

# Impact of Humic Substances on Toxicity of Polycyclic Aromatic Hydrocarbons and Herbicides

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## ABSTRACT

The impact of humic substances (HS) of different origins (soil, peat, water) and fractional composition [humic (HA) or fulvic (FA) acids] on toxicity of polycyclic aromatic hydrocarbons (PAHs) and herbicides was investigated. Acute toxicity tests were carried out for pyrene (Py), fluoranthene (Flt), anthracene (An), atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), and trifluralin (2,6-dinitro-*N,N*-dipropyl-4-trifluoromethyl-aniline). Twenty-one of 26 samples of HS studied reduced toxicity of PAHs, whereas toxicity of PAHs was unaffected by all four samples of soil FAs. The most pronounced effect was observed for the more hydrophobic PAHs—Py and Flt. The impact of HS on the toxicity of less hydrophobic An was much less than that of Py and Flt. The greatest detoxifying ability was characteristic of soil HAs, mixtures of HAs and FAs isolated from peat, and Aldrich HA (Aldrich Chemical, Milwaukee, WI). The impact of HAs isolated from seven different soils on toxicity of atrazine and trifluralin also varied greatly, depending on the origin of the sample. The greatest decrease in toxicity of both herbicides was observed in the presence of HAs from chernozems. The least detoxifying impact or no effect was characteristic of HAs isolated from podzols. It is concluded that (i) detoxification is the predominant effect of HS on toxicity of organic xenobiotics, and (ii) the magnitude of the impact strongly depends on the origin and composition of the HS.

## 14-1 INTRODUCTION

Humic substances are major constituents of the bulk organic matter in soil and water ecosystems. They can be considered to be irregular polymers of aromatic polyhydroxyl carboxylic acids (Hayes et al., 1989). Peculiarity of the structure of HS arises from coexistence of both polar and hydrophobic environments in the same molecule. As a result, HS can bind some polar and most hydrophobic xenobiotic organic compounds. The binding to HS causes a change in speciation of organic xenobiotics followed by a change in their toxicity and bioaccumulation.

The mediating impact of HS on the biological effect of organic xenobiotics has been extensively studied in the last decade. However, the results are very contradictory. The majority of authors have reported mitigating effects of HS on the biological activity of organic chemicals (Landrum et al., 1985; McCarthy & Jimenez, 1985; Morehead et al., 1986; Oris et al., 1990; Day, 1991; Perminova et al., 1996), but contrasting observations have been reported as well (Leversee et al., 1983; Stewart, 1984; Oikari et al., 1992; Steinberg et al., 1992).

The contradictory results are apparently a consequence of the immense complexity of the phenomenon under consideration. In addition to the possible influence of aquatic chemistry and peculiar features of each experimental design adopted by the investigators, differences in results are caused by the substantial variability in properties of HS of different origin (soil, peat, water) and the large differences in the properties of HS of the same origin. The latter is a consequence of irregularity and chemical heterogeneity of humic macromolecules. The situation becomes even more complex when, instead of purified and structurally well-characterized isolates from water, soil, peat, or coal, commercial HS are used for bioassays (the mostly widely used representative of which is Aldrich HA) or the whole pool of dissolved organic matter (DOM) or natural water itself. In the latter case, some

concomitant ions and organic compounds incorporated into commercial HS during its production, or water hardness can modify toxicity of the chemicals tested even more than the interaction with the HS (Kukkonen, 1995).

The objective of the current study was to investigate the impact of HS of different origins (soil, peat, water) and fractional composition (HAs or FAs, or compositions of those) isolated with standard techniques on the toxicity of PAHs and herbicides.

## 14-2 MATERIALS AND METHODS

### 14-2.1 Sources and Isolation of the Humic Substances Samples

In bioassays with PAHs, 26 HS samples of different origins (soil, peat, and fresh water) and fractional composition (HA, FA, and HA + FA) were used.

In bioassays with herbicides, seven soil HAs were used that were isolated from sod-podzolic (HBW, HBP, HBG), gray wooded (HGW, HGP), and chernozemic (HS and HST) soils. Short descriptions of the sources of HS samples and the isolation techniques used are given in Table 14-1.

### 14-2.2 Chemicals

#### 14-2.2.1 Polycyclic Aromatic Hydrocarbons

The PAHs used were Py (Aldrich, 97% pure), Flt (Aldrich, 97% pure) and An (Aldrich, 98% pure).

#### 14-2.2.2 Herbicides

The herbicides used were atrazine (Dr. Ehrenstorfer Ltd., Augsburg, Germany, 98% pure) and trifluralin (Dr. Ehrenstorfer Ltd., 99.7% pure).

#### 14-2.2.3 Preparation of Water Solutions of Polycyclic Aromatic Hydrocarbons

A batch technique was used to prepare water solutions of the PAHs (Boehm & Quinn, 1973). The PAHs were dissolved in acetone in a 1-L flask. The amount of each PAH added was less than its water solubility. The acetone was then evaporated. One liter of water prepared for culturing *Daphnia magna* (see below) was added and the flask was shaken overnight. The concentrations of PAHs in the final solutions were determined with laser fluorimetry. They were  $1.7 \times 10^{-7}$ ,  $5 \times 10^{-7}$ ,  $7 \times 10^{-7}$  M for An, Py, and Flt, respectively. The solutions were stored in the dark.

