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Toxicity of Phenolic Compounds to Sediment Bacteria

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Biodegradation of organic compounds plays an important role in remediation of polluted environments. Several factors influence the rate and extent of biodegradation: number of degrading organisms, adequate supply of nutrients, adequate availability of a suitable electron acceptor. One important factor is the toxicity of the organic chemical itself. Very often chemicals may be susceptible to biodegradation at low concentrations, yet may be toxic to the degrading population at higher concentrations, thus inhibiting their own biodegradation.

Phenolic compounds are known to exhibit toxicity to bacteria. Under batch conditions, a strain of Pseudomonas could degrade 0.1% phenol in approximately 48 hr, while taking over 130 hr to degrade 0.15% phenol (Bettmann and Rehm 1984). A concentration of 0.2% phenol was inhibitory to the cells, killing 50% of the inoculum within 10 d. Activated sludge bacteria maintained in pulse fed batch reactors were inhibited by concentrations of phenol in excess of 50 mg/L (Autenrieth et al. 1991). Biodegradation of 4-chlorophenol by Alcaligenes sp. A 7-2 showed a similar concentration effect, being inhibited at concentrations above 160 mg/L in fed-batch culture (Balfanz and Rehm 1991).

The present report is part of an investigation on the biodegradation of phenol in contaminated soils and sediments in northwestern Indiana. Phenol has been found in groundwater aquifers which drain into the Grand Calumet watershed (Banaszak and Fenelon 1988), and in groundwater contaminated by a Superfund site (Dean-Ross 1989). Data on phenol toxicity were needed in order to assess the potential for in situ biodegradation of phenol and related compounds in sediments of the Grand Calumet River.

MATERIALS AND METHODS

Two sampling sites were selected on the Grand Calumet River, in Gary, Indiana, one at Bridge Street and the other at Kennedy Street (See Fig. 1). These sites were selected because a previous study had indicated the presence of phenolic contamination (Simmers et al 1991). For comparison purposes, two sediment

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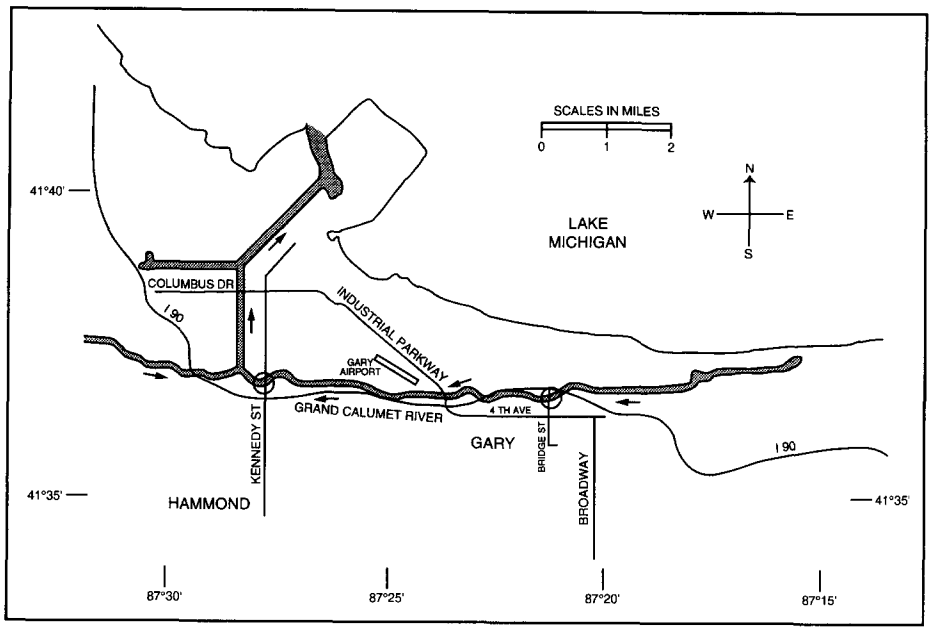

Figure 1. Map of northwestern Indiana showing sampling sites (circled).

samples were collected from Fort Wayne, Indiana; one site was on the St. Joseph River at the Indiana University-Purdue University at Fort Wayne campus, the other was from the Maumee River at its junction with Harvester Ditch. Sediment samples for bacteriological analysis were collected using aseptic techniques and stored in sterile bottles until return to the laboratory. The sediment samples were subdivided in the laboratory and a portion was taken for total phenol analysis performed by the 4-aminopyrine colorimetric method as described by Mort and Dean-Ross (1994).

