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Toxicity of Selected Arsenical Compounds in Short Term Bacterial Bioassays

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Use of bioassays to determine the toxicity of pollutants in the environment is an important component of tier testing. Most bioassays procedures utilize fish, invertebrates, algae or a combination of species from several trophic levels as indicators (LITTLE 1978). With the notable exception of bacterial mutagen tests (SLATER et al. 1971, AMES et al. 1975, GREEN et al. 1977) very few toxicity studies utilize bacteria as the indicator organism. However, as the first step in the food chain, the ecological importance of bacteria cannot be ignored. The purpose of this study, therefore, was to determine the toxic effect of selected arsenical compounds using a bacterial population as test species.

The few reports concerning bacterial toxicity testing include the response of marine bacteria to copper (VACCARO 1977) and oils (HODSON et al. 1977). DIETZ et al. (1976) studied several pollutants in flow through systems for their effect on numbers of heterotrophs, bacterial productivity, ATP and chlorophyll A concentrations. BRINGMANN & KUHN (1977) determined the toxicity threshold for 190 compounds based on growth rates of the bacteria Pseudomonas putida and the algae Scenedesmus quadricauda. A toxicity index, based on the ratio of algae and bacteria threshold values was compiled.

MATERIALS AND METHODS

The bacteria Citrobacter frundii, Aeromonas sp. and Klebsiella sp., isolated from soil and identified by the method of COWAN & STEEL (1970) served as the test organisms. These were grown in mixed culture in nutrient broth for 18 h prior to inoculation. The test media contained nutrient broth and one of three arsenicals: monosodium methanearsonate (MSMA), sodium arsenate or sodium arsenite. MSMA concentrations ranged from 10,000 µg/mL to 80,000 µg/mL in 10,000 µg/mL increments. Sodium arsenate and sodium arsenite were used in logarithmic series from 100 µg/mL to 100,000 µg/mL and from 10 µg/mL to 10,000 µg/mL, respectively. Test compounds were sterilized by filtration through a 0.45 µ filter and added aseptically to previously autoclaved nutrient broth. One mL of the 18 h bacterial culture was then added to 100 mL aliquots of the test media at

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each herbicide concentration. Test flasks were incubated aerobically in a shaker bath at 23°C for 24 h. Non-challenged control cultures were grown in similar manner. After incubation, numbers of viable cells were determined using the standard plate count technique (STANDARD METHODS 1975). All studies were done in duplicate.

RESULTS AND DISCUSSION

Results of this short term static bacterial bioassay are given in Figure 1. The LC_{50} for MSMA was 27,000 µg/mL after 24 h indicating good tolerance on the part of the bacteria to this herbicide. The LC_{50} for the two inorganic arsenicals, however, was considerably lower. Sodium arsenate produced an LC_{50} of 1300 µg/mL while sodium arsenite showed an LC_{50} of 270 µg/mL. The reduction in growth as compared to controls occurred proportionately across the three species tested as opposed to marked toxicity and reduced growth to one species alone.

The LC_{50} data show that the pentavalent organic arsenical is far less toxic than the inorganic forms tested, and that the trivalent inorganic form is more toxic than the pentavalent inorganic counterpart. This same pattern of toxicity has been described in growth inhibition testing with Lactobacillus leichmannii in a basal medium (LOY et al. 1961) and in toxicity testing of arsenate and arsenite using unidentified bacteria (WOOLSON 1975).

If the bioassay proceeds to 48 h and 96 h, (as in acute toxicity testing in animals) adaptation of the microorganisms may become manifest by the way of enzyme induction or transport of the compound. This may result in growth of the test culture in excess of controls indicating increasing tolerance to the test compounds with time. Figure 2 illustrates this phenomenon using MSMA. The LC_{50} data for 24, 48 and 96 h were 28,000 µg/mL, 60,000 µg/mL, 220,000 µg/mL respectively.

Figures 1 and 2 indicate that the short term bacterial bioassay could be a simple and effective screening technique to rapidly assess the toxicity of compounds to microorganisms. The method measures cell numbers by direct count rather than monitoring viability through oxygen uptake (VEITS 1978) or dehydrogenase activity (SIMON & DORE 1978). The 24 h incubation could be used to measure short term toxicity, while incubation to 48 or 96 h would give an indication of the adaptive capacity of the population to the chemical in question.

