APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1979, p. 922-927
0099-2240/79/11-0922/06$02.00/0
Vol. 38, No. 5

Alkaline Phosphatase Assay for Freshwater Sediments: Application to Perturbed Sediment Systems

GARY S. SAYLER,* MARLA PUZISS, AND MARTIN SILVER

Department of Microbiology and The Graduate Program in Ecology, The University of Tennessee, Knoxville, Tennessee 37916

Received for publication 5 September 1979

The *p*-nitrophenyl phosphate hydrolysis-phosphatase assay was modified for use in freshwater sediment. Laboratory studies indicated that the recovery of purified alkaline phosphatase activity was 100% efficient in sterile freshwater sediments when optimized incubation and sonication conditions were used. Field studies of diverse freshwater sediments demonstrated the potential use of this assay for determining stream perturbation. Significant correlations between phosphatase and total viable cell counts, as well as adenosine triphosphate biomass, suggested that alkaline phosphatase activity has utility as an indicator of microbial population density and biomass in freshwater sediments.

In the course of investigations of the microbial ecology of chemically contaminated freshwater sediments, it was desirable that an estimation of sediment phosphatase activity be made, to more fully circumscribe microbial processes in diverse sediment ecosystems. Phosphatases are a group of orthophosphorus mono- and diester phosphohydrolases, enzymes capable of cleaving orthophosphate groups from organic phosphate compounds. These enzymes have been characterized as alkaline, neutral, or acid phosphatases, based on the optimum pH for the observed hydrolytic cleavage of the esterified phosphate group. In most cases these enzymes are rather unspecific with respect to the organic phosphate substrate as well as to the pH at which the hydrolysis occurs (8, 17). Although these enzymes act as extracellular enzymes, they are associated with specific membrane-cell wall components and are localized, in gram-negative bacterial cells, in the periplasmic space (4) or on the cell wall (6). Free phosphatase results from the natural loss of phosphatase from the periplasmic region and environmental stress such as cation or osmotic shock.

The presence and activity of phosphatases in natural systems relates directly to phosphorus cycling and its availability to other organisms such as algae and macrophytes. Phosphatase activity in natural systems is modulated by mineral forms of phosphorus as well, as specific inhibitors or repressors of phosphatase production and activity (7, 18, 19). The occurrence of phosphatases in aquatic, terrestrial, and sewage systems has been documented (1, 2, 5, 8, 10, 13, 16). In addition, the relative activity of phosphatase has been suggested as a potential measure of microbial biomass and general trophic conditions of aquatic environments (11). However, the activity and significance of phosphatases in aquatic sediments has received little attention. It was the objective of this study to develop a phosphatase assay and to define its potential use in freshwater sediments.

MATERIALS AND METHODS

Sediment samples. Sediments used in examining the recovery efficiency and optimization of alkaline phosphatase activity were obtained from either Melton Hill Reservoir, a moderately polluted reservoir located 20 km from Knoxville, Tenn., or a small unpolluted pond on the Oak Ridge Reservation, Oak Ridge, Tenn. Pond sediments were employed in establishing aquatic microcosm sediment communities (9). These samples were predominantly composed of clay and fine silts and were inhabited by aquatic macrophytes. Macrophyte root tissues were excluded from sediment subsamples used for phosphatase assays. A third group of sediment samples examined was collected from Saucon Creek and its confluence with the Lehigh River at Bethelehem, Pa. These samples were collected above and below a coking plant effluent stream, a point source of contamination. Saucon Creek is a shallow, fast-flowing stream, and its sediment substrates are composed of sand and small gravel, whereas the Lehigh River samples were predominately compact silt and clay. Neither group of samples had a macrophytic flora. Walker Branch, an uncontaminated stream on the Oak Ridge Reservation, was used as a source for samples of fine particulate organic matter, which were siphoned from the bottom substrate of quiescent pools. Suspended particulates, harvested by membrane filtration, and Aufwuch communities (rock scrapings) were also obtained from Walker Branch. Walker Branch and Saucon Creek share similar structural and hydrodynamic characteristics, al-

though they are separated by a distance of approximately 1,200 km.

