Extraction of bioavailable phosphorus in soils and sediments using an ultrasonic washing machine

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ABSTRACT

For improving the management of watershed eutrophication, methods for measuring bioavailable phosphorus (BAP) are more important than measurements of total phosphorus (TP). BAP in particulate form (P-BAP) is an important substance that promotes eutrophication, especially during rainy seasons. Only a portion of particulate phosphorus (PP) is taken up by algae that contribute to eutrophication. Erosion and runoff associated with rainfall transport PP bound to sediments and soil particles to surface waters, thus increasing PP concentration. This research evaluated an extraction method using an ultrasonic washing machine for extraction time and frequency. Extraction at a frequency of 28–45 kHz and an extraction time of 1 min resulted in extracted P concentrations almost the same as concentrations extracted using conventional methods. This new method requires less time and is more efficient than conventional methods because it extracts P from multiple samples in a single step. Results indicate that extraction using an ultrasonic washing machine is a promising method for rapidly obtaining BAP from sediments and soil particles.

Key words | bioavailable phosphorus, particulate phosphorus, soil and sediments, ultrasonic washing machine

HIGHLIGHTS

- Extraction of BAP is evaluated by using ultrasonic washing machine.
- Frequency of 28–45 kHz with extraction time of 1 min is suitable for extraction.
- This new method is highly correlated with conventional method.
- Ultrasonic washing machine extracts P from multiple samples in a single step.
- Extraction using this machine is a promising method for rapidly obtain BAP from suspended solids.

INTRODUCTION

Eutrophication is a common problem in rivers and lakes, and also in closed coastal areas. This phenomenon causes the degradation of aquatic ecosystems and accompanying losses in biodiversity and economic resources (Huang et al. 2017). Eutrophication is triggered by excessive input of phosphorus (P) and nitrogen (N) (Serrano et al. 2017), typically derived from human activities in the watershed. Excess nutrients stimulate rapid growth of harmful algae and aquatic plants (Dupas et al. 2015; Liu et al. 2015; Wang et al. 2018). P is usually the growth-limiting nutrient because it is normally in short supply in freshwater systems (Serrano et al. 2017; Zhu et al. 2018). Phosphorus is not present in the atmosphere, and aquatic organisms rely on phosphorus from soils, fertilizers or phosphorus-containing compound to maintain a balanced ecosystem (Antikainen et al. 2004). In nature, most phosphorus is found in the form of phosphate ions. Phosphate compounds found in soils, fertilizers and other compound may be carried in surface runoff to rivers, lakes, and oceans, where they are taken up by aquatic organisms.

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Total phosphorus (TP) is one of the water quality parameters used to assess environment health and manage eutrophication in Japan (Japanese Ministry of the Environment). However, only certain types of TP are bioavailable for algae to contribute to eutrophication. TP can be divided into dissolved phosphorus (DP) and particulate phosphorus (PP). DP is readily available for algal uptake, but PP is only partially bioavailable. The P fraction which is readily available for algal uptake and contributes to eutrophication is termed bioavailable phosphorus (BAP) (Ellison & Brett 2006). P has a strong affinity for particulate matter. Thus, PP bound to sediment and soil particles comprises the majority of P in surface runoff (Inoue & Ebise 2019). Erosion and runoff transports PP bound to sediments and soil particles to surface waters leading to a predominance of PP in these systems (Cavalcante et al. 2018).

Methods for measuring BAP in particulate form are thus more important than TP for management of eutrophication. However, current methods for estimating BAP are time-consuming. BAP is currently measured using one of two methods, biological or chemical. The algal growth potential test (AGP) (Ngoc et al. 2017b), for determining algal yield is the most direct biological method for estimating bioavailability of PP. The AGP test requires an incubation time of more than 14 days and is not suitable for evaluating BAP in large numbers of samples. Chemical methods, such as a single extraction with 0.1 M NaOH has been suggested by Sharpley et al. (1991). This method is often used to measure P-BAP, even though extraction requires 17 h.

