

BAP MEASUREMENT USING SUCCESSIVE EXTRACTION BY ACID AND ALKALI

Jun Nakajima*, Takuya Okubo**

*Department of Environmental Systems Engineering, Faculty of Science and Engineering, Ritsumeikan University,
1-1-1 Nojihigashi, Kusatsu, 525-8577, Japan, jnakajim@nisiq.net

**Lake Biwa Research Institute, 1-10 Uchidehama, Otsu, 520-0806, Japan, okubo@lbri.go.jp

ABSTRACT

It is important for management of lake water quality to estimate the amount of not only PO₄-P but also bioavailable phosphorus (BAP) in suspended solids entering lake especially from non-point sources. AGP test or extraction by 0.1N NaOH is widely used to measure BAP although the former takes a long period and the latter measures rather high percentage of TP. In order to measure more easily soluble fraction, i.e. highly bioavailable phosphorus (HBAP), we introduced a successive extraction method with 0.1N HCl and 0.1N NaOH. HBAP was measured as extractable phosphorus by 0.1N HCl during three minutes. This fraction was verified to be easily released from sediments in one day incubation. The successive extraction method exhibited a good repeatability and improved sensitivity, which was applied to HBAP and BAP measurement of SS in the river water entering Akanoi Bay in Lake Biwa and bottom sediments in the bay. From the results of the seasonal changes, HBAP and BAP in SS exhibited a large influence by cultivation of paddy field in the basin. BAP and HBAP in the sediment of Akanoi Bay that were accumulated in the surface layer were also influenced by phosphorus entering the bay.

KEYWORDS: Bioavailable phosphorus, eutrophication, lake water management, HBAP, phosphorus extraction, agriculture runoff

INTRODUCTION

Although phosphorus amount entering a lake is usually estimated as total phosphorus (TP), it is more important to estimate the phosphorus amount which is utilized by algae and causes lake eutrophication. Dissolved orthophosphate (PO₄-P) is well known to be able to be utilized by algae. Some fraction in particulate phosphorus which is able to be utilized by algae is measured as bioavailable phosphorus (BAP). It is one of the important substances causing lake eutrophication. Although the sum of PO₄-P and particulate bioavailable phosphorus should be utilized by algae, we name BAP in this paper only for bioavailable phosphorus in particulate fraction. BAP has been measured by two methods; biological method and chemical method (Okubo, 1996). Cultivation of green algae (mainly *Selenastrum*) has been carried out in the biological method of BAP measurement (Hosomi and Sudo, 1979; Williams *et al.*, 1980; Dorich *et al.*, 1980, 1985; Ekholm and Krogerus, 1998). This method is superior that it measures the potential of algal growth directly as AGP (algal growth potential) test. However it takes several weeks to get to the maximum growth of the algae. Mainly 0.1N NaOH extraction is used in the chemical method of BAP measurement based on the fractionation of soil phosphorus (Dorich *et al.*, 1985; Butkus *et al.*, 1988; Parker, 1991; Sharpley *et al.*, 1991, 1992). Phosphorus extracted by this method is thought to be iron combined or aluminum combined amorphous phosphate substances (Chang and Jackson, 1957; Williams *et al.*, 1967). Good relationships were shown between the chemical method and the biological method (Williams *et al.*, 1980; Dorich *et al.*, 1980, 1985; Butkus *et al.*, 1988; Sharpley *et al.*, 1991).

Sharpley *et al.* (1991; 1992) proposed a chemical method to measure BAP in water sample containing SS by 17 hours extraction after adding NaOH solution to adjust the concentration in the sample to be 0.1N. We followed the Sharpley's method to use 0.1N NaOH to extract BAP in this study. Moreover we attempted to improve the sensitivity of the measurement by collecting SS by filtration procedure. Because the ratio of non-apatite inorganic phosphorus is high in Japanese soil (Sekiya, 1973), the BAP / TP ratio is expected to be rather high. Therefore we also attempted to measure more easily soluble and bioavailable fraction (HBAP; highly bioavailable phosphorus) in BAP measurement. Salt solution (NH₄Cl) or weak acid (2.5% acetic acid) have been used in the successive extractions of soil phosphorus fractions (Chang and Jackson, 1957; Williams *et al.*, 1967; Sekiya, 1973). Besides, successive 0.1N HCl and 0.5N NH₄OH extraction has been used to measure organic phosphorus in soil and lake sediment (Pearson, 1940; Nakajima *et al.*, 1979; Nakajima and Kaneko, 1988). The inorganic phosphorus extracted by 0.1N HCl was estimated to be metal combined phosphorus or polyphosphate by applying this successive extraction to activated sludge (Nakajima and Kaneko, 1987).