Chemicals and enzyme sources. Alkaline phosphatase type III (Sigma Chemical Co., St. Louis, Mo.), an orthophosphorus monoester phosphohydrolase, was employed for all studies using purified enzyme. Lypholyzed, alkaline phosphatase-rich *Escherichia coli* K-12 cells (Sigma) were used as a standard source of unpurified enzyme for those studies using whole-cell suspensions of bacterial phosphatase. *p*-Nitrophenylphosphate (p-NPP; Sigma) was employed as the substrate for demonstrating phosphatase activity. *p*-Nitrophenol (p-NP; Sigma) was used in preparing standard curves for quantitating p-NPP hydrolysis. Aroclor 1254, a commercial polychlorinated biphenyl mixture (Monsanto Chemical Co., St. Louis, Mo.); certified phenanthrene, a tricyclic condensed aromatic hydrocarbon (Eastman Organic Chemicals, Rochester, N.Y.); and $HgCl_2$ (Fisher Scientific Co., Norcross, Ga.) were used in studies examining the effect of environmental contaminants on alkaline phosphatase activity. Tris(hydroxymethyl)aminomethane (Tris; Fisher) was used in the preparation of buffer solutions.

Phosphatase assay. The general phosphatase assay used in this study was a modification of the assay described by Bessey et al. (3). In this assay, the phosphate group of *p*-NPP is hydrolytically cleaved. The products of this cleavage are orthophosphate and p-NP, a colored compound at an alkaline pH, which can be spectrophotometrically quantitated.

The routine assessment of sediment phosphatase activity followed a standard format. Sediment samples (0.5 to 1.0 ml) were placed in 15-ml graduated conical centrifuge tubes. The volume of the samples was adjusted to 4.0 ml with 1 M Tris-hydrochloride buffer (pH 8.6), and the samples were sonicated for 45 s (to release cell-bound phosphatase) employing an Artex sonic dismembrator (Artex power supply and microprobe at 30% relative output). The samples were placed in a 37°C water bath, and, at timed intervals, 1.0 ml of p-NPP (1 mg ml^{-1}) in 0.2 M Tris buffer (pH 7.6) was added. Samples were immediately mixed for 2 to 3 s in a Vortex mixer and were then returned to the water bath for a 1-h incubation. At the end of the incubation 1.0 ml of 1 N NaOH was added to the samples, and they were again mixed (2 to 3 s) to stop the reaction and develop color. The samples were centrifuged in a table-top centrifuge at 3,000 rpm for 10 min to pellet the sediment. After centrifugation, a 0.5-ml sample of supernatant was withdrawn and diluted 1:10 in 1 M Tris buffer (pH 8.6). The absorbance of the diluted sample was measured at 418 nm on a Bausch and Lomb Spectronic 70 spectrophotometer. Absorbance readings were converted to nanomoles of p-NP per milliliter by comparison to a p-NP standard curve ranging from 1.0 to 100.0 nmol of p-NP ml^{-1}. Spectrophotometric blanks were prepared by deleting the p-NPP from the reaction mixture and replacing it with 1.0 ml of 0.2 M Tris buffer (pH 7.6).

Estimation of sediment biomass and bacterial population densities. Sediment adenosine triphosphate (ATP) biomass and total viable cell counts of sediment bacterial populations were assessed by cold-sulfuric acid extraction (D. M. Karl and P. A. LaRock, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, G202, p. 53) (ATP quantitation was performed on a DuPont 760 fluorescent biometer; I. E. Dupont De Nemours and Co., Inc., Wilmington, Del.) and by spread plate inoculation on YEPG agar (14) (incubation time, 4 weeks at 25°C). Benthic algal communities were not observed in these studies, and no attempt was made to estimate other biological components of the samples examined.

Factors affecting phosphatase activity. Time-course sonication and incubation time experiments were performed to optimize the recovery of phosphatase activity from natural sediments. In addition, in vitro assays were performed to determine the effects of sonication and chemical environmental contaminants ($HgCl_2$, Aroclor 1254, and phenanthrene) on the activity of the enzyme itself.

