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Biological Stability of Toxaphene in Estuarine Sediment

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Toxaphene has until recently been the most widely used organochlorine insecticide in the United States (CASIDA et al. 1974). Recent evidence indicates that this compound is a carcinogen (HOOPER et al. 1979). The environmental fate of toxaphene is therefore of great interest. Although laboratory studies with aquatic organisms have shown a wide variety of effects due to toxaphene, including bioaccumulation (SCHIMMEL et al. 1977, SCHOETTGER & OLIVE 1961, SANBORN et al. 1976), field studies of incidents of toxaphene contamination of estuarine habitats show that no long term effects occur (KEISER et al. 1973, GINN & FISHER 1974, REIMOLD et al. 1973).

Recent evidence indicates that microorganisms in estuarine sediments may play a role in the removal of toxaphene from contaminated habitats (WILLIAMS & BIDLEMAN 1978, CLARK & MATSUMURA 1979). Other than the work of PARR & SMITH (1976) which showed a decrease in the amount of toxaphene present in soil incubated anaerobically and supplemented with alfalfa meal, however, little work has been done to quantitate the disappearance of toxaphene from the environment or to determine the toxicity of metabolic endproducts. The goals of the present research were to isolate from estuarine sediments microorganisms capable of metabolizing toxaphene, to quantitate the disappearance of toxaphene from laboratory cultures of the isolates, and to determine the toxicity of metabolic endproducts.

MATERIALS AND METHODS

Four 125-mL flasks containing 50 mL of SY medium (GRAY 1963) with 100 mg/L toxaphene were inoculated with a small amount of estuarine sediment collected at low tide from Neponset River Estuary, Neponset, Massachusetts. These flasks, along with four sterile control flasks of SY medium with toxaphene were incubated aerobically at 27C on a shaker bath at 100 oscillations per min. Anaerobic incubations were conducted in 10 mL of SY medium in 16X20 mm test tubes in anaerobe jars at 30C. At intervals of 0, 2, 4, and 8 days, 10 mL aliquots from one inoculated and one sterile control flask were analyzed for their toxaphene content using a semiquantitative thin-layer chromatographic technique. This method is a modification of that presented by ISMAIL & BONNER (1974), using commercially prepared aluminum oxide coated glass plates and spraying the plates with a 5% ethanolic solution of silver nitrate after developing. Exposure to an ultraviolet light for one hour caused toxaphene to appear as

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a single, concise, grayish-green spot. The amount of toxaphene present in the samples was estimated by comparison of the color intensity of spots from standard solutions of toxaphene to the color intensity of spots from samples. The accuracy of this method was tested in a blind experiment and found to be reliable to within 0.5 μg.

At the end of the incubation period, streak plates were made on SY medium containing 1.5% agar to isolate organisms present in the cultures. Two isolates were obtained which were used to inoculate twenty-one 125-mL flasks containing 10 mL of basal nutrient medium (GRAY 1963) with 100 mg/L toxaphene which were incubated aerobically as before. Twenty-one similar flasks were maintained as sterile controls. After 0, 1, 3, 6, 9, 12, and 21 days each of incubation, 3 inoculated and 3 sterile control flasks were selected at random and the toxaphene extracted with hexane and analyzed by thin-layer chromatography. This experiment was repeated and the toxaphene content of duplicate cultures and sterile controls measured after 3, 6, 9, 12, and 21 days. Extracts from 21-day-old cultures from the second experiment were also subjected to bioassay using 7 day old Artemia salina as the test organism. These assays were conducted for 24 h in 50mm X 30mm glass petri dishes in which 10 shrimp in each dish were exposed to artificial seawater (MICHAEL et al. 1956) containing residue dissolved in acetone from cultures and sterile control extracts. Five replications of each test were performed. Control dishes contained either artificial seawater only, extracts from 21-day sterile controls, or extracts from 21-day-old cultures of the isolates in basal nutrient medium containing no toxaphene. The latter was a control for toxic hexane-extractable medium components, cellular products, and acetone which was used as a carrier for toxaphene in all media and seawater. TLC data from both experiments were pooled for statistical analysis by two-way analysis of variance. Bioassay data were compared by Student's t-test.

The ability of the isolates to use toxaphene as a sole carbon source was tested by streak plating on toxaphene minimal medium (basal nutrient medium with no glucose, with 100 mg/L toxaphene added) and by measuring the oxygen consumption of the isolates in the presence of toxaphene using a Gilson differential respirometer. The effect of toxaphene on the oxygen consumption of estuarine sediment was also tested. Dilutions of marsh sediment were plated on toxaphene minimal medium to test for the presence of toxaphene utilizing organisms.

