46 Humic Substances as Natural Detoxicants

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ABSTRACT

Influence of humic substances (HS) of different origins on the toxicities of heavy metals (cadmium, copper, and lead), polyaromatic hydrocarbons (phenanthrene and fluoranthene), and herbicides (atrazine and Glean) in relation to *Chlorella vulgaris*, *Daphnia magna* and higher plants, respectively, is assessed quantitatively by the method of biotesting. A reduction in the toxicity in the presence of HS is registered in all the tested systems. A conclusion is made that humics-containing materials can be used as natural detoxicants for the contaminated aqueous and soil media.

INTRODUCTION

The role of humic substances (HS) - major constituents of natural organic matter (NOM) - in mediating biological activity (toxicity, bioaccumulation, uptake) of anthropogenic chemicals in the environment is still poorly understood. Although they are, in general, considered to have a mitigating impact on the biological activities of heavy metals and organic chemicals (Giesy et al., 1983; Vymazal, 1984; McCarthy & Jimenez, 1985; Oris et al., 1990), HS are known also to have contrary effects (Ramamoorthy & Blumhagen, 1984; Stewart, 1984; Oikari et al, 1992). Given the ubiquity and abundances of HS in the environment, it is important to:

1. Develop principles for the quantitative estimation of the impacts of humic acids (HAs) on the biological activities of the main classes of harmful chemicals released into the environment [heavy metals, anthropogenic biocidal compounds, petroleum hydrocarbons, and polyaromatic hydrocarbons (PAH)]. Biological activities of the chemicals are greatly influenced by the environment of their surroundings. Thus it is no longer possible to predict such activities in natural sourroundings from experiments conducted in distilled water and pure sand media. Extended studies on the interactions of the chemicals with NOM (the major component in the alteration of the biological activity of exotoxicants in the environment) can give better indications of the 'maximum permissible levels' and allow more meaningful predictions to be made of the dangers from the release of the chemicals to the environment.

2. Develop a better awareness of the detoxifying impact of peat HAs. There are large natural sources of HS, such as peat and sapropel (lake bottom sediments), and there is also quite a well developed industry of both humic fertilizers and biostimulators. Based on the known detoxifying impact of peat HAs, and because of the fact that certain specific activities can be related to the kind of source peat, it should be possible to develop the use of peat as a humics-enriched material to be used as detoxicants for soil media. Information about the affinities between well characterized peat HAs and certain classes of organic chemicals can be used in designing the synthesis of specific detoxifying agents based on peat humics. This approach would bring awareness of the potential detoxifying properties of HAs into the field of practical agricultural research.

Based on these considerations, we believe that the experimental results presented here can serve as an initial step in investigations of the roles of HS as natural detoxicants.

MATERIALS AND METHODS

Materials

The HS used as model humic materials in the experiments were as follows:

Riverine fulvic acid (FA) (PAHs experiments). Extraction from Moscow River water by sorption on diethylaminoethylcellulose, elution using 0.1M NaOH, and demineralizing by discharging through a cation-exchanger. The emerging solution was evaporated to dryness and redissolved in distilled water. Details of the procedure are in Perminova (1987).

Riverine FA (heavy metals experiments). Extraction from the acidified Moscow River water using XAD-2 resin, followed by the same operations involving elution, demineralizing and desiccating. The technique is similar to that described by Thurman and Malcolm (1981).

Peat and soil HA. Isolation by alkali extraction following preliminary extraction of peat samples using a mixture of ethanol-benzene, and further separation of FA and HA according to IHSS methods (Kuwatsuka et al., 1992; Lowe, 1992).

Marine FA was kindly provided by Dr. G.R. Harvey; the isolation technique is described by Harvey et al. (1983).

Peat and sapropel was provided by Dr. M.V. Popov (Tver Polytechnical Institute).

Oxyhumate and hydrohumate were commercially available preparations and were kindly provided by Prof. G.V. Naumova (Institute for Natural Resources and Ecology

of Belorussian Academy of Sciences).
All the chemicals used in the experiments were reagent grade.

Methods

Toxicity of cadmium (Cd), copper (Cu), and lead (Pb) was examined by the method of algological biotesting. The green algae *Chlorella vulgaris* was used as a biotarget organism. Photosynthetic activity as a test-function was determined by a fluorimetric method according to Lyadsky et al. (1987) and Polynov (1992). The composition of the test solutions was: KNO_3, 0.5 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.25 g L^{-1}; HS, 0 to 25 mg L^{-1}. The four types of the test systems (pure nutrition medium, nutrition medium plus heavy metal, nutrition medium plus HAs, and nutrition medium plus heavy metal plus HAs) were measured for toxicity during four hours of incubation.

Toxicity of PAHs was controlled by registering grazing activity of the crustacean *Daphnia magna* by use of a fluorimetric technique. The details of the procedure are described in Matorin et al. (1990). Grazing activity of the cladocera was used as a test parameter. It was determined by a decrease in concentration of the algae cells registered via the intensity of the chlorophyll fluorescence of the solution after 24 h of incubation.