These in vitro assays were performed using 1 M Tris buffer (pH 8.6) (4.0 ml) without sediment or with ca. 0.5 to 1.0 ml of autoclaved sediments. One microliter of a 1:100 dilution of purified enzyme, ca. 0.03 U (1 U will hydrolyze 1 μmol of p-NPP min^{-1} h^{-1} at pH 10.4, 37°C), or 5 μl of phosphatase-rich *E. coli* suspension (0.1 g ml^{-1}), approximately 0.05 U, was added to appropriate tubes. Reaction mixtures were incubated as previously described; however, various combinations of no treatment, sonication, contaminant dosing, and inclusion of sediments were examined to determine their effects on the recovery of phosphatase activity.

In situ alkaline phosphatase activity. Alkaline phosphatase activity was assessed in natural sediment samples collected from experimental microcosms, Saucon Creek-Lehigh River samples, and Walker Branch samples. Microcosm and Walker Branch samples were assayed within 0.5 to 3 h after sample collection. Saucon Creek or Lehigh River samples were processed on site in July 1978. In December 1978, Saucon Creek-Lehigh River samples were flown to the Knoxville campus before processing. In both instances total sample processing, from the point of collection to final analysis, was completed within 15 to 18 h.

Data analysis. All assays were subjected to computerized analysis of variance; a posteriori examination of sample means was performed using the Student-Newman-Kuels least-significant-range procedure (15). Type I error was set at $\alpha = 0.05$. Multiple correlation analysis was performed on a DEC-10-IBM 360 computer system with subprograms derived from *Statistical Package for the Social Sciences* (12).

RESULTS

In vitro alkaline phosphatase assessment. Minimal phosphatase activity was demonstrated in sediment samples incubated with p-NPP for 15 to 30 min; however, a 13.5-fold increase in phosphatase activity was observed after a 45-min incubation, relative to the 30-min incubation (Fig. 1). Prolonged incubation, 60 and 90 min, resulted in insignificantly greater phosphatase activity as compared to 45 min of incubation, 2.8% and 3.7% relative increase, respectively. Phosphatase activity demonstrated a plateau at 30 s of sonication, with 5.0% and 7.6% relative increases in phosphatase activity at 45

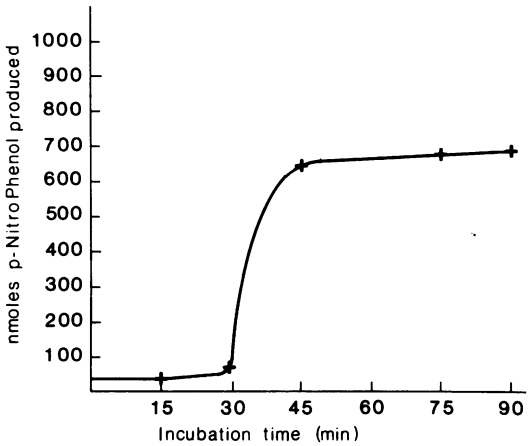

FIG. 1. *Time-course development of phosphatase activity in untreated microcosm sediments. Nanomoles of p-NP produced per milliliter of reaction mixture per hour; mean values of three observations; coefficient of variation, 10%.*

and 60 s of sonication, respectively (Fig. 2). All further assays were performed using a 45-s sonication period and 60 min of incubation to allow maximum development of phosphatase activity and to avoid phosphatase assessment at time points that were subject to rapid rates of change in activity, which would magnify minor technical errors.

The effects of a 45-s sonication on purified alkaline phosphatase, the mixing of the enzyme with sediment, or the combination of the two treatments were insignificant as compared to unsonicated buffer-enzyme mixtures (Table 1). Whole-cell alkaline phosphatase activity was insignificantly stimulated by sonication. However, the effect of both sediment alone and sediment plus sonication significantly stimulated the recovery of alkaline phosphatase from whole-cell suspensions, 38% and 47%, respectively.