RESULTS AND DISCUSSION

Semiquantitative TLC analysis of the toxaphene in the preliminary cultures in SY medium showed no change in the sterile controls while the amount of toxaphene present in both aerobic and anaerobic cultures appeared to decrease with time. Two organisms, designated T1 and T2 were isolated from these cultures and characterized as belonging to the genus Vibrio. Since these preliminary results indicated that aerobic metabolism of toxaphene was as significant as anaerobic metabolism, all further studies were limited to

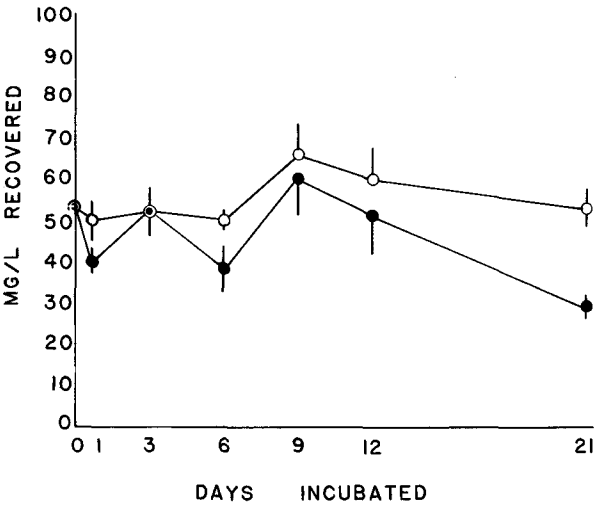

Fig. 1. Toxaphene recovered by hexane extraction and TLC analysis after incubation of cultures (●) and sterile controls (○).

aerobic conditions. This was done because of the convenience of working with aerobic systems and because at that time no aerobic metabolism of toxaphene had been reported.

Further experiments also showed a slight although statistically non-significant decrease in toxaphene content of cultures over time (Figure 1). A possible explanation for this decrease is that presented by PARIS and co-workers (1975), who observed rapid sorption of toxaphene to fungi, bacteria, and algae with distribution coefficients as high as 10^4. Differences in the amount of growth and therefore in the amount of surface area available for sorption may account for the observed results.

The lack of significance of the decreases in toxaphene content of cultures over time correlates with growth and respirometry results. No growth was observed on toxaphene minimal medium for either of the isolates alone, a mixed culture of the isolates, or dilutions of estuarine sediment. Toxaphene had no effect on oxygen consumption by mixed cultures, pure cultures, or sediment (Table 1). Bioassay results also show no significant change in the toxicity of toxaphene to A. salina after exposure to the action of microorganisms for 21 days (Figure 2).

Although no significant degradation of toxaphene was observed in the present research, results of other work (CLARK & MATSUMURA 1979, WILLIAMS & BIDLEMAN 1978) showed a change in the polarity with an increase in the water soluble fractions of the toxaphene mixture as a result of both microbial metabolism and physical factors. These results are not contradicted here since the TLC technique used in the present work does not separate toxaphene components and is specific for chlorinated compounds. The solvent system employed

TABLE 1. Oxygen consumption rates of the isolates and marsh sediment in the presence of various substrates. Values are expressed as μL oxygen consumed/mL/h (cells) or μL oxygen consumed/g. dry wt. sediment/h (sediment). Each value is the average of two determinations. 10 and 100 mg/L are concentrations of toxaphene to which sediment was exposed.

	Substrate			
Sample	Endogenous	Glucose	Toxaphene	Acetone
T1	0.5	11.4	2.0	4.4
T2	1.2	4.1	1.5	1.5
T1&T2	0.7	8.2	1.6	1.4
Sediment	2.4	---	2.3 (10 mg/L)	2.4
			2.8 (100 mg/L)	

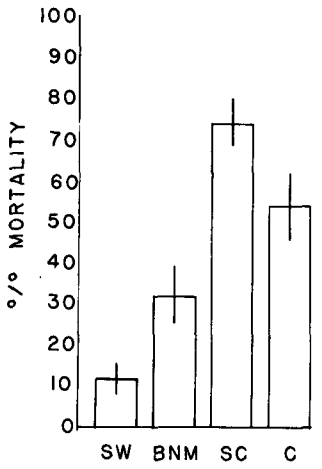

Fig. 2. Toxicity of culture and control extracts to Artemia salina after 21 days of incubation. BNM: basal nutrient medium. SW: seawater. SC: sterile control. C: culture.

may not be sensitive enough to detect small changes in polarity, as is evidenced by the fact that the TLC system cannot distinguish between the components of the toxaphene mixture itself. Therefore, although the total amount of toxaphene does not appear to diminish, dechlorination of some of the components of the toxaphene mixture cannot be ruled out on the basis of these results.

Microbial metabolism of a complex mixture such as toxaphene is hardly significant if the total amount of the compound is not reduced and its toxicity is not affected. The observed lack of significant long-term effects of toxaphene contamination on estuarine environments (KEISER et al. 1973, GINN & FISHER 1974, REIMOLD et al. 1973) can, however, be accounted for in the light of this and other research. The complete breakdown of toxaphene by microorganisms in marsh sediment or soil seems unlikely. A more likely mechanism for the removal of toxaphene from the environment is an increase in the water solubility of toxaphene due to microbial

action followed by leaching out of sediments as a result of tides and rainfall. The role of microorganisms in the removal of toxaphene from estuaries, therefore, seems to be to accelerate the leaching process which is known to occur for many insecticides (LICHTENSTEIN et al. 1966). Further research is neeeded to establish this role and determine its significance.

The results obtained in this work indicate that any microbial metabolism of toxaphene by the two isolates from estuarine sediment which may occur does not reduce the total amount of chlorinated hydrocarbon present nor does it reduce the toxicity of toxaphene to the brine shrimp, A. salina. Growth and respirometry data also show that no organisms capable of utilizing toxaphene as a carbon source were present in the samples taken. Work is now in progress to study further the interactions between estuarine microorganisms and toxaphene as well as other insecticides.

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