Toxicity of the herbicides Glean and atrazine was studied in the vegetation experiments using corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) plants as biotargets, respectively. Duration of the vegetation period was 50 days for corn and 40 days for wheat. Twelve seeds of wheat or 5 seeds of corn were planted per pot containing 400 g of sod-podzolic soil of the Moscow region. Peat and sapropel was introduced by mixing with the whole soil prior to planting. Preparations of oxyhumate and hydrohumate were added by pouring these into a 1-cm depth of the upper soil layer. Watering the plants was conducted by running water. Dry biomass of the crop was used as a test function.

For the estimation of the detoxifying ability of the tested HS, a parameter 'percentage of detoxification', was introduced. This was calculated by the formula:

$$D(\%) = \left(1 - \frac{R_o(R_d - R_{d+t})}{R_d(R_o - R_t)}\right) x\ 100$$

where:

D = percentage of detoxification;
R = biological response, for green algae -- photosynthetic activity, for *Daphnia magna* -- grazing activity; for corn and wheat plants -- dry biomass of the crop;
R_o = biological response registered in the control experiments -- test medium without either toxicant (heavy metals, PAHs, atrazine, and Glean) or detoxicant (preparations of HS);
R_d = biological response in the presence of detoxicant;
R_t = biological response in the presence of toxicant;
R_{d+t} = biological response in the presence of both detoxicant and toxicant in the same test medium.

Use of the given formula allows the evaluation of a pure detoxification effect. The influence of the possible fertilization effect caused by introducing humic materials into the test media is eliminated by the appropriate calculation.

RESULTS AND DISCUSSION

As can be seen from the results of the experiments conducted in aqueous media contaminated with heavy metals (Fig. 1), the toxic impact of the metals on algae in the presence of HAs is greatly decreased. This general trend is valid for all four types of the tested HS preparations. However, while the detoxification efficiencies of peat, soil, and riverine HS are very similar, the marine FA stands out, exposing a much weaker detoxification impact on Cu, as well as Cd. This can be explained by the structural peculiarities of marine HS, which are characterized by the predominance of aliphatic structural units (Harvey et al., 1983), followed by the decrease in chelating ability of these substances in comparison with more highly aromatic substances such as peat, soil, or fresh water humics. The conclusion which can be drawn from these results is that the marine environment can be considered to be much more vulnerable to the toxic impact of heavy metal contamination than fresh water or soil ecosystems. It means that at the same level of pollution, the ecological consequences for the marine environment can be much more severe. This fact demonstrates that the existence of the same maximum permissible levels of heavy metals for fresh water and marine ecosystems is illogical in terms of the real dangers which these contaminants pose to the environment.

Figure 2 demonstrates the reduction in toxicity of phenanthrene (Phen) and fluoranthene (Fl) to *Daphnia magna* in the presence of riverine FA. These results are in general agreement with the data of other researchers with regard to the lowering of the bioavailability of PAHs in humics-containing solutions due to association between the molecules of PAHs and FA. The fact that in our experiments, reduction in toxicity of Phen and Fl was directly proportional to an increase in the concentration of FA in the test solution also confirms the above mentioned mechanism of PAHs detoxification via associate formation.

Figure 3a shows the concentration dependence of the detoxification efficiency of two types of peat in relation to atrazine. Addition of both types of peat (lowland and highland) to the contaminated soil caused an essential increase in the dry biomass of the crop. The mitigating impact was strengthened with increasing dosage of peat to the soil. Given the well established (Hayes, 1970; Choudhry, 1983) high affinity of atrazine for binding with HS, the observed decrease in its phytotoxicity in the presence of humics-enriched material such as peat can be considered to be relatively satisfactory. It is of interest that both types of peat tested (lowland and upland) were characterized by a relationship that was practically identical for the value of the percentage detoxification and the dosage of peat introduced into the contaminated soil. This can be used to indicate that there is no significant correlation between the type of peat and its mitigating ability in relation to atrazine. However, in order to extend this conclusion to practical needs of agriculture, many more different types of peat must be tested.

