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Purification of PCB Contaminated Water by Chitosan: A Biological Test of Efficiency Using the Common Barbel, *Barbus barbus*

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A recent survey of the distribution and amount of persistent toxics in the Belgian environment emphasized that PCBs were certainly among the most hazardous xenobiotics (Thomé 1984). The aquatic ecosystem appeared to be particularly contaminated by highly chlorinated PCBs characterized by more than 4 chlorines per molecule. In that view, commercial blends such as Aroclor 1254 and 1260 (Monsanto, USA) are particularly abundant according to their previously large utilization, their high lipophility, their low solubility and their low biodegradability (Haque et al. 1974; Hützinger et al. 1974).

Standard methods for water purification used in water softening plants remained largely ineffective to eliminate these toxics. Up to now, expensive treatments leading only to an incomplete elimination such as adsorption onto activated charcoal or synthetic resins have been used (Tucker and Saeger 1975). There is thus a real need for more efficient and cheaper treatments of waste water polluted by highly persistent toxics like PCBs.

Chitosan, the deacetylated form of chitin, a natural polymer, perhaps could fulfil these requirements. Indeed, among industrial applications proposed for chitosan, its use in water purification appears as a very promising one (Muzzarelli and Tanfani 1981). In waste water treatment, chitosan can be used as a floculent and a coagulating agent for organic matter (Bough et al.1976). Furthermore, chitosan possesses the advantage of being simultaneously an ion chelating agent for heavy metals and an adsorption medium for acid pesticides occasionally present in polluted water (Davar and Wightman 1981; Masri and Friedman 1972; Shigeno et al. 1980). In all these applications, chitosan is often more effective or more attractive than other more expensive polymers--synthetic resins (Muzzarelli 1971), activated charcoal (Davar and Wightman 1981), cellulosic derivatives (Koshijima et al. 1973), chitin and synthetic polyelectrolytes (Muzzarelli 1977). Chitosan can be produced indeed at lower cost from chitinous wastes originating in crustacean meat industries or fungi grown in fermentation processes (Muzzarelli 1977, 1981).

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The present paper deals with adsorption capabilities of chitosan towards highly chlorinated PCBs. The impact of that purification on the aquatic environment is tested by the analysis of the PCB accumulation in young specimens of the sensitive teleost *Barbus barbus* kept in PCB contaminated water filtered or unfiltered through chitosan.

MATERIALS AND METHODS

Chitosan at a high purity level was prepared in our laboratory following a procedure modified from Jeuniaux (1955) and Asano and Hamakawa (1978). Crude shrimp shells (*Crangon crangon*) from local sea food stores were decalcified by treatment during 24 h in two successive baths of 10% HCl. Shells were then washed in distilled water before deproteinization by two successive baths of 5% NaOH for 6 h and a last wash with distilled water. The purified chitin so obtained was deacetylated for 10 h in 40% NaOH. The residue was then dried at 85°C in an oven for 12 h. The resulting chitosan was ground to 10 ASTM mesh size.

The PCB adsorption capabilities of chitosan were tested using a flow-through system (Figure 1) composed of 50-L glass tanks (A, B, C) in which water containing the dissolved PCB (Aroclor 1260) was replaced at a constant rate (36 L/day). Daily, tank A was filled with 50 L of pond water previously filtered through activated charcoal in a separate 300-L tank (E). Water of tank A was daily spiked with 5 mL Aroclor 1260 in acetone (5 µg/mL) to obtain a final concentration in water of 0.5 µg/L. Regular replacement of water in experimental tanks (B, C) was achieved by means of a peristaltic pump at a flow rate of 36 L/day.Adsorption of PCB onto the inner surface of glass was prevented by treatment with dimethylchlorosilane (DMCS 10% in hexane). Water originating in tank B was filtered through chromatographic columns (30 x 1 cm) filled with 1 or 10 g of chitosan powder. Chitosan was replaced every day. Control experiments were carried out using 5 mL acetone without PCB.

Thirty barbels aged one year (mean weight = 1.365g;SD = 0.55 g) and originating from experimental fish farms of Liège University were placed in tanks B, C and T. Analysis of PCB concentrations in water and fish of each experimental tank were carried out as a function of time in 100 mL water samples and in 3 fish specimens treated separately.

