/hour). Mean +/– standart deviation.

HP concentration, mass %	Value of CO_2 emission from soil samples		Ecophysiological parameters	
	SIR	MR	C_{mic}	qCO_2
Background				
0	0.054 ± 0.005*a*	0.058 ± 0.005*a*	44.89 ± 1.33*ac*	5.24 ± 0.63*a*
Urbanozem (control)				
0	0.047 ± 0.001*b*	0.076 ± 0.005*b*	42.33 ± 1.09*a*	7.05 ± 0.54*ab*
Urbanozem treated with nanomagnetitohumate				
0.0025	0.051 ± 0.002*a*	0.060 ± 0.004*ad*	45.89 ± 1.73*a*	5.09 ± 0.70*ac*
0.01	0.050 ± 0.008*a*	0.063 ± 0.008*adc*	44.91 ± 7.81*a*	5.45 ± 1.34*a*
1	0.050 ± 0.002*a*	0.060 ± 0.006*a*	45.13 ± 2.33*a*	5.21 ± 1.88*ac*
Urbanozem treated with lignohumate				
0.0025	0.063 ± 0.003*c*	0.069 ± 0.001*c*	57.17 ± 1.56*b*	4.67 ± 0.24*ac*
0.01	0.054 ± 0.003*a*	0.073 ± 0.001*bc*	48.66 ± 3.31*c*	5.81 ± 0.62*a*
1	0.046 ± 0.001*b*	0.070 ± 0.002*bc*	42.07 ± 2.67*a*	5.21 ± 1.43*ac*

improving soil properties as a habitat for soil microorganisms [23, 25].

In high concentration (1%), humic preparations did not exert any noticeable effect on the soil respiration intensity or the ecophysiological parameters of soil biota. We may suppose that their effect at small rates (0.0025 and 0.01%) is caused by their own physiological activity rather than by the influence of C_{org} addition as a nutrient for microorganisms.

Enzyme activity. The influence of HP was manifested in the alteration of biochemical parameters of the soils, in particular, the activity of urease and cata-

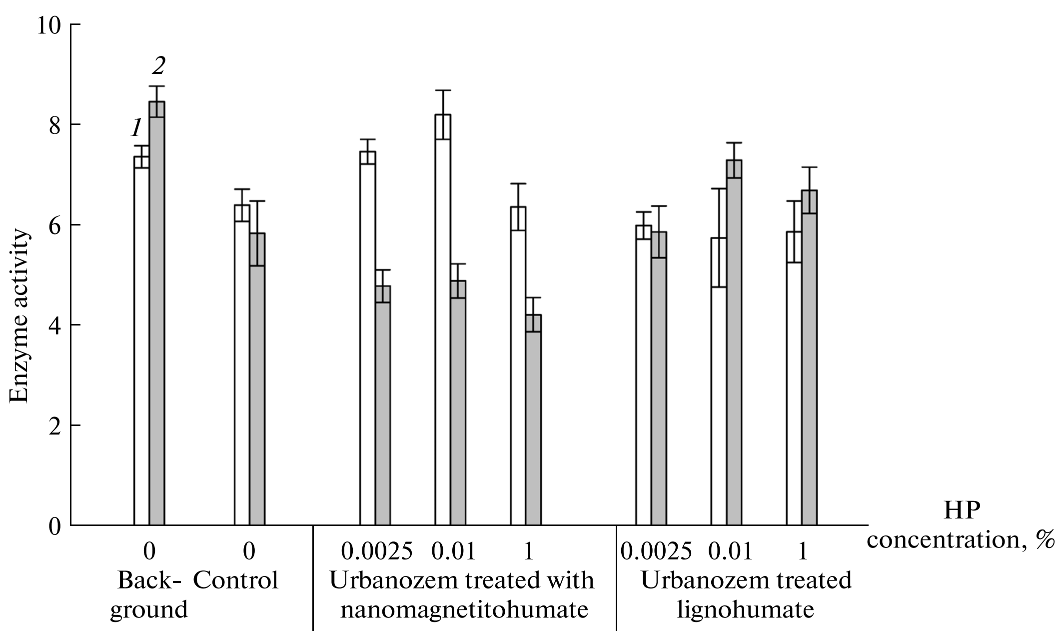

Fig. 3. Influence of humic preparations on urease (*1*, mg NH_3/10 g soil) and catalase (*2*, mL O_2/min/g soil) activities in urbanozem samples. Vertical bars indicate standart deviation.