### 14-2.3 Toxicity Tests with Polycyclic Aromatic Hydrocarbons

Acute toxicity tests were carried out using procedures described previously (Matorin et al., 1990; Matorin & Venediktov, 1990; Polynov, 1992). Acute toxicity of PAHs to *Daphnia magna* (water fleas) was estimated by means of measurements of their nutrition activity.

Table 14-1. Sources and isolation techniques of humic substances (HS) samples.

HS sample	Source	Isolation technique
<u>Soil HA† and HS</u>		
HBW	sod-podzolic soil (forest, Moscow)	Extraction with 0.1 M NaOH, acidification to pH 2, centrifugation and dialysis of precipitate (Orlov & Grishina, 1981).
HBWN	sod-podzolic soil (forest, Novgorod)	
HBP	sod-podzolic soil (plough, Moscow)	
HBG	sod-podzolic soil (garden, Moscow)	
HGW	gray wooded soil (forest, Tula)	
HGP	gray wooded soil (plough, Tula)	
HS	ordinary chernozem (Voronezh)	Preliminary treatment of the soil sample with sulfuric acid (decalcification) and further procedure as described above.
HST	typical chernozem (Voronezh)	
<u>Soil FA‡</u>		
FBW	sod-podzolic soil (forest, Moscow)	Extraction with 0.1 M NaOH, acidification to pH 2, centrifugation, discharge of the supernatant through XAD-2 resin, elution of FAs with 0.1 M NaOH, followed by desalting with cation-exchanger (Swift, 1996).
FBG	sod-podzolic soil (garden, Moscow)	
FBP	sod-podzolic soil (plough, Moscow)	
FGW	gray wooded soil (forest, Tula)	
FST	typical chernozem (Voronezh)	
<u>Peat HA + FA</u>		
T1	highland Sphagnum-Fuscum peat	Extraction of peat sample with ethanol-benzene mixture, and follow by extraction with 0.1 M NaOH.
T5	highland Sphagnum peat	
T6	highland sedge peat	Desalting of the extract with cation-exchanger (Loewe, 1992).
T7	highland woody peat	
HTL	highland woody-herbaceous peat	
TTL	lowland woody-herbaceous peat	
<u>Aquatic HA + FA</u>		
FMX	the Moscow River	Sorption on XAD-2 resin from the acidified (pH 2) natural water. Elution with 0.1 M NaOH and desalting with cation-exchanger (Thurman & Malcolm, 1981).
WM3X	the North Dvina River	
SWA	swamp water	
FMC	the Moscow River	Sorption on DEAE-cellulose, others as described above.
<u>Others</u>		
HTW	highland peat "H"	Water extract from peat without further treatment.
SEL	typical chernozem (Stavropol)	Soil sum of HA and FA obtained by alkaline (0.1 M NaOH) extraction of soil and desalted with cation-exchanger.
Aldrich	Aldrich Humic Acid	Commercial preparation purchased from Aldrich Chemical Co.

†HA, humic acid.

‡FA, fulvic acid.

*Daphnia magna* were obtained from a stock maintained at the Division of Hydrobiology of Lomonosov Moscow State University. Waterfleas were cultured at 20°C with a light/dark rhythm of 16 h/8 h in tap water that was previously filtered through activated charcoal and stored for 2 wk under ambient conditions. Suspensions of green microalgae *Chlorella pyrenoidosa* were used to feed *D. magna* daily. For bioassays only 5- to 6-d-old organisms were used.

Six daphnids were transferred into a 50-mL glass beaker containing 25 mL of test solution. Each experimental series included four types of test solutions: water (control), water + HS, water + PAH, water + PAH + HS. Three replicates were made for each assay. Water pH, checked at the beginning and at the end of the test, was  $7.5 \pm 0.2$  in all assays; the temperature was  $20 \pm 2^\circ\text{C}$ . Daphnids in test solutions were kept for 24 h without feeding. Then organisms were fed with a culture of algae, *Chlorella pyrenoidosa*. Intensity of chlorophyll fluorescence of the algae in the test solution was measured immediately after addition of the algal suspension and at the end of the feeding period, which was set at 1 h in all assays.

Grazing activity (GA) of *D. magna* was determined by a decrease in the concentration of algal cells during the feeding period and described by the following equation (Matorin & Venediktov, 1990; Polynov, 1992):

$$GA = [\text{Ln}(kF_0F_t^{-1})]V(nt)^{-1} \quad [1]$$

where,  $F_0$  and  $F_t$  are the intensity of fluorescence of the test solution at the beginning and at the end of feeding, respectively;  $t$  is the duration of the feeding period, h;  $V$  is the volume of the test solution, mL;  $n$  is the number of *D. magna*; and  $k$  is the coefficient accounting for a change in fluorescence intensity of the algae itself during the feeding process. The  $k$  is calculated as a ratio of fluorescence intensities of the control water with and without the introduced algal suspension (without daphnids), measured at the beginning and end of the feeding period.

