United States
Environmental Protection
Agency

Environmental Research Laboratory
Corvallis OR 97333

Research and Development

EPA-600/S3-83-017 June 1983

EPA

Project Summary

Bacterial Bioassay for Level 1 Toxicity Assessment

Kenneth J. Williamson and Peter O. Nelson

Nitrifying bacteria were tested to determine their applicability as Level 1 bioassay organisms. Level 1 testing involves general bioassay and analysis procedures that will identify the presence of toxic material in a given waste stream.

The toxicity of five metals and three organic toxicants to the nitrifying bacteria *(Nitrobacter* and *Nitrosomonas)* were determined and compared to other common bioassay organisms. In general, the bacteria exhibited comparable sensitivity for toxicants with affected substrate metabolism, but lower sensitivity for specific toxicants such as pesticides that affect the activity of nerve cells.

The application of the bacterial bioassay was shown for two cases of Level 1 testing: a field study of a toxic industrial waste and its pretreatment, and an assessment study of the potential leachate problems for a flue-gas scrubber solid waste.

This Project Summary was developed by EPA's Environmental Research Laboratory, Corvallis, OR, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Bioassay tests are used to detect biologically harmful chemicals whose effects can be manifested as cellular, genetic, behavioral, or metabolic damage. The U.S. Environmental Protection Agency (EPA) has developed a three-phased bioassay approach to performing assessment of the toxicity of aqueous solutions (including solid waste leachates); this approach is divided into Level 1, 2, and 3 tests. Level 1 involves general bioassay analysis procedures that will identify the presence of toxicity in a given waste stream. Level 2 tests attempt to identify and quantify the specific compounds associated with the toxicity found in the Level 1 test. In Level 3 tests, more details concerning chronic health and ecological effects of the stream components are sought.

The focus in the Level 1 tests is a complementary series of bioassay tests for acute toxicity, mutagenicity, cytotoxicity, and soil microbiological inhibition. These tests provide no specific identification of the toxicant, but serve as signals for a wide range of potentially toxic responses.

The primary difficulty with the application of these tests is the complexity that results in high costs. The tests require highly trained personnel, modern laboratories, and long time periods. An attractive alternative to reduce costs is the use of lower level organisms, especially bacteria. Such a bacterial bioassay would be supplemental to the proposed Level 1 bioassay tests and other health and toxicity tests and, hopefully, correlative. The advantage of using bacteria as compared to the other Level 1 bioassay organisms would be greater simplicity, shorter testing times, and lower cost. Such a test could be accomplished within a few hours by chemical technicians and would involve minimal laboratory facilities.

Project

This research was conducted to determine the potential applicability of using mixed cultures of the autotrophic orga-

nisms *Nitrosomonas* and *Nitrobacter* to detect toxic compounds under Level 1 type testing. Toxicity was measured by reducing nitrogen oxidation. In addition, it was desired to know the relative sensitivity of these two bacterial genera to other bioassay organisms. Specifically, the objectives were to:

1. Determine the toxic concentrations of a wide range of known toxicants to *Nitrobacter* and *Nitrosomonas* and compare these concentrations to known toxic levels for other health and ecological bioassays presently used.
2. Demonstrate the use of nitrifying bacteria to estimate toxicity and the type of toxicant present in complex wastewaters.

The Test Procedure

Enriched cultures of *Nitrobacter* or *Nitrosomonas*—*Nitrobacter* were grown in a downflow column packed with polyethylene beads. Bacteria were removed from the column and freeze-dried. Rehydration was done with a solution of approximately 5 mg NO_2-N/l for *Nitrobacter* and 5 mg NH_4^+-N/l for *Nitrosomonas*. The bioassay procedure was:

1. Fifty ml of 15 mg/l of $NaNO_2$-N or $(NH_4)_2SO_4$-N was prepared with the selected concentrations of wastewater and was placed in a similar flask as the control.
2. An equal volume of *Nitrobacter* or *Nitrosomonas* suspension was placed in each flask and the flasks were shaken at a constant temperature for several hours.
3. One ml of each solution was taken out periodically and measured for NO_2^- or NH_4^+ spectrophotometrically.
4. At the end of the experiment, dry-weight of the biomass was determined by filtration of the solution and by drying at 105° for one hour.

NO_2^--N or NH_4^+-N concentrations versus time for each flask were plotted and the slope determined by a best squares fit. A comparison of the calculated slope of each line with the slope obtained for the control yielded the relative metabolism rate of the test solution. These rates are plotted versus concentration of the wastewater or toxicant; a reduced rate of metabolism confirms a toxic response from the wastewater sample.

Sensitivity of Nitrosomonas *and* Nitrobacter

The 50 percent and 90 percent relative metabolisms were extrapolated from dose response curves to estimate the range of maximum sensitivity (Figures 1 and 2). The ranges varied from about 10^1 to 10^2 μg/l for silver to 10^5 to 10^6 μg/l for zinc, and from 10^4 to 10^6 μg/l for the organics.

These values were compared to literature values for static bioassay using *Daphnia,* trout, fathead minnow, and algae. Qualitative comparisons of the bacterial bioassay are shown in Table 1. These comparisons show that the nitrifying bacteria exhibit a range of less to increased sensitivity for the metal toxicants, but less sensitivity to all the toxic organics.

Level 1 Type Testing

To demonstrate the applicability of the bacterial bioassay to Level 1 type testing, *Nitrobacter* were used to study the effect of pretreatment upon toxicity of a toxic industrial waste and the effect of various leachates upon the toxicity of a leachate from a flue-gas scrubber solid waste. In all three cases, the simplicity and low cost of the *Nitrobacter* bioassay allowed extensive monitoring of the effect of a wide range of variables upon the resulting toxicity that probably would not have been possible with alternative bioassay organisms.