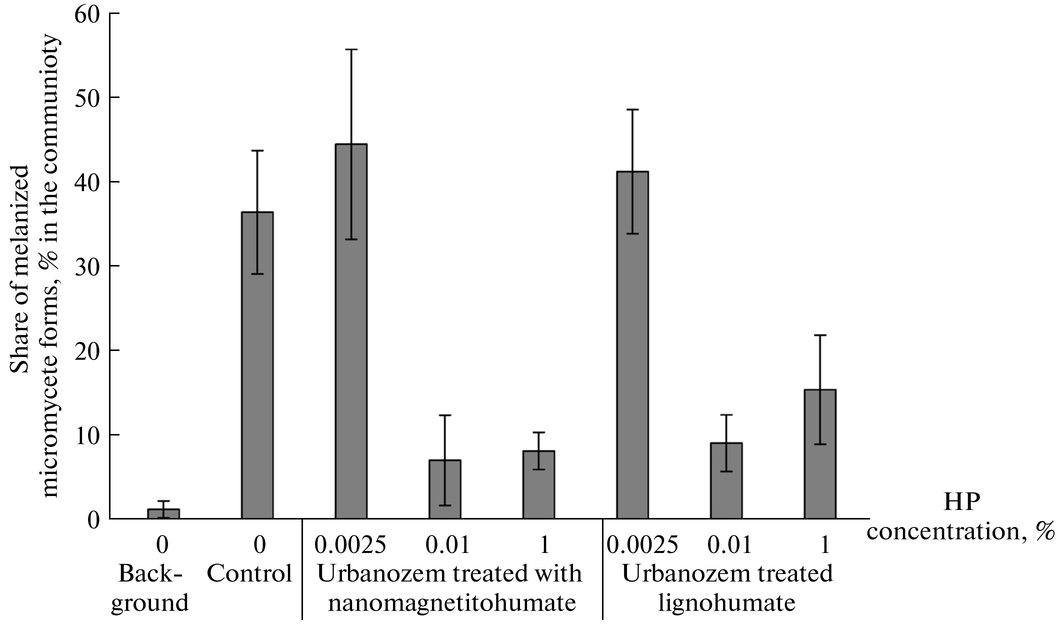

Fig. 4. Influence of humic preparations on the share of dark-colored micromycetes in the community. Vertical bars indicate standart deviation. Vertical bars indicate standart deviation.

lase (Fig. 3). Application of nanomagnetitohumate in concentrations of 0.0025 and 0.01% exerted the maximal effect on the urease activity. For these treatments, the urease activity is permanently higher than that for the control. Biostimulation of urease activity may be caused by the excessive content of total nitrogen after the soil treatment with nanomagnetitohumate.

With the introduction of nanomagnetitohumate, the tendency was revealed to the decrease of the catalase activity in soil as compared to the background and control urbanozem samples. The presence in the preparation of highly active magnetite nanoparticles in macroligands of humic acids could contribute to the formation of different-ligand coordination centers with the participation of both humic acids and the catalase enzyme (with trivalent iron included in its active center). The enzyme-inhibiting complexes formed may show a higher stability and may suppress the activity of enzymes [8].

The introduction of lignohumate did not exert any significant impact on the urease activity index; however, it favored the growing catalase activity in urbanozem, with the highest effect observed at a concentration of 0.01%.

To our opinion, the rearrangement of soil micromycetal community, namely reduction of the share of dark-colored micromycetes in the community for the experiment options with 0.01 and 1% introduced humic preparations, was an interesting result of applying humic preparations. (Fig. 4).

Assessment of remediation effect of humic preparations by the triad-based approach. As proceeds from the vegetation experiment, the introduction of humic substances influences the mobility of some heavy metals, as well as the content of plant nutrients, manifestation of phyto- and ecotoxic properties, and structural and physiological parameters of soil biocenosis functioning. To integrate the data on the effect of humic substances on urbanozems, we calculated the indices of soil status after its treatment with humic preparations according to the chemical, biological, and toxicological indices (ISch, ISb, and ISt), as well as the integral index of soil status using the triad method.

