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Detoxifying Capacity of Humic Acids toward the Triphluraline Herbicide

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Abstract—Ability of humic acids (HA) of the soil to detoxify triphluraline increases in the zonal-genetic soil sequence from soddy-podzolic soils to chernozems. The cultivation of soddy-podzolic and gray forest soils increases the detoxifying potential of HA toward the triphluraline. The structural parameters of HA that affect their detoxifying properties are determined.

Humic acids (HA) belong to the largest class of specific compounds in the organic matter of the soil. The presence in the molecule of the proper "humic framework," aromatic carbonic skeleton substituted by alkyl and functional groups, and a large periphery presented by the fragments of polysaccharide and polypeptide character is characteristic of the chemical composition of HA [4, 11]. The ratio of "framework" and peripheral parts in molecules depends significantly on the conditions of their formation and can be considered as one of the diagnostic features that allows one to judge the source of HA. It is well known that HA with a more developed framework part enriched with aromatic fragments are formed in chernozemic soils with high microbiological activity, whereas HA with a more developed aliphatic periphery formed by carbohydrate–protein complex are formed in soddy-podzolic soils where processes of biodegradation are not so intense [3, 4]. The increasing degree of development of soddy-podzolic soil contributes to the increase of HA content in humus and the formation of HA with an enlarged aromatic part [1, 6].

One can expect that the character and the efficiency of reaction capacity of HA will depend on the ratio of framework and peripheral parts, because of the principal difference of their chemical nature. HA with a large peripheral part (HA of peat, tundra, soddy-podzolic, gray forest, and some other soils) will be characterized by the increased ability to hydrolytic and oxidizing transformations typical for the polysaccharide-polypeptide complex. At the same time, we can expect higher reaction capacity for metals and organic compounds with the formation of intra- and intermolecule complexes from HA with the developed framework enriched by aromatic structures with carboxyl, hydroxyl, carbonyl, and other substituents.

Many authors studied the contribution of HA to the processes of herbicide inactivation in soils [8, 12, 16], but they underestimated the fact that differences in the character of reaction ability of HA can be especially important for the assessment of their role in herbicide inactivation in the soil. For example, the decrease of the toxic effect of herbicide results in the chemical reaction with HA, which occurs, depending on the nature of herbicide, by the mechanism of ionic, donor–acceptor, or hydrogen binding [10, 16]. The higher is the concentration of reaction centers in molecules of HA (the contribution of the framework part of HA to the common structure of the molecule is higher) the higher is their potential ability to bind the herbicide. According to this assumption, detoxifying activity of HA for the herbicides will significantly depend on the specificity of their chemical composition.

From the aforesaid it follows that this work is aimed at the determination of the interrelation between the structure of HA extracted from the different soils and agricultural use and their detoxifying ability to the triphluraline herbicide.

OBJECTS AND METHODS

Soil sampling. The following soil samples (A horizon, 3–20 cm) were taken for extraction preparations of HA: in the educational–research soil–ecological center of Moscow State University Chashnikovo in the Moscow oblast, soddy-podzolic loamy soil under mixed forest dominated by spruce, arable soil after rye cultivation, and garden soil (Pgar.); in the Tula oblast, gray forest loamy soil in under broad-lived forest and arable soil under forage grasses; in the Voronezh oblast, typical arable chernozem and meadow chernozemic soil under hay field.

Extraction of HA preparations. Preparations of humic acids were extracted by the method [6] which specified the following stages: decalcification by 0.1 M H_2SO_4, extraction by 0.1 M NaOH (the ratio soil : solution = 1 : 6), acidification of obtained alkaline extract by sulfuric acid to pH 2 for precipitation HA, and their subsequent centrifuging and salt elimination through dialysis. The obtained suspensions of HA were dried under temperature 60°C.

