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Vertical Profiles of Alkaline Phosphatase Activity in Dam Reservoirs and its Relation with Microbial Parameters

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The alkaline phosphatase activity (APA) of two dam reservoirs and inflowing streams were measured monthly in 2000. During summer months in 2001, the vertical profiles of APA and related parameters were also examined in one of the reservoirs. The APA was relatively high during the summer season in the epilimnion while it was almost invariable in the hypolimnion. A small increase in APA was observed at just above the bottom. The APA fluctuation was independent of the concentration of soluble reactive phosphorus. It was assumed that APA is not indicative of the phosphorus availability status. An examination of size-fractionated samples suggested that APA in reservoirs was attached to particles larger than 0.4 μm, whereas in streams it existed in a dissolved form. There was a positive significant correlation between chlorophyll *a* concentration and APA in the photic zone. In the aphotic zone, APA correlated positively with the colony count of heterotrophic bacteria, but not with microscopic total bacterial counts.

Key words : alkaline phosphatase activity, dam reservoir, photic and aphotic zones, chlorophyll *a*, heterotrophic bacteria

INTRODUCTION

Alkaline phosphatase, which is produced by various microorganisms in the limnological environment (Berman, 1970; Stewart and Wetzel, 1982; Francko, 1983), is considered to play an important role in the phosphorus cycle (Reichardt *et al*., 1967). It is commonly regarded that the enzyme is produced in response to the acute reactive phosphorus depletion (Thingstad, 1988). Therefore alkaline phosphatase activity (APA) has been considered to indicate phosphorus nutrient status of the aquatic microorganisms (Chrost *et al*., 1984). However, some studies in France (Jamet *et al*., 2001) and in Japan (Hirotani *et al*., 2001, 2004) raised doubts on this recognition. The aim of this study is to examine the relation of APA with phosphorus availability and microbial parameters.

MATERIALS AND METHODS

The study was done mainly in the upper reaches of the Ishite River, which originates from low peaked mountains ca. 1100 m above sea level, and in the Souja River which originates from the same mountain range and flows through the adjacent watershed (Fig. 1). Coniferous plantations mainly covered the surrounding mountains and there are multipurpose dams in both watersheds. The total storage volume and watershed area of Ishitegawa Dam (Ishite River) and Tamagawa Dam (Souja River) are 1.3×10^7 m^3, 72.6 km^2, 9.9×10^6 m^3, and 38.1 km^2, respectively. These two dam reservoirs are located only 14 km apart, so that the climatic and geological condi-

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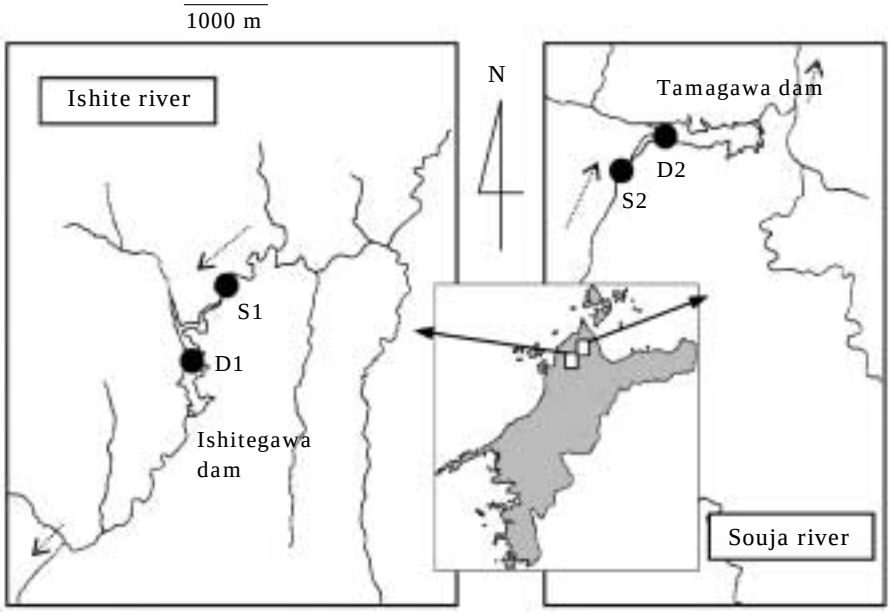

Fig. 1. Map of two watersheds and sampling sites. Broken arrows indicate the direction of flow.

tions are assumed identical. Water samples were collected monthly in 2000 at all stations from the surface, and from various depth including half of the water depth (1/2D) and 1 m above the bottom (D−1) at Station D1 during summer months in 2001.