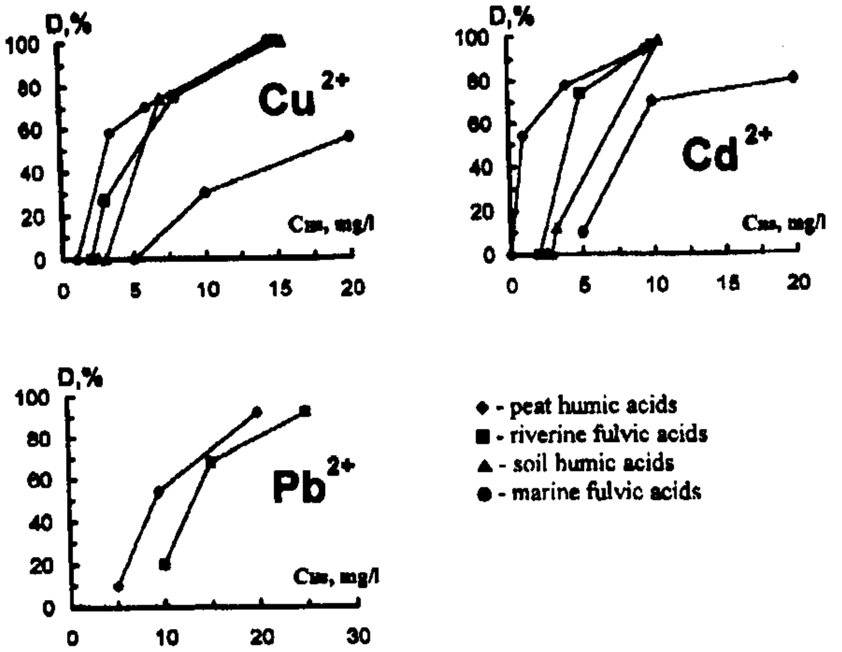

Fig. 1. Detoxification of heavy metals by humic substances of different origins in relation to *Chlorella vulgaris:* C_{Cu} = 50 µg L^{-1}, C_{Cd} = 800 µg L^{-1}, C_{Pb} = 2000 µg L^{-1}.

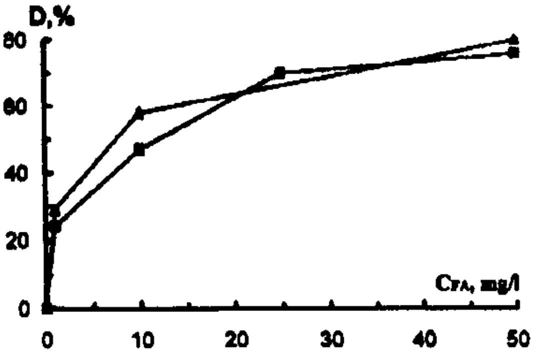

Fig. 2. Detoxification of PAHs by riverine fulvic acid in relation to *Daphnia magna* ▲ = phenanthrene, 0.5 mg L^{-1}; ■ = fluoranthene, 0.13 mg L^{-1}.

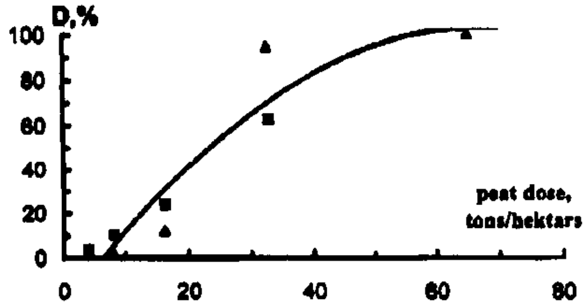

Fig. 3a. Detoxification of atrazine by different peat samples. Dose of atrazine = 1 kg ha^{-1}; ▲ = peat 7, ■ = peat 8.

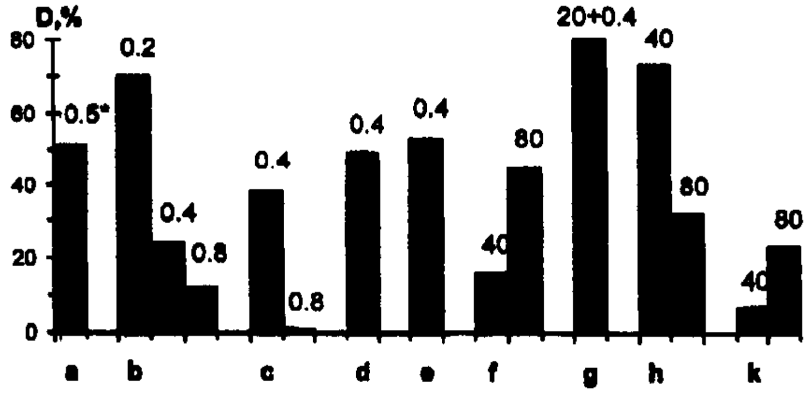

Fig. 3b. Detoxification of the herbicide Glean by different humic substances-containing materials. Dose of Glean = 10 g ha^{-1} (vegetation experiments).
a = activated charcoal; b = oxyhumate; c = hydrohumate; d = oxyhumate by sprinkling; e = hydrohumate by sprinkling; f = sapropel; g = sapropel + hydrohumate; h = peat 3; k = peat 10. Numbers above the columns are doses of detoxicant in Mg ha^{-1}.

Figure 3b demonstrates the detoxification efficiency of the different humics-enriched materials of natural and man-made origin in relation to the herbicide Glean. Despite the high variability of the registered effects, all of these were on the same level or higher than that of the activated charcoal, a quite efficient agricultural ameliorant (Lebedeva et al., 1991). The diversity of the effects observed apparently originates from the influence of components in these materials that are other than HS. Nonetheless, the conclusion can be made that humics-containing materials can be used as natural detoxicants for the contaminated soil (as well as aqueous) media, but additional research is needed to deal with the selectivity and efficiency of their action on the different groups of the contaminants.

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