PCBs were extracted from water by filtration through C_{18} microcolumns (Bond Elut C_{18}, Analytichem International, Harbor City, California, U.S.A.). In a previous work, it has been established that these cartridges were particularly efficient in the extraction of organochlorinated pesticides (Marcelle and Thomé 1983). Maximum efficiency was achieved by successive treatments with 1 ml hexane, acetone and methanol in water (1:1). Water samples (100 mL) were then passed through the column so treated to receive nonpolar compounds dissolved in polar solvents. Most interferring polar compounds retained in the cartridge were

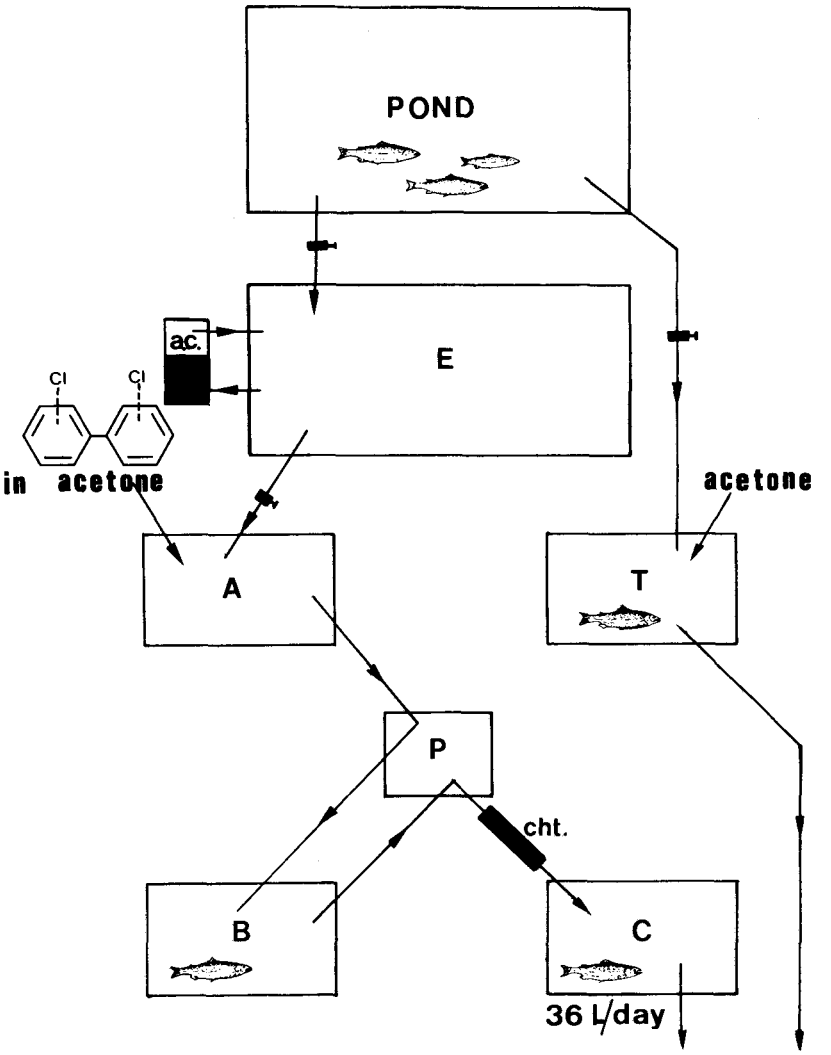

Figure 1. Flow through system used to test the efficiency of chitosan adsorption towards PCB. Explanations in the text.
a.c. = activated charcoal; cht. = chitosan

eliminated by elution with 0.2 mL of methanol/water (1:1). PCBs were then eluted with 1 mL hexane and analysed by gas chromatography. This extraction method appeared to result in high performance and rapid purification of dissolved PCB. The mean recovery percentages reached indeed 97 $\pm$ 4 % within an overall procedure ranging 10 min.

Individual fish was killed, weighed and homogenized in 5 mL acetone containing 1 g anhydrous Na_2SO_4 by means of an Ultra-turrax homogenizer (Janke & Kunkel Gmbh, Ika werk, Staufen i.B, W-Germany). After centrifugation at 1750 g for 15 min, the

resulting pellet was treated similarly with 5 mL acetone. The supernatants from both centrifugations were pooled and evaporated to dryness under a gentle stream of nitrogen. Dry extracts were solubilized in 1 mL hexane and further cleaned up by filtration through Florisil Waters microcartridges (Waters Associates, Inc. Milford, Masachusetts, USA). The PCBs were eluted by means of 4 mL hexane. The extract was then concentrated to 1 mL under a gentle stream of nitrogen. One microliter sample was injected in the splitless mode (gas holded time= 30 s; injector temperature = 260°C) using a Carlo Erba Fractovap 4130 capillary gas chromatograph (Carlo Erba, Milano, Italy) equipped with a Ni^{63} ECD (detector temperature = 275°C). Samples were analysed on a 25m x 0.25 mm I.D. glass capilalry coated with 0.15 µm of chemically immobilized SE-52. This analytical column was prepared according to Grob (1980) and was kindly provided by M.Termonia, IRC, Tervueren, Belgium. The column was temperature programmed from 60°C (initial hold = 1 min) to 240°C at 7.5°C/min with a hydrogen carrier gas flow rate of 1 mL/min and a Ar/CH_4 (90:10) make up gas flow rate of 30 mL/min. The peaks were identified by retention time and PCB amounts were expressed as Aroclor 1260.