Studies on the effects of Aroclor 1254, phenanthrene, and $HgCl_2$ demonstrated no significant effect upon the activity of purified alkaline phosphatase (Table 2). Aroclor 1254 insignificantly stimulated the recovery of alkaline phosphatase from whole-cell suspensions. Phenanthrene and $HgCl_2$ significantly increased the recovery of whole-cell alkaline phosphatase by 43% and 35%, respectively, as compared to undosed controls.

Alkaline phosphatase activity of chemically stressed freshwater sediments. Samples collected from Saucon Creek before the diversion of a coking plant effluent from the creek to the Lehigh River (July 1978) demonstrated a significant point source (sites 2 and 3) effect of the coking plant effluent on sediment alkaline phosphatase activity (Table 3).

Phosphatase activity at site 2 (temporary coking effluent discharge during effluent diversion) and site 3 (0.25 km below the chronic effluent discharge) was significantly depressed as compared to site 1 (0.5 km upstream) and site 4 (1 km downstream from site 3). Lehigh River samples (site 5c) collected 25 m below the confluence were an order of magnitude greater than the average phosphatase activity of either site 1 or 4 on Saucon Creek.

In December 1978, 6 months after the effluent diversion, Saucon Creek and Lehigh River samples were again examined for phosphatase activity, total viable heterotrophic bacterial counts

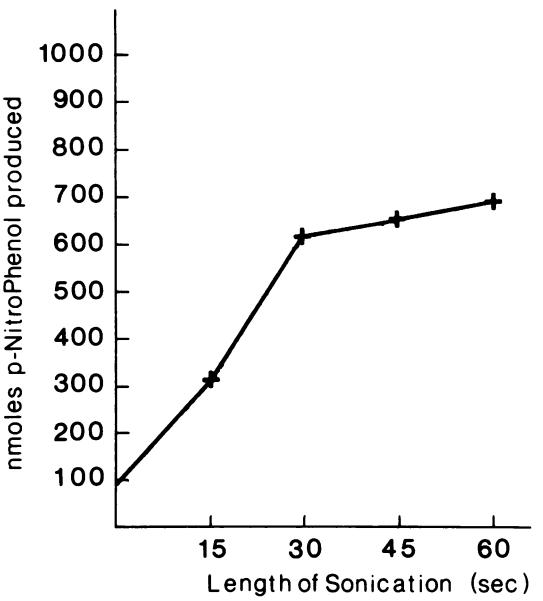

FIG. 2. *Effect of sonication time on the liberation of alkaline phosphatase from untreated microcosm sediments. Nanomoles of p-NP produced per milliliter of reaction mixture per hour; mean value of three observations; coefficient of variation, 7.8%.*

TABLE 1. *Effect of sonication and sediment mixing on in vitro alkaline phosphatase activity*[a]

Treatment	p-NP production (nmol)	
	Enzyme	Cell suspension
None	488	324
Sonication	469	374
Sediment	477	448[b]
Sonication and sediment	516	475[b]

[a] Purified enzyme (0.03 U) or phosphatase-rich *E. coli* K-12 cells (0.05 U) in sterile buffer or 0.5-ml sediment samples.

[b] Indicates those values significantly different from the untreated samples, $\alpha = 0.5$.

TABLE 2. *Effect of chemical contaminants on in vitro alkaline phosphatase activity of sediment mixtures*[a]

Treatment[b]	p-nitrophenol production (nmoles hr^{-1} g^{-1} dry wt sediment)	
	Enzyme	Cell suspension
None	6,900	2,017
Aroclor 1254	6,940	2,488
$HgCl_2$	6,940	2,891[c]
Phenanthrene	7,303	2,784[c]

[a] Purified enzyme or phosphatase-rich *E. coli* K-12 in sterile 0.5-ml sediment samples.

[b] All phosphatase assays were performed according to the general method as described in text. Toxicant concentrations: Aroclor 1254, $HgCl_2$, and phenanthrene, 1.0, 10, and 1.0 μg g^{-1}, respectively, for free enzyme; 5.0, 10, and 5.0 μg g^{-1}, respectively, for whole cells.