Recently, ultrasonication has been used for extraction of various elements from food and environmental samples. The ultrasonic extraction is based on the principle of acoustic or ultrasonic cavitation, combined with their ability to transfer energy to attached substances into the extractant (Dolatowski et al. 2007). This method requires less time and shows high efficiency (Song et al. 2013). Ngoc et al. (2017a) proposed a method to measure BAP from PP by ultrasonication that requires only a 1-min extraction, much faster than the 17 h required for the conventional chemical method (Ngoc et al. 2017a, 2017b). BAP is extracted from sediments by using 0.1 M NaOH combined with ultrasonication produced by an ultrasonic horn (Ngoc et al. 2017b). This extraction method processes a single sample in each operation, which limits the number of samples that can be extracted and makes the method unsuitable for routine laboratory analysis. Therefore, an ultrasonic extraction method using an ultrasonic washing machine was investigated in this paper. This technology is typically used for medical devices, electronics, and foods that require an extremely high degree of cleanliness (Niemczewski 2009). Most experimental laboratory apparatus are cleaned using an ultrasonic washing machine: cavitation generated by the machine offers complete and rapid removal of dirt and debris (Tangop & Thongri 2019). Use of a machine thus requires less time and provides higher efficiency than conventional methods, quickly processing multiple samples in a single step. To develop a simple and convenient method, suitability of an ultrasonic washing machine to quantify BAP in particulate form was investigated using soil and sediments from rivers and lakes.

**METHODS**

**Sample collection**

Soil samples were collected from the surface to a depth of 5 cm at a household garden. This soil showed the lowest P concentrations. Lake sediment (standard sediment: NIES CRM No. 31) collected from Lake Kasumigaura in Ibaraki Prefecture was purchased from the National Institute for Environmental Studies, Japan to control the accuracy of pretreatment and instrumental analysis. River sediment was collected from the River Shio in Aichi Prefecture in Central Japan. This river shows the highest concentrations of bioavailable phosphorus among rivers that empty into Mikawa Bay, based on preliminary measurements. River sediment was collected from the surface to a depth of 5 cm at riverbed by using a scoop to fill a clean sampling bottle. Every bottle was tightly capped and immediately transported to the laboratory.

The soil and river sediment were air-dried at 4 °C for 3 days and then sieved through a 0.149-mm-mesh screen to remove larger particles such as plant fibers. All samples were stored at 4 °C before analysis.

**Particulate organic carbon (POC), particulate organic nitrogen (PON) analysis**

POC and PON concentrations were measured by combusting a known amount of target sample using an nitrogen-carbon analyzer (Sumigraph NC-22A, Sumika Chemical Analysis Service, Ltd, Tokyo, Japan).

**BAP extraction**

**Sharpley’s chemical extraction method (control 1)**

Sharpley’s extraction method (Dorich et al. 1985; Sharpley et al. 1991) was used as Control 1 to quantify BAP
concentrations in all samples in a single-step chemical extraction. This method effectively estimates BAP in agricultural runoff at a ratio of target samples to extractant of 2 mg mL\(^{-1}\).

Fifty mg of dried sample was placed in a 50-mL centrifuge vial that contained 25 mL of 0.1 M NaOH. Vials were then shaken mechanically for 17 h on a multi-shaker (MMS-210 Orbid Multi-Shaker, EYELA, Tokyo, Japan) at 160 rpm.

Ngoc’s ultrasonic method (control 2)

Ngoc’s ultrasonic method (Ngoc et al. 2017a, 2017b) was used as Control 2 to quantify BAP concentrations in all samples. Dried samples were extracted with 0.1 M NaOH combined with ultrasonication (Ultrasonic Disruptor Sonifier II, model W-450, Branson, CT, USA) at an intensity of 30 W. The ratio of NaOH to target sample was 1 mg mL\(^{-1}\), 25 mg of dried sample in 25 mL of 0.1 M NaOH. Sonication time was 1 min. During ultrasonication, the ultrasonic horn, which has a fixed operating frequency of 20 kHz, was placed 1 cm from the bottom of centrifuge vial and did not contact vial walls to prevent generator overload and broken vials. During treatment, the centrifuge vial was fully immersed in an ice-water bath to avoid heating.

Proposed method

A new extraction method uses an ultrasonic washing machine commonly found and used for laboratory analysis. Experiments employed different frequency and power output (Table 1) to optimize extraction conditions. Two appliances, Machine A (Ultrasonic cleaner Mus-10D, EYELA, Tokyo, Japan) and Machine B (Compact 3 frequency desktop ultrasonic cleaner W-113, Honda, Tokyo, Japan) were used. Both machines have the frequency range of 20 kHz–45 kHz, commonly used for cleaning labware. The proposed method placed 25 mg of dried sample in a 50 mL centrifuge vial with 25 mL of 0.1 M NaOH as an extractant. Centrifuge tubes were then immersed inside ultrasonic washing machines for ultrasonic treatment. At various frequencies and intensities. The water level in machines maintained at the same levels as solutions in extraction vials.