Table 1. Elementary composition of humic acids

HA	Concentration, at.%				Atomic ratio		
	C	H	N	O	O/C	H/C	C/N
Soddy-podzolic under forest	38.20	37.63	3.33	20.85	0.55	0.99	11.48
Soddy-podzolic arable	36.90	38.91	3.14	21.04	0.57	1.05	11.73
Soddy-podzolic under garden	36.29	39.98	2.68	21.05	0.58	1.10	13.54
Gray forest	36.70	38.30	3.21	21.80	0.59	1.04	11.45
Gray forest arable	37.16	37.04	3.15	22.65	0.61	1.00	11.79
Typical chernozem	48.73	28.53	3.08	19.66	0.40	0.59	11.82
Meadow-chernozemic	45.93	33.87	3.40	16.80	0.37	0.74	13.52

Characteristic of HA preparations

Element analysis. Concentrations of C, H, N in preparations of HA were measured in the element analyzer Carlo Erba Strumantazione. Ash and moisture contents were determined in order to calculate the results for dry ashless matter. Oxygen concentrations were calculated by difference. The obtained results are presented in Table 1.

^{13}C-NMR spectroscopy. The composition of the carbon skeleton in molecules of HA was studied by the method of ^{13}C-NMR spectroscopy using NMR-spectrometer VXR–400 (Varian). The samples were prepared by dissolving the weighed portion (100 mg) in NaOD/D_2O. The specter was registered under the working frequency 100 MHz, lag time 3 s. Quantitative analysis of the specter was performed through integration by the following intervals of chemical shares (in mass shares relative to tetramethylsilane): 0–50 (carbon of alkyl groups, C_{Alk}), 50–108 (carbon of carbohydrates, primary alcohols, acetals, C_{Alk-O}), 108–165 (aromatic carbon, C_{Ar}), 165–185 (carbon of carboxyl groups, C_{COOH}). The contribution of aliphatic carbon to the skeleton of HA was estimated by the summation of the integral intensities of C_{Alk} and C_{Alk-O}. The contribution of aromatic fragments to the structure of the studied HA as compared with aliphatic ones was determined by the ratio $C_{Ar}/(C_{Alk} + C_{Alk-O})$ (Table 2).

Gel chromatography. Mean molecular masses of HA were determined with the help of gel chromatography. The separation of HA was carried out in the tower filled by the sorbent TOYOPEARL–50HW(S), Japan. The sample was dissolved and eluted by a 0.028 M phosphate buffer. The detection was carried out by the spectrophotometer with wave length 254 nm by the concentration of dissolved organic carbon in eluate (Graentzel, Germany). The tower was calibrated by polydextrans.

Reverse titration. The total acidity of HA was characterized by the barite method in modification [7]. To do this, 15 mg of HA preparation were placed in a plastic vessel and 20 ml of 0.06 M $Ba(OH)_2$ were added. The obtained suspension was agitated for two days. Aliquots of the solution were taken after clarification and titration by 0.1 M HCl in the presence of phenolphhtalein. The total acidity (mg-equiv/g HA) was calculated by the volume of acid. The results are presented in Table 2.

Biotesting of seedlings. Herbicide triphluraline 2,6-dinitro-*N,N*-dipropyl-4-trifluoromethyl-aniline (active material of trephlane) was used in the toxicological experiment. The toxic effect of the herbicide was in the inhibiting of root growth, namely, in the disturbance of cell division [17]. Triphluraline is quickly decomposed in the light and in water with low oxygen concentration, and is slightly metabolized by plants [2].

To prepare the solution of herbicide, place the weighed portion of a chemically pure substance in a small volume of ethyl alcohol with subsequent finishing to volume with distilled water.

Solutions of HA were prepared from finely dispersed air-dried preparations in the following order: the weighed portion of HA necessary for making the mother solution was dissolved in a small amount of 0.1 M KOH, neutralized by sulfuric acid to pH 7, and finished to the required volume by distilled water.

