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SOIL BIOLOGY

Humic Preparations and the Assessment of Their Biological Activity for Certification Purposes

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Abstract—The diversity of responses of test organisms (higher plants, microalgae, protozoa, crustaceans, bacteria, and mammal cell cultures in vitro) to the action of humates produced industrially from different raw materials was discussed in relation to the urgent problem of the certification of structural and functional properties of commercial humates. It was proposed to include higher plants (for assessing the effect of stimulation) and a set of biotests using test organisms of different trophic levels (for determining toxic concentrations) in the program of assessing the biological activity of humic preparations by laboratory testing methods.

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INTRODUCTION

The resilience of the biosphere to strong anthropogenic impacts and its self-restoring capacity are largely determined by the presence of humic substances (humus) in the soil. By their genesis, humic substances represent a particular final stage of the physical, chemical, and microbiological transformation of organic matter in nature. The uniqueness of their properties and structure are determined by soil-forming processes and soil fertility, as well as the decomposition of hard rocks and minerals and fixation, concentration, dispersion, and redeposition of chemical elements [6, 35]. Natural humic substances regulate the growth of plants; improve the physicochemical properties of soil; stimulate the activity of microorganisms; affect the migration of nutrients; and stimulate soil respiration, synthesis of proteins and carbohydrates, and enzymatic activity.

In the last years, interest has increased in different aspects of the application of humic substances in crop growing, cattle breeding, protection of natural environments from pollution, and some industries [5, 7, 8, 19, 25]. Industrial humic preparations produced from natural resources (coal, peat, bottom sediments, large-tonnage organic waste, etc.) largely inherit the properties of humic substances from the original raw materials and, hence, act as ameliorants and preparations for the detoxification, remediation, and reclamation of degraded and polluted soils and as plant growth regulators.

The detoxification technologies of polluted soils and sediments with the use of humic and humic–mineral substances are mainly based on the inactivation of pollutants upon the application of humates to polluted soils and sediments by the fixation of heavy metal ions, their transformation into immobile (water-insoluble) forms, and the deactivation of organic ecotoxicants during their sorption on humic matrices [18, 24, 29]. The use of humic preparations for the microbial and phytoremediation of soils is primarily related to the fact that humates have physiological activity with respect to plants and some microbial species, which results in the stimulation of native soil microbiota. It is also known that humates are capable of affecting the toxicity of inorganic pollutants (primarily heavy metals) and some organic compounds [9, 22, 38].

Humic preparations have the widest use as plant growth stimulators in agriculture. Experiments with different higher plant cultures showed that the use of commercial sodium, potassium, and ammonium humates, regardless of their sources, at the optimum rates appreciably stimulates the germination of seeds, improves the respiration and nutrition of plants, increases the length and biomass of seedlings, enhances the enzymatic activity, and reduces the input of heavy metals and radionuclides into plants [19, 21, 26, 39]. This effect is especially manifested at the early developmental stages of plants; however, in some cases, it is visible during the entire ontogenesis, including the crop yield.

The biological activity of natural humic substances and commercial humic preparations is observed with respect to not only higher plants; this is a largely universal phenomenon for living organisms: bacteria [10, 37], fungi [11], algae [12, 13, 16], fodder yeasts [3, 23], fish, warm-blooded animals, and birds [8, 34]. The introduction of humic substances into nutrient solutions or nutrient budgets of animals, birds, and fish

affects the availability and accumulation of metals and antibiotics in their tissues [32, 33] and levels the physiological consequences of stresses [4, 15, 27]. This makes commercial humic preparations suitable for use as feed additives in cattle breeding, aquarium husbandry, and the hydrolysis industry.

At the same time, the acting mechanisms of humic substances on living organisms are scarcely understood. It is taken for granted that humic substances have a stimulating effect in the range of relatively low concentrations (10^{-2}–10^{-4}%) and an inhibiting effect at higher concentrations. This response of living organisms is typical of biologically active substances. Although the attempts to identify hormones in humic substances have failed, the hormone-like activity of both natural humic substances and commercial humic preparations leaves no doubt [17, 20, 28, 34].