### Centrifugation and pH adjustment

Extractions from all methods were then centrifuged at 1,000 rpm (Table-top centrifuge model 5100 with an RS-4 universal swing rotor, Kubota, Tokyo, Japan) for 5 min. The clear supernatant (0.1 M NaOH extract) in each vial was collected for P measurement. Residues were washed twice with 25 mL of 0.1 M NaOH for 10 min each using an orbital mechanical shaker. Clear supernatants from these washes were collected after centrifugation at 1,000 rpm for 5 min. These extracts were neutralized with 1 M HCl or 1 M NaOH for P analysis. For all samples, analyses were performed in triplicate.

### Analysis of phosphorus

The concentration of P was measured colorimetrically in supernatants using the molybdenum blue method (Flow injection analyzer, OG-FI-300S, Ogawa & Co., Ltd, Japan). TP was measured by adding 10 mL of distilled water to samples along with potassium persulfate (K\(_2\)S\(_2\)O\(_8\)). Samples were digested in an autoclave (MLS-3750, Sanyo, Osaka, Japan) at 120 °C for 30 min to solubilize all phosphorus.

### Statistical analysis

Two-way analysis of variance (ANOVA) and t-tests were used to compare extraction performance of washing machines with controls. Linear regression analysis was performed to address similarities between P concentrations extracted by control groups and the proposed method under various conditions. Assuming no significant difference between methods, the significance level was set at 5% to obtain more accurate results. A probability (p-value) of <0.05 was regarded as statistically significant.

### RESULTS AND DISCUSSION

#### Properties of samples

POC and PON are important elemental components of particulate organic matter in surface water that influence the availability of P-BAP (Inoue & Ebise 1991; Ellison & Brett 2006). Fractions of POC, PON, and PP in samples are shown in Figure 1.
POC in the soil was lowest among three samples, 1.36%. POC in lake and river sediment were 3.18% and 3.20%, respectively. These samples contain virtually identical levels of organic matter. Furthermore, PON in the lake and river sediment was also similar, 0.37% and 0.41%, respectively. The amount of organic matter in the soil was less than sediments and similar between sediments. C to N ratios show little difference among samples (Figure 2). The C:N ratio from soil, lake sediment, and river sediment were 6.6, 8.6, and 7.8, respectively. C:N ratio is used to characterize origins of organic matter in rivers. Phytoplankton is rich in nitrogen compounds and low C:N ratios of sediments indicate a dominance of marine organic matter. Therefore, three target samples had low C:N ratio which are thought to indicate planktonic sources.

PP content in the target samples was: soil, 0.04%; standard sediment, 0.05%; and river sediment, 0.19%. Available phosphorus content of river sediment was thus about four times higher than availability in soil and lake sediment. Phosphorus in the river system is mainly present as phosphate ion, which chemically bonds to calcium, iron, and aluminum to form PP. This P tends to accumulate in river sediment. Further, during rain events, increase river flow entrains more particulate matter, including particulate carbon and nitrogen associated with organic matter (Saunders & Lewis 1998; Mbabazi et al. 2019). Thus, river sediment often shows relatively high levels of phosphorus. C:P ratios calculated were: soil, 36.4; lake sediment, 62.6; river sediment, 17.1 (Figure 2). The C:P ratio in river sediment is lowest, indicating the highest relative P content.

Based on Redfield ratio (Tyrrell 2019), the C:N:P ratio remains relatively constant in marine phytoplankton, and this ratio in primary producers constrains cycling of all elements. For phytoplankton, a ratio of 41:7.2:1 is applicable (Richards 1965; Beiras 2018). Since C:P ratio of organic matter in samples is constant, inorganic phosphorus is higher than organic phosphorus in river sediment, and organic carbon is higher than phosphorus in lake sediment.

**Extraction time**

The effect of the extraction time on the performance of ultrasonic washing machines was evaluated by using Machine A with a fixed frequency of 38 kHz /120 W (Table 1) because it is commonly used for laboratory analysis. Extraction times were 0, 1, 5, and 10 min. Extracted P concentration in soils and lake sediments increased as the extraction time increased (Figure 3). In the river sediments, P concentrations extracted for 1 and 5 min was essentially the same, 1.082–1.083 mg/g. However, it increased to 1.177 mg/g when the extraction time is 10 min.