RESULTS AND DISCUSSION

In a preliminary series of experiments, the quality and the accuracy of the contaminating experimental set-up have been tested. The results presented in Figure 2 show that PCB concentrations in the water of the "clean" tank B and in the absence of any fish specimen exponentially increased up to 0.5 µg/L after 120 h and then leveled off. According to Lafontaine and Delforge (1979), a concentration of 0.5 µg PCB/L of natural water corresponds to a high pollution level. These experimental data are in quite good agreement (χ^2 test = $P < 0.05$) with the theoretical curve resulting from estimations based on the system dynamics (entry and loss of pollutant at the 36 L/day flow rate) (Figure 2). It can therefore be concluded (1) that no PCB is lost in the flow-through system and (2) that the analytical method of PCB concentration determination is quite accurate despite numerous extraction and analysis steps.

In the presence of 30 fish (total wet weight = 41 g) in tank B, the change of PCB concentration in environmental water as a function of time (as shown in Figure 3) was quite similar to the curve shown in Figure 2. This emphasizes the efficiency of a flow-through system quite able to sustain a constant level of PCB contamination despite the presence of fish. The results shown in the lower curve of Figure 3 have been obtained after filtration of the PCB contaminated water on chitosan just before tank C feeding. In such conditions, the maximum concentration of PCB in water was 0.2 µg/L. PCBs leveled off at that value for more than 500 h. It appears to always remain much lower than the theoretical curve expected in the absence of chitosan filter. From this, it can be concluded that the adsorption of PCB by chitosan powder is efficient enough to retain up to 60% PCB in

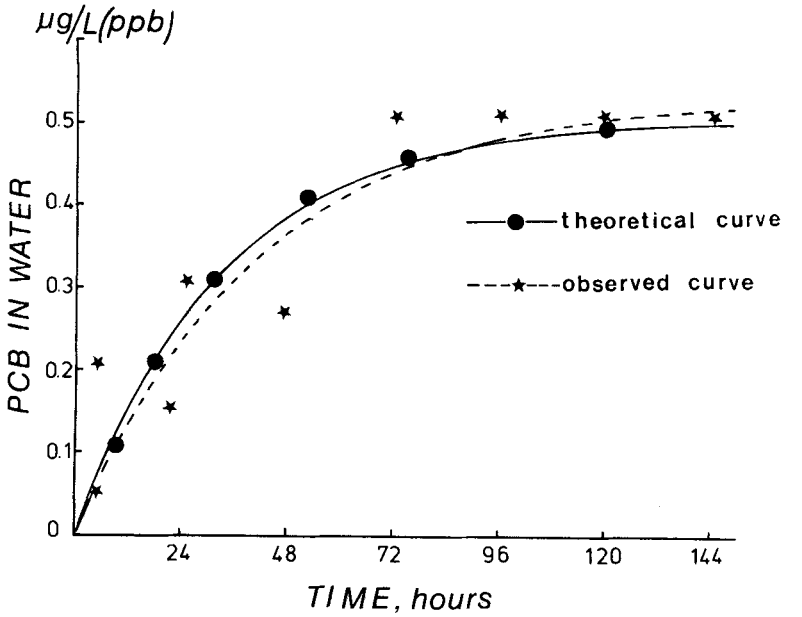

Figure 2. PCB concentrations in the water of the "clean" tank B in the absence of any fish(see details in the text).