## 14-2.4 Toxicity Tests with Herbicides

### 14-2.4.1 Atrazine

The method of (Vavilin et al., 1995) was used to measure acute toxicity. Photosynthetic activity of the green unicellular algae *Chlorella pyrenoidosa* was measured.

*Chlorella pyrenoidosa* (thermophilic strain CALU-175 from the collection of the Biology Institute, Saint-Petersburg State University, Russia) was grown in 20% Tamiya medium (pH 6.8; Tamiya et al., 1961) at  $32^\circ\text{C}$ , under  $60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  continuous irradiance and bubbling with moisturized air. *Chlorella pyrenoidosa* was maintained in log growth phase by daily dilution with fresh media to maintain a cell density of about 1 to  $10 \times 10^5$  cells  $\text{mL}^{-1}$ . For bioassays, algae were concentrated by centrifugation and resuspended to a final concentration of 5 to  $7 \times 10^5$  cells  $\text{mL}^{-1}$  in the 10% Tamiya medium without phosphates and EDTA. Each experimental series included four types of test solutions: water (control), water + HA, water + atrazine, water + atrazine + HA. No mortalities were observed in the presence of HA.

After 3 h of growth, photosynthetic activity of algae was measured. For this purpose, the algae were adapted to darkness for 30 s. Chlorophyll fluorescence induction was recorded for 300 s using a fluorometer. The fluorescence detected immediately after complete opening of the shutter yielded a value of initial fluorescence ( $F_0$ ). The value of maximum fluorescence ( $F_m$ ) corresponded with the maximum on the fluorescence induction curve.

Photosynthetic activity ( $F$ ) was then calculated as follows:

$$F = (F_m - F_0)(F_m)^{-1} \quad [2]$$

Three replicates were made for each experimental point.

#### 14-2.4.2 Trifluralin

Acute toxicity tests were carried out using wheat seedlings (*Triticum aestivum* L.) and root length as a target-function according to (Department of Agriculture of USSR, 1988). Germinated seeds of *T. aestivum* were placed on petri plates (10 seeds per plate) filled with 35 g of substrate (volcanic sand). Each experimental series included four types of test media: sand with distilled water (control), sand + HA solution, sand + trifluralin, and sand + trifluralin + HA. Plates with seeds were placed in an incubator at  $25 \pm 0.5^\circ\text{C}$  for 48 h. The root length ( $L$ ) of each seedling was measured and an average value was determined for each plate. Three replicates were made for each assay.

### 14-3 RESULTS AND DISCUSSION

#### 14-3.1 Impact of Humic Substances on the Toxicity of Polycyclic Aromatic Hydrocarbons

*Daphnia magna* were exposed, in the absence of HS, to five concentrations of each of the model PAHs in the ranges of  $1$  to  $5 \times 10^{-7}$  M for Py,  $0.5$  to  $7 \times 10^{-7}$  M for Flt, and  $0.2$  to  $1.7 \times 10^{-7}$  M for An. Further toxicological experiments used maximal concentrations of PAHs, that is,  $5 \times 10^{-7}$  M,  $7 \times 10^{-7}$  M, and  $1.7 \times 10^{-7}$  M for Py, Flt, and An, respectively; these produced the most remarkable and reproducible toxic effects. These concentrations of Py, Flt, and An caused a reduction in grazing activity of *D. magna* down to  $(54 \pm 6)$ ,  $(58 \pm 5)$ , and  $(45 \pm 7)\%$  of the control, respectively ( $P < 0.05$ ).

The toxicity of PAHs in the presence of HS (at 3, 6, 12, and 24 mg C L<sup>-1</sup>) was measured at the above concentrations of Py, Flt, and An. Typical effects of PAHs on nutrition activity of *D. magna* vs. concentration of the HS of different origins are given in Fig. 14-1. An increase in the nutrition activity of *D. magna* as the concentration of HS increased was observed, except for the soil FAs. As a rule, the nutrition activity increased up to a concentration of HS of about 6 to 12 mg C L<sup>-1</sup>. At a greater concentration of HS, the nutrition activity reached a maximum rate. To compare the impacts of HS of different origins on toxicities of PAHs, the nutrition activities of *D. magna* were measured in the presence of 6 mg C L<sup>-1</sup> for all the HS samples (Fig. 14-2). None of the HS caused an increase in the toxicities of the PAHs. Most of the HS samples (21 out of 26) decreased the toxicities of Py, Flt, and An. The effect was greatest for Py and least for An. These results are probably related to hydrophobicity. Anthracene ( $\text{Log}K_{\text{ow}} = 4.4$ ) is the least hydrophobic, whereas Flt and Py ( $\text{Log}K_{\text{ow}} = 5.2$  and  $5.2$ , respectively) are more hydrophobic (Leo et al., 1971).

Among the HS samples of natural origin, the greatest detoxification of PAHs was obtained for soil HAs and peat HA + FA. Toxicities of PAHs were reduced to a lesser extent by aquatic HAs + FAs and were unaffected by soil FAs, except for the preparation isolated from typical chernozemic soil (FST). The commercial preparation of Aldrich HA used for comparison had the greatest detoxifying effect of all samples; its effect exceeded that of both the peat HA + FA and the soil HA.