Conclusions

Nitrifying bacteria can be used as Level 1 bioassay organisms to detect acute toxicity of aqueous solutions. The bacterial bioassay is simple, rapid, and low cost

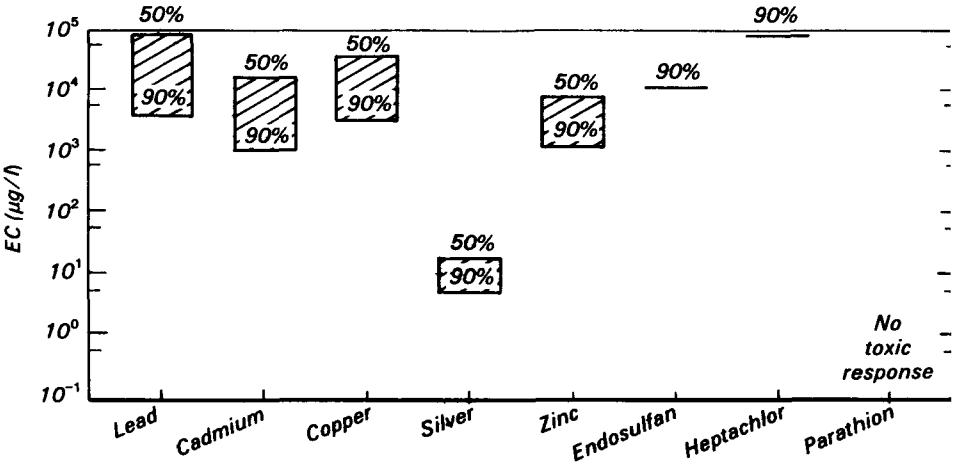

Figure 1. Summary of relative metabolism of Nitrobacter *for various toxicant levels.*

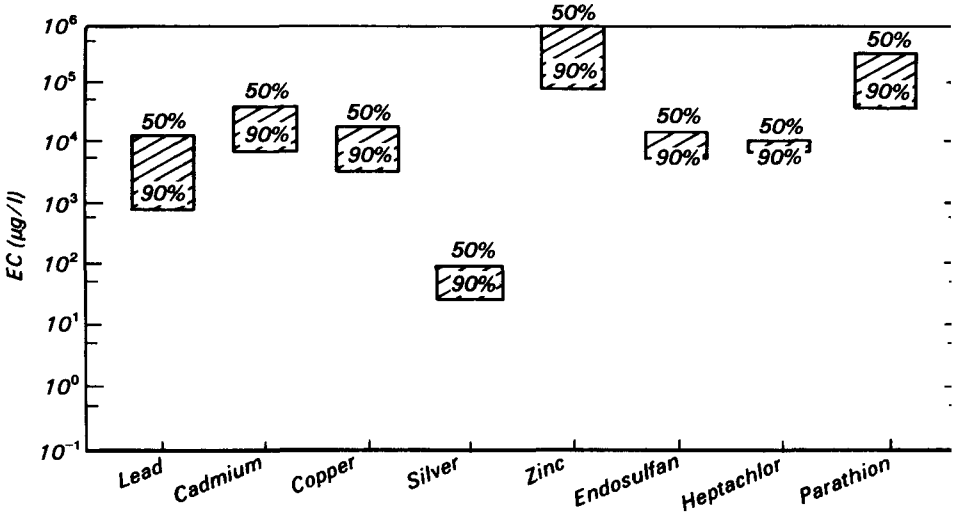

Figure 2. Summary of relative metabolism of Nitrosomonas *for various toxicant levels.*

Table 1. *Comparison of Sensitivity of* Nitrobacter *and* Nitrosomonas *to Other Bioassay Organisms**

	Bioassay Organism			
Toxicant	Daphnia	*Trout*	*Fathead Minnow*	*Algae*
Lead	*0*	+	+	*0*
Cadmium	-	-	*0*	-
Copper	-	-	-	*0*
Silver	+	+	+	+
Zinc	-	-	-	-
Endosulfan	-	-	-	*x*
Heptachlor	-	-	-	-
Parathion	-	-	-	-

**+, more sensitivity; 0, comparable sensitivity; -, less sensitivity; x, no data available.*

and could be standardized using freeze-dried organisms. It may be useful in cases where comparative toxic levels of a large number of samples are required, or for field application with limited equipment and personnel.

The bacterial bioassay with either *Nitrobacter* or *Nitrosomonas* exhibits approximately somewhat less sensitivities for general metabolic toxicants (such as heavy metals) compared to other common bioassay organisms, but exhibits dramatically lower sensitivity for specific toxicants (such as pesticides).

The bacterial bioassay can be used for Level 1 type testing as shown by its successes in studies to optimize pretreatment of toxic wastewaters, and to assess toxicity of solid waste leachates under various leaching conditions.

K. J. Williamson and P. O. Nelson are with Department of Civil Engineering, Oregon State University, Corvallis, OR 97331.
***David T. Tingey** is the EPA Project Officer (see below).*
The complete report, entitled "Bacterial Bioassay for Level 1 Toxicity Assessment," (Order No. PB 83-182 287; Cost: $11.50, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Environmental Research Laboratory
U.S. Environmental Protection Agency
Corvallis, OR 97330

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Postage and
Fees Paid
Environmental
Protection
Agency
EPA 335

Official Business
Penalty for Private Use $300