[c] Indicates those values significantly different from the untreated control samples, $\alpha = 0.05$.

(TVC), and ATP-biomass (Table 4). It was apparent that alkaline phosphatase activity had recovered at site 3, which had experienced the greatest perturbation due to the coking effluent. However, site 5c, which was now receiving direct effluent perturbation, exhibited a significant twofold reduction in alkaline phosphatase activity.

During both sampling periods, TVC and ATP biomass estimates followed patterns similar to those observed for alkaline phosphatase activity. Correlation coefficients (r) were calculated for those three estimates and were found to be significantly correlated: $r = 0.82$ (ATP-phosphatase), 0.82 (ATP-TVC), and 0.53 (TVC-phosphatase) ($\alpha = 0.05$), and $r = 0.43$ (ATP-phosphatase, $\alpha = 0.10$), 0.73 (ATP-TVC), and 0.87 (TVC-phosphatase) ($\alpha = 0.05$), respectively, for the August and December sampling periods.

Phosphatase activity of stressed microcosm sediments. Phosphatase activity of control microcosms and microcosms dosed with a water-soluble extract of synthetic oil was examined 2 months after the toxicant perturbation (Table 5). No distinct toxicant effects on phosphatase activity were observed. Phosphatase activity was also reflected in similar trends in TVC and ATP levels for the microcosms. Correlation coefficients for ATP-phosphatase, TVC-phosphatase, and ATP-TVC were highly significant ($r = 0.98$, 0.87, and 0.95, respectively), which indicated a much greater sample homogeneity in the microcosm sediments than in the Saucon Creek-Lehigh River sediments. Alkaline phosphatase activity of the microcosm sediments was approximately an order of magnitude higher than phosphatase activity observed for Saucon Creek and Lehigh River samples (Tables 3 and 4). However, TVC and ATP were comparatively similar to the microcosms and the Saucon Creek-Lehigh River samples.

Phosphatase activity of unstressed freshwater sediments. A final group of samples collected from Walker Branch were examined for phosphatase activity. These samples included the fine particulate organic matter sediments of quiescent pools; suspended particulates, collected by membrane filtration; and rock scrapings of Aufwuch communities. Phosphatase activity of these samples ranged upwards two orders of magnitude greater than any other samples previously examined (Table 6). Aufwuch samples were approximately an order of magnitude greater than either the fine particulate organic matter or suspended particulates. Although Saucon Creek and Walker Branch reflect very similar aquatic habitats, direct comparison of phosphatase activity cannot be made due to the differences in the sample types. The Walker Branch samples do indicate the high level of phosphatase activity that can be encountered in uncontaminated freshwater sediments.

DISCUSSION

Alkaline phosphatase assays of water and soil frequently employ solvents to shock phospha-

TABLE 3. *Comparative alkaline phosphatase activity, TVC, and ATP of Saucon Creek-Lehigh River sediment samples prior to coking plant effluent diversion, July 1978*

Sample site[a]	Alkaline phosphatase[b]	TVC (per g [dry wt] of sediment)	ATP (fg g^{-1} [dry wt] of sediment)
1a	135.8 ± 30.6	$1.9 \pm 0.5 \times 10^7$	$4.9 \pm 0.4 \times 10^6$
1b	180.4 ± 14.1	$1.0 \pm 0.3 \times 10^7$	$1.5 \pm 0.3 \times 10^6$
1c	85.2 ± 18.7	$6.9 \pm 1.2 \times 10^6$	$1.2 \pm 0.2 \times 10^6$
2a[c]	38.6 ± 0.0	$3.5 \pm 0.5 \times 10^5$	$6.3 \pm 1.2 \times 10^6$
2b	51.0 ± 3.3	$1.8 \pm 0.4 \times 10^6$	$1.7 \pm 0.7 \times 10^6$
2c	55.4 ± 6.2	$9.1 \pm 1.5 \times 10^6$	$2.0 \pm 1.0 \times 10^6$
3a[d]	5.6 ± 2.6	$1.2 \pm 0.4 \times 10^6$	$2.1 \pm 1.4 \times 10^6$
3b	7.8 ± 2.8	$1.1 \pm 0.6 \times 10^6$	$2.2 \pm 0.6 \times 10^6$
3c	43.2 ± 40.5	$3.5 \pm 0.2 \times 10^6$	$1.2 \pm 0.7 \times 10^6$
4a	384.6 ± 113.1	$7.5 \pm 0.6 \times 10^5$	$2.5 \pm 0.7 \times 10^7$
4b	17.6 ± 3.7	$3.2 \pm 0.3 \times 10^6$	$5.0 \pm 1.8 \times 10^7$
4c	86.5 ± 4.7	$3.5 \pm 0.4 \times 10^6$	$4.3 \pm 0.4 \times 10^7$
5c	2,106.2 ± 121.1	$3.4 \pm 0.2 \times 10^7$	$6.7 \pm 2.9 \times 10^7$