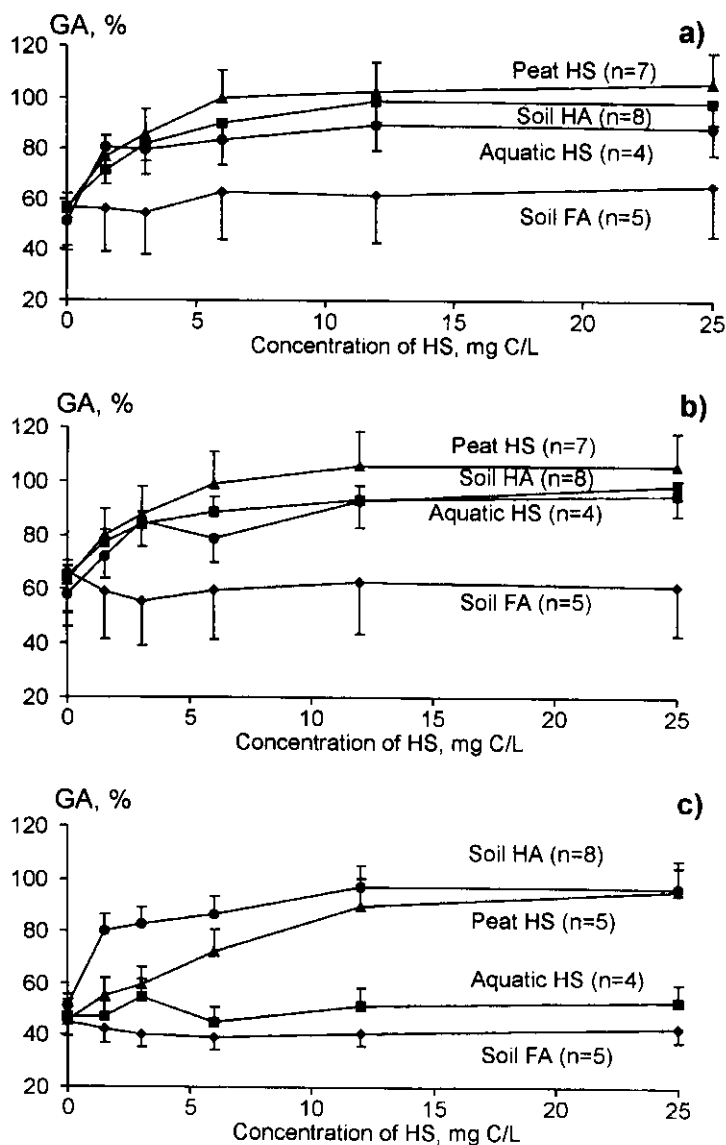


Fig. 14-1. The grazing activity (GA) of *Daphnia magna* (in % of control) averaged by humic substances (HS) samples of the same origin (soil, peat, water). Bars show  $\pm 1$  standard deviation for each group of HS samples. (a) pyrene, (b) fluoranthene, (c) anthracene.

In general, the HS samples could be ranked with regard to their abilities to detoxify PAHs as follows: Aldrich HA and chernozemic HA > sod-podzolic and gray wooded soil HA  $\approx$  peat (HA + FA) > aquatic (HA + FA)  $\approx$  chernozemic FA > sod-podzolic and gray wooded soil FA.

The data are in good agreement with numerous investigations on the influence of HS on bioaccumulation of PAHs. The main effect of HS is a decrease in bioconcentration of PAHs (Spacie et al., 1983; McCarthy et al., 1985) or no effect (Landrum et al., 1985). A lack of effect is usually observed for PAHs with low  $\text{Log}K_{ow}$  values (naphthalene, phenanthrene, An). This suggests a linkage between the structure of HS and their ability to detoxify PAHs. Hence, loss in toxicity of PAHs