The aforementioned facts explain the necessity of developing an efficient methodology for the integrated ecological diagnosis of humic preparations abundant in the present-day market. The degree of ecotoxicity is one of the essential features of their quality, because the hormone-like effect on living organisms can both stimulate and inhibit the development of biota representatives. It is known that living organisms differ in their sensitivity to the action of humates [16, 31, 36]. This can be more or less related to the chemical structure of humic preparations produced from different substrates.

The development of a program for the certification of structural and functional features of commercial humic preparations is an important challenge. The sound selection of informative methods, including those for the biological assessment of humic preparations, is a difficult problem to solve. Methodological programs for assessing the bioactivity and ecotoxicity of humic preparations produced from different raw materials are already actively and thoroughly discussed not only by ecotoxicologists but also by chemists and specialists involved in the production of commercial humic preparations. Such issues were already brought up at the round table session specially organized under the initiative of Prof. S.N. Chukov, President of the Subcommission on Soil Organic Matter of the Dokuchaev Soil Science Society, within the framework of the V All-Russian Conference "Humic Substances in the Biosphere" (March 1–4, 2010, St. Petersburg); an earnest dialogue about some methodological aspects of the assessment of the bioactivity and chemical parameters of humic preparations took place.

DIVERSITY OF COMMERCIAL HUMATES

Commercial humic preparations are produced by many enterprises in Russia and some other countries. Commercial humates are very diverse; their effect is not confirmed by certificates; therefore, customers can determine the quality of these stimulators only after their procurement. The composition and properties of humic preparations vary depending not only on the source of raw material (peat, coal, etc.) but also on the features of the deposit and production technology. If the flowchart for the industrial production of humic preparations corresponds to the laboratory methods of their isolation and the commercial preparations contain no artificial admixtures (e.g., mineral elements), the composition of commercial humates and their humic substances reflects the genesis of organic raw material and conditions of humification.

The diversity of humic products is illustrated in Table 1, in which the descriptions and main properties of some Russian and foreign humic preparations are given. The preparations were produced using industrial technologies from different humic resources: carbonaceous materials, peat, bottom sediments, and organic waste; these are liquid or powdered sodium or potassium humates with different additives: microelements, mineral fertilizers, and silicic acid. Fulvic acid (FA) preparations are also produced, although more rarely.

Preparations from carbonaceous materials (oxidized low-calorific brown coal or lignite) prevail in the global production of humic preparations. Leonardite and humalite are also weathered oxidized brown coals frequently associated with carbon shales. Producers of humic products from Russia and Europe sometimes use these terms for raw materials, although, in the strict sense, leonardite originates from a specific deposit of brown coal in North Dakota (USA), and humalite originates from Alberta (Canada). Humic preparations from peat and sapropel are the most popular on the Russian market and in the countries rich in peat deposits.

A specific feature of humic resources is that peat and sapropel are the youngest caustobioliths retaining structural fragments of plant tissues. Humic preparations from peat and sapropel usually contain more nitrogen than coal preparations by a factor of 1.5–2, probably owing to the presence of residual proteins, and similar amounts of humic acids (HAs) and substances from the acid-soluble fraction (ASF) of organic matter, including FAs, owing to the presence of residual saccharides. Carbonaceous materials pass through the deep stages of humification and carbonification in the course of diagenesis, which results in the accumulation of condensed aromatic structures and loss of proteins, carbohydrates, and aliphatic fragments. Therefore, the preparations from coals contain the largest amount of carbon and the smallest amounts of hydrogen and nitrogen compared to the humic preparations from peat, and HAs prevail in their organic matter [40]. As for humic preparations from organic waste, their properties are mainly determined by the composition of raw material and production technology.