In this study, therefore, we firstly attempted to introduce 0.1N HCl extraction procedure before 0.1N NaOH extraction in BAP measurement in order to extract phosphorus loosely combined with metal salts on the surface of SS and sediments, and examined to use the 0.1N HCl extract fraction as HBAP. Secondly from the results of this examination a new chemical method to measure BAP as well as HBAP was proposed. Thirdly we applied this method to measure HBAP and BAP of suspended solid (SS) in the rivers entering a bay in Lake Biwa and sediment in the bay.

MATERIALS AND METHODS

Samples

Surface water samples for SS measurement were taken at 6 points (W2 -W7) in rivers and channels entering Akanoi Bay in Lake Biwa at 18 times during October 2000 to September 2001. A lake water sample at Yabase Bridge (W1) in the southern part of Lake Biwa was also taken on the same days. The water samples brought back to the laboratory in polyethylene bottles were immediately filtered through a glass fiber filter (Advantek GS25). The SS collected on the filter

was taken as the wet sample and the dried sample was made by drying the wet sample with the filter at 105 C. Sediment core samples of 30cm depth from the surface were collected at three points (M1, M2, M3 from west to east) in Akanoi Bay every two months during June 1999 to May 2000 by using core sampler. M1 and M2 were inside the bay and M3 was close to the mouth of the bay. The core samples at the mouth of Tenjin River (M4) entering the bay was also collected at April and May 2000. The core samples were divided to portions of 0-2cm depth, 2-4cm depth, 6-8cm depth, 10-12cm depth, 14-16cm depth and 18-20cm depth from the surface. Each portion well mixed was taken as the wet sample and the dried sample was made from the wet sample by drying at 105C and grinded. Soil samples in the paddy fields in the Akanoi Bay basin at five points were also taken and grinded after drying at 105C.

Extraction method

HBAP and BAP were extracted from the samples fundamentally according to Pearson (1940) as follows. A portion of the sample (SS sample attached to a filter or 1g of sediment) was put in a 140mL PTFE bottle. 50 mL of 0.1N HCl was added in the bottle and after shaken for 3 minutes the acid extract was obtained by filtration (Advantek No.5C). HBAP was determined as PO₄-P in the acid extract after neutralization. The residue of the filtration with the filter was put into the bottle again and 100 mL of 0.1N NaOH was added in the bottle. After shaken 17 hrs the alkali extract was obtained by filtration. NaOH-P was determined as PO₄-P in the alkali extract after neutralization. BAP was obtained as the sum of HBAP and NaOH-P. HBAP and BAP were also expressed as P content in dry weight (mg/g) as well as P concentration in water (mg/L) for SS sample.

Examination of the extraction condition

For the examination of the sample preparation wet samples and dried samples made from four SS samples and four sediment samples were used and extracted to determine HBAP and BAP. Triplicate examination was carried out for each sample. For the examination of the extraction time two kinds of dried sediment samples were used. One gram of the sample was put in the bottle and extracted during 3 minutes, 10 minutes, 30 minutes, 1 hour, 4 hours and 17 hours using 50 mL of 0.1N HCl or 100 mL of 0.1N NaOH. Duplicate examination was carried out for each extraction. For the examination of the amount of the solvent two kinds of dried sediment samples were used. One gram of the sample was put in the bottle and extracted by 50 mL, 100mL, 200mL and 500mL of 0.1N HCl or 0.1N NaOH during 3 minutes for 0.1N HCl and during 17 hours for 0.1N NaOH, respectively. Triplicate examination was carried out for each condition.

Phosphorus release examination

Two wet sediment samples were used for the examination of phosphorus release in water. One gram of sample and 100mL of distilled water were put in a polyethylene bottle and the bottle was incubated by shaken in a dark place at 25C. The liquor in the bottle was filtered using a glass fiber filter (GS25) at the beginning and after 1, 3, 5, 7, 14 days. The sediment sample on the filter was dried at 105 C and HBAP and BAP in the dried sample was determined. Duplicate sample was used for each incubation period.