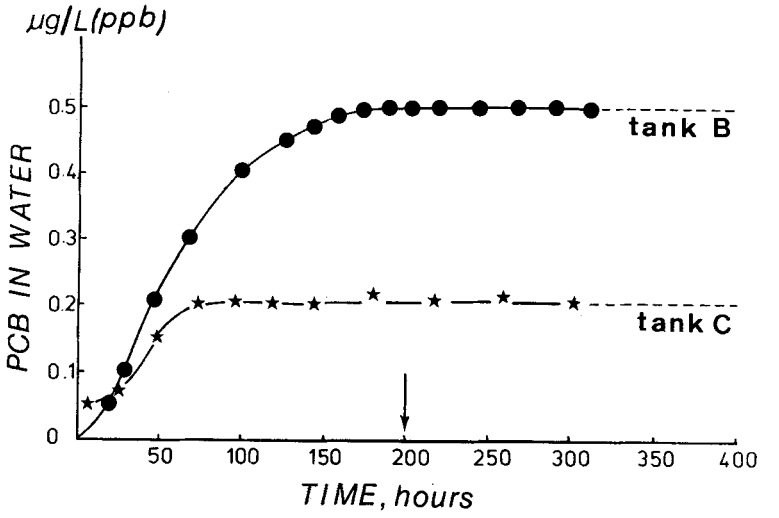

Figure 3. PCB concentrations in water of tank B(●) receiving contaminated water (0.5 ppb) and in water of the tank C(★) after filtration through chitosan. Filtration was performed through 1 g chitosan until 200 h experiment and then through 10 g (arrow).

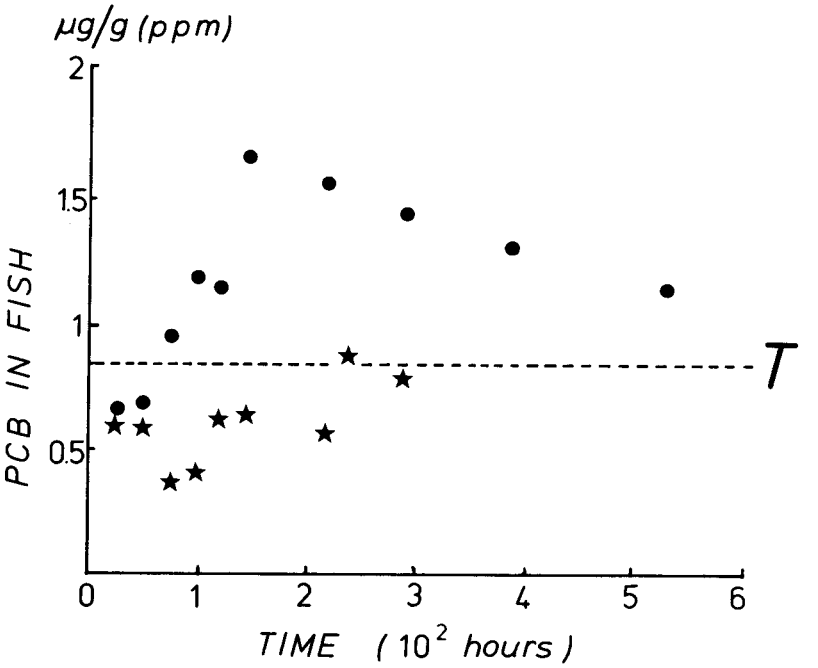

Figure 4. PCB concentrations in fish maintained in tank B (●) receiving contaminated water (0.5 ppb) and in tank C (★) after filtration of water through chitosan. T = level of contamination of control fish.

our experimental conditions. The data plotted in Figure 3 clearly demonstrate that filtration through larger amount of chitosan (up to 10 g) does not lead to a more efficient water purification.

Figure 4 shows that, in tank B, the amount of pollutant in fish linearly and immediately increased during 120 h of progressive contamination, reached a maximum mean value (1.6 µg/g wet weight) at time 120 h and then slowly resumed a lower level of about 1.0 µg PCB/g fish wet weight. Our results are in good agreement with the data reported by Poels et al. (1979) on rainbouw trout (*Salmo gairdneri*) maintained for 18 mo in Rhine river (Netherlands) water which "naturally " contains significant amounts of PCB. After filtration of such a contaminated water (at o.5 ppb level) through chitosan, fish contamination always remained lower than 1 µg/g, i.e., significantly lower than what has been found in tank B animals. In fact, these data do not significantly differ from control values obtained with "clean" fish.

These results are of significant interest when considered in the context of the work done by Poels and Strik (1975; see also Poels et al. 1979). These authors indeed reported serious

metabolic diseases when fish intoxication exceeded 1 µg/g (growth inhibition, liver and kidney volume increase, decrease of haemoglobin ratio in blood). Beneath the 1 µg/g threshold, these symptoms did not appear. In reasonable agreement with these observations, our results indeed suggest that filtration of PCB contaminated water through chitosan should be sufficient to "protect" efficiently fish against damaging intoxication.

These results suggest that the purification of PCB contaminated water using chitosan filter could find favorable application to environment preservation. Moreover, because of its low cost price due to natural distribution and easy production combined with its high level of efficiency, chitosan appears as a very competitive and powerful decontaminating agent the use of which might certainly be more widespread.

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