Thus, along with some common features, humic preparations produced from raw materials formed

Table 1. Composition and properties of some Russian and foreign commercial humic preparations

Preparation, country	Source	Indicated composition	pH*	C	N
				% of dry matter	
Preparations from carbonaceous materials					
Gumat-80, RF	brown coal	Na/K humate	10.5	43	0.3
Gumat 7+, RF	″	K humate + microelements	10.1	35	0.7
Energen-ekstra, RF	″	K humate	9.9	49	0.3
Energen-Na, RF	″	Na humate	10.1	43	2.2
Energum, RF	″	Na humate	9.4	39	2.4
Gumi, RF	″	Na/K humate	8.2	43	0.4
USA, USA	lignite	Na/K humate	9.0	40	0.4
ION-14, USA	″	humate with monosilicic acid	8.1	38	0.4
Sakhalinskii, RF	leonardite	Na/K humate	8.9	35	1.2
Humisol, Italy	″	80% humate + fulvate	7.8	41	0.5
Pow-Humus, Germany	″	K humate	9.9	43	1.0
HPA WDG 70, USA	″	dry 70% HA	7.6	43	0.6
HPA WP 80, USA	″	dry 80% HA	7.8	41	1.0
Soluble product -IL Humic G-F-P-K, USA	″	humate with addition of mineral fertilizers	7.5	27	0.6
Soluble product -IL Humic G-K, USA	″	humate with addition of mineral fertilizers	7.2	32	0.3
SP-85, USA	″	85% soluble humate	8.3	45	1.0
SP-100, USA	″	100% soluble Na/K humate	8.1	48	1.2
BorreGro HA-1,USA	″	acid-soluble humate	7.4	35	0.8
Dry-soluble, USA	″	humate	8.0	38	0.8
Liquid Fulvic, USA	″	liquid FA	2.5	Nd	
Organo Liquid Humic, USA	″	liquid HA	8.8	″	
Preparations from peat and bottom sediments					
Plodorodie, RF	peat + sapropel	K/Na humate	9.9	33	1.5
Bigus, RF	sapropel	K humate	8.9	32	1.7
Edagum, RF	peat	humate with monosilicic acid	11.3	38	1.9
Skarabei, RF	″	HA	2.7	48	1.4
Fleksom, RF	″	K humate	8.9	42	1.1
EkoOrganika, RF	″	K humate	8.1	36	1.5
Preparations from organic waste					
Lignogumat (Na), RF	lignosulfonate	Na humate	10.2	37	0.5
Lignogumat A (K), RF	″	K humate	9.9	36	0.3
Lignogumat AM (K), RF	″	K humate + microelements	9.5	34	0.2
Gumistar, RF	vermicompost	biohumus	9.0	44	1.7
Preparations from unknown sources					
Chinese Humate, China	no data	80% Na/K humate	9.4	53	0.6
Humisolve-CZ, Czech Republic	″	60% K humate	9.1	42	0.7
India 90% soluble, India	″	no data	8.6	25	0.2
Tha	″	K humate	7.6	27	0.8
Sol 80% HA + K powder, USA	″	80% K humate	8.9	45	0.2
70% FA powder, USA	″	FA	7.1	33	2.5

Note: (Nd) not determined.

* pH was determined at a preparation concentration of 1 g/l.

under different conditions of biomass transformation and humification have individual properties. Therefore, the effect of humic preparations on living test systems will depend not only on the sensitivity of organisms to the specified experimental conditions but also on the composition of humic preparations, which in turn is largely determined by the genesis of organic raw material.

NECESSARY AND POSSIBLE APPROACHES TO ASSESSING THE BIOLOGICAL ACTIVITY OF COMMERCIAL HUMATES

In our opinion, the experimental testing scheme containing different phytotests with higher plants (from laboratory to microplot methods) should be implemented for the reliable estimation of biological activity of humic preparations primarily used in agriculture. However, it is also important to confirm the ecological safety (ecotoxicity characteristic) of commercial preparations for living organisms (representatives of the main trophic levels) ensuring the sustainable functioning of natural ecosystems and agrocenoses.

Biotesting is a necessary stage of assessing the stimulating activity and ecological safety of humic preparations used in plant growing and agriculture, as well as a useful procedure for characterizing their detoxifying effect when used for the remediation of natural and technogenic objects: polluted soils, waters, or toxic waste [9, 18].

It is assumed that biotesting provides information on ill-being before the appearance of obvious (indicative) changes in natural ecosystems. Biotesting methods are usually highly sensitive; they can detect lower concentrations than analytical sensors. When used as a method supplementing bioindication and analytical procedures, biotesting has some undoubted merits, including the integral response of biological systems, [14]. The information value of these systems for predicting the consequences of harmful impact on the environment exceeds that of the physicochemical methods of analysis. The test response integrates the effects of all biologically harmful factors, including physical and chemical impacts, as well as the impacts of the present biotic factors.