Neutral substrate perlite was placed in Petri dishes (35 g per dish). Solutions of humic acids and herbicide were introduced through mixing with the whole volume of the substrate. HA were introduced in doses 10, 25, 50, 100, and 200 mg per kg of the substrate, triphluraline was introduced in dose 0.5 mg/kg (this concentration of herbicide caused a 50% decrease of biotest response, the length of seedling roots, in the estimation of the scale of herbicide toxicity). Perlite in the control dishes was moistened by neutral solution of KOH + H_2SO_4. Ten germinated seeds of wheat *Triticum aestivum* were placed into the furrow in the middle of the Petri dish and exactly oriented by the seedling to the upper part of the dish and by the root to the lower part of the dish. The testing was made in triplicate. The scheme of the experiment was following:

(1) Control (perlite without HA and triphluraline), (2) Triphluraline (T) 0.5 mg per kg of perlite, (3) HA 10 mg per kg of perlite, (4) HA 25 mg/kg, (5) HA 50 mg/kg, (6) 100 mg/kg, (7) 200 mg/kg, (8) HA

Table 2. Distribution of carbon (C) in molecules of HA (results of ^{13}C-NMR spectroscopy), total acidity, and molecule masses (MM) of HA

HA	The share of C in composition of fragments, %				$\frac{C_{Ar}}{C_{Alk}+C_{Alk\text{-}O}}$	Total acidity, mg-equiv/g HA	Molecular mass, Dalton
	C_{COOH}	C_{Ar}	$C_{Alk\text{-}O}$	C_{Alk}			
Soddy-podzolic under forest	12	46	16	22	1.24	7.55	17900
Soddy-podzolic arable	15	45	18	17	1.28	6.92	25900
Soddy-podzolic under garden	14	46	15	18	1.38	5.94	24800
Gray forest	12	46	19	18	1.23	8.17	24300
Gray forest arable	14	47	15	19	1.36	6.33	23700
Typical chernozem	15	54	11	14	2.13	8.49	14900
Meadow-chernozemic	14	55	11	15	2.12	7.12	15500

10 mg/kg + T, (9) HA 25 mg/kg + T, (10) HA 50 mg/kg + T, (11) HA 100 mg/kg + T, (12) HA 200 mg/kg + T.

The Petri dishes were placed to thermostat for 48 h under 25°C. The mean length of the central root of the seedling was used as test response (R).

Detoxifying ability of HA (D) was calculated by the results of the experiment according to the following formula [13]:

$$D = 1 - \frac{R_0}{R_0 - R_t}\frac{R_d - R_{d,t}}{R_d},$$

where R_0 is the test response in the control, R_t is the test response in the presence of triphluraline, R_d is the test response in the presence of HA, and $R_{d,t}$ is the test response in the presence of HA and triphluraline.

RESULTS AND DISCUSSION

Effect of soil and land use type on the structure of the forming HA. The structure of HA formed in the soils of different types and under different crops was studied in all extracted preparations by the methods of element and functional analysis, gel chromatography, and ^{13}C-NMR spectroscopy. The results obtained are presented in Tables 1 and 2.

As seen from the data on elementary composition Table 1), the effect of the zonal factor appeared in the ower ratio H/C and O/C observed in HA of cher-ozems as compared with soddy-podzolic and gray for- soils. The decrease of the H/C ratio usually indicates increase in the content of aromatic fragments in the ucture of HA, whereas the decrease in O/C attests to decrease of their oxidation rate [3].

The study carried out by the method of ^{13}C-NMR spec-copy confirmed the observed trends. For example, ble 2) maximum concentrations of aromatic carbon –55%) were observed in HA of chernozems in com-ison with HA of soddy podzolic and gray forest soils –47%). Minimum concentrations of the carbon of carbohydrate structures (11%) were also observed in chernozems. A significant difference in the relative contribution of aromatic fragments to the structure of HA in soddy-podzolic and gray forest soils was not found. A significant difference was not found in the total acidity of HA preparations extracted from of different zonal soils (7.12–8.17 mg-equiv/g).

The study of molecular-mass composition had shown (Table 2), that the lowest values of molecular mass, 1500 Dalton, were observed in HA of chernozems, whereas HA of soddy-podzolic and gray forest soils had molecular masses 18000 and 24000 Dalton, respectively. This fact leads to the assumption that macromolecules of HA in gray forest and soddy-podzolic soils have in their composition less transformed polysaccharide chains, whereas the strongest degradation of the carbohydrate complex is reached in chernozemic HA, as the result of high microbiological activity in chernozems.

The obtained structural information is in agreement with the well known idea that chernozemic HA represent the product of deeper humification with less chemical heterogeneity [4]. This phenomenon appears at a molecular level in the enrichment of chernozemic HA with aromatic fragments and in lower molecular masses.

