

EFFECT OF HUMIC ACID ON THE BIOAVAILABILITY OF γ -HEXACHLOROCYCLOHEXANE IN *MARSILEA MINUTA* (L.)

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Abstract. Effect of various concentrations of humic acid (0.2 to 1%) on the bioavailability of γ -HCH in vegetative clones of the aquatic fern *Marsilea minuta* was studied in a static experimental bioassay system on different photoperiods. Addition of humic acid showed the reduction in the bioavailability of γ -HCH in all the photoperiods (72 hr light to 144 hr light) at the interval of 16 hr light (L) and 8 hr dark (D) in both aerial and submerged portion as compared to control indicating its protective role in toxicity.

Keywords: bioavailability, γ -HCH, humic acid, photoperiods

1. Introduction

Pesticides applied for agriculture reach the sediments in the aquatic environment through soil runoff and leaching. Sediment plays a crucial and complex role in deposition, release and distribution of contaminants in aquatic environment (Solomons *et al.*, 1987). Sediments with higher concentration of TOC have greater capacity to absorb non-polar organic compounds, thereby reducing toxicity. Environmental physicochemical factors can influence the toxicity of xenobiotics on aquatic organisms by modifying its specific molecular properties, its fate in the medium or the receptivity of its biological targets (Goodrich *et al.*, 1991; Lores *et al.*, 1993). Humic substances represent a major fraction (60–80%) of the total dissolved organic matter in natural waters, sediments, soils and anthropogenic effluents (Rav-Acha and Rebhun, 1992). The binding strengths between the pollutants and humic molecules may vary, depending upon the nature of pollutants and on the general composition of the aquatic environment (Misra *et al.*, 1996a, b). The presence of humic acid and fulvic acid in lakes and streams may stimulate the growth of phytoplankton (Tan, 1994; Vrana and Votruba, 1995). Information regarding how humic acid levels influence the toxic risks to aquatic macrophyte is scanty. Aquatic macrophytes are known to have great importance, forming a substantial component of the primary production in many aquatic habitats and thus offer a convenient test system to study the uptake, storage translocation, and toxicity of pollutants under experimental conditions and for extrapolation to ecological field studies (Charpentier *et*



al., 1987; Brix and Schierup, 1989; Guilizzoni, 1991). The ability of aquatic macrophytes to bioaccumulate and in some cases, biotransform toxic compounds into less toxic forms is largely unexplored. However, some recent 'phytoremediation' studies have documented great potential for the aquatic plants to bioaccumulate nutrients, metals and organics from contaminated sediments and/or water (Salt *et al.*, 1995; Cunningham *et al.*, 1996). Living at the boundary between the sediment layer and the water column, rooted plants have great potential for biomonitoring toxic metals and organochlorine contaminants (Manny and Kenaga, 1991; BERNACKI *et al.*, 1997; Lovett-Doust *et al.*, 1994a,b). We have selected the aquatic fern *Marsilea minuta* as test species due to the ease of developing vegetative clones from the same plant for comparative experimental studies. Also, pteridophytes have received comparatively very little attention from environmental toxicologists. Since *Marsilea* is an emergent plant with roots anchored to the sediments and is capable of amphibious existence it could prove to be an ideal material for biological monitoring and ecotoxicological evaluation of environmental pollutants. *Marsilea sp.* was found to bioconcentrate mercury to different degree. The pathomorphological manifestations of Cd toxicity on *Marsilea minuta* as studied by transmission electron microscopy indicated damage to chloroplasts and tonoplasts (Singh *et al.*, 1991).

Since studies on the influence of natural organic substances on the bioavailability of organic micropollutants are sparse, the results of a study of the influence of humic acid on the bioavailability of γ -hexachlorocyclohexane (γ -HCH), the commonly used pesticide in aquatic macrophyte *Marsilea minuta* was studied in the present manuscript to understand the behaviour of γ -HCH in the fresh water ecosystems using sediment-water-biota compartment on different photoperiods.

2. Materials and Methods

2.1. CHEMICALS

γ -HCH (99% purity) was purchased from Sigma Chemical Company, U.S.A. Pesticide reference standards of Reidel-de-Haen (West Germany) were a gift of RIVM (Netherlands). Other chemicals used in the experiment were from British Drug House (England) and E. Merck (Germany). Humic acid (sodium salt) from Aldrich Chemical Company was used in all present studies.

2.2. PREPARATION OF THE COATED SAND

Pure acid washed calcinated sea sand (E. Merck) with particle size of 0.1–0.3 mm was used as simulated sediment by incorporating known amounts of the desired organics for environmental dynamics studies. The sand was coated homogeneously with γ -HCH (500 $\mu\text{g/g}$) by continuous shaking with desired amount in n-hexane.