It should be noted that the multiplicity of forms and applications of humic preparations complicates the adequate selection of a test system for assessing the safety of these substances. The standardized biotesting procedures recommended by the state bodies of environmental control for the toxicological control of soils, waters, and other objects can be considered as the basis for the assessment of the biological safety of commercial humic preparations and their effect on the natural environments. The current list of updated biotesting procedures certified as national standards includes only about ten items. The federal registers of Russia include about ten toxicological measurement procedures and corresponding biotest systems recommended for use in nature conservation and environmental control. They are based on the test reactions of bacteria, protozoa, crustaceans, mammals, and microalgae; i.e. they cover all the main links of the trophic biocenotic chain (destructors, consumers, and producers).

Our studies showed that the variability of responses of standardized cultures can pose a significant problem in the development of a program for the environmental assessment of humic preparations. Factors determining the differences in the responses of test organisms of different taxons to the impacts of humic preparations are insufficiently understood. Nonetheless, the available data suggest an effect of exposure conditions, including the saturation of culture medium with nutrients, on the response of test organisms and a correlation between the sources of commercial humates (raw materials) and the test reactions of organisms [12, 13, 36].

ANALYSIS OF EXPERIMENTAL RESULTS

We performed a comparative study of the toxicity of humic preparations taken from the collection of Russian and foreign preparations of the Department of Soil Chemistry, Moscow State University. The humic preparations under study were produced industrially from three groups of organic raw materials: carbonaceous materials with different degrees of oxidation (brown coal, leonardite, and lignite), lacustrine bottom sediments (sapropel), and organic industrial waste (lignosulfonate) (Table 2). All of them are used as plant growth stimulators and soil amendments.

Preparations strongly differ in the composition and proportions of HAs and FAs in mixtures with other substances of the ASF (Tables 2, 3). Humic preparations from coals (BC-EnNa, Le-PhK, Li-Ion) contain the largest amount of carbon and the smallest amount of nitrogen; HAs prevail in the water extract (75–80% of water-soluble organic matter). The Sa-Plod preparation from sapropel contains the largest amount of nitrogen and similar amounts of HAs and ASF substances. The humic preparation from organic waste was prepared by so-called artificial humification of lignosulfonate; it inherits a very low content of nitrogen, a high content of sulfur (about 4%), and a strong predominance of FAs and ASF substances over HAs: 90 and 10%, respectively.

These compositional features of humic preparations can result in different effects on test organisms, because the ecotoxicity mechanism of humic substances is apparently related to their chemical structure, which is in turn determined by their genesis. The results of biotesting experiments showed that some test cultures responded similarly to the impacts of all humates, and the sensitivity of other test cultures to the presence of humic preparations of different origin varied significantly.

Table 2. Origin and some properties of humic preparations

Source of raw material	Humic preparation	pH	Ash	N_{tot}	C_{tot}	S_{tot}
			%			
Brown coal	BC-EnNa	9.0	18.4	1.13	43.6	0.6
Leonardite	Le-PhK	9.5	28.5	0.87	39.0	2.7
Lignite	Li-Ion	10.2	36.1	0.79	34.8	0.7
Sapropel	Sa-Plod	9.5	28.3	1.81	34.6	0.6
Organic waste	Ow-LhNa	10.1	32.2	0.35	34.6	3.7

Table 3. Carbon and humic substances in water extracts from humic preparations

Preparation	C_{H_2O}	C_{HA}	C_{ASF}	C_{H_2O}/C_{tot}	C_{ASF}/C_{tot}	C_{ASF}/C_{tot}
BC-EnNa	$\frac{18.1}{100}$	$\frac{13.5}{75}$	$\frac{4.6}{25}$	0.42	0.31	0.11
Le-PhK	$\frac{23.3}{100}$	$\frac{18.6}{80}$	$\frac{4.7}{20}$	0.60	0.48	0.12
Li-Ion	$\frac{15.0}{100}$	$\frac{11.9}{79}$	$\frac{3.1}{21}$	0.43	0.34	0.09
Sa-Plod	$\frac{18.1}{100}$	$\frac{9.5}{52}$	$\frac{8.6}{48}$	0.52	0.27	0.25
Ow-LhNa	$\frac{24.2}{100}$	$\frac{2.3}{10}$	$\frac{21.9}{90}$	0.70	0.07	0.63