[a] Three transect samples were taken at each site; each analysis was performed in triplicate. Mean and standard deviation are reported for each assay.

[b] Nanomoles of p-NP produced per gram (dry weight) of sediment per hour.

[c] Effluent point source discharge during diversion construction.

[d] Former effluent point source discharge prior to diversion construction. Phosphatase activity at site 3 was significantly lower than site 1, control, and downstream sites 4 and 5 (Student-Newman-Kuels, $\alpha = 0.05$).

TABLE 4. *Comparative alkaline phosphatase activity, TVC, and ATP of Saucon Creek-Lehigh River sediment samples 6 months after effluent diversion, December 1978*

Sample site[a]	Alkaline phosphatase[b]	TVC (per g [dry wt] of sediment)	ATP (fg g^{-1} [dry wt] of sediment)
1a	282.1 ± 23.1	$1.1 \pm 0.2 \times 10^7$	$4.5 \pm 2.8 \times 10^7$
1b	73.9 ± 24.1	$4.4 \pm 0.1 \times 10^6$	$4.1 \pm 0.1 \times 10^7$
1c	187.5 ± 8.0	$2.6 \pm 0.1 \times 10^6$	$2.2 \pm 0.3 \times 10^7$
3a	251.7 ± 23.6	$1 \pm 0.1 \times 10^7$	$4.6 \pm 2.9 \times 10^7$
3b	119.9 ± 8.1	$3.6 \pm 0.3 \times 10^6$	$3.3 \pm 1.3 \times 10^7$
3c	307.3 ± 22.1	$2.1 \pm 0.1 \times 10^7$	$1.6 \pm 0.1 \times 10^8$
4a	229.2 ± 29.5	$2.3 \pm 0.1 \times 10^7$	$1.6 \pm 0.6 \times 10^8$
4b	696.6 ± 35.7	$4.8 \pm 0.8 \times 10^6$	$3.1 \pm 3.0 \times 10^7$
4c	125.0 ± 19.6	$1.0 \pm 0.1 \times 10^7$	$5.0 \pm 2.7 \times 10^7$
5b[c]	0.0	$6.1 \pm 0.2 \times 10^6$	$5.8 \pm 0.3 \times 10^6$
5c	800.0 ± 282.8	$3.6 \pm 0.6 \times 10^7$	$1.5 \pm 0.6 \times 10^8$
6c[d]	148.5 ± 22.7	$3.0 \pm 0.9 \times 10^6$	$7.5 \pm 0.27 \times 10^7$

[a] Triplicate transect samples were taken for each site; mean value and standard deviation are reported. Site 2 was lost during completion of the diversion construction.

[b] Nanomoles of p-NP produced per gram (dry weight) of sediment per hour.

[c] Lehigh River sites 5b and c were situated 10 m below the new coking plant effluent discharge; alkaline phosphatase activity at site 5c was significantly lower than the preceding sampling period (Table 3) (Student-Newman-Kuels, $\alpha = 0.5$).

[d] Site 6c was located 25 m above the confluence of Saucon Creek and the Lehigh River.

