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Effects of Environmental Toxicants on Metabolic Activity of Natural Microbial Communities

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Two methods of measuring microbial activity were used to study the effects of toxicants on natural microbial communities. The methods were compared for suitability for toxicity testing, sensitivity, and adaptability to field applications. This study included measurements of the incorporation of ^{14}C-labeled acetate into microbial lipids and microbial glucosidase activity. Activities were measured per unit biomass, determined as lipid phosphate. The effects of various organic and inorganic toxicants on various natural microbial communities were studied. Both methods were useful in detecting toxicity, and their comparative sensitivities varied with the system studied. In one system, the methods showed approximately the same sensitivities in testing the effects of metals, but the acetate incorporation method was more sensitive in detecting the toxicity of organic compounds. The incorporation method was used to study the effects of a point source of pollution on the microbiota of a receiving stream. Toxic doses were found to be two orders of magnitude higher in sediments than in water taken from the same site, indicating chelation or adsorption of the toxicant by the sediment. The microbiota taken from below a point source outfall was 2 to 100 times more resistant to the toxicants tested than was that taken from above the outfall. Downstream filtrates in most cases had an inhibitory effect on the natural microbiota taken from above the pollution source. The microbial methods were compared with commonly used bioassay methods, using higher organisms, and were found to be similar in ability to detect comparative toxicities of compounds, but were less sensitive than methods which use standard media because of the influences of environmental factors.

The addition of industrial, municipal, and agricultural pollutants to receiving waters has led to great interest in the effects of such activities on the life in those waters. Many bioassays have been developed in an attempt to quantify the toxic effects of an effluent (28). Most have focused on the use of eukaryotic test organisms such as phytoplankton (18, 21, 25, 29), zooplankton (2, 14, 39), benthic macroinvertebrates (9), and fish (2, 16).

Because of the importance of microbial activities in biogeochemical cycling and on other trophic levels, the effects of pollution on microbial life in receiving waters is possibly of even greater interest. Microbial communities respond to environmental perturbations in many ways. Some recent examples are the changes in cell numbers reported by Bewley and Campbell (5) and Lavoie (22) in response to heavy metals and carbide wastes. Orndorff and Colwell (32) found that cell numbers and heterotrophic uptake of labeled amino acids are affected by kepone, and others have shown that enzyme activity is affected by industrial effluents (35). Evolution of CO_2 by soil microorganisms is affected by plutonium (43), and the activity of decomposing communities is affected by acid (27) and petroleum hydrocarbons (26). Shifts in community structure occur after exposure to heavy metals (20), gasoline (19), as a result of predation (42; T. W. Federle, R. J. Livingston, D. A. Meeter, and D. C. White, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, I172, p. 168), and after manual cleaning of metal surfaces (31).

Cell numbers or growth rates have often been used to measure toxic effects (4, 33). These methods require relatively long exposure times which allow changes to occur within the microbial population or community being tested. Resistant organisms could be selected for in a population with a short generation time; thus, the effects on these selected organisms, rather than the effects on the indigenous organisms, would be tested.

Measurements of the metabolic activity of a microbial community give a more immediate indication of the effects of perturbations on the community as it is found in nature. Therefore,

methods measuring the indigenous activities of the microbiota should give a more realistic view of the reaction of a particular community to toxicants. Many methods of measuring microbial activity have been applied to the study of microbial response to toxic environmental conditions. Among these are measurements of mineralization rates (15), luminescence (8), nitrogen fixation (17), and heterotrophic potential (1).

The purpose of this study was to develop methods by which the effects of environmental toxicants on the microbiota of receiving streams could be measured. It was desired that methods be quick, easy to perform, and nonselective, and that they provide a close estimate of the activity of indigenous microorganisms. Measurements of acetate incorporation and glucosidase activity fulfilled these criteria. Acetate incorporation has been used to study the effects of oil (26) and acid (27) on a decomposer community. Measurements of glucosidase activity have been used to study microbial succession on decomposing litter in a semitropical estuary (30) and have been shown to correlate with ATP, respiration, and weight loss in a decomposer community (40).