The concentration of BAP extracted by machine A, extraction time 1 and 10 min were: 0.178 mg/g and 0.239 mg/g for soil; 0.302 mg/g and 0.355 mg/g for lake
sediment; and 1.082 mg/g and 1.177 mg/g for river sediment (Table 2). In overall, concentrations of extracted P increased significantly with extraction time. Increased extraction time generates heat due to the long period of cavitation (Niemczewski 2009). Also, as temperature increases, molecular vibration of phosphorus adsorbed to suspended matter increases, which will enhance desorption (Tao et al. 2020). Some non-bioavailable phosphorus is more likely to be extracted under these conditions. Therefore, an extraction time less than 10 min is appropriate.

A significant difference between control and proposed method depending on extraction time. At 10 min, a significant difference was confirmed between the proposed method and both Control 1 and Control 2 on the soil sample with the lowest concentration of BAP. No significant differences were found using extraction times of 1 and 5 min. To increase the accuracy of the proposed process, a shorter extraction time of 1 min is appropriate. In support, linear regression analysis between controls and P concentration extracted in 1 min by Machine A showed slopes of 1.03 for control 1 and 0.98 for Control 2. R² was 0.990–0.996 for regression (Figure 4). This strong statistical relationship indicates that extractable phosphorus using an ultrasonic washing machine is almost identical to phosphorus extracted in Control 1 and Control 2 using a conventional chemical method and an ultrasonic horn, respectively. Moreover, no statistically significant differences were found with an extraction time of 1 min. These results show that effective extraction of BAP can be accomplished in 1 min using ultrasonication.

**Frequency**

The effect of ultrasonic frequency on the concentrations of extracted P was evaluated using Machine B that allows frequency adjustments of 28, 45, and 100 kHz. Extraction time was 1 min. Concentrations of BAP extracted by Machine B for frequencies of 28, 45 and 100 kHz were: 0.197 mg/g, 0.213 mg/g and 0.208 mg/g for soil; 0.309 mg/g, 0.270 mg/g and 0.237 mg/g for lake sediment; and 1.059 mg/g, 1.019 mg/g and 0.938 mg/g for river sediment (Table 2). Extracted P concentration increased with decreasing frequency (Figure 5). Extracted BAP concentrations were higher at 28 and 45 kHz because low frequency generates strong cavitation responsible for P extraction. At increased frequency, the oscillation period of cavitation and maximum bubble radius decreased, causing pressure inside the bubble to decrease (Ye et al. 2019). Bubble radius and pressure inside the bubble in part determine the strength of cavitation. The larger the radius and the higher the pressure, the stronger the cavitation effect (Zhang et al. 2017; Ye et al. 2019). High frequency thus generates less cavitation energy than low frequency. Hence, 100 kHz is not suitable for use at BAP extraction. Fortunately, the frequency used in many commercial ultrasonic washing machines is around 40 kHz. Such machines are available at an affordable price.

Linear regression analysis for Controls 1 and 2 and P concentration extracted by Machine B showed slopes after extraction at 28 kHz of 1.02 for Control 1 and 0.96 for

### Table 2 | Average concentration of BAP

<p>| Concentration of phosphorus (mg/g) | Soils (0.374) | Lake sediments (0.508) | River sediments (1.871) |</p>
<table>
<thead>
<tr>
<th>Sample TP</th>
<th>Control 1</th>
<th>Control 2</th>
<th>Machine A extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>0.172</td>
<td>0.170</td>
<td>0.126</td>
</tr>
<tr>
<td>Control</td>
<td>0.291</td>
<td>0.294</td>
<td>0.231</td>
</tr>
<tr>
<td>Machine A extraction time</td>
<td>0 min</td>
<td>1 min</td>
<td>5 min</td>
</tr>
<tr>
<td>0.178</td>
<td>0.200</td>
<td>0.239</td>
<td></td>
</tr>
<tr>
<td>0.302</td>
<td>0.347</td>
<td>0.355</td>
<td></td>
</tr>
<tr>
<td>1.082</td>
<td>1.083</td>
<td>1.177</td>
<td></td>
</tr>
<tr>
<td>Machine B frequency</td>
<td>28 kHz</td>
<td>45 kHz</td>
<td>100 kHz</td>
</tr>
<tr>
<td>0.197</td>
<td>0.213</td>
<td>0.208</td>
<td></td>
</tr>
<tr>
<td>0.309</td>
<td>0.270</td>
<td>0.237</td>
<td></td>
</tr>
<tr>
<td>1.044</td>
<td>1.059</td>
<td>0.938</td>
<td></td>
</tr>
</tbody>
</table>
Control 2. R2 was 0.980–0.990 for both comparisons (Figure 6). At 45 kHz, slopes were 1.03 for Control 1, 0.96 for Control 2. R2 was 0.966–0.974 (Figure 6). Extraction at both frequencies shows a strong statistical relationship with control groups. Phosphorus extracted by Machine B is almost identical to Controls 1 and 2. Also, no significant difference was observed at 28 kHz or 45 kHz between controls and the proposed method. A low frequency should be used for P extraction.