Phosphorus determination

PO₄-P was determined by colorimetric method using ascorbic acid as a reductant (Harwood *et al.*, 1969a). Total phosphorus (TP) was determined by potassium persulfate oxidation method for water samples (Menzel and Corwin, 1965) and by hydrogen peroxide digestion method for sediment samples (Harwood *et al.*, 1969b).

RESULTS AND DISCUSSION

Condition of the extraction

In order to discuss the influence of drying sample before the acid and alkali extraction, the amounts of phosphorus determined using wet samples or dried samples were compared. For HBAP and BAP in SS samples the difference between the cases of using the wet sample and the dried sample was small. The difference between the two cases was not significant in t-test ($P > 0.05$) except 3 cases among 12 examinations. The difference between the wet sample and dried sample was also not significant in t-test ($P > 0.05$) for the sediment samples except 3 cases among 10 examinations. These conclude that sample drying does not influence the amount of extracted phosphorus significantly in the case of SS in the river water and the lake sediment. Therefore we decided to use dried samples because the extraction procedure is able to follow the measurement of SS.

The change of extracted phosphorus by 0.1N HCl with time showed that a part of phosphorus fraction was extracted rapidly in the first 3 to 10 minutes. The ratio of this rapidly extracted fraction to the total extracted phosphorus after 17 hours extraction ranged from 33% to 60% and suggested to be changed greatly with samples. This fraction was considered to be amorphous metal phosphate salt or co-precipitated phosphate with metal hydroxides. On the contrary phosphorus extracted slowly in several hours by 0.1N HCl seemed to include crystal structure calcium phosphate such as apatite. Because the apatite type phosphorus is known to be hard to be dissolved in water, the 0.1N HCl extraction time should be short not to measure apatite type phosphorus. Therefore we decided the extraction time by 0.1N HCl to be 3 minutes.

The change of extracted phosphorus by 0.1N NaOH with time exhibited a rapid increase followed by slow increase. The rapidly extracted fraction seemed to be almost same as 0.1N HCl extract fraction for 3 minutes. Because the increase became slow after 17 hours, we decided the extraction time by 0.1N NaOH to be 17 hours according to Pearson(1940) and Sharpley *et al.* (1991).

The amount of extracted phosphorus from 1g of the sediment samples by 50 ml - 500 ml of 0.1N HCl and 0.1N NaOH tended to increase slightly according to the increase of the amount of the solvent. However the increase of extracted

phosphorus was small compared to the increase of the solvent. Therefore we concluded that 50 ml of 0.1N HCl and 100 ml of 0.1N NaOH was enough for the extraction.

PROPOSAL OF SUCCESSIVE EXTRACTION BY ACID AND ALKALI

The extract condition was decided as described above. By using this extraction procedure the reproducibility of phosphorus determination in the extract was good and the relative error was almost less than 10%. The detection limit for SS sample that depends on the volume of the sample is 0.002 mg/L and 0.003 mg/L for 0.1N HCl and 0.1N NaOH extraction respectively when 1 L of sample is used. The detection limit will be improved by increasing the sample volume. The detection limit for sediment sample is around 0.01 mg/g for both of 0.1N HCl and 0.1N NaOH extraction if the sample weight and the portion volume for detection of PO₄-P are 1g and 5mL - 10mL, respectively. This value is usually sufficiently lower than actual extractable phosphorus in sediments and soils. Sharpley *et al.* (1991) proposed the extraction of BAP in SS by adding NaOH solution to set sample water concentration to 0.1N. A detection limit of this method is around 0.03 mg/L. It is therefore clear that the proposed method with collection SS by filtration is superior to the Sharpley's extraction in sensitivity of detection.

Table 1 shows the comparison of extracted phosphorus by 0.1N HCl - 0.1N NaOH successive extraction and 0.1N NaOH - 0.1N HCl successive extraction. Although the amount of phosphorus extracted by 0.1N NaOH after 0.1N HCl extraction was large, the amount of phosphorus extracted by 0.1N HCl after 0.1N NaOH extraction was small. This indicates that the phosphorus extractable by 0.1N HCl is able to be extracted by 0.1N NaOH during 17 hours. Therefore the sum of phosphorus extracted by 0.1N HCl extraction and 0.1N NaOH extraction by our proposed condition described above must be almost same as BAP by Sharpley's method. Hence it is reasonable to determine BAP as the sum of 0.1N HCl and 0.1N NaOH extraction phosphorus in our proposed extraction condition.