The following trends can be observed in the structural characteristics of HA extracted from the soils of different agricultural development. An insignificant increase in the atomic ratio H/C and O/C and a relatively significant increase in C/N ratio were observed in the series of soddy-podzolic soils under forest, cropland, and garden (Table 1). This fact attests to the impoverishment of HA extracted from the garden soil, with nitrogen. The decrease of nitrogen content in comparison with the native variant was also determined in HA from the arable gray forest soil. The change of the value C_{Ar}/C_{Al} in the studied series of soddy-podzolic and gray forest soils suggests that the increase in the

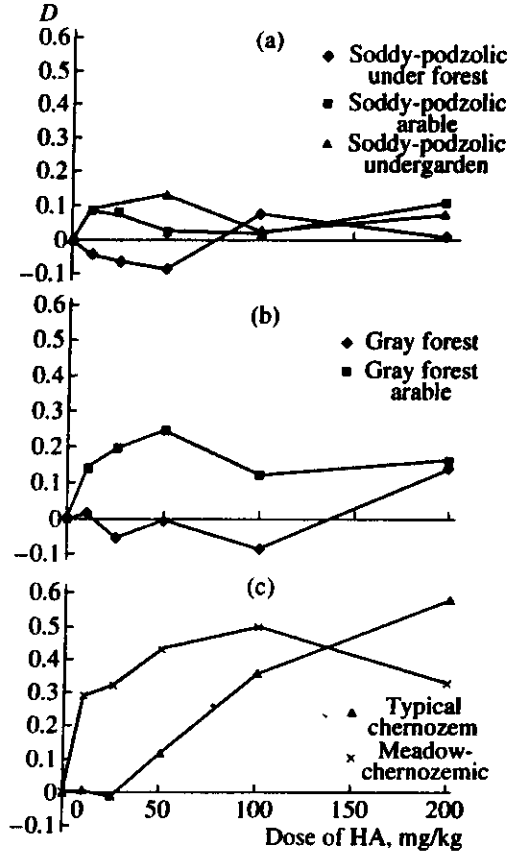

Fig. 1. The detoxifying ability (*D*) of HA extracted from different soils to trifluraline: (a) soddy-podzolic soil, (b) gray forest soil, (c) chernozem.

degree of soil cultivation is accompanied by the increase in the share of aromatic carbon (Table 2).

The study of molecular-mass composition (Table 2) had shown a significant increase of mean molecular mass of HA in cultivated soddy-podzolic soils (from 18 000 Dalton in forest soil to ~25 000 Dalton in arable and garden soils). At the same time, HA in virgin and cultivated variants of gray forest soil were characterized by approximately similar values of mean molecular mass.

Detoxifying ability of HA to triphluraline. The study of the detoxifying ability of all above-characterized preparations of HA to herbicide triphluraline was performed by a method of biotesting in the seedlings. Corresponding curves are presented in Fig. 1.

HA in virgin soddy-podzolic and gray forest soils actually exerted no detoxifying effect to triphluraline under all studied doses. The detoxifying effect of other preparations appeared under the applying doses 10 to 50 mg/kg. Maximum detoxification of triphluraline (50%) was obtained under the application of HA from meadow chernozemic soil at a dose of 100 mg/kg.

Humic acids are lined up (Fig. 2) in order of increa ing total (under all doses of HA application) detoxif ing capacity (S_D) toward triphluraline calculated as th area under the curve *D*–HA dose. The presented da showed that humic acids formed zonal series in whic the lowest value of S_D was characteristic for HA soddy-podzolic and gray forest soils under forest so and meadow chernozemic soil with the highest valu for HA. The comparison of detoxifying capacity of HA extracted from virgin and arable soddy-podzolic and gray forest soils showed that HA of cultivated soil decreased the toxicity of triphluraline much more efficiently than HA of virgin soils.

The interconnection of the structure and detoxifying capacity of HA to triphluraline. Correlation analysis was carried out on the structure data presented in Tables 1 and 2 and total detoxifying ability (S_D) determined for all studied preparations of HA, in order to find significant interconnections between detoxifying ability of HA and their structural parameters.