MATERIALS AND METHODS

Study sites and sampling. Soil was collected from just below the litter layer in a forested area in Cincinnati, Ohio, on 2 April 1982. Compost was obtained from Paygro, Inc., a composter in South Charleston, Ohio, on 11 February 1982. Stream water and sediments were collected at: two locations on the industrially polluted Mill Creek near its confluence with the Ohio River in Cincinnati (sites 1 and 2); at a site approximately 6 miles upstream on the Mill Creek (site 3); and on the Ohio River near Anderson Ferry (site 4).

Studies on the effect of a point source of pollution on the receiving stream water and sediments were conducted at three different sites. At site 5, the clean water sample was taken from Gunpowder Creek just above its confluence with Utterback Creek in Florence, Ky. The impacted sample was taken from Utterback Creek, which flows into Gunpowder Creek and receives the effluent from both an asphalt production plant and a wastewater treatment plant. This site was sampled on 20 September 1982. Site 6 was a location on the Great Miami River in Dayton, Ohio, where the river is impacted by the effluent from a wastewater treatment plant that serves the city of Dayton. Samples were collected from above and below this outfall on 24 September 1982.

At site 7, samples were taken from above and below the outfall of the wastewater treatment plant on Utterback Creek in Florence, Ky. The downstream sample was taken above the outfall of the asphalt production plant, and therefore only the effect of the treatment plant was measured. This site was sampled on 5 November 1982 and again on 7 November 1982.

Microcosms were created from water and sediment collected from the Little Miami River near Cincinnati, Ohio, on 2 December 1982. Sediments were placed into 10- and 25-gallon aquaria, overlaid with water which was continuously aerated, and allowed to equilibrate for 13 days before use. Microcosms were maintained at 24°C in a darkened room to inhibit algal growth.

Sample preparation. Water samples were used directly or were filtered onto 0.45-μm Millipore filters. Sediment samples were taken with a KB corer (5-cm diameter) from a boat. Soil, compost, and sediment samples were mixed in L-salts medium (23) in an Oster blender for 30 s, were allowed to settle for 1 min, and then were reblended for 15 s. Activity, biomass, and dry weight samples were taken while the blend was being stirred. Sediments were extruded from the cores, and the top 2.0 to 2.5 cm was removed and blended with 200 ml of L-salts.

Activity measurements. (i) Acetate incorporation into microbial lipids. Microbial activity was measured as the incorporation of ^{14}C-labeled acetate into microbial lipids (26). A 1-ml volume of water or blend was added to 1 ml of toxicant solution in a 20-ml glass scintillation vial and incubated for 0.5 h at the appropriate temperature. A 100-μl volume of ^{14}C-labeled acetate (2.022 μCi/ml, 57.9 mCi/mmol) was added, and the mixture was incubated for 1 h. A 5-ml volume of methanol and 2.5 ml of chloroform were added to stop the reaction and extract the labeled lipids. The samples were extracted for at least 2 h, at which time 2.5 ml of chloroform and 2.5 ml of distilled deionized water were added to cause phase separation. After complete separation, the chloroform phase (bottom) was removed by aspiration, placed into a clean vial, and allowed to dry by evaporation. A 10-ml volume of scintillation cocktail [0.40% 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazolyl)benzene in toluene] was added, and the vials were counted in a Packard Tri-Carb scintillation counter and corrected for quenching by the channels-ratio method. Results were expressed as disintegrations per minute per micromole of lipid phosphate per hour.

(ii) Glucosidase activity. Glucosidase activity was measured by a modification of the method used by Morrison et al. (30). A 1-ml volume of the sample was incubated with 1 ml of toxicant solution in a glass test tube at the appropriate temperature for 0.5 h. A 2-ml volume of substrate solution (5 mM *p*-nitrophenyl β-D-glucopyranoside, 25 mM $NaHCO_3$ [pH 9.0]) was added, and the solution was incubated for 1 to 6 h until a yellow color adequate for measurement by spectrophotometry had developed. The reaction was stopped by putting the tubes on ice. A 2-ml volume of 50 mM $NaHCO_3$ (pH 9.0) was added to enhance the color, the solutions were filtered on 0.45-μm Millipore filters, and the filtrate was analyzed in a Bausch & Lomb Spectronic 20 spectrophotometer at 410 nm. Standards were prepared by dissolving *p*-nitrophenol in the reaction buffers, and a standard curve was constructed.