TABLE 5. *Comparative alkaline phosphatase activity, TVC, and ATP of chemically stressed microcosm sediments*

Microcosm no.[a]	Alkaline phosphatase[b]	TVC (per [dry wt] of sediment)	ATP (fg g^{-1} [dry wt] of sediment)
EE2	1,067 ± 623	$8.4 \pm 1.0 \times 10^6$	$6.3 \pm 4.5 \times 10^7$
EE3[c]	1,758 ± 195	$1.4 \pm 0.5 \times 10^6$	$7.6 \pm 1.5 \times 10^7$
EE4[c]	1,156 ± 598	$1.6 \pm 0.4 \times 10^6$	$4.8 \pm 2.3 \times 10^7$
EE6	3,268 ± 235	$2.8 \pm 0.5 \times 10^7$	$2.1 \pm 0.8 \times 10^8$

[a] Six replicate samples were removed for each analysis. Mean and standard deviation are reported for each parameter assessed.

[b] Nanomoles of p-NP produced per gram (dry weight) of sediment per hour.

[c] Microcosms EE3 and EE4 were undosed control microcosms.

tase from the microbial cell and extract it from the medium (2, 16). The efficiency of p-NP extraction may exceed 99% (16); however, interfering organic substances may also be extracted by such treatments, limiting the sensitivity of the assay. Although direct comparisons were not made, the sonication procedure employed in this study was found to be essentially 100% efficient in recovering purified phosphatase activity from sediment samples as compared to the enzyme in buffer above. Increased recoveries of whole-cell phosphatase (*E. coli*) activity in mixtures of sediment, with or without sonication (Table 1), most likely represented physical as well as chemical shocking of the phosphatase from the microbial cell. The purification enzyme demonstrated a high degree of stability to sonication and was not affected by three relatively common environmental contaminants: polychlorinated biphenyl, phenanthrene, and Hg^{2+}. The lag in phosphatase activity during sediment incubation was attributed to particulate-bound enzyme-sediment interactions rather than enzyme synthesis, since phosphatase is not induced by p-NPP.

A most significant result of this study is the correlation of phosphatase activity with estimates of bacterial population densities and biomass. These results indicate the microbial nature of the observed phosphatase activity. In a greater sense, they demonstrate that changes in phosphtase activity are a function of the microbial biomass and its activity in sediment systems, rather than an inhibition or repression of the enzyme itself. This observation was further supported by the high phosphatase activity of Walker Branch samples. Standing crop biomass estimates of selected Walker Branch samples (estimated from ash-free dry weight of sediment) were one to two orders of magnitude greater than either the Saucon Creek or microcosm samples. Unfortunately, ATP estimates were not obtained for the Walker Branch samples, and consequently direct comparisons with Saucon Creek and microcosm samples cannot be made.

The results of this study suggest that the variability of phosphatase activity in a group of samples is directly related to the heterogeneity of the samples within a given site. The observation is derived both from the phosphatase

TABLE 6. *Alkaline phosphatase activity of Walker Branch samples*

Sample type	Streams reference number	Alkaline phosphatase activity[a]
FPOM[b]	2	13,530 ± 0
	4	6,030 ± 340
	11	18,390 ± 750
Suspended particulates[c]	2	6,750 ± 350
	4	12,000 ± 449
	11	9,250 ± 224
Aufwuch	459	268,000 ± 17,500
	430	271,000 ± 16,900
	306	87,000 ± 15,300

[a] Mean of three observations; nanomoles of p-NP produced per gram (dry weight) of sediment per hour.

[b] Fine particulate organic matter, bottom sediments.

[c] Suspended particulates recovered by filtering 1.0 liter of water through a 0.4-μm membrane filter.

activity of transect sites as compared to general sites (Saucon Creek samples) and from variability in microcosm phosphatase activity (Table 5). A conclusion that can be drawn from the Saucon Creek studies (assuming that the among-site variability is not attributable to temporal cycles) is that the assessment of alkaline phosphatase activity can be used as an index of stream perturbation and recovery. This conclusion is subject to verification by continuing studies on the recovery of Saucon Creek.