Note: Results expressed in percent of dry matter and percent of C_{H_2O} are given above and below the line, respectively

Biotests of water solutions of humic preparations were performed in a wide concentration range (5–10 000 mg/l) to reveal their stimulating and inhibiting effects on higher plants (radish *Raphanus sativa*), warm-blooded animal cells in vitro, the simplest crustaceans (*Daphnia magna*), protozoa (*Paramecium caudatum*), bacteria (the Biotox analytical system), and microalgae (*Chlorella vulgaris* and *Scenedesmus quadricauda*). Generalizing the obtained results (partially reported earlier), we may state the following.

More or less predictable results were obtained in phytotests on higher plants under laboratory conditions, which included the treatment of seeds with solutions of humic preparations, their germination in the dark at 27°C, and the recording of biometric parameters of 3-day-old seedlings. Relatively low concentrations of humic preparations had a stimulating effect on the germination of seeds and the growth of roots. No stimulation was observed at high concentrations of humic preparations, and inhibition of plant growth was noted in some cases. However, the dynamics of test response for different humic preparations was described by different dose–effect curves (Fig. 1I, A). No clear correlation was revealed between the composition of humic preparations and the response of plants [39]; however, the effect of individual properties of preparations is obvious.

The test of humic preparations in a warm-blooded cell culture in vitro based on the procedure of measuring the toxicity index from changes in the mobility of mammal gametes with a video analyzer (FR.1.31.2009.06301) in plant-stimulating concentrations (50–200 mg/l) showed their safety for warm-blooded animal cells. The toxicity index (TI) was within the range specified in the procedure, which corresponded to the optimum mobility of sperm cells ($80 < TI < 120$) (Fig. 1I, B). This test culture showed similar responses to all humic preparations: it was not sensitive to the impact of humates from coals and other sources in the studied concentration range.

No toxic effect of humic preparations in the concentration range 5–100 mg/l was observed for the test culture of protozoa (ciliate *Paramecium caudatum*). Ciliates showed a good survival in the presence of humic preparations: the TI value was no higher than 50%, which indicated the absence of a harmful effect (FR.1.39.2006.02506) in accordance with the established criteria (Fig. 1I, C).

The standard estimation of acute toxicity with the use of *Daphina magna* (FR.1.39.2007.03222) showed