The procedure was modified during the study to avoid the task of filtering each tube. The reaction was carried out in 15-ml polypropylene screw-capped centrifuge tubes, and the addition of 50 mM $NaHCO_3$ was replaced by the addition of 2 ml of acetone. This allowed the solutions to be cleared by centrifugation rather than by filtration. New standards were prepared with acetone, and a new standard curve was constructed.

Biomass measurements. Lipids were extracted in glass separatory funnels by the method of Bligh and

Dyer (7). Phosphate analyses were performed on the digested lipids as described by White et al. (41).

Toxicity tests. Microbial communities were exposed to toxicants in four or five 10-fold concentrations ranging from 0.1 mg/liter to 1 g/liter. For each test, a positive control to which no toxicants were added and a killed control to which $HgCl_2$ was added to a concentration of 250 mg/liter were run. These controls and all treatments were carried out in triplicate. Preincubation with the toxicants was for 0.5 h, and the length of the subsequent incubation with the substrate depended on the method used and the activity of the sample. The activity values for the treatments were then expressed as a percentage of the control by subtracting the killed control value from all treatments and from the positive control and dividing all treatments by the positive control value. The percentages of control values were plotted on semilog paper, with percent on the arithmetic axis and toxicant concentration on the logarithmic axis. From these plots, a 50% effective concentration (EC_{50}) was graphically determined for each toxicity test (2).

Toxicants. The metals tested (Cd, Hg, Zn, Cr, Cu, and Ni) were administered as chloride salts, and concentrations were calculated as milligrams of metal ion per liter. Organic compounds tested included the surfactants sodium deoxycholate and sodium dodecyl sulfate, the herbicide diquat, sodium pentachlorophenate, and phenol. Phenol was administered in microliters per liter.

Point source studies. Point sources of pollution were studied in two ways, using the acetate incorporation method. The sensitivity to toxicants of communities from above and below the outfall were compared by exposing them to Cd and diquat or Cd and sodium pentachlorophenate, and EC_{50} values were calculated as described above. The sensitivities of upstream communities to the conditions found downstream were measured by exposing 1 ml of upstream samples to 1 or 0.5 ml of a water or sediment filtrate taken from below the outfall. These results were expressed as a percentage of the control.

Reagents. *P*-Nitrophenyl β-D-glucopyranoside and *p*-nitrophenol were purchased from Sigma Chemical Co.; ^{14}C-labeled acetate was from Amersham Corp.; and β-glucosidase was purchased from Calbiochem. Diquat and sodium pentachlorophenate were supplied by Cornelius Weber of the U.S. Environmental Protection Agency, Cincinnati, Ohio. Other chemicals were reagent grade and were purchased from Fisher Scientific Co. All chloroform used in this study was redistilled before use. Distilled deionized water was obtained by passing distilled water through a Barnstead Ultrapure demineralizing column.

RESULTS

Development of methods. Toxicity tests consisted of a 0.5-h preincubation of the sample with the toxicant solutions, followed by incubation with the test substrate. A 0.5-h preincubation was chosen because maximum toxic effect of cadmium and diquat was demonstrated within 15 min by the glucosidase assay, but variation among replicates was much reduced after 30 min. Similar results were obtained when the same experiment was run with the acetate incorporation method. The length of incubation with the substrate varied depending on the method used and the activity of the sample.

Acetate incorporation into lipids. The amount of radioactivity incorporated into sediment lipids increased linearly with time during the 1-h incubation period. Replication for this method was approximately 3%, and sensitivity was 2.08 mmol of acetate per nmol of lipid phosphate per hour.

Glucosidase activity. Standard curves for the production of *p*-nitrophenol were constructed by dissolving *p*-nitrophenol into the reaction buffers used in the assay. A separate standard curve was produced for the original method in which sodium bicarbonate was added and for the modified method in which acetone was used, and the curves were linear from 0.55 to 30 nmol of *p*-nitrophenol per ml. Production of *p*-nitrophenol progressed linearly over the course of a 4.5-h incubation. Replication was about 5.4%, and sensitivity was 0.12 nmol of *p*-nitrophenol per mg (dry weight). The addition of acetone allowed tubes to be cleared by centrifugation rather than by filtration and also was shown to be effective in stopping the reaction. Both the substrate and the product of the glucosidase assay were tested for their toxicity to microbes, using the microbial activity assay. Neither was found to be toxic at the concentrations encountered in the assay procedure.