Table 1 Extracted phosphorus by 0.1N HCl (1 hour) - 0.1N NaOH (17 hours) and 0.1N NaOH (17 hours) - 0.1N HCl (17 hours) successive extractions using four sediment samples (A - D).
average \pm standard deviation (mg/g)

	0.1N NHCl extract (1 hour)	-->	0.1N NaOH extract (17 hours)			0.1N NaOH extract (17 hours)	-->	0.1N HCl extract (17 hours)
A	0.31 \pm 0.05	-->	0.60 \pm 0.02		C	0.49 \pm 0.02	-->	0.10 \pm 0.001
B	0.07 \pm 0.003	-->	0.14 \pm 0.01		D	0.22 \pm 0.01	-->	0.06 \pm 0.003

The amount of phosphorus extracted by 0.1 N NaOH has a high percentage of T-P in many SS and sediment samples. Moreover a high content of phosphorus extracted by 0.1 N NaOH in sediment samples indicates that a large part of BAP sinks into the lake bottom without uptake by microorganisms. Because it seems to be necessary to measure a fraction in BAP that is more easily available by microorganisms in water, we propose HBAP as highly bioavailable phosphorus by measuring phosphorus that is extracted in 3 minutes with 0.1N HCl. HBAP is a part of BAP that is most easily extractable fraction by 0.1N HCl as well as 0.1N NaOH. This fraction is considered to be amorphous metal phosphate salt or phosphate coprecipitated with metal hydroxide.

In order to verify that HBAP is easily soluble in water, phosphorus release examination was carried out using sediment samples. The percentages of HBAP and NaOH-P remained in the samples is shown in Fig. 1 during 14 days incubation. HBAP decreased rapidly to 30 - 40% in one day incubation and decreased gradually after that. On the contrary the decrease of NaOH-P in one day incubation was small compared with HBAP and a high percentage of NaOH-P was remained even after 14 days incubation. From this result showing that most part of HBAP (60 - 70%) was released in one day, it is clear that HBAP represents phosphorus fraction easily soluble in water. Therefore it is reasonable to determine HBAP as phosphorus extracted by 0.1N HCl for 3 minutes as the easily soluble fraction seems to be easily bioavailable.

Fractionation of phosphorus in river water

It is possible to fractionate phosphorus in river water simply and rapidly as shown in Fig. 2 by applying the successive extraction by acid and alkali. Sample water (0.5 L - 1.5 L) is filtered through a glass fiber filter (GS25) and phosphate phosphorus in the filtrate is determined as PO₄-P. Dissolved phosphorus (DP) in the filtrate is determined by potassium persulphate digestion method and dissolved organic phosphorus (DOP) is determined as the difference between DP and PO₄-P. On the other hand, the filter is dried at 105C and SS is measured. After measuring SS the filter is put into a PTFE bottle and extracted by 0.1N HCl during 3 minutes to measure HBAP. The residue is extracted with 0.1N NaOH during 17 hours to measure NaOH-P. BAP is determined as the sum of HBAP and NaOH-P. TP in the water sample is measured by potassium persulfate digestion method and particulate phosphorus (PP) is determined as the differences between TP and DP. Residual phosphorus (Res-P) is determined as the difference between PP and BAP.

Phosphorus in water sample is fractionated into 5 fractions; PO₄-P, DOP, HBAP, NaOH-P and Res-P by using this fractionation. This fractionation is based on solubility but also considered on bioavailability. The bioavailability is considered in order of PO₄-P, HBAP, and NaOH-P (or BAP).