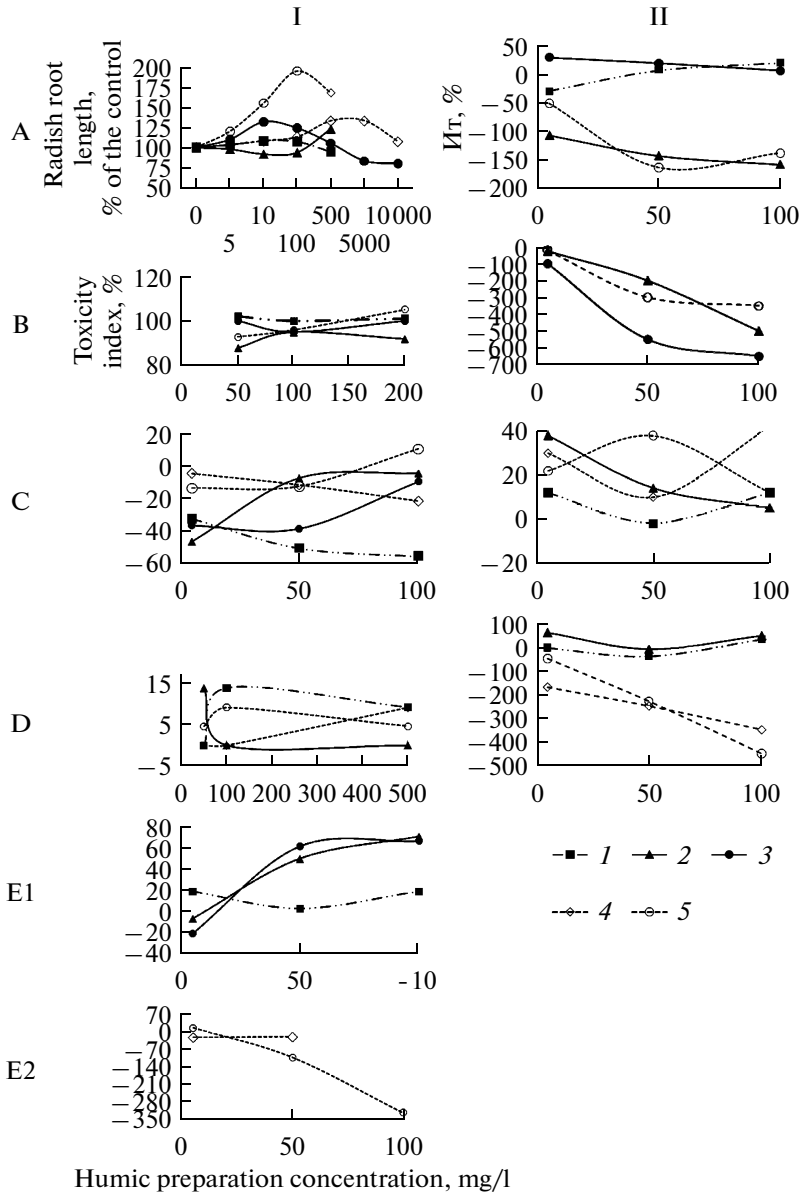

Fig. 1. Dose–effect curves for the action of humic preparations on (I) different test cultures: (A) higher plants (radish seeds); (B) mammal (bull) gametes in vitro; (C) protozoa (*Paramecium*) (D) daphnids; (E1) and (E2) bacteria (gene-modified luminescent culture) and (II) protococci: (A) chlorella in 2% Tamiya medium; (B) chlorella in 25% Tamiya medium; (C) Scenedesmus in 100% Uspenskii medium; (D) Scenedesmus in 10% Uspenskii medium; (*1*) BC-EnNa; (*2*) Li-Ion; (*3*) Le-PhK; (*4*) Sa-Plod; (*5*) OW-LhNa.

that the TI limit specified in the procedure (50% mortality of individuals) was not exceeded in the study of humic preparations in the concentration range of 50–500 mg/l, which indicated the absence of acute toxicity in the samples (Fig. 1I, D).

The effect of humic preparations on bacteria was determined from changes in the luminescence intensity of a gene-modified culture (PND F T 14.1:2:3:4.11-04) using an Ecolum biosensor. According to this procedure, estimates were ranked in three groups: nontoxic (IT < 20), toxic (20 < IT < 50), and highly toxic (IT > 50). Bacterial cultures were found to be more sensitive to humic preparations; they showed selective responses depending on the composition (origin) of humates. The humic preparation from brown coal, BC-EnNa, had no toxic effect on the test culture (IT < 20) in the entire concentration range (5–100 mg/l); two other preparations from analogous raw material, Le-PhK and Li-Ion, showed no toxicity only at the lowest concentration and were highly toxic (IT > 50) at higher concentrations (Fig. 1I, E1). A similar toxic effect of coal HA solutions at concentrations of 60–120 mg/l was reported for these bacterial strains [2]. In contrast to the humic preparations from coals, humates from

younger raw materials (Sa-Plod from sapropel and especially OW-LhNa from lignosulfonate) showed quite another effect on bacterial culture: observed was not only the absence of toxicity at all concentrations tested (IT < 20) but also the stimulation of luminescence of the bacterial preparation against the control (Fig. 1I, E2). This could be related to the differences in the chemical structure of humic preparations, including the increased content of the ARF substances in the preparations from sapropel and organic waste.

