Bull. Environ. Contam. Toxicol. (1985) 34:75–79


Bulletin of Environmental Contamination and Toxicology

# Rapid Assessment of Effluent Toxicities Using *E. coli* as a Bioindicator

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Both chemical and biological assay techniques are required in order to assess the environmental impact of effluents and set quality criteria for them. Chemical techniques can only be used to determine concentrations of specific known toxicants so that it is possible to miss other toxic substances which could be present. Furthermore, chemical studies alone cannot be used to quantify synergistic or antagonistic effects which may result with mixed effluents. The use of bioindicator organisms is therefore essential to determine the effects of effluents in the environment.

Bacteria satisfy the criteria for indicator organisms (Phillips 1977). Their culture in the laboratory is comparatively simple and test results can be obtained rapidly using a minimum of sophisticated equipment. The use of mixed bacterial cultures as bioindicators has been described by Bauer et al. (1981) who measured biological oxygen demand (BOD) in a rapid bioassay method.

An alternative method involving an agar-diffusion technique to determine the relative toxicities of selected metal-rich effluents is described here. A pure culture of Escherischia coli (NTC 10418) was used in order to avoid anomalous results which can be obtained when mixed cultures are employed because of the different resistance levels of various bacterial species to individual toxicants.

## MATERIALS AND METHODS

Effluent toxicities were assessed using the agar diffusion method for the determination of the toxicities of metals (Thompson & Watling 1983; 1984). Stainless steel cylinders, porcelain beads and wells cut into the agar surface were tested as alternatives to the paper discs. All these methods gave smaller zones of inhibition of bacterial growth and less reproducible results and consequently the method using paper discs was used for the comparative tests.

In order to optimize the method for effluent testing, nine different media (Table 1) were prepared in duplicate tests in 100 ml aliquots

TABLE 1: Composition of test media

| Media No | Agar Make | % Agar | % Tryptone | % NaCl | % Other Nutrients |
|---|---|---|---|---|---|
| 1 | Oxoid | 1.5 | - | 0.5 | Peptone 0.5, Yeast extract 0.2, Lablemco 0.1 |
| 2 | Noble | 1.2 | - | - | - |
| 3 | Noble | 1.2 | 0.5 | 0.5 | - |
| 4 | Noble | 1.2 | 0.25 | 0.5 | - |
| 5 | Noble | 1.2 | 0.1 | 0.5 | - |
| 6 | Biolab* | 1.2 | - | - | - |
| 7 | Biolab* | 1.2 | 0.5 | 0.5 | - |
| 8 | Biolab* | 1.2 | 0.25 | 0.5 | - |
| 9 | Biolab* | 1.2 | 0.1 | 0.5 | - |

*High Purity

with either 0.5 ml or 1.0 ml of an overnight culture of E. coli added to 5 ml of 1% tryptone water prior to assay.

A 200 ml volume of effluent was evaporated to near dryness in a rotary evaporator. The evaporate was dissolved in a known volume of sterile distilled water and 100 µl of this solution were transferred to a paper disc which was then placed on an agar plate.

RESULTS AND DISCUSSION

Ten effluent samples were tested (Table 2) and these were also analysed for selected metals (Table 3). Zone sizes listed in Table 2 are measured across the outer edge of the zone of inhibition of bacterial growth and include the diameter of the paper disc.

Optimum results were obtained when 0.1% tryptone and 0.5% sodium chloride were added to Noble agar or Biolab High Purity agar (Table 1, media 5 and 9) and the inoculum was 0.5 ml of an overnight culture of E. coli in 5 ml of 1% tryptone water per 100 ml media.

With the exception of samples E3, E8, E9 and E10, which are predominantly mixed urban and industrial effluents, all other effluents tested produced zones of inhibition of bacterial growth. Zones of inhibition of bacterial growth were always produced by effluents which contained increased concentrations of toxic metals. (The relative degree of effluent preconcentration prior to testing must be taken into account when the results for the metal contents of individual effluents are compared with the respective zone sizes obtained under the test conditions).