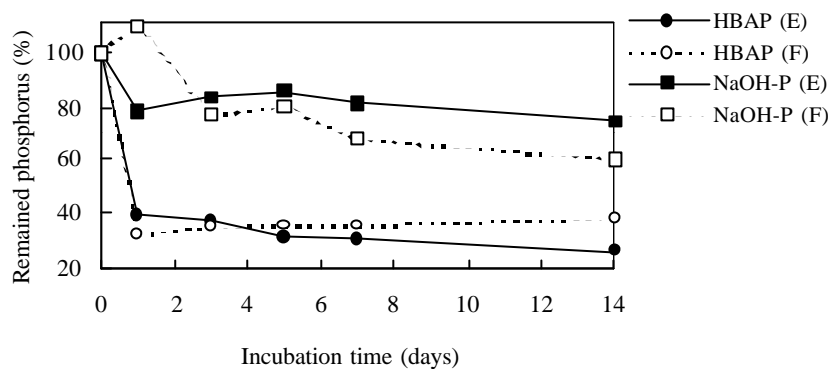


Fig. 1 Changes in the remained phosphorus ratio in the release examination using two sediment samples (E and F).

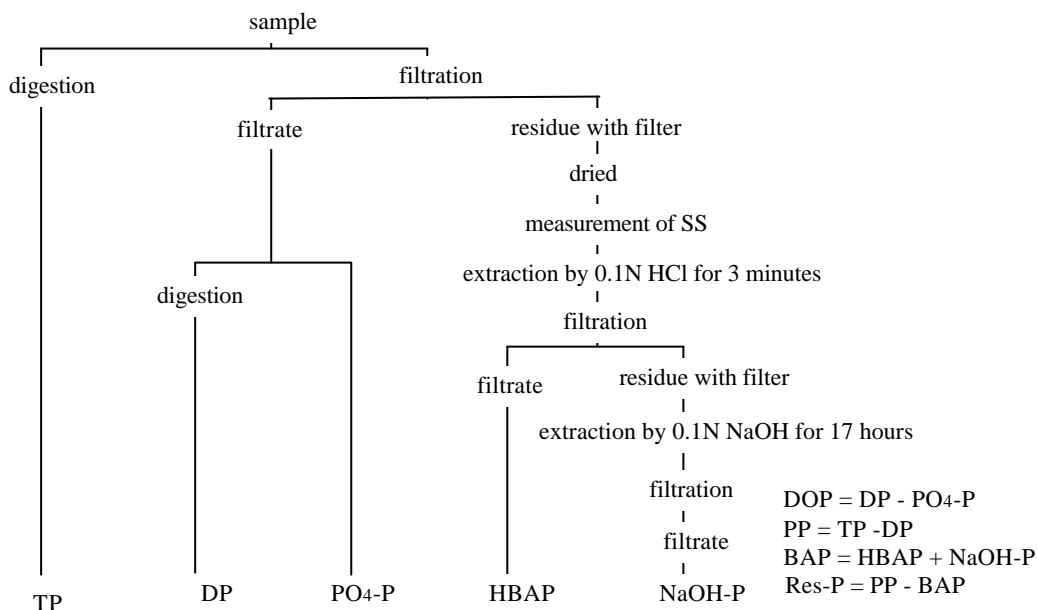


Fig. 2 Fractionation flow of phosphorus in water.

HBAP AND BAP IN RIVER WATER ENTERING AKANOI BAY

The concentration ranges of HBAP and BAP were 0.002 - 0.081 mg/L and 0.005 - 0.29 mg/L, respectively, and the difference by the river and its seasonal change were large. The paddy field area is large in the Akanoi Bay basin and the water quality of the rivers is influenced by the farm works in the paddy fields (Okubo *et al.*, 1999). Since the water quality increased at the term of tilling, water filling and rice planting, the 18 sampling periods were divided into three terms; A: tilling, water filling and rice planting term (the last week of April - the first two weeks of May), B: the other irrigation term (May - August) and C: non-irrigation term (September - April) according to the local agricultural time table (Konan Agricultural Management Center, 2001). Moreover, the sampling point were classified into three groups; the point (W2, W4, W6, W7) where the influence of the agricultural effluent from paddy field was clear, the point (W5) where the influence of an agricultural effluent and drainage from the household was clear and the point (W3) whose influence from an agricultural effluent was small. As an example of the point where the influence of an agricultural effluent was seen, the changes in SS, TP, PO₄-P, HBAP, and BAP in W2 are shown in Fig. 3. A remark increase was seen in SS and the phosphorus fractions from the end of April to the beginning of May. It was clear that SS increased because of tilling, filling water and rice planting at that period (Okubo *et al.*, 1999). The average concentrations were higher in order of the term A>B>C. The same tendency was recognized at W4, W5, W6, and W7. The increase of SS and TP at the A term had been well known and our results showed the corresponding increase of bioavailable phosphorus such as HBAP and BAP. On the other hand, the difference among A, B and C terms was small at W3 where the influence of an agricultural effluent was small. PO₄-P was always high at W5 and it was because of the influence of household wastewater. DOP and Res-P did not show apparent tendency of the seasonal changes. The percentages of HBAP and BAP to T-P were 1.0 - 24%, and 5.8 - 54%, respectively. The percentages did not show apparent seasonal change. HBAP and BAP in W1 that did not influenced by agricultural effluent exhibited seasonal change because of the change of SS component such as planktons.