The γ -HCH level was up to saturation limits. The solvent was evaporated to dryness under controlled conditions (temperature and pressure) to avoid vaporization of γ -HCH. In order to account for any evaporation loss, HCH content of the soil was analysed prior to the experiment.

2.3. MARSILEA CULTURE

Plants of *Marsilea minuta* L. were collected from fresh water ponds, washed repeatedly with distilled water and cut into 4.0 cm long pieces of stolons with intact rhizome, apical bud, leaves and roots. These clones were maintained in 3% Hoaglands solution (EPA, 1975) for the development of second generation leaves and roots which were used for experiments. The new generation was clipped off to keep the cultures generating. Throughout the experiment, cultures were maintained under standard physiological conditions at $25 \pm 0.5^\circ\text{C}$ in BOD incubator. The 16 hr light and 8 hr dark photoperiod was adjusted by Program Timer. During illumination, light was provided by a fluorescent white light (Philips) with a flux of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$.

2.4. HCH-HUMIC ACID INTERACTION

Acid washed sand was coated with $500 \mu\text{g}$ of γ -HCH per gram sand. 8 g γ -HCH coated sand was taken in each 250 mL beaker and divided into control and experimental. Experimental sets contained 6 nos. of *Marsilea minuta* plant in 60 mL of 3% Hoagland solution containing 0.2, 0.5 and 1.0% humic acid whereas control contained all the constituents similar to experimental except humic acid. 4 sets of experiments were performed in triplicate for photoperiods 16 hr (L) and 8 hr (D). The plants were taken out at 0 hr, 72 hr (L), 80 hr (D), 96 hr (L), 104 hr (D), 120 hr (L), 128 hr (D), 144 hr (L).

Aerial (shoot and leaf) and submerged (root) part of *Marsilea minuta* was separated, dried on filter paper and weighed. Homogenates of both the portions were prepared by cutting into small pieces and homogenizing in a Potter Elvehjem glass homogenizer.

2.5. ESTIMATION OF γ -HCH

γ -HCH from the homogenate was extracted with 2 portions of 50 mL AR n-hexane in a separating funnel. The extracts were dried over anhydrous sodium sulphate and filtered through glass wool. The filtered extracts were concentrated to 3.0–4.0 mL under controlled conditions to avoid loss of γ -HCH by vaporization. Finally the volume was made up to 5.0 mL. The extracts were subjected to gas chromatographic analysis on a Varian Vista 6000. A $1.8 \text{ m} \times 4 \text{ mm}$ column packed with chrome WHP mesh size 80/100 coated with 1.5% OV-17 and 1.95% OV-210 was used. Conditions (all temp. in $^\circ\text{C}$); injector 250, column 180, detector 250; Carrier gas: nitrogen 60 mL/min, Electron Capture Detector ^{63}Ni was used.

3. Results

Humic acid reduced the bioavailability of γ -hexachlorocyclohexane in all the photoperiods in both aerial and submerged portion. Addition of humic acid from 0.2 to 1.0% in the system caused the decrease in the concentration of γ -HCH in aerial part in the range from 16 to 41% in 72, 96, 120 and 144 hr light phase while in the dark phase at 80, 104 and 128 hr it caused 38 to 50% reduction as compared to control (Table I). Higher concentration of humic acid did not show much effect. Similar was the trend with submerged portion. Here again addition of humic acid from 0.2 to 1.0% caused the decrease of γ -HCH in submerged portion from 43 to 80% in 72, 96, 120 and 144 hr light phase while dark phase at 80, 104 and 128 hr it caused the reduction of 34 to 49% as compared to control (Table II). Higher concentrations of humic acid in this case was ineffective.