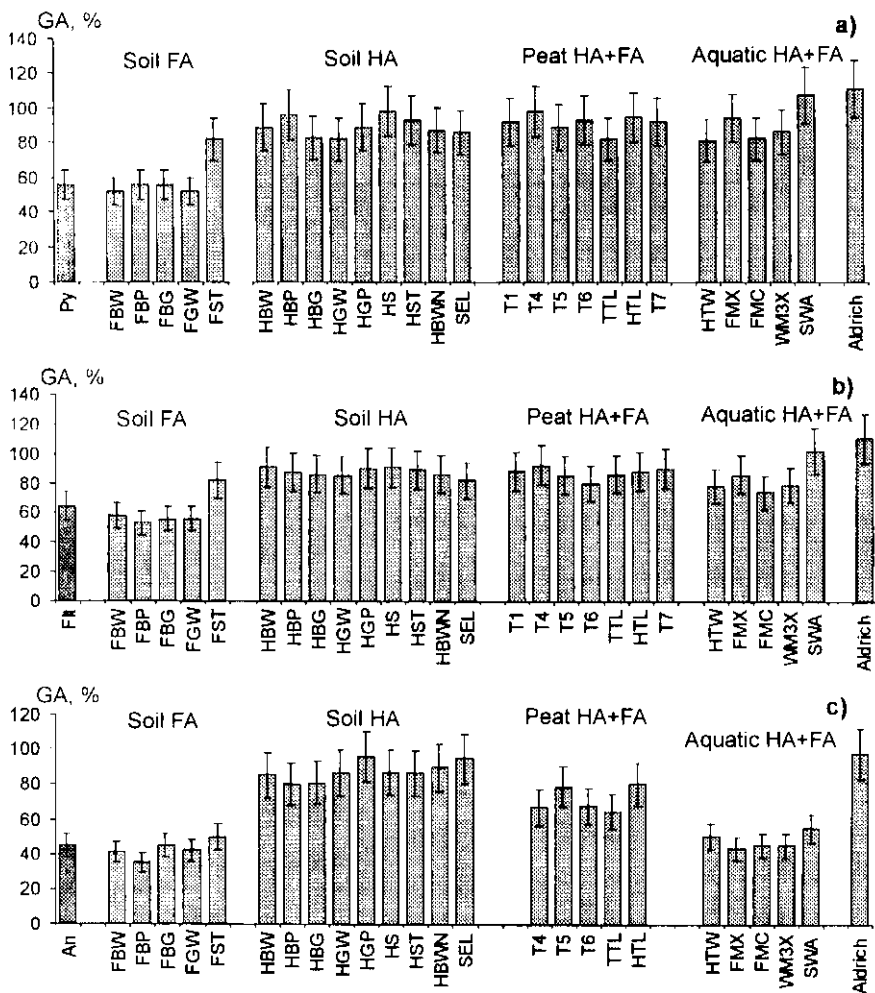


Fig. 14-2. Grazing activity (GA) of *Daphnia magna* in the presence of 6 mg C L<sup>-1</sup> of humic substances (HS) of different origins at a concentration of  $5 \times 10^{-7}$  M Py,  $7 \times 10^{-7}$  M Flt, and  $1.7 \times 10^{-7}$  M An. Darker shaded column is control solution with PAHs, but without HS. Bars show  $\pm 1$  standard deviation. (a) pyrene, (b) fluoranthene, (c) anthracene.



released into water or soil media containing HS depends strongly not only on the content, but also on the quality of HS.

To determine if the observed relationship between the quality of HS and its effect on the toxicities of PAHs is also valid for the other classes of organic xenobiotics, the following experiments were conducted with two herbicides—representatives of *s*-triazines and substituted anilines.

### 14-3.2 Impact of Soil Humic Acids on the Toxicity of Herbicides

An impact of HS on the toxicity of atrazine and trifluralin was determined for HAs isolated from seven different soils (Table 14-1). The HA sample isolated from the sod-podzolic soil near Novgorod (HBWN) was not used.

The concentration of atrazine in all bioassays with *Chlorella pyrenoidosa* was  $2.2 \times 10^{-7} M$ . The corresponding toxic effect and its confidence interval ( $P = 0.05$ ,  $n = 3$ ) resulted in  $85 \pm 3\%$  of the photosynthetic activity of the control. To elucidate a relationship between the quality of HA, determined by its source, and its impact on the toxicity of atrazine, the mean photosynthetic activity of *Chlorella pyrenoidosa* was plotted against the concentration of the HA present (Fig. 14-3). The mean photosynthetic activity was calculated by averaging the measurements at a concentration of  $2.2 \times 10^{-7} M$  atrazine in the presence of HA from a specified soil source.

Photosynthetic activity of *Chlorella pyrenoidosa* increased substantially with an increase in concentration of HA from chernozemic soils. The presence of HA from gray wooded soil reduced the toxicity of atrazine, whereas HA from sod-podzolic soils had no effect on the toxicity of atrazine. Thus, the HA of three different soil types diverged substantially in their abilities to decrease the toxicity of atrazine.

By plotting photosynthetic activity of *Chlorella pyrenoidosa* at the same concentration of all seven HAs (Fig. 14-4), an essential variation is seen in the detoxifying effects produced by HA samples isolated from the same soil type. Thus, HA

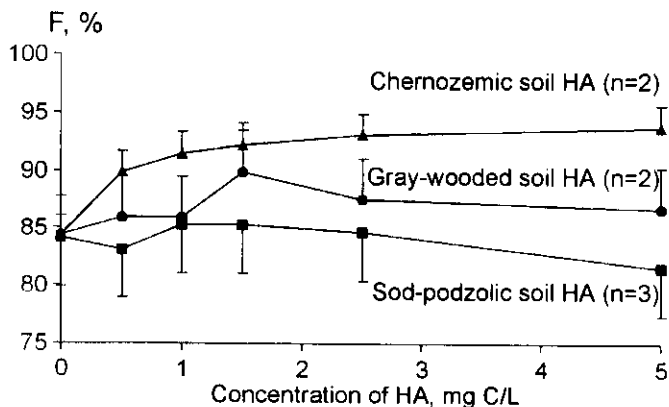


Fig. 14-3. Mean photosynthetic activity (F, % of control) of *Chlorella pyrenoidosa* averaged by humic acid (HA) samples isolated from soils of the same type vs. a concentration of humic acid (HA) at a content of atrazine of  $2.2 \times 10^{-7} M$  ( $n =$  number of samples averaged; bars show  $\pm 1$  standard deviation).