Toxicity tests of natural communities. Ionic interactions have been shown to affect the toxicity of metals (13), and it was of some concern that the mineral salts solution in which sediment, soil, and compost samples were blended might affect the observed toxicities of metals. Sediment samples were blended in L-salts (23) and in distilled deionized water, and their response to cadmium toxicity was tested by using the glucosidase activity method. There was no difference in the response of communities blended in a completely defined mineral salts medium (L-salts) from that of those blended in distilled deionized water.

The effects of various toxicants on various natural microbial communities were tested by using the acetate incorporation and glucosidase activity methods (Table 1). In every case, glucosidase activity appeared to be the more sensitive indicator of toxicity, but this did not hold true in later experiments. Cadmium appeared to be much less toxic to sediment communities than to water communities taken from the same site (sites 1 and 3).

A wide range of toxicants was tested on the sediment communities of the Little Miami River microcosms, using the acetate incorporation and glucosidase activity methods (Table 2). The two

TABLE 1. Effect of toxicants on natural microbial communities, using the acetate incorporation and glucosidase assays

Environment	Toxicant	EC_{50} (mg/liter)	
		Acetate incorporation	Glucosidase activity
Soil	Hg	ND[a]	38.1
Compost	Hg	15.2	0.2
	Zn	3.3	ND
Stream water (site 1)	Cd	1.9	0.6
	Diquat	0.3	0.001
Stream sediment (site 1)	Cd	100	40
	Diquat	0.18	0.07
Stream sediment (site 2)	Cd	100	100
	Diquat	0.64	0.29
Stream water (site 3)	Cd	16	1.65
Stream sediment (site 3)	Cd	49	3.8
River sediment (site 4)	Cd	18	ND

[a] ND, Not determined.

detergents tested, sodium deoxycholate and sodium dodecyl sulfate, showed no toxic effects with either method. The EC_{50} values for metals were in the same order of magnitude regardless of which method was used. For the organics sodium pentachlorophenate and phenol, the glucosidase method showed no effect, whereas the acetate incorporation method indicated significant inhibition of the community.

The effects of two metals and two organic compounds on the activity of pure glucosidase enzyme were then tested, and the organics sodium pentachlorophenate and phenol had no effect on the enzyme (Table 3). Mercury directly inhibited the pure enzyme as it had the community, but copper had no effect on the pure enzyme, although it had inhibited the activity of the microcosm community.

Based on the microcosm studies, the metals tested were ranked as to their relative toxicities. These rankings are shown in Table 4 and are compared with the rankings obtained by other commonly used bioassay methods.

The effects of a point source of pollution on the microbiota of the receiving stream were studied by comparing the sensitivity to toxicants of water and sediment organisms taken from above the point source to those of organisms taken from below. Table 5 shows the effects of cadmium and diquat or cadmium and sodium pentachlorophenate on these communities. EC_{50} values were in most cases 2 to 100 times higher for communities taken from below the outfall than for those taken from above.

Organisms from above and below the outfall at site 7 were also tested for their sensitivity to cadmium by plating them on nutrient agar that contained 0 and 10 mg of cadmium per liter. The ratio of cadmium-resistant to total CFU was 0.0267 for upstream organisms and 0.163 for downstream organisms.

The effects of the actual point source effluent on the microbiota were studied by exposing water or sediment communities from above the outfall to water or sediment filtrates from below. Table 6 shows responses ranging from 96% inhibition of activity to 20% stimulation.

TABLE 2. Effects of toxicants on microcosm sediment communities, using the acetate incorporation and glucosidase activity assays

Toxicant[a]	EC_{50} (mg/liter)	
	Acetate incorporation	Glucosidase activity
Cd	>100	100
Hg	23	26
Cr	91	100
Cu	6	10
Zn	60	100
Ni	68	60
SDOC	>1,000	>1,000
SDS	>1,000	>1,000
PCP	2	>100
Phenol	245[b]	>10,000[b]

[a] SDOC, Sodium deoxycholate; SDS, sodium dodecyl sulfate; PCP, sodium pentachlorophenate.
[b] Microliters per liter.