Concentration of extractable P

Concentrations of BAP extracted by the proposed method were investigated using both machines A and B frequencies around 28–45 kHz and an extraction time of 1 min (Figure 7). Average concentrations of BAP obtained using machine A were 0.178 mg/g (soils), 0.302 mg/g (lake sediments), and 1.082 mg/g (river sediments) (Table 2). Concentrations obtained by machine B were 0.197 mg/g and 0.213 mg/g (soils), 0.309 mg/g and 0.270 mg/g (lake sediments), 1.059 mg/g and 1.019 (river sediments), respectively, for frequencies of 28 kHz and 45 kHz (Table 2). These percentages are comparable to the concentrations of 0.172 mg/g and 0.170 mg/g (soils), 0.291 mg/g and 0.294 mg/g (lake sediments), 1.024 mg/g and 1.058 mg/g (river sediments), respectively, for Control 1 and Control 2.

Moreover, ratios of extracted P to total PP, soil: 45–60%; lake sediments: 50–60%, and river sediments: 50–60%, are...
consistent with the proportion of BAP in PP in agricultural streams ranging from 37% to 83% which depended on the type of agriculture in the watershed of the stream (Sharpley et al. 1991). However, the C:P ratio for three samples varies from 20% to 65%. The lower the C:P ratio, the higher the inorganic phosphorus in the samples. In this case, C:P ratio indicates the ratio between carbon and total phosphorus. Carbon also represents organic matter in the samples. Hence, the content of organic matter is higher as the carbon in the target samples increases. In addition, the relative P content increases as the C:P ratio decreases. Since the ratio between carbon and organic phosphorus is constant, the inorganic phosphorus of the total P contents is increases when the C:P ratio is low.

For each sample, ANOVA (Table 3) was performed to identify the similarity of washing machine method and Controls 1 and 2. Concentrations of extracted BAP using washing machines were not significantly different from conventional methods. The proposed extraction method examined in this study is an appropriate method for estimating BAP in soils and sediments.

CONCLUSION

In this study, BAP extraction using an ultrasonic washing machine was used to extract BAP from soils and sediments quickly and easily. Among samples, organic matter content of soil was lowest, and lake and the river sediments showed higher and equal content. TP was higher in river sediments than lake sediments due to a greater proportion of inorganic phosphorus in the river sediment. Extraction time and frequency on the concentrations of extracted P using two ultrasonic washing machines, A and B, showed...
that frequencies of 28–45 kHz and an extraction time of 1 min achieved results highly consistent with bioavailable P concentrations extracted by conventional methods. Use of higher frequencies decreased extraction of P due to a decrease in cavitation. Further, increasing extraction time released more P into the solution. This increase was likely due to release of non-bioavailable P caused by heat generation during the longer extraction period. Short extraction times are needed for accurate results. Ultrasonic washing machine is suitable for BAP extraction from 28 kHz to 45 kHz/100 W with an extraction time of only 1 min. The ratio of soil to extractant in the new method is also lower, 1 mg mL$^{-1}$. The condition of this method is immersed the centrifuge tubes inside washing machines with the water level in machines maintained at the same levels as solutions in extraction vials. Results using this machine are highly correlated with results from conventional methods using the same samples. The benefits of the proposed method are: (1) extraction of multiple samples in a single operation; (2) requirement for only a ultrasonic washing machine with a low frequency range between 28 kHz and 45 kHz; (3) ultrasonic washing machines are common laboratory equipment, available at an affordable price which is 10 times cheaper than ultrasonic sonifier used at Control 2; (4) no requirement for potassium persulfate ($K_2S_2O_8$) digestion which saves time during analysis.

**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

**REFERENCES**