APA was measured using *p*−nitrophenyl phosphate as a substrate (Reichardt *et al*., 1967; Obst, 1985). Stock solution (25 mg L^{-1}) of *p*−nitrophenyl phosphate was mixed 1 : 6 with sample water. After incubation for 6 h at 30°C, *p*−nitrophenol was measured spectrophotometrically at 405 nm. Samples treated in the same manner but without the substrate were measured as control blanks.

Soluble reactive phosphorus (SRP) of the 0.45 µm membrane filter filtrate was measured by the molybdenum blue colorimetric method (Koroleff, 1983). Chlorophyll *a* (Chl−*a*) was measured spectrophotometrically by grass−fiber filtration and acetone extraction (Lorenzen, 1967). Direct count (DC) of bacterial cells was done by staining the cells with 4′−6−diamidino−2−phenylindole (25 µg mL^{-1}, DAPI) followed by filtration with Isopore membrane filters (0.2 µm pore size, Millipore Corp., Bedford, MA), and counting the cells using epifluorescence microscope (Porter and Feig, 1980). Heterotrophic plate counts (HPC) were determined by the pour plate method using R2A (Difco, Detroit, MI) (AHPA−AWWA−WPCF,

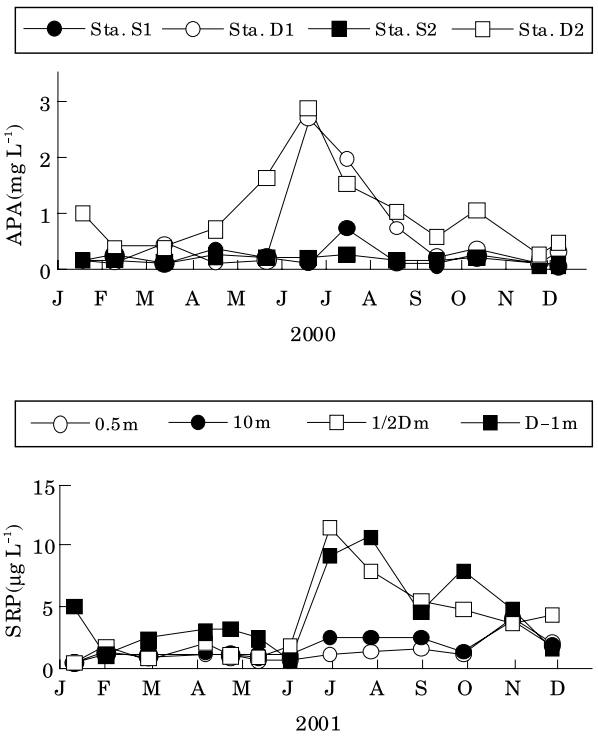

Fig. 2. Fluctuation of alkaline phosphatase activity (APA) in dam reservoirs and inflowing streams in 2000 (top) and soluble reactive phosphorus (SRP) in the water column at Sta. D1 in 2001 (bottom). 1/2D m indicates half of the water depth and D−1 m indicates 1 m above the lake bottom.

