Mercury-Resistant Bacteria and Petroleum Degradation

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The concentration of mercury in water and sediment and in the oil extracted from water and sediment was determined for samples collected in Colgate Creek, located in Baltimore Harbor of the Chesapeake Bay. The concentration of mercury in the oil was 4,000 times higher than in sediment and 300,000 times higher than in water samples. The mercury-resistant bacterial populations of the samples studied have been shown to degrade oil, suggesting these bacteria to be a significant factor in the degradation of oil in Colgate Creek.

The full extent of the effects of petroleum on biological communities is not yet fully known (2), although the fact that petroleum persists in the natural environment is universally accepted (1). Recently, Matsumura et al. (7) reported that alkaline pH, organic content of sediments, and microorganisms affect release of mercury into water. Our own studies in the Chesapeake Bay have shown that greater populations of petroleum-utilizing and mercury-resistant bacteria occur in areas where high concentrations of petroleum and mercury are found (9, 14). We were aware that high concentrations of pesticides have been shown to be associated with oil slicks (10). The fortuitous discovery in our laboratory that the concentration of mercury in benzene-extractable material of Chesapeake Bay sediment was greater than in unextracted Chesapeake Bay sediment led us to examine the mercury and oil relationship with respect to microbial degradation of petroleum. One aspect of the research underway in our laboratory is the problem of petroleum degradation carried out by the autochthonous microbial populations of the natural environment (3, 14). Mobilization of mercury and the cycling of this heavy metal in the environment are also being investigated (8, 9). This paper reports the degradation of oil by mercury-resistant bacteria.

Methods for isolating, culturing, and identifying mercury-resistant bacteria from Colgate Creek in Baltimore Harbor have been described elsewhere (9, 14). The surface sediment and water samples examined in this study were collected in Colgate Creek by using a ponar grab and Niskin sampler, respectively. The concentration of mercury in sediment, water, and benzene-extracted water and sediment samples was measured by using flameless atomic absorption spectrophotometry, as described by Hatch and Ott (4), with modifications according to Nelson et al. (8). Additional surface sediment samples were collected from Baltimore Harbor by using a gravity corer, and these samples were extracted by using a Soxlet method, with hexane as the solvent. Preliminary experiments demonstrated that hexane and benzene extracted similar hydrocarbons, quantitatively and qualitatively. The hexane extracts were analyzed for lead, zinc, copper, nickel, and manganese, in addition to mercury, by atomic absorption spectrophotometry. Cadmium and copper content were measured by atomic absorption spectrophotometry, utilizing a graphite atomizer. Benzene extracts of the sediment samples were loaded into a 0.75-inch (3.39-cm) capillary tube closed at one end. Samples were tared prior to and after the analysis. Low-resolution mass spectrometry scanning to about m/e 500 was employed. A CEC 21-103C mass spectrometer was used for the analysis.

From the results shown in Table 1, the concentration of mercury in the extracted oil was 4,000 times that found in the sediment and 300,000 times that of the water column. Laboratory studies have demonstrated microbial conversion of mercuric chloride to methyl mercury (11) and to inorganic mercury (11–13), as well as conversion of phenylmercuric acetate to di-

| Sample                      | Mercury conc (ug/gm) |
|-----------------------------|----------------------|
| Sediment                    | 0.67                 |
| Benzene extract of sediment | 2,795.02             |
| Water                       | <0.01                |
| Benzene extract of water    | 2,960.56             |
phenyl mercury (6) and inorganic mercury (8). However, the nature of the mercury compounds associated with oil is not known and is presently under investigation in our laboratory and also as part of a cooperative study with the Inorganic Chemistry Section, National Bureau of Standards, Washington, D.C. Evidence obtained by mass spectrometric analysis of the extracted oil suggests that the oil is a complex mixture of hydrocarbons (Table 2). Measured concentrations of mercury probably represent the amount present in the oil in Colgate Creek rather than that which is preferentially extracted with benzene. The concentrations of mercury detected in benzene extracts of Colgate Creek sediment and water are significantly higher than the levels normally present in crude oil. Preliminary results indicate that the mercury is associated with specific organic complexes in the oil.