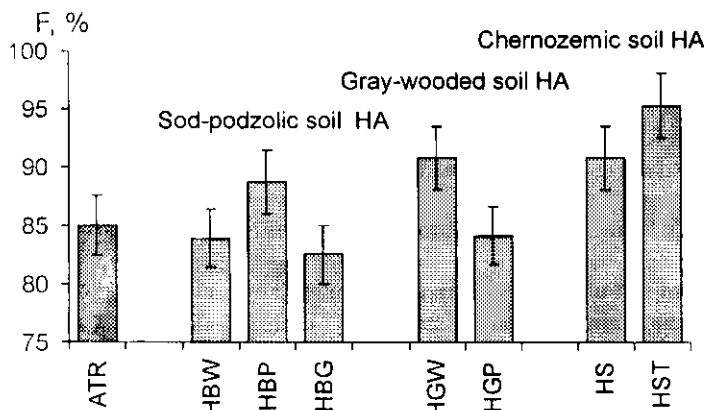


Fig. 14-4. Photosynthetic activity (F, in % of control) of *Chlorella pyrenoidosa* in the presence of 2.5 mg C L<sup>-1</sup> of humic acids (HA) at a concentration of atrazine of  $2.2 \times 10^{-7}$  M. Darker shaded column represents atrazine without HA; bars show  $\pm 1$  standard deviation.

samples from two chernozemic soils detoxified atrazine (13–15%), whereas HAs from sod-podzolic and gray wooded soils had either no effect or caused a slight decrease (0–7%) in its toxicity.

A similar investigation was undertaken for trifluralin. To estimate the impact of soil HA on toxicity of trifluralin, all bioassays with wheat seedlings were carried out at an application rate of 0.5 mg trifluralin kg<sup>-1</sup> of sand. The toxic effect produced by this rate of herbicide decreased root length of the seedlings to  $22 \pm 3\%$  of the control ( $\pm$  shows confidence interval at  $P = 0.05$ ,  $n = 30$ ). All seven soil HAs were introduced into the sand with trifluralin at rates of 25, 50, 100, and 200 mg HA kg<sup>-1</sup>. To elucidate a relationship between the quality of HA, determined by its source, and its impact on the toxicity of trifluralin, the mean root length of the

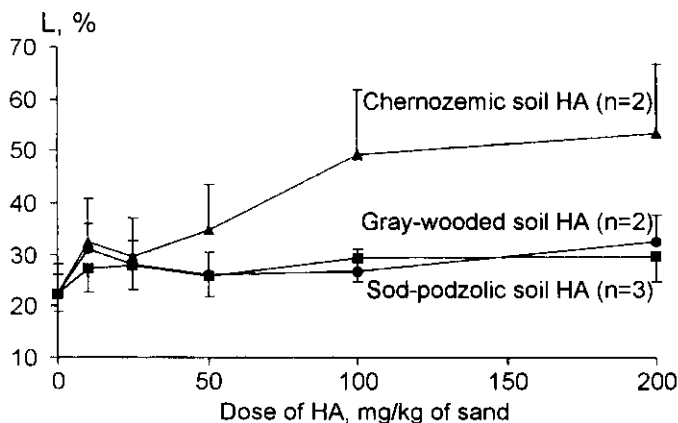


Fig. 14-5. Mean root length (L) of wheat seedlings (in % of control) averaged by humic acid (HA) samples isolated from soils of the same type vs. a dose of HA at a content of trifluralin of 0.5 mg kg<sup>-1</sup> ( $n$  = number of samples averaged; bars show  $\pm 1$  standard deviation).

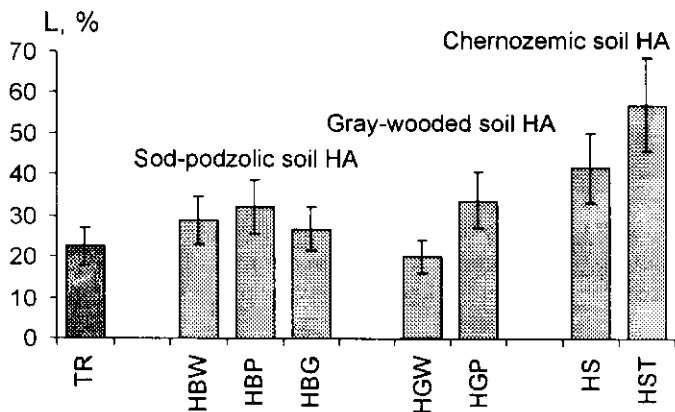


Fig. 14-6. Root length ( $L$ ) of wheat seedlings (in % of control) in the presence of  $100 \text{ mg kg}^{-1}$  humic acids (HA) at a content of trifluralin of  $0.5 \text{ mg kg}^{-1}$ . Darker shaded column represents trifluralin without HA; bars show  $\pm 1$  standard deviation.

seedlings was plotted against the rate of HA (Fig. 14-5). The mean root length of the seedlings was calculated by averaging the measurements at a rate of  $0.5 \text{ mg trifluralin kg}^{-1}$  in the presence of HA of the same soil type.