The effect of humic preparations on two microalgal species (*Chlorella vulgaris* and *Scenedesmus quadricauda*) was assessed for concentrations of 5–100 mg/l by the direct calculation of the increase in cell number in populations, with modification in some cases of the standard methods for the determination of toxicity from the optical density of chlorella alga culture (PND F T 14.1:2:3:4.10-04) and the determination of toxicity from chlorophyll fluorescence and the number of alga cells (FR.1.39.2007.03223).

In the tests with algae, as in the tests with bacterial culture, humic preparations showed different effects depending on their genesis and the composition of nutrient medium (Fig. 1II). The growth of chlorella cultivated upon the addition of humic preparations under different conditions (2% Tamiya medium) was usually stimulated above the toxicity boundary (TI < –30), although in some cases it was either inhibited (TI > 20 for the Le-PhK preparation from coal) or within the range of permissible variation (Fig. 1II, A). When the saturation of the Tamiya medium with nutrients was increased to 25%, humates further stimulated the development of the culture; the highest stimulating effect of the Le-PhK preparation inhibiting the development of cells under standard conditions was observed at an excess of nutrients (Fig. 1II, B). A direct relationship between the concentration and stimulating effect was also observed for all humic preparations.

In contrast to the chlorella response, *S. quadricauda* did not respond to the addition of humic preparations by the enhancement of growth under standard cultivation conditions (Uspenskii medium no. 1); on the contrary, toxicity was manifested in some cases (Fig. 1II, B). No clear relationship was observed between the concentration of the humic preparations studied and their effect on the alga culture; neither was a difference in the effects of humic preparations of different origin revealed. However, when the nutrient value of the medium was decreased by tenfold dilution of the Uspenskii medium, a more differentiated response of the test culture on humic preparations from different raw materials was manifested: the BC-EnNa and Li-Ion humic preparations from coals enriched with HAs had the highest stimulating effect at a rate of 50 mg/l and an inhibiting effect at rates of 5 and 100 mg/l, while the humic preparations from young raw materials enriched with the ARF substances (Sa-Plod and OW-LnNa) stimulated the development of microalgal cells in direct relationship to the concentration of humates (Fig. II, D).

The results of these experiments, which showed that the result of a biotest is affected by at least two factors (the nutrient value of the medium and the composition of the humic preparation), were reported in detail by Fedoseeva et al. [12, 13]. Thus, it follows from the data analyzed that the biotesting of humic preparations of different origin using a set of standard test systems revealed different sensitivities of test organisms to humic preparations; separate biotest systems had differentiated responses to humic preparations of different genesis depending on the degree of saturation of the medium with nutrients.

CONCLUSIONS

The presented published and experimental data show that test organisms differ in their sensitivity to humic preparations. Biotesting with the use of six test systems revealed the specific responses of organisms of different trophic levels.

Test systems with the use of higher plant seeds usually show auxin-like effects of different degrees of manifestation. Therefore, laboratory methods with the use of a universal plant species (or set of species) can be recommended as a reliable tool for the objective comparison of the biological activities of humic preparations with respect to the stimulation of higher plant growth. A set of three plant species containing monocotyledonous and dicotyledonous plants is proposed in foreign standards [30].

Test systems with the use of warm-blooded animal cells, infusoria, and daphnids were found to be not very sensitive to the action of humic preparations in the studied concentration range; differentiated responses to the action of humic preparations of different genesis were observed in the bacterial test system, which was apparently related to the chemical structure of humates.

In test systems with microalgae, different responses were recorded depending not only on the test culture species but also on the degree of saturation of the medium with nutrients and the genesis of humic preparations.

Thus, programs for assessing the biological activity of humic preparations should include sets of biotests formed from test systems based on the response of higher plants and some standardized test organisms of other trophic levels.

In addition, the responses of test cultures of different species should be further studied with the view of optimizing the testing conditions of humic preparations of different origin with consideration for the specific impact of humates from young and mature raw materials on living organisms and the dependence of species response on the composition of the medium. To compare the effects stimulating the development of

higher plants and assessment of ecotoxicity, a kind of standard humic preparations from the traditional and most common raw materials (coal, peat, sapropel) should be developed in order to have definite reference points for the characterization of new humic preparations.

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SPELL: 1. Humic, 2. Invertase, 3. Pizzeghello