To determine whether mercury-resistant bacteria are capable of degrading petroleum, representative strains of several genera of bacteria were examined for ability to utilize petroleum (Table 3). Of the six representative strains examined, only an Enterobacter sp. (strain 85) was unable to utilize petroleum as a sole source of carbon. Utilization of petroleum by Pseudomonas spp. may prove important since most of the mercury-resistant bacteria isolated from the Chesapeake Bay have been identified as Pseudomonas spp. (9). In addition to screening mercury-resistant bacteria for petroleum-degrading ability, a number of petroleum-degrading bacteria were screened for mercury (HgCl) resistance. Since mercury resistance is dependent upon the strain, species, and genus of bacteria examined, a wide range of tolerances to mercury was expected. In fact, petroleum-degrading bacteria were resistant to concentrations of mercuric chloride ranging from 2 to 30 mg/liter with a Pseudomonas sp. being most resistant. The fact that these bacteria were resistant to such high concentrations of mercury is of ecological importance, since most bacteria require adaptation to mercury resistance. Since these petroleum-degrading bacteria were isolated from the mercury- and oil-rich water and sediment of Colgate Creek, the observed mercury resistance may be due to the continuous exposure to mercury concentrated in the oil found in this environment.

To determine whether a high concentration of heavy metals, including metals other than mercury, was associated with increased oil content in the sediments of Chesapeake Bay, sediment samples collected in Baltimore Harbor were examined for oil and heavy metal content (Fig. 1). The results obtained indicated that with an

### Table 2. Composition of benzene-extractable material from Colgate Creek sediment

| Compound type               | Percent |
|-----------------------------|---------|
| Paraffins                   | 20.0    |
| Monocycloparaffins          | 26.0    |
| Dicycloparaffins            | 8.5     |
| Tricycloparaffins           | 7.2     |
| Tetracycloparaffins         | 2.4     |
| Pentacycloparaffins         | 0.5     |
| Hexacycloparaffins          |         |
| Alkylbenzenes               | 28.8    |
| Benzylocycloparaffins       | 0.6     |
| Benzenecycloparaffins       | 0.2     |
| Alkylnaphthalenes           | 3.1     |
| Alkylacenaphthene           | 1.0     |
| Fluorenes                   | 1.0     |
| Phenanthrenes               | 0.5     |
| Cyclopentaphenalenenes      | 0.4     |

### Table 3. Mercury-resistant strains of bacteria isolated from samples collected in the Chesapeake Bay and tested for ability to degrade petroleum

| Genus                 | Strain no. | Maximum HgCl tolerance (mg/liter) | Petroleum utilization |
|-----------------------|------------|----------------------------------|-----------------------|
| Pseudomonas sp.       | 94         | 24-40                            | +                     |
| Arthrobacter sp.      | 72         | 4-5                              | +                     |
| Flavobacteria sp.     | 119        | 20-24                            | +                     |
| Vibrio sp.            | 639        | 12-16                            | +                     |
| Pseudomonas sp.       | 244        | 50-60                            | +                     |
| Pseudomonas sp.       | 127        | 20-24                            | +                     |
| Citrobacter sp.       | 132        | 40-50                            | +                     |
| Pseudomonas sp.       | 187        | 16-20                            | +                     |
| Enterobacter sp.      | 85         | 12-14                            | -                     |

* Data from Nelson et al. (8).
* Days.

**Fig. 1. Relationship of oil and heavy metal concentrations in sediment samples collected in Baltimore Harbor.**
increased oil content, i.e., ≥1%, an increase in zinc, chromium, lead, copper, nickel, cadmium, and mercury, but not manganese, can be expected.

In summary, we suggest that in a heavy metal-enriched environment, mercury and those heavy metals soluble in oil may concentrate in the oil and that the mercury-resistant microorganisms present in the mercury-rich, oil-laden samples are capable of utilizing oil. This phenomenon may prove important in the biological transfer of both mercury and oil in the food chain.

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