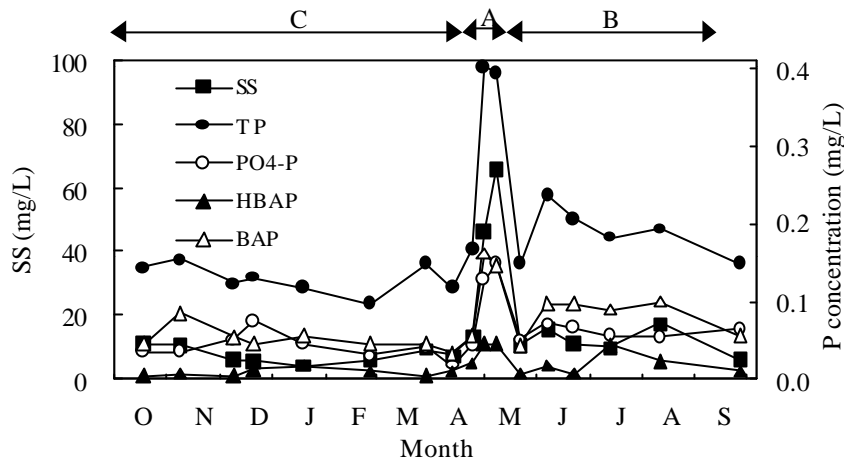


Fig. 3 Seasonal changes in the fractionated P and SS concentrations at W2. A: the term of tilling, filling water and rice planting, B: the other irrigation term and C: non-irrigation term.

The HBAP and BAP contents in SS ranged from 0.20 - 8.6 mg/g and 0.69 - 14 mg/g, respectively. Their contents had the tendency of A<B and C that indicated the seasonal difference of SS component. They decreased at the term of tilling, filling water and rice planting especially in W2, W4, W6 and W7. This is because the increase of SS concentration was larger than the increase of HBAP and BAP concentrations. The increase of SS was resulted from the flows of the paddy soil and the phosphorus content of the paddy soil was low compared with SS particles as shown in Table 2. SS particles contains not only soils but also planktons, detritus of plants and/or SS came from urban sewage whose phosphorus content were suggested to be higher than soils. Although HBAP and BAP contents will decrease at the term of tilling, filling water and rice planting, their loadings to Akanoi Bay and Lake Biwa will increase because of the increase of SS loading.

Table 2 HBAP and BAP content in SS (W1 - W7) and paddy soils.

		W1	W2	W3	W4	W5	W6	W7	Paddy Soils
HBAP	average	0.47	1.46	0.72	0.97	2.98	1.90	2.04	0.15
(mg/g)	SD	0.16	1.11	0.50	0.49	1.27	1.12	1.92	0.04
BAP	average	1.40	6.36	3.98	4.40	6.89	6.95	4.52	0.61
(mg/g)	SD	0.47	2.73	2.36	2.00	1.69	2.79	3.33	0.14