The obtained results made it possible to determine significant correlations between the integral detoxifying ability (S_D) of HA and such structural parameters as C_{Ar}/C_{Al} ($r = 0.95$), O/C ($r = -0.87$), and molecular mass ($r = -0.71$). The maximum correlation of the detoxifying ability of HA to triphluraline was obtained for C_{Ar}/C_{Al} (C_{Ar}/C_{Al} dependence of S_D is presented in Fig. 3). This fact can be considered as substantiation of the hypothesis on the leading role of the framework of HA in interactions with organic compounds that are accompanied by the formation of weak intermolecular π-π–complexes.

The molecule of trifluraline possesses clearly pronounced acceptor character that is caused by the presence of strong acceptor substituents (two nitro- and one triflourinomethyl- group) in aromatic cycle. In this case, the donor ability of trisubstituted nitrogen in the aniline group of trifluraline is significantly weakened because of the spatial difficulties. The aromatic system of HA possesses much more charge density in comparison with the described structure of the herbicide and can act as a donor toward the acceptor ring of trifluraline. The ability of HA to manifest acceptor or donor character depending on the reaction ability of the partner was theoretically substantiated and corroborated by Ziechmann *et al.* [11, 19]. This interaction can result in the formation of weak intermolecular π-π-complexes trifluraline–HA and, consequently, in decreasing herbicide activity of trifluraline. The more developed is the aromatic system of HA, the higher is their combining and, respectively, detoxifying capacity for trifluraline. It should be noted that other authors also observed a high contribution of aromatic fragments to adsorption capacity of HA toward symtriazines that are characterized depending on the conditions by both donor–acceptor and hydrogen binding with molecules of HA [14].

Several works were devoted to the problems of behavior and transformation of trifluraline in soils and

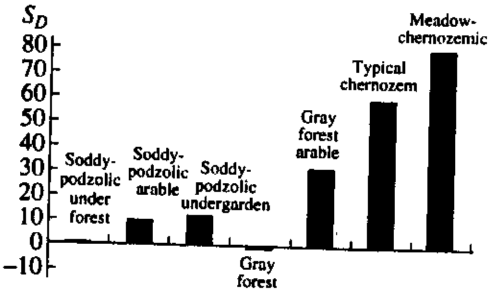

Fig. 2. Integral detoxifying ability (S_D) of HA preparations to trifluraline depending on the soil type and character of its use.

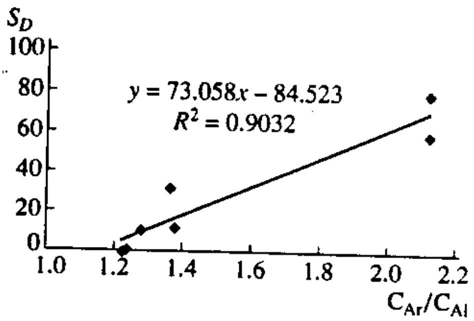

Fig. 3. C_{Ar}/C_{Al} dependence of summary detoxifying ability (S_D) of HA preparations.

substrates with different concentrations of mineral and organic components [9, 15, 18]. These works also emphasized the significant decrease of herbicide toxicity in soils with a high content of organic matter and substrates enriched with industrial preparations of HA. However, the multi-component character of the soil, the weakness of intermolecular donor–acceptor combining, and its dependence on the conditions of the reaction (pH, ionic strength, temperature, etc.) require additional experiments to assess the role of interactions "trifluraline–HA" in the processes of inactivation of this erbicide in the soil.

CONCLUSIONS

The detoxifying capacity of HA extracted from even soils of different type and land to trifluraline was udied. The detoxifying ability of HA increased in the ries: soddy-podzolic under forest ~ gray soil under rest < arable soddy-podzolic < garden soddy-podlic < arable gray forest < typical chernozem < meadow-rnozemic soil.

The existence of a significant correlation between toxifying ability of HA and their structural parame- was found. The highest correlation ($r = 0.95$) was erved between the integral detoxifying capacity of HA and their enrichment with aromatic fragments (C_{Ar}/C_{Al}).

The suggestion has been made about the mechanism of trifluraline detoxification by HA.

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