Effluent E10 gave rise to a zone of increased bacterial growth around the disc. This effluent consisted of domestic sewage, paint factory, oil refinery and paper mill effluents, together with by-products from the sugar cane industry. It is possible that this bacterial enrichment has been caused by the mixing of the effluents

Table 2: Zone sizes for various test effluents

| Sample No | Conc. Factor | Source of Effluent | (Media) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 40 | Wood treatment | 45.0 | 55.4 | NT | 45.0 | 45.0 | NT | NT | 45.0 | 48.0 |
| 2 | 40 | Electroplating | 18.0MS | 31.4 | NT | 20.1 | 23.5 | NT | NT | 16.6 | 24.0 |
| 3 | 13 | Paper products | NZ | NZ | NT | NZ | NT | NZ | NZ | NZ | NZ |
| 4 | 40 | Motor manufacture | NZ | 15.8 | NT | NZ | 16.3 | 19.6 | NZ | NZ | 14.7MS |
| 5 | 20 | Battery manufacture recycled water | NZ | 31.5 | NT | 19.4 | 23.1 | 32.0 | NT | 19.3 | 24.2 |
| 6 | 20 | Battery manufacture effluent water | 30.0 | 57.5 | NT | 47.0 | 50.0 | NT | 38.5 | 43.4 | 55.4 |
| 7 | 10 | Tannery | 31.7 | 50.7 | NT | 36.6 | 38.2 | 49.0 | 36.9 | 37.0 | 40.2 |
| 8 | 40 | Sewage | NZ | NZ | NT | NZ | NZ | NZ | NZ | NZ | NZ |
| 9 | 10 | Paper mill | NZ | NZ | NZ | NZ | NZ | NZ | NZ | NZ | NZ |
| 10 | 10 | Sewage | NZ | NZ | NZ | NZ | NZ | NZ | NZ | NZ | NZ |

NZ = No zone NT = Not tested MS = Moderately sensitive

Table 3: Metal concentrations (μg/l) in test effluents

| Sample No | Cu | Pb | Zn | Fe | Mn | Cd | Cr | Ni | Co |
|---|---|---|---|---|---|---|---|---|---|
| E1 | 2100 | 400 | 380 | 700 | 100 | 200 | 1685000 | 700 | 6 |
| 2 | 800 | 100 | 630 | 600 | 200 | 70 | 6000 | 11700 | 4 |
| 3 | 1700 | 200 | 2000 | 4000 | 200 | 10 | 1000 | 200 | 3 |
| 4 | 200 | 200 | 2700 | 600 | 400 | 6 | 500 | 100 | 4 |
| 5 | 200 | 7800 | 1400 | 1100 | 200 | 6 | 400 | 100 | 4 |
| 6 | 200 | 845000 | 300 | 18000 | 100 | 10 | 700 | 100 | 6 |
| 7 | 300 | 200 | 2800 | 800 | 60 | 50 | 3720000 | 100 | 5 |
| 8 | 100 | 50 | 100 | 600 | 50 | 40 | 300 | 100 | 4 |
| 9 | 100 | 200 | 700 | 4500 | 700 | 6 | 200 | 50 | 2 |
| 10 | 300 | 600 | 1200 | 32000 | 2200 | 16 | 100 | 60 | 3 |

because the domestic sewage and paper mill effluents tested individually do not give rise to this phenomenon.

From the above study it is apparent that the main application of this test is in the quality control screening of metal-rich effluents over a prolonged period of time. Samples of effluent, taken at different periods, can be tested and the resulting zone sizes will indicate a relative increase or decrease in toxicity. If a quantitative result is required, then diffusion gradient curves can be produced for the specific toxic component of the effluent (Thompson & Watling 1984). The zone sizes can then be compared with the diffusion curve to give toxicant concentration in $\mu g\ l^{-1}$.

A further application of the test is in the determination of synergistic and antagonistic effects of combined effluents. To do this, zone size measurements are obtained from individual test effluents. The tests are then repeated placing discs of the test effluents adjacent to each other on a new test plate. The discs are placed in such a way that after incubation, should there be no interaction between the two effluents, the edges of the zones of inhibition of growth will just touch. Where there is interaction between the effluents the resultant effects will be readily observed as distortions of otherwise uniformly circular zones.

The use of a bacterial effluent testing system lends itself to rapid routine testing of metal containing effluents. Synergistic and antagonistic effects of effluent mixtures can be quickly estimated and the actual concentration of selected components quantified at each dilution stage by reference to the diffusion gradient graphs for that compound. In this way it is possible to maintain a close watch on variations in effluent composition and hence toxicity with a minimum of equipment and trained personnel.

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Received February 27, 1984; accepted March 18, 1984