ACKNOWLEDGMENTS

We thank J. Giddings for his assistance in the microcosm studies.

These investigations were supported by Public Health Service grant no. ESO-1521-2 from the National Institutes of Health; by Union Carbide, Oak Ridge National Laboratory subcontract 7182; by U.S. Department of the Interior, Office of Water Research and Technology grant no. 14-34-0001-9096; and by the National Science Foundation, contract DEB 78-03012.

LITERATURE CITED

1. **Berman, T.** 1969. Phosphatase release of inorganic phosphorus in Lake Kinnert. Nature (London) **224**:1231-1232.
2. **Berman, T.** 1970. Alkaline phosphatases and phosphorus availability in Lake Kinnert. Limnol. Oceanogr. **15**:663-674.
3. **Bessey, O. A., O. H. Lowry, and J. J. Brock.** 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. **164**:321-329.
4. **Bhatti, A. R.** 1978. Alkaline phosphatase of *Serratia marcescens:* cytochemical localization. Microbios Lett. **4**:83-88.
5. **Browman, M. G., and M. A. Tabatabai.** 1978. Phosphodiesterase activity of soil. J. Soil Sci. Soc. Am. **42**:284-290.
6. **Cheng, K. J., J. M. Ingram, and J. W. Costerton.** 1971. Interaction of alkaline phosphatase and the cell wall of *Pseudomonas aeruginosa*. J. Bacteriol. **107**:325-336.
7. **Flint, K. P., and J. W. Hopton.** 1977. Substrate specificity and ion inhibition of bacterial and particle associated alkaline phosphatases of waters and sewage sludge. Eur. J. Appl. Microbiol. **4**:195-204.
8. **Flint, K. P., and J. W. Hopton.** 1977. Seasonal variation in the phosphatase activity of waters and sewage sludge. Eur. J. Appl. Microbiol. **4**:204-215.
9. **Giddings, J. M., and G. K. Eddleman.** 1979. Some ecological and experimental properties of complex aquatic microcosms. Int. J. Environ. Stud. **13**:119-123.
10. **Jones, J. G.** 1972. Studies of freshwater bacteria: association with algae and alkaline phosphatase activity. J. Ecol. **60**:59-75.
11. **Jones, J. G.** 1972. Studies on freshwater microorganisms: phosphatase activity in lakes of differing degrees of eutrophications. J. Ecol. **60**:777-791.
12. **Nie, N. H., C. H. Hull, J. G. Jenkins, K. Stenbrenner, and D. H. Bent (ed.).** 1970. Statistical package for the social sciences, p. 280-286, 320-360, 411-421. McGraw-Hill, New York.
13. **Reichardt, W., J. Overbeck, and L. Steubing.** 1967. Free dissolved enzymes in lake water. Nature (London) **216**:1345-1347.
14. **Sayler, G. S., L. C. Lund, M. P. Shiaris, T. W. Sherrill, and R. E. Perkins.** 1979. Comparative effects of Aroclor 1254 (PCB) and phenanthrene on glucose uptake by freshwater microbial populations. Appl. Environ. Microbiol. **37**:878-885.
15. **Sokal, R. R., and F. J. Rohlf.** 1969. Biometry. p. 226-246. W. H. Freeman and Co., San Francisco.
16. **Tabatabai, M. A., and J. M. Bremner.** 1969. Use of p-nitrophenyl phosphate assay for soil phosphatase activity. Soil Biol. Biochem. **1**:301-307.
17. **Torriani, A.** 1960. Influence of inorganic phosphate in the formation of phosphatases by *Escherichia coli*. Biochim. Biophys. Acta **38**:460-469.
18. **Tyler, G.** 1974. Heavy metal pollution and soil enzymatic activity. Plant Soil **41**:303-311.
19. **Tyler, G.** 1976. Heavy metal pollution, phosphatase activity, and mineralization of organic phosphorus in forest soils. Soil Biol. Biochem. **8**:327-332.