4. Discussion

Factors like hydrophobic interactions may influence environmental persistence, inter compartmental distribution kinetics, bioavailability, biomagnification and target species and tissue effects (Bruggeman, 1982; Jaffe, 1991). The environmental fate of inorganic and organic pollutants (metals and pesticides) are affected by the presence of humic acid in the aquatic environment. Aldrich humic acid is a terrestrially derived humic material and appears to partition organic contaminants 4 to 20 fold more strongly than natural aquatic humic and fulvic acid (Landrum *et al.*, 1985; Chiou *et al.*, 1987). Humic materials can enhance the water solubility of organic compounds through complexation and binding (Means and Wijayratne, 1982). The humic acid pesticide complex must be too large or too polar to penetrate across biological membranes (Day, 1991). Reduction in the bioavailability of γ -HCH in all the photoperiods in both aerial and submerged portion of *Marsilea minuta* in the present study could be due to the complex formation as a result of binding between humic acid and γ -HCH and the free radical generated from humic acid as a result of exposure at different photoperiods could be one of the contributing factors responsible for binding. Production of reactive oxygen species by humic acid through photochemical changes induced by UV radiation is known to derive photo oxidation of organic contaminants (Cooper *et al.*, 1989; Canonica *et al.*, 1995). Reduction in the rate of photosynthesis because of the dark brown colour of the complex in water could be another factor responsible for reduction in the bioavailability of γ HCH. The natural humus water can reduce the bioavailability of selected trichlorophenols and lindane towards fish. Several authors have demonstrated a reduction in the toxic activity of pyrethroid containing media in the presence of humic acid in various aquatic species. This effect was assigned to the adsorption of pyrethroid by humic acids (Day, 1991, Ortego and Benson, 1992; Muir *et al.*, 1994). Based on spectral characterization and fast atom bombardment

TABLE I

Effect of various concentrations of humic acid on the bioavailability of γ -HCH in the aerial portion of *Marsilea minuta* (L)

| Conc. of Humic acid | Conc. of γ -HCH in $\mu\text{g/g}$ | | | | | | |
|---------------------------|---|------------------|------------------|------------------|------------------|------------------|------------------|
| | 72 hr (L) | 80 hr (D) | 96 hr (L) | 104 hr (D) | 120 hr (L) | 128 hr (D) | 144 hr (L) |
| Control | 0.896 \pm 0.15 | 0.923 \pm 0.17 | 0.949 \pm 0.23 | 1.515 \pm 0.28 | 1.468 \pm 0.26 | 1.478 \pm 0.27 | 1.164 \pm 0.16 |
| 0.2% | 0.749 \pm 0.13 | 0.743 \pm 0.12 | 0.752 \pm 0.16 | 0.705 \pm 0.25 | 1.182 \pm 0.18 | 0.755 \pm 0.23 | 0.904 \pm 0.12 |
| 0.5% | 0.698 \pm 0.11 | 0.675 \pm 0.10 | 0.745 \pm 0.15 | 0.687 \pm 0.20 | 1.097 \pm 0.17 | 0.640 \pm 0.21 | 0.882 \pm 0.18 |
| 1% | 0.535 \pm 0.10 | 0.586 \pm 0.11 | 0.500 \pm 0.12 | 0.636 \pm 0.15 | 0.778 \pm 0.21 | 0.627 \pm 0.19 | 0.612 \pm 0.21 |

Values are represented as arithmetic mean of three replicates \pm S.E.

TABLE II

Effect of various concentrations of humic acid on the bioavailability of γ -HCH in the submerged portion of *Marsilea minuta* (L)

| Conc. of Humic acid | Conc. of γ -HCH in $\mu\text{g/g}$ | | | | | | |
|---------------------------|---|------------------|------------------|------------------|------------------|------------------|------------------|
| | 72 hr (L) | 80 hr (D) | 96 hr (L) | 104 hr (D) | 120 hr (L) | 128 hr (D) | 144 hr (L) |
| Control | 2.135 \pm 0.25 | 2.246 \pm 0.27 | 2.444 \pm 0.35 | 2.548 \pm 0.32 | 2.636 \pm 0.37 | 2.455 \pm 0.35 | 2.568 \pm 0.30 |
| 0.2% | 1.225 \pm 0.18 | 1.310 \pm 0.21 | 1.245 \pm 0.20 | 1.725 \pm 0.24 | 1.190 \pm 0.19 | 1.428 \pm 0.19 | 1.341 \pm 0.21 |
| 0.5% | 0.719 \pm 0.14 | 1.238 \pm 0.19 | 0.627 \pm 0.17 | 1.430 \pm 0.21 | 0.617 \pm 0.15 | 1.382 \pm 0.17 | 0.698 \pm 0.12 |
| 1% | 0.540 \pm 0.11 | 1.156 \pm 0.16 | 0.491 \pm 0.11 | 1.372 \pm 0.21 | 0.486 \pm 0.10 | 1.275 \pm 0.13 | 0.663 \pm 0.15 |

Values are represented as arithmetic mean of three replicates \pm S.E.

mass spectrometry records we have obtained indication for sequestration of γ -HCH by humic acid (Misra *et al.*, 1998). Thus, it could be considered that the humic acid have a protective role when the toxicant is introduced into the medium but whether it would modify the eventual long term toxicity of the toxicant can not be decided by the present data and needs further study.

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