HBAP AND BAP IN SEDIMENTS IN AKANOI BAY

The average contents of BAP and HBAP in the surface sediments were 0.08 - 0.42 mg/g and 0.31 - 2.4 mg/g, respectively. HBAP as well as BAP showed higher at the river mouth (M4) and lower at the mouth of the bay (M3). The horizontal distribution of HBAP and BAP in the surface sediment suggested that phosphorus entered the bay from the river, settled at the river mouth and was accumulated in the sediment. On the contrary low contents in the sediment at M3 suggested that HBAP and BAP concentrations were diluted by the lake water outside the bay. The vertical profiles of HBAP and BAP contents at M1 and M2 were almost similar as shown in Fig. 4 as an example in October. They decreased rapidly from the surface to the 10 cm depth and almost stable in the depth of 10-20 cm. HBAP was about 0.1-0.2 mg/g in the surface layer and 0.05 mg/g in the lower layer. BAP was about 0.6 - 1.0 mg/g in the surface layer and 0.2 mg/g in the lower layer. The accumulation of HBAP and BAP suggested phosphorus supplied from the lake water to the surface sediment. On the other hand the difference between the surface sediment and the lower layer was small at M3 (also shown in Fig.4). M3 that tends to be influenced by lake water outside the bay and winding up the sediment seemed to result in small change of the vertical profile of HBAP and BAP. The accumulation of BAP was much higher than HBAP in the surface sediments. This suggested that HBAP had been already released from SS before sedimentation or easily released from sediment to lake water after sedimentation while BAP (especially NaOH-P) tended to be retained in SS and sediment. It also supports our proposal that HBAP is easily soluble and bioavailable fraction in BAP. In M4 HBAP and BAP were extremely high and it indicated quick sedimentation of particulate phosphorus which flowed into the bay at the river mouth.

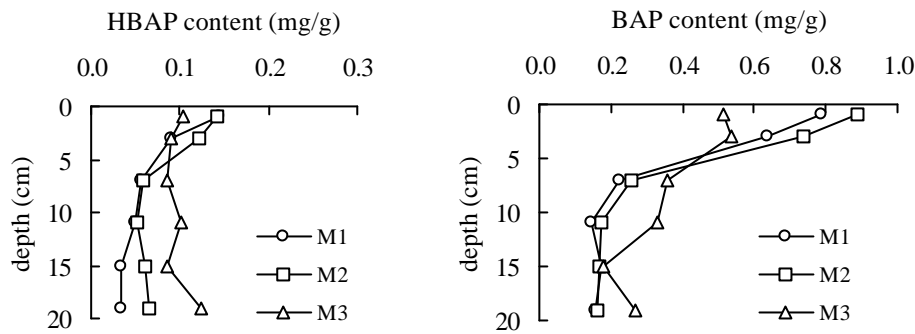


Fig. 4 Vertical profiles of HBAP and BAP at M1, M2 and M3 in Akanoi Bay (October, 1999).

The seasonal change of HBAP and BAP contents in the sediments was not clear except HBAP in the surface sediments that showed slight increase at May in M1 and M2 as well as M3. The increase of BAP however did not detected in this period. This period was just after the term of tilling, filling water and rice planting. Therefore the surface sediment seemed to be influenced by the erosion from a paddy field. This influence was apparent not in BAP but in HBAP. It suggests that HBAP indicates the increase of phosphorus load entering lake more sensitively than BAP.

CONCLUSIONS

- (1) By using the successive extraction by acid and alkali, BAP and HBAP which is most easily soluble fraction in BAP were simply determined.
- (2) From examination of extraction conditions, HBAP was measured by extraction with 50mL of 0.1N HCl during 3 minutes followed by extraction with 100mL of 0.1N NaOH during 17 hours to measure NaOH-P successively. BAP was obtained as the sum of HBAP and NaOH-P.
- (3) By collecting SS on a filter the detection limit was improved to be 0.002 mg/L and 0.003 mg/L for BAP and NaOH-P, respectively using 1L of sample water.
- (4) HBAP was verified to be more easily dissoluble fraction by phosphorus release examination using sediment samples.
- (5) New fractionation method of phosphorus in river water was proposed using the successive measurement of HBAP and BAP. The bioavailability is supposed to be high in order of PO₄-P, HBAP and BAP by using this method.
- (6) HBAP and BAP in SS in the rivers entering Akanoi Bay of Lake Biwa were high in order of the tilling, water filling and rice planting term, the other irrigation term and the non-irrigation term. Not only PO₄-P but also HBAP and BAP increased by the effluent from paddy fields.
- (7) BAP was more highly accumulated in the surface sediment in Akanoi Bay than HBAP. This suggested that HBAP had been already released from SS before sedimentation or easily released from sediment.

ACKNOWLEDGMENTS

This research is partially supported by Lake Biwa Research Institute, Shiga Prefecture. The authors also thank to Yukiko Ohya, Naomi Akita, Michiko Sakamoto and Toshihiro Nakamura for their help to the study.

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