The soil status was characterized according to the Russian system in ranking the quality indices by five categories (I–V) [2, 20], distinguishing the following ranges of integral state index: (EnvRI = 0) background; (0 ≤ EnvRI ≤ 0.30) slightly disturbed; (0.30 < EnvRI ≤ 0.50) disturbed; 0.50 < EnvRI ≤ 0.79) strongly disturbed; and (0.79 < EnvRI ≤ 1) irreversibly disturbed. The calculation results based on the experimental data are shown in Figs. 5 and 6.

Summarizing the data obtained on the influence of humic substances on soil biota status, plant growth, and biotest reaction, we may point out that the positive effect is most pronounced at HP concentrations of 0.0025 and 0.01%. With these concentrations applied, the ISb and ISt decreased noticeably in respect to the control approaching the background values. Since HP did not exert any significant effect on the content of mobile HM species (lead, chromium, cadmium, and nickel), this may prove that their own biological activity, rather than their ability to bind toxicants, is the main factor controlling the remediation effect of

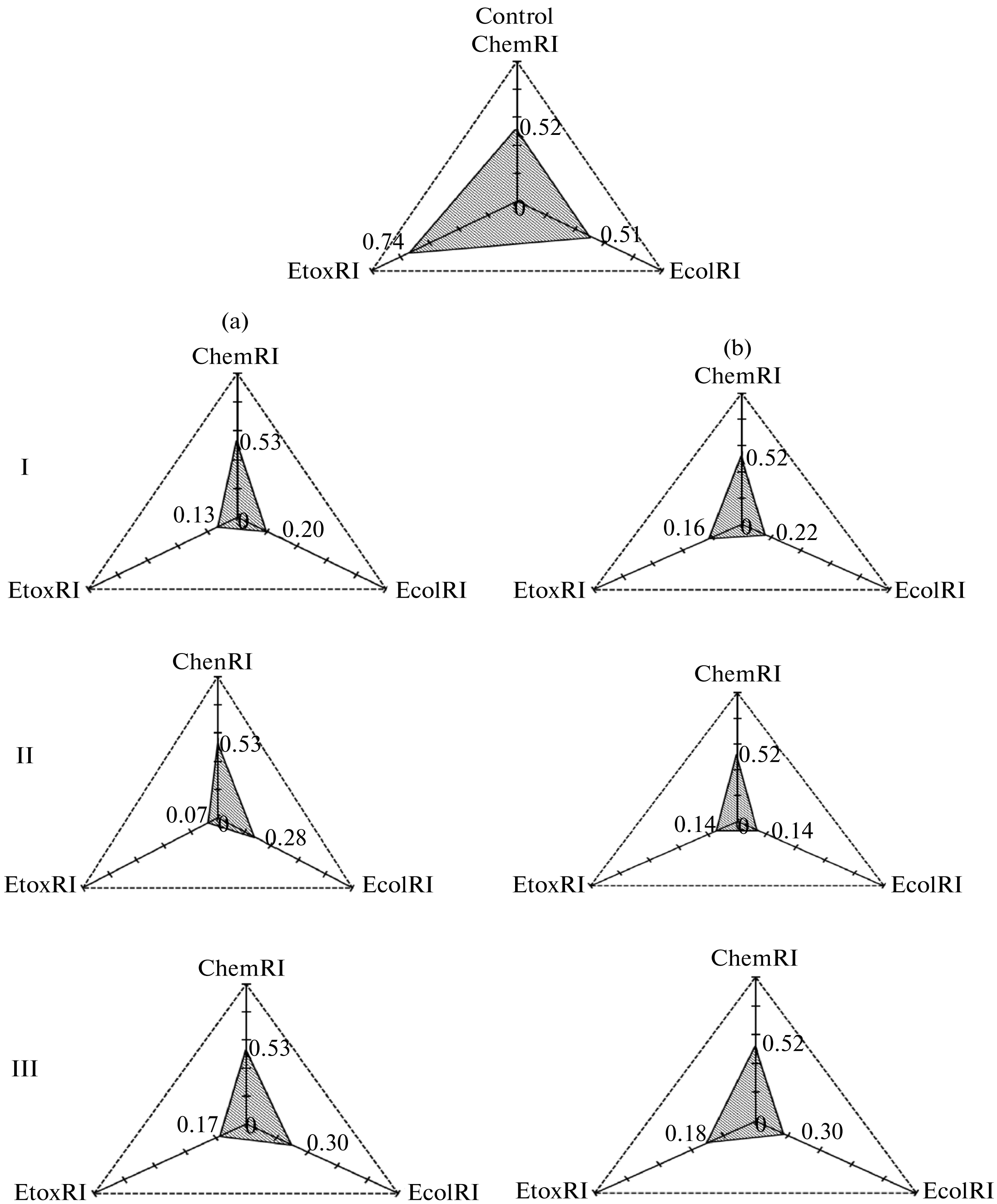

Fig. 5. Graphic interpretation of the results on assessing the ecological state of urbanozems treated by humic preparations using the triad method (values by ChemRI, EtoxRI and EcolRI axes correspond to the soil state indices calculated by the chemical, ecological (bioindicative), and toxicological data; 0 is the background composition; the dark triangle square stands for the soil disturbance degree; rates of HP applied, %: I—0.0025, II—0.01; and III—1.0). Soil treatments: (a) nanomagnetitohumate and (b) lignohumate.

nanomagnetitohumate and lignohumate Similar facts were described earlier in other studies. Chukov et al. [18, 19] showed a decrease in the toxicity of copper and nickel ions for corn and chlorella upon introduction of humic acids, with the total sorption capacity of introduced HP being 5–6 times lower that the concentration of cations. This led them to the conclusion that the bioprotective mechanism of HP is rather related to the direct physiological stimulation of adaptive processes than to the direct adsorption of heavy metal ions.

Thus, in correlating the integral assessment results obtained for chemical, biological, and toxicological properties samples with the soil quality scale [2, 22],

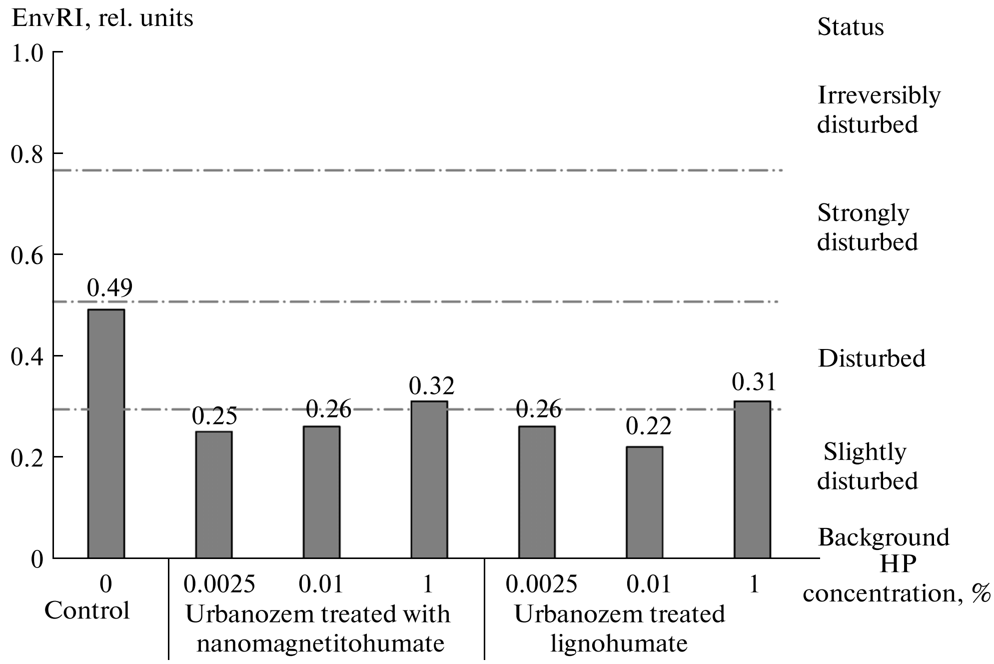

Fig. 6. Integral Environmental Risk Index calculated by the triad method, and the condition of urbanozems after the treatment with humic preparations.

we may note the change in soil ecological status after treatment with humic preparations in low concentrations: the soils passed from the category “disturbed” to the category “slightly disturbed”.

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