Root length of the seedlings increased steadily and very remarkably (from 22–55% of the control) as the rate of HA increased only for HA from chernozemic soils. The HAs from sod-podzolic and gray wooded soils had no effect on the toxicity of trifluralin at any application rate ( $P > 0.05$ ). Thus, the HAs of three different soil types diverged substantially in their abilities to decrease the toxicity of trifluralin.

By plotting root length of the seedlings at the same application rate of all seven HAs (Fig. 14-6), an essential variation is seen in the detoxifying effects produced by HA samples isolated from the same soil type. Like atrazine, the most pronounced and uniform effects were obtained for HAs from chernozemic soils. Effects of HAs from the various sod-podzolic and gray wooded soils were slight and more variable.

Thus, no increase in toxicity of atrazine and trifluralin occurred in the presence of different concentrations of soil HAs in test media. Moreover, HAs from chernozemic soils produced a substantial detoxifying effect on both atrazine and trifluralin.

This finding is in general agreement with the results of other studies on the impacts of HAs on the phytotoxicity and bioconcentration of herbicides (Draber et al., 1991; Manthey et al., 1993; Genevini et al., 1994) as well as with the data on  $ED_{50}$  determinations for herbicides on the soils of different types. The latter showed that the greatest  $ED_{50}$  and the largest amount of bound residues are characteristic for chernozemic soils (Wheeler et al., 1979; Barriuso & Calvet, 1992; Lunyov, 1992). The authors usually relate this phenomenon to a quantitative factor—a much greater content of organic matter in chernozems. The results of the current study show the importance of the quality of organic matter in its ability to affect the toxicity of herbicides.

## 14-4 CONCLUSIONS

Detoxification was the major effect of HS on the organic xenobiotics studied: three PAHs (Py, Flt, An) and two herbicides (atrazine and trifluralin). No increase in toxicity of the chemicals listed above was observed in the presence of HS. The magnitude of detoxification was strongly dependent on the source and structure of the HS. This encourages application of quantitative structure-activity relationships (QSAR) analyses to this field of the HS research. Development of the QSAR model for prediction of the mediating impact of HS on biological activity of organic xenobiotics from the known structure of HS is our immediate goal.

## 4-5 ACKNOWLEDGMENTS

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## REFERENCES

- Barriuso, E., and R. Calvet. 1992. Soil type and herbicides adsorption. *Int. J. Anal. Chem.* 46:117-128.
- Boehm, P.D., and J.G. Quinn. 1973. Solubilization of hydrocarbons by the dissolved organic matter in sea water. *Geochim. Cosmochim. Acta* 37:2459-2477.
- Day, K.E. 1991. Effects of dissolved organic carbon on accumulation and acute toxicity of fenvalerate, deltamethrin and cyhalothrin to *Daphnia magna* (Straus). *Environ. Toxicol. Chem.* 10:91-101.
- Draber, W., K. Tietjen, J. Kluth, and A. Trebst. 1991. Herbicides in photosynthesis research. *Angew. Chem. Int. Ed. Engl.* 30:1621-1633.
- Genevini, P.L., G.A. Sacchi, and D. Borio. 1994. Herbicide effect of atrazine, diuron, linuron and prometon after interaction with humic acids from coal. p. 1291-1296. *In* N. Senesi and T.M. Miano (ed.) *Humic substances in the global environment and implication on human health*. Elsevier Science B.V., Amsterdam, The Netherlands.
- Hayes, M.H.B., P. MacCarthy, R.L. Malcolm, and R.S. Swift. 1989. Structures of humic substances: the emergence of 'forms'. p. 689-734. *In* M.H.B. Hayes et al. (ed.) *Humic substances II: In search of structure*. John Wiley and Sons, Chichester, UK.
- Kukkonen, J. 1995. The role of natural organic material on the fate and toxicity of xenobiotics in aquatic environment. p. 95-108. *In* M. Munavar and M. Luotola (ed.) *The contaminants in the Nordic ecosystem: Dynamics, processes and fate*. Ecovision World Monogr. Ser. SPB Acad. Publ., Amsterdam, the Netherlands.
- Kukkonen, J., and A. Oikari. 1987. Effects of aquatic humus on accumulation and toxicity of some organic micropollutants. *Sci. Total Environ.* 62:399-402.
- Landrum, P.F., M.D. Reinhold, S.R. Nihart, and B.J. Fadie. 1985. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoyi* in the presence of humic and fulvic materials and natural dissolved organic matter. *Environ. Toxicol. Chem.* 4:459-467.
- Leo, A., C. Hansch, and D. Elkins. 1971. Partition coefficients and their uses. *Chem. Rev.* 71:525-616.
- Leversee, G.J., P.F. Landrum, J.P. Giesy, and T. Fannin. 1983. Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons. *Can. J. Fish. Aquat. Sci.* 40:63-69.
- Loewe, L.E. 1992. Studies on the nature of sulfur in peat humic acids from Fraser river delta, British Columbia. *Sci. Total Environ.* 113:133-145.
- Lunyov, M.I. 1992. Pesticides and protection of phythocenoses. (In Russian.) Kolos Publ., Moscow.
- Manthey, M., M. Faust, S. Smolka, and L.H. Grimme. 1993. Herbicide bioconcentration in algae: studies on lipophilicity-sorption-activity relationships (LSAR) with *Chlorella fusca*. *Sci. Total Environ.* 114:453-459.
- Matorin, D.N., D.V. Vavilin, and P.S. Venediktov. 1990. Application of the chlorophyll luminescence techniques for investigation of nutrition activity in Crustacea. (In Russian.) *Biol. Sci. (USSR)* 1:146-151.