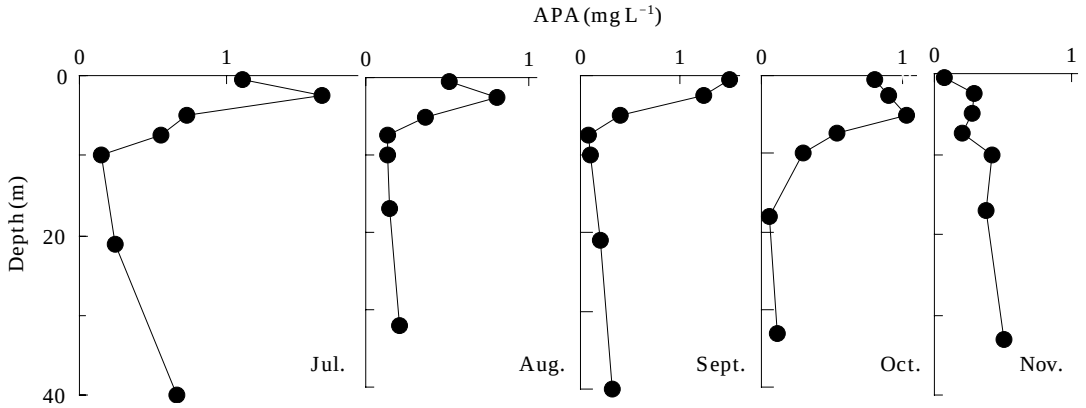

Fig. 3. Vertical profiles of alkaline phosphatase activity (APA) at Sta. D1 during July to November, 2001.

1995). The plates were inverted and incubated at 25°C for 5 d. The plate counts were expressed as colony forming units (CFU) per milliliter. The microbial parameters were indicated in logarithmic scales.

For the size-fractionation, the sample waters were filtered aseptically through Isopore membrane filters of pore sizes of 0.2, 0.4, 1.2, 5, or 10 µm. Each filtrate was subjected to APA, Chl-*a*, and HPC analyses. Data from each filtrate were subtracted by data from filtrate by the smaller pore size filter.

RESULTS AND DISCUSSION

In the monthly field study in 2000, the APA in the reservoirs indicated a seasonal cycle, with a peak during the summer season; and in the inflowing streams the activities were relatively invariable all the year round (Fig. 2). The APA fluctuation in the reservoirs located in the two adjacent watersheds showed similar dynamics. SRP in the epilimnion remained almost constant in both reservoirs (Hirotani *et al*., 2001).

The mean SRP concentrations at Sta. D1 in 2001 were 1.4, 1.7, 3.6, and 4.3 µg L^{-1}, at 0.5 m, 10 m, in the intermediate water (1/2D), and at the bottom (D-1), respectively (Fig. 2). The monthly variation in SRP concentration at 0.5 m and 10 m remained constant, whereas there was an increase in SRP concentrations in July at deeper layers.

For the vertical profiles of APA in a dam reservoir (Sta. D1) (Fig. 3), the maximum of the APA

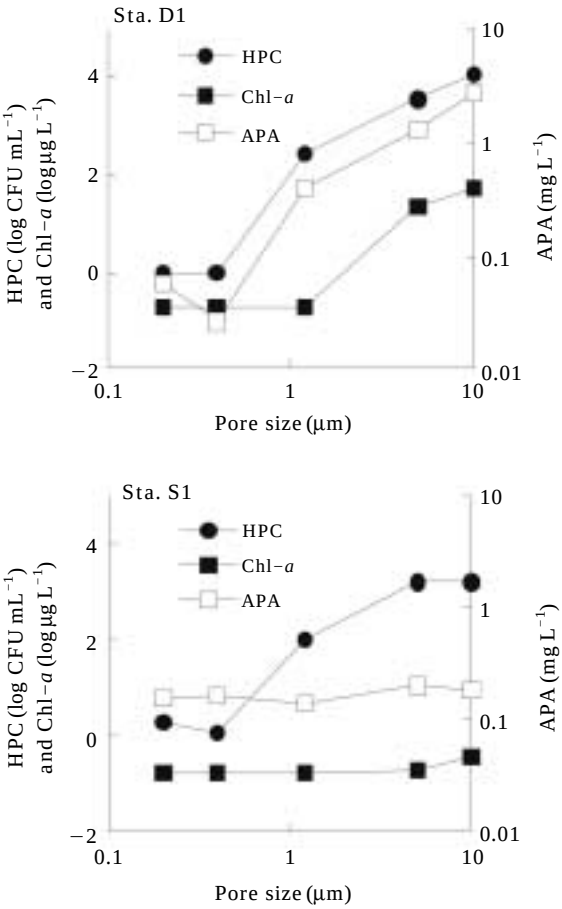

Fig. 4. Microbial parameters including alkaline phosphatase activity (APA) in size fractionated sample from Sta. D1 (top) and Sta. S1 (bottom).

was observed near the surface between 0.5 and 7.5 m-depth and gradually declined downwards