The procedure of Liebert and Barkay (1988) was used to measure the number of bacteria tolerant to phenol and related compounds. In this procedure, sediment bacteria are exposed to nalidixic acid, an antibiotic which acts by inhibiting cell division. Cells continue to grow and may reach several times the usual length. These enlarged cells are counted under the microscope after acridine orange staining and represent the number of viable cells in a sample. As suggested by these authors, preliminary studies were conducted to optimize the conditions for direct counting of resistant cells. Various concentrations of yeast extract and nalidixic acid were tested in order to determine the optimum conditions for the assay. The procedure was standardized as described below.

Sediment suspensions were prepared by taking 5 g (wet weight) of sediment and adding it to 45 mL of sterile sodium pyrophosphate (0.1%) followed by incubation with shaking for 2 hr to detach cells from sediment particles. At the

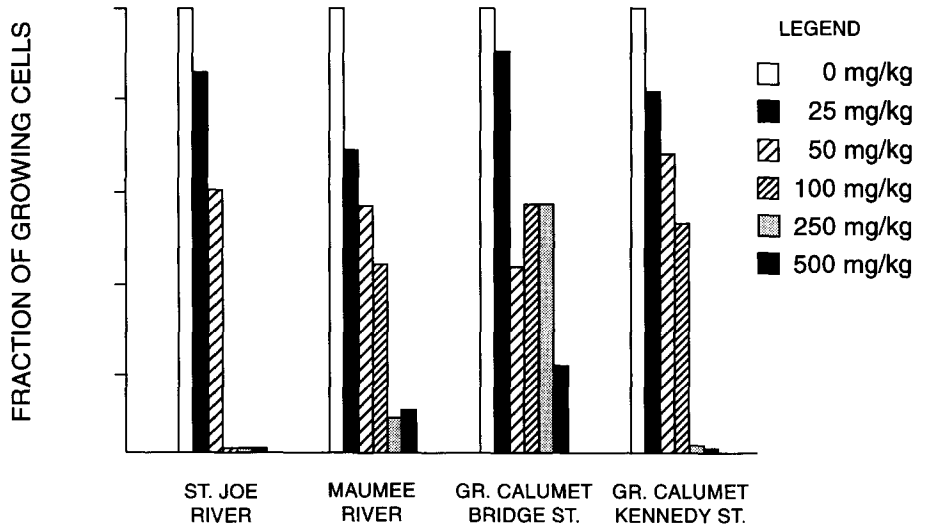

Figure 2. Response of the sediment bacterial communities to phenol.

end of this time, the sediment was further diluted by adding 5 mL of suspension to 45mL of a solution containing yeast extract (300 mg/L), nalidixic acid (150 mg/L) and amounts of phenol (Fisher Chemical, >99% purity), 4-methylphenol or 4-chlorophenol (both from Aldrich Chemical, >99% purity) to give final concentrations in the range of 25 to 500 mg/L. One dilution from each suspension was prepared with no added toxicant to serve as a control. After 24 hr incubation, aliquots of the suspension were stained with acridine orange, filtered and observed using epifluorescence microscopy according to the procedure of Dean-Ross (1988). Enlarged cells were counted. The fraction of phenol-resistant cells was determined by dividing the number of elongated cells at particular phenol concentrations by the number of elongated cells in the control flask receiving no added phenolic compound.

RESULTS AND DISCUSSION

The concentration of phenolics in these sediments was 0.81, 4.5, 9.6 and 7.3 mg/kg for the St. Joe River, Maumee River, Grand Calumet-Bridge St. and Grand Calumet Kennedy St. sites, respectively. Thus the site selected as a relatively nonpolluted site had the lowest level of total phenols of the four sites. The other three sites had approximately one order of magnitude greater concentration of total phenols, with the Grand Calumet-Bridge Street site demonstrating the highest level of total phenols.

Results of the toxicity determinations are illustrated in Fig. 2-4 for phenol, 4-methylphenol and 4-chlorophenol, respectively. Bacteria present in sediment from the St. Joe River showed the greatest sensitivity to phenol, exhibiting 40%