DISCUSSION

The observed toxicity of a compound is dependent on many environmental factors. Many toxicants can be chelated by clay particles and organic compounds (3), thereby reducing their toxicity. The effect of sediments on cadmium and diquat toxicity were demonstrated in this study by the higher EC_{50} values observed in

TABLE 3. Effect of toxicants on activity of pure glucosidase enzyme and on glucosidase activity of a natural community

Toxicant[a]	EC_{50} (mg/liter)	
	Pure enzyme	Microcosm community
Hg	1.22	26
Cu	>100	10
PCP	>100	>100
Phenol	>10,000[b]	>10,000[b]

[a] PCP, Sodium pentachlorophenate.
[b] Microliters per liter.

TABLE 4. Comparative toxicities of tested compounds[a] measured by various bioassay methods[b]

Relative toxicity	Compound in following assay:					
	Microbial activity (EC_{50})	Glucosidase activity (EC_{50})	*S. capricornutum*[c] (96-h LD_{50})	*D. magna*[d] (48-h LD_{50})	*P. promelas*[e] (48-h LD_{50})	Rat[f] (oral LD_{50})
Least	SDOC, SDS, Cd					Cr
		Phenol, SDOC, SDS, PCP				SDOC
					Phenol	SDS
	Phenol		Cu	Cr	Cr	Phenol
	Cr	Cd, Cr, Zn	Cr	Ni	Ni	Zn
	Ni		Zn	Zn	Cd	PCP
	Zn	Ni	Ni	Cd	Zn	Cu
	Hg	Hg	Cd	Cu	Cu	Ni
	Cu	Cu		Hg		Cd
Most	PCP					Hg

[a] SDOC, Sodium deoxycholate; SDS, sodium dodecyl sulfate; PCP, sodium pentachlorophenate.
[b] LD_{50}, 50% lethal dose.
[c] See reference 10.
[d] See reference 6.
[e] See reference 34.
[f] See reference 24.

sediment samples than in water samples taken from the same sites (Tables 1 and 5). Cadmium and diquat have both been shown to bind strongly to humic material (37).

The sensitivity of a method to a wide variety of toxicants is important for its use in environmental studies. For many microbial communities, glucosidase activity was a more sensitive indicator of toxicity than was acetate incorporation (Table 1). In studies of the microcosm community, however, EC_{50} values for metals were in the same order of magnitude regardless of which of the two methods was used, and the acetate incorporation method was much more sensitive to the effects of organic toxicants than was the glucosidase activity method (Table 2). It was thought that these data indicated a direct effect of the metal ions on enzyme activity, as metal ions can inhibit enzyme activity by replacing an ion normally found at the active site or by binding at a site other than the active site, thereby disrupting the structure of the active site (12).

Mercury inhibited activity of pure glucosidase enzyme but copper had no inhibitory effect (Table 3). However, inhibition of metabolism by copper was detected by the glucosidase activity method. This does not explain the lack of sensitivity to the effects of organic toxicants but does indicate that for metals that do not directly interfere with enzyme activity, the effect on metabolism can be measured.

The variation in response to organic toxicants by the two methods suggests that more than one test may be necessary to thoroughly evaluate toxic effects. A study by Dutka and Kwan (11) compared the results of four different methods of detecting toxicity to bacteria and also found that each method had its own sensitivity pattern to toxicants. They proposed that a battery of three or four different tests would provide the best estimates of toxicity.

The toxicants that we tested with the microcosm community were ranked according to their toxicity, and these rankings were compared with those obtained with other methods of toxicity testing (Table 4). The relative toxicities of the compounds compared very well whether the acetate incorporation or the glucosidase activity method was used. The microbial assays compared well with results obtained for the *Daphnia magna* (cladoceran) (6) and *Pimephales promelas* (fathead minnow) (34) tests and compared in

TABLE 5. Effects of toxicants on natural microbial communities taken from above and below various sewage outfalls, measured as acetate incorporation

Environment	Toxicant	EC_{50} (mg/liter)	
		Above outfall	Below outfall
Stream water (site 5)	Cd	0.49	1.85
	Diquat	0.00001	0.06
River water (site 6)	Cd	0.39	0.66
	Diquat	0.002	0.005
River sediment (site 6)	Cd	8.9	34.0
	Diquat	0.05	0.095
Stream sediment (site 7)	Cd	52	50
	PCP[a]	0.66	10.2
Stream sediment (site 7)	Cd	14	45
	PCP	0.14	7.8

[a] PCP, Sodium pentachlorophenate.