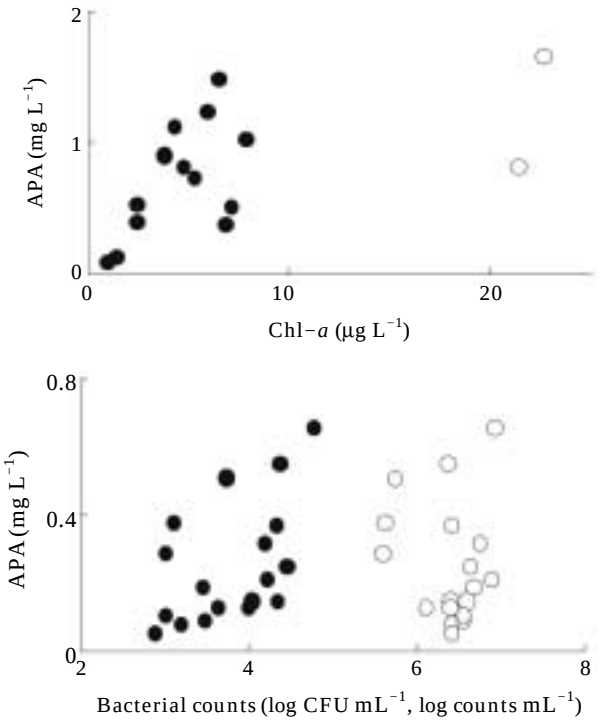

Fig. 5. Correlation between chlorophyll *a* (Chl–*a*) and alkaline phosphatase activity (APA) when Chl–*a* were above 1 μg L^{-1} (top, open symbols indicate the outlying data ($>2\sigma$) measured in July and August), and correlation between direct count (open, log counts mL^{-1}) and heterotrophic plate count (closed, log CFU mL^{-1}) and APA in aphotic zones (bottom).

with a slight increase near the bottom. In November, during the destratification of the water column, the vertical profile of APA became unclear.

One may think that high APA near the surface would be due to the biological response to the shortage of available phosphorus. However, APA at 10 m depth where SRP was at similar level to that of 0.5 m depth was lower than the surface and APA at 10 m depth was often detected as minimum. Therefore, it is likely that the APA does not indicate phosphorus availability of aquatic microorganisms. This agrees with our former studies (Hirotani *et al*., 2001, 2003, 2004).

In size–fractionated samples, high APA was detected in >0.4 μm filtrates (Fig. 4), suggesting that the enzyme is attached to particles (Hirotani *et al*., 2001). In the streamwater sample, APA evenly distributed for all size–fractions, suggesting that the enzyme has a soluble form.

A statistically significant positive correlation (n = 15, r = 0.54, P–value = 0.036) was found between APA and Chl–*a* in the photic zone (>1 μg L^{-1} Chl–*a*) (Fig. 5). Thus, in the photic zone APA may be derived from the algae. In the aphotic zone, a significant correlation was found between APA and HPC (n = 18, r = 0.50, P–value = 0.033), but not between DC (n =18, r = –0.12, P–value = 0.63). Further studies are needed to explain the discrepancy between these bacterial parameters.

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＜**국문적요**＞

댐 저수지에서 alkaline phosphatase 활성의 수직변화와 미생물 요인들과의 상관관계

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2000년 두 댐 저수지와 유입 하천에서 월별 alkaline phosphatase activity (APA)을 측정하였다. 또한 2001년 여름 동안은 한 댐 저수지에서 APA의 수직변화와 관련된 요인들을 조사하였다. APA는 여름 동안 저수지 표층에서 상대적으로 높게 나타났으나 심층에서는 거의 변화가 없었다. 그러나 바닥층 바로 윗부분에서는 APA의 미미한 증가 관찰되었다. APA 변화와 용존무기인 농도 변화와는 상관관계가 없었으며, 이 결과 조사 대상 저수지에서 APA는 인 이용성의 상태를 의미하지는 않는 것으로 추정되었다. 시료의 size-fractionation 분석 결과, 조사 대상 저수지들에서 APA는 0.4 μm보다 큰 입자들에 흡착된 반면, 유입 하천에서는 용존형태로 존재하는 것으로 판단되었다. 저수지 유광층에서 엽록소-*a*의 농도와 APA간에 유의한 양의 상관성이 나타났다. 무광층에서 APA는 세균의 colony count와 양의 상관성을 나타냈으나 총 세균수와는 상관성이 없었다.