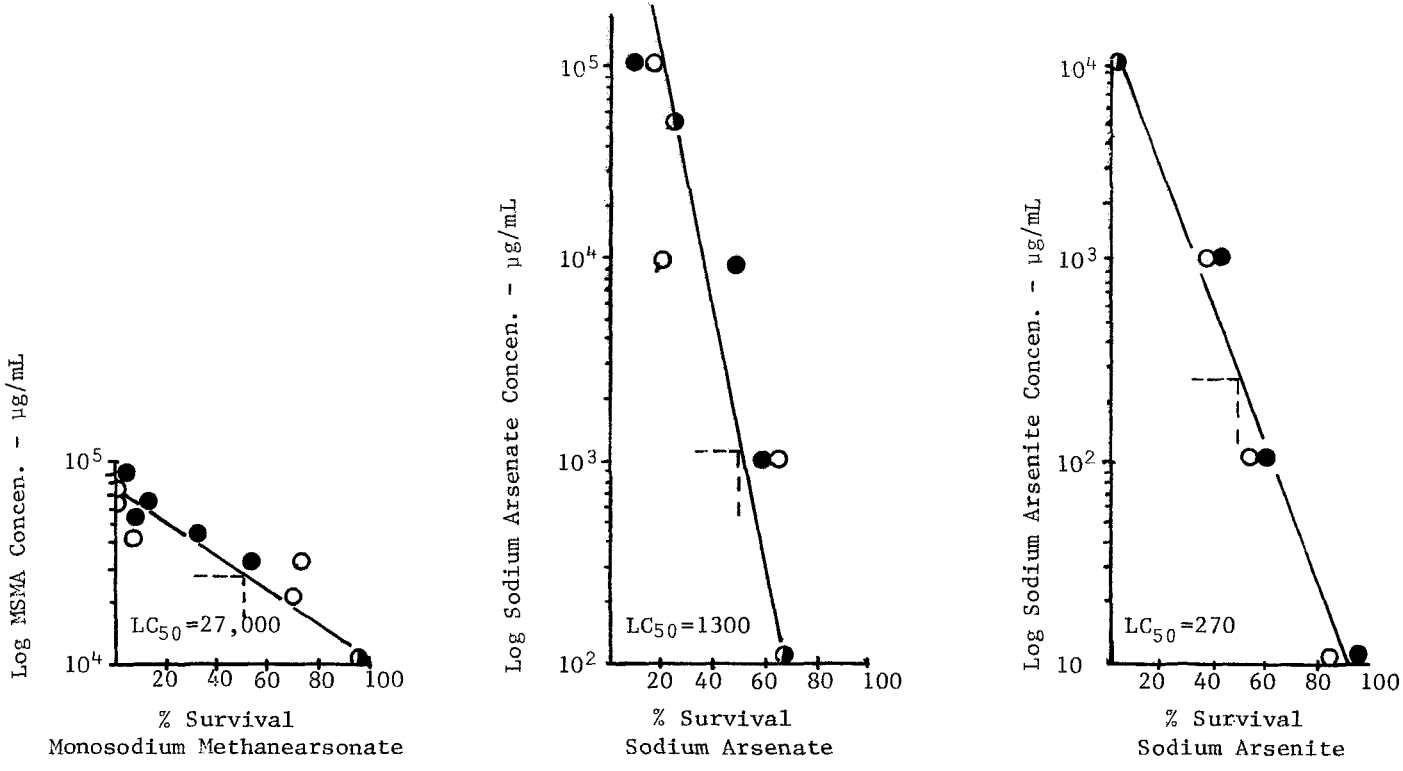

Figure 1. Toxicity of Selected Arsenical Herbicides to a Mixed Bacterial Population Following 24 hours of Exposure

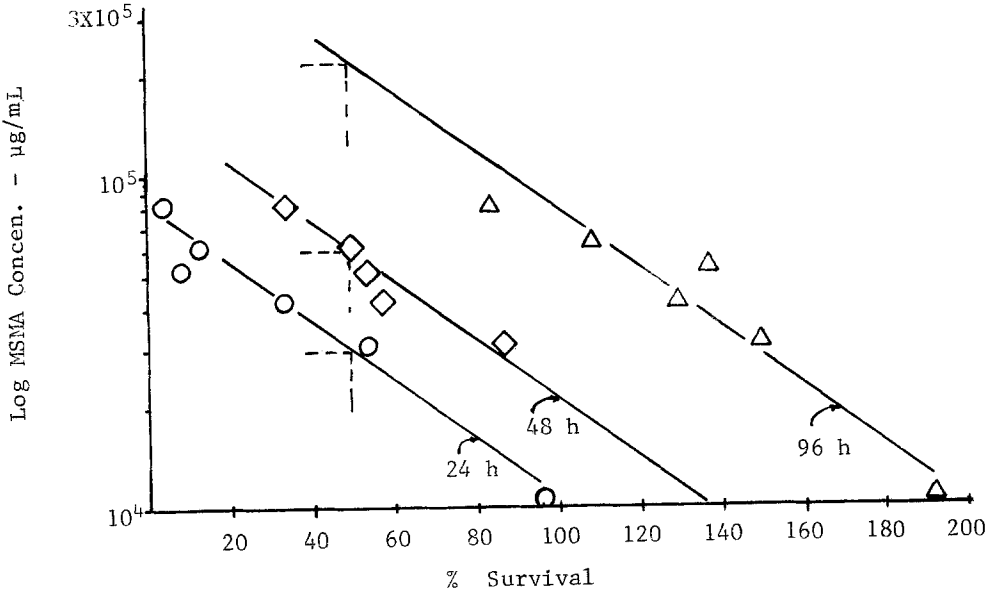

Figure 2. Response of a Bacterial Population to MSMA Over Time

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