- Matorin, D.N., and P.S. Venediktov. 1990. Chlorophyll luminescence in microalgae culture and natural phytoplankton populations. (In Russian.) *Itogy Nauki i Tekh. Ser. Biofiz. (Moscow)* 40:40–101.
- McCarthy, J.F., and B.D. Jimenez. 1985. Reduction in bioavailability to bluegills of polycyclic aromatic hydrocarbons bound to dissolved humic material. *Environ. Toxicol. Chem.* 4:511–521.
- McCarthy, J.F., B.D. Jimenez, and Th. Barbee. 1985. Effect of dissolved humic material on accumulation of polycyclic aromatic hydrocarbons: structure–activity relationships. *Aquat. Toxicol.* 7:15–24.
- Morehead, N.R., B.J. Eadie, B. Lake, P.F. Landrum, and D. Berner. 1986. The sorption of PAH onto dissolved organic matter in Lake Michigan waters. *Chemosphere* 15:403–412.
- Oikari, A., J. Kukkonen, and V. Virtanen. 1992. Acute toxicity of chemicals to *Daphnia magna* in humic waters. *Sci. Total Environ.* 117/118:367–377.
- Oris, J.T., A.T. Hall, and J.D. Tylka. 1990. Humic acids reduce the photo-induced toxicity of anthracene to fish and daphnia. *Environ. Toxicol. Chem.* 9:575–583.
- Orlov, D.S., and L.A. Grishina. 1981. Praktikum on humus chemistry. MGU Publ. (In Russian.) Moscow State Univ. Publ., Moscow.
- Perminova, I., D. Kovalevsky, N. Yashchenko, N. Danchenko, A. Kudryavtsev, D. Zhilin, V. Petrosyan, N. Kulikova, O. Philippova, and G. Lebedeva. 1996. Humic substances as natural detoxicants. p. 399–406. In C.E. Clapp et al. (ed.) *Humic substances and soil organic matter in soil and water environments: Characterization, transformation and interactions*. IHSS Inc., St. Paul, MN.
- Polynov, V.A. 1992. Development and application of fluorescent methods of biotesting of natural and waste waters. Ph.D. diss. Lomonosov Moscow State Univ., Moscow, Russia.
- Spacie, A., P.F. Landrum, and G.J. Leverset. 1983. Uptake, depuration and biotransformation of anthracene and benz(a)pyrene in bluegill sunfish. *Ecotoxicol. Environ. Safety* 7:330–341.
- Steinberg, C.E.W., A. Sturm, J. Kelbel, S.K. Lee, N. Hertkorn, D. Freitag, and A.A. Kettrup. 1992. Changes in acute toxicity of organic chemicals to *Daphnia magna* in the presence of dissolved humic material (DHM). *Acta Hydrochim. Hydrobiol.* 20:326–332.
- Stewart, A.J. 1984. Interactions between dissolved humic materials and organic toxicants. p. 505–521. In K.E. Cowser (ed.) *Synthetic fossil fuel technologies*. Butterworth Publ., Boston, MA.
- Swift, R.S. 1996. Organic matter characterization. p. 1011–1069. In *Methods of soil analysis*. Part 3. SSSA Book Ser. 5. SSSA and ASA, Madison, WI.
- Tamiya, H.K., K. Morimura, M. Yokota, and R. Kunieda. 1961. The mode of nuclear division in synchronous culture of *Chlorella*: Comparison of various methods of synchronization. *Plant Cell Physiol.* 2:383–403.
- Thurman, E.M., and R.L. Malcolm. 1981. Preparative isolation of aquatic humic substances. *Environ. Sci. Technol.* 15: 463–466.
- Vavilin, D.V., V.A. Polynov, D.N. Matorin, and P.S. Venediktov. 1995. Sublethal concentrations of copper stimulate photosystem II. Photoinhibition in *Chlorella pyrenoidosa*. *J. Plant Physiol.* 146:609–614.
- Wheeler, W.B., G.D. Stratton, R.R. Twilley, Ou Li-Tse, D.A. Carlson, and J.M. Davidson. 1979. Trifluralin degradation and binding in soil. *J. Agric. Food Chem.* 27:702–705.