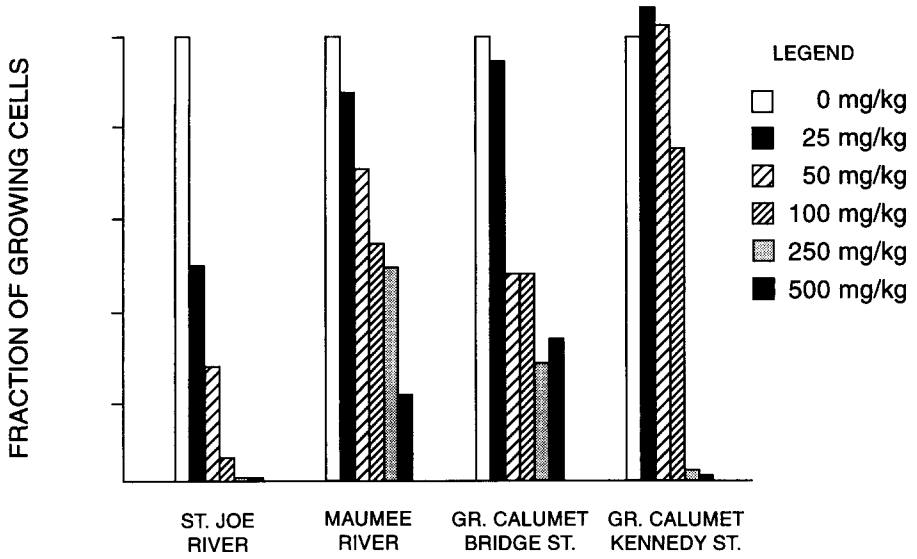

Figure 3. Response of the sediment bacterial communities to 4-methylphenol.

inhibition at 50 ppm and almost complete inhibition at concentrations of 100 ppm and above. The response of this bacterial community to 4-methylphenol and 4-chlorophenol was similar, except that concentrations of 25 and 50 ppm of these two phenolics were slightly more toxic than was phenol.

The bacterial community in sediments from the Grand Calumet River at Kennedy Street showed greater tolerance to the phenolics than did the St. Joe River sediment. The response of the sediment bacteria to 25 and 50 ppm phenol was similar to that of the St. Joe River response, but 100 ppm phenol only produced about 35% inhibition, instead of the almost complete inhibition shown by the St. Joe River bacteria. As with the St. Joe River sediment, concentrations of 250 and 500 ppm completely inhibited growth of the Grand Calumet River, Kennedy Street community. Response of the community to 4-chlorophenol was similar to that of phenol, except that 4-chlorophenol at a concentration of 100 ppm was substantially more toxic than was phenol at the same concentration. 4-Methylphenol was less toxic to the community at concentrations between 25 and 100 ppm, although at the higher concentrations it produced complete inhibition of growth as did the other two phenols.

Sediment bacteria from the Maumee River and Grand Calumet River, Bridge St. showed greater tolerance to the three phenolics in that concentrations of 250 and 500 ppm did not completely inhibit growth of the bacterial communities.

Differences in response of the two sediment communities were observed among the three phenols with respect to concentration. A concentration of 250 ppm

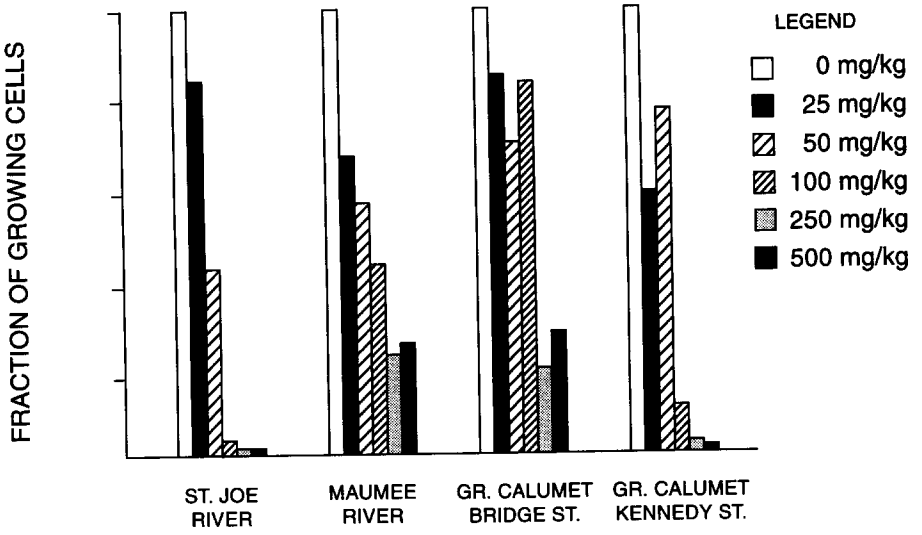

Figure 4. Response of the sediment bacterial communities to 4-chlorophenol.

phenol did not inhibit the Grand Calumet-Bridge St. community to the same extent as the Maumee River community. The response to 250 ppm of 4-methylphenol was reversed. With 4-chlorophenol, markedly less toxicity was observed in sediments from the Grand Calumet-Bridge St. site than the Maumee River site.

In conclusion, the site with the least exposure to phenol, the St. Joe River site, demonstrated the least tolerance to the phenolics. The remaining sites showed greater exposure and greater tolerance to the phenolic compounds, although the response to individual phenolics varied with concentration from site to site. Thus bacterial sediment communities show the capacity to adapt to ambient concentrations of pollutants such as phenol.

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