TABLE 6. Acetate incorporation of natural aquatic communtities taken from above a sewage outfall after incubation with filtrates taken from below the pollution source

Site	Environment	ml of filtrate added	% control ± SD
5	Water	1.0	3.6 ± 0.5
		0.5	9.4 ± 1.7
	Sediment	1.0	120.4 ± 11.3
		0.5	121.3 ± 12.7
6	Water	1.0	59.7 ± 11.4
		0.5	64.4 ± 9.3
	Sediment	1.0	10.7 ± 1.8
		0.5	20.7 ± 2.9
7	Sediment	1.0	20.0 ± 9.8
		0.5	34.0 ± 16.7
	Sediment	1.0	60.9 ± 29.2
		0.5	67.5 ± 32.4

a general way with results obtained from rat tests (24), but rankings were not similar to that obtained for the *Selenastrum capricornutum* (green alga) test (10). Tests of rank correlation were attempted by using both the Kendall tau and Spearman rank correlation coefficient (36) analyses, but small sample sizes made them inconclusive.

General trends across all organisms tested were seen in the extreme toxicity of copper and mercury and the relative lack of effect of chromium. Organic compounds were generally less toxic than metals. An obvious discrepancy is seen in the data from the *S. capricornutum* tests. This test was done after the standardized algal assay bottle test (38), in which 300 μg of EDTA per liter is added to culture media. The investigators also conducted the same toxicity tests in media lacking EDTA and found that copper was the most toxic metal and cadmium was one of the least, more closely agreeing with the results from other organisms. Differential chelation of metals by EDTA apparently caused differences in the toxicities measured by this method.

The microbial activity tests showed the same responses to toxicity as did other methods, but they proved to be much less sensitive than others. *S. capricornutum* (10) and *D. magna* (6) tests were from two to five orders of magnitude more sensitive to toxicants than were the microbial activity methods. Tests with the fathead minnow (34) were from two to three orders of magnitude more sensitive. This is easily explained by the fact that our microbial tests were run under natural conditions, whereas the others were run under optimal conditions in standard media. Chelation and adsorption of toxicants are reduced to a minimum. These other tests measure the potential toxicity of an effluent; the microbial tests measure the actual toxicity under conditions similar to those found naturally, and, therefore, the sensitivities of the tests cannot really be compared.

Point sources of pollution. With the acetate incorporation method, toxic compounds were more inhibitory to microbial communities taken from above a sewage treatment plant outfall than to those taken from below (Table 5). There are two possible explanations for this. Much organic material is added to the stream with the treatment plant effluent, and this could cause chelation of the toxicants. Also, the organisms below the plant could be more resistant to perturbation due to selection since they are more continuously exposed to more toxic compounds than are those from above. This could be especially true of the sediment community since it is less mobile than the water column community. Although chelation of toxicants at the downstream site is sure to be involved, there is also some evidence in favor of the sensitivity theory. When organisms from above and below an outfall were plated on media with and without 10 mg of cadmium per liter, the ratio of cadmium-resistant organisms to total organisms was one order of magnitude higher in samples from below the outfall than in samples from above.

The actual effect of a point source of pollution on the microbiota of the receiving stream was demonstrated by the exposure of organisms taken from above the outfall to filtrates taken from below (Table 6). This method could detect both inhibition and stimulation of the microbiota by the effluent. The testing was done under conditions close to those found in nature, and, therefore, the environmental factors influencing toxicity at that site are present. Synergistic and antagonistic interactions among toxicants in the effluent are thus accounted for.

The acetate incorporation and glucosidase activity methods have been shown to be useful in evaluating the response of natural microbial communities to toxic stress. They react to toxicants in much the same way as do other methods, but are not directly comparable to these methods since toxicity is measured under nearly natural conditions and the sensitivity is decreased. Therefore, the use of these microbial methods must differ from that of the other methods. These methods are best suited to studies of the effect of a particular toxicant or effluent on a particular microbial community. Many of the environmental influences that complicate the interpretation of tests run under standard conditions are incorporated into the microbial methods, and accurate predictions of an effluent's effects in the stream to which it is being added are possible. Extrapolation of the results of these tests can be made to higher

organisms because these tests react similarly to those which use higher organisms and because the health of the ecosystem as a whole depends on the health of the microbiota.

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