

Ultrasonic treatment for quantification of bioavailable phosphorus in soils and suspended sediments

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ABSTRACT

Several bioassays and chemical extraction methods have been proposed to quantify bioavailable phosphorus (BAP), but procedural and theoretical limitations have hindered their use. In this paper, we developed a method for P extraction from agricultural soil and suspended sediment by combining ultrasonic treatment with extraction by 0.1 molL⁻¹ NaOH solution. The extraction process is less time-consuming and technically simpler than alternative conventional chemical methods. The high correlation ($r = 0.97$) between P extraction with the ultrasonic treatment and the conventional extraction methods suggests the new technique is a promising method for quantifying BAP in agricultural soils and suspended sediments. The method should be tested in soils and sediments that possess different characteristics to confirm the range of its applicability.

Key words | bioavailable phosphorus, NaOH extraction, particulate phosphorus, ultrasonic treatment

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ABBREVIATIONS

BAP Bioavailable phosphorus

P Phosphorus

TP Total phosphorus

DP Dissolved phosphorus

PP Particulate phosphorus

INTRODUCTION

Phosphorus (P) is vital for living organisms. However, its excessive presence in aquatic environments is a major cause of water pollution, and can lead to severe eutrophication and harmful algal or cyanobacterial blooms (Krenkel & Novotny 1980). Eutrophication results in the mortality of many aquatic organisms and threatens human health. Total phosphorus (TP) has therefore been adopted in the water quality standards developed by the Ministry of the Environment – Government of Japan (1982), the United States Environmental Protection Agency (1986), and the Canadian Council of Ministers of the Environment (2004). However, TP cannot accurately represent the severity of eutrophication because it includes complex P forms that

either do not feed algae and cyanobacteria or contribute to eutrophication (Ellison & Brett 2006). Thus, the bioavailable P (BAP) provides a better indication than TP of the severity of the eutrophication and its likely impacts on algal growth.

P exists in bodies of water in two main forms: dissolved phosphorus (DP) and particulate phosphorus (PP). DP, usually in the orthophosphate form, is generally considered to be readily available for algal uptake, whereas PP is only partially bioavailable (Ellison & Brett 2006). PP is bound to sediment and soil particles, and comprises the majority of the P in surface runoff (overland flow) into bodies of water (about 88% of the total; Inoue & Ebise 1991). This is due to P's strong affinity for particulate matter. The PP concentration increases when the flow increases during rain or floods (Inoue & Ebise 1991). Therefore, eutrophication processes can be better understood by quantifying both the PP in soils and the PP in suspended sediments.

Diffuse sources of P such as agricultural fields are often the largest cause of excess P in bodies of water. Intensive agricultural activities require phosphate-containing fertilizers to provide nutrients and improve soil quality for crop production. Over time, this practice results in P

accumulation in soils, increasing the risk of P transport into nearby bodies of water through leaching, erosion, and runoff. Such transport can lead to high levels of P in suspended sediments, and is the major source of excess P in water bodies (Bochove et al. 2006).

Several methods have been used to measure PP bound to soils, including bioassays using *Microcystis aeruginosa* (Okubo et al. 2011), sequential extraction schemes (Wang et al. 2013), and single-step extraction using 0.1 molL^{-1} NaOH (Sharpley et al. 1991). However, these conventional methods are too time-consuming; they require longer than 14 days for incubation in the bioassays, four days for the sequential extraction scheme, or 17 hours for the single-step extraction, respectively. In addition, there are theoretical limitations to these methods; for example, the use of strong solvents such as HCl and NaOH for a long time can alter the chemical structure of the PP, thereby decreasing the test's accuracy. Hence, researchers have been seeking a new method for accurately determining PP concentrations in water bodies that requires less time than conventional methods.

Recently, ultrasonic treatment has been studied because it represents an efficient technique for extracting various elements or chemicals from food and environmental samples that takes less time than previous methods, thereby improving sampling yields and the quality of the extracts (Karina & Clara 2008; Pico 2012). This technique is based

on the penetration of ultrasonic waves into materials, combined with their ability to transfer energy to attached substances such as P, thereby weakening the bonds with particulate matter and increasing release of the substances into the extractant. Despite the technique's potential, its application to quantitative studies of P has been limited; we therefore chose to test its ability to rapidly extract P from soils and suspended sediments in an agricultural area. Our goal was to examine the optimal working conditions for the ultrasonic treatment that would obtain BAP similar to those estimated by conventional methods.

METHODS

Sample collection and preparation

The Umeda River (about 22 km in length, with a basin area of approximately 86 km^2) is a secondary river that flows into Mikawa Bay through Toyohashi City, in the Aichi Prefecture of central Japan's main island (Figure 1). The watershed includes forest (1.6% of the land area) in the upstream reaches and intensively managed agricultural fields (97.5%) and 'other' land (including wasteland and urban areas; 0.9%) in the middle and downstream reaches. Phosphate fertilizer, in the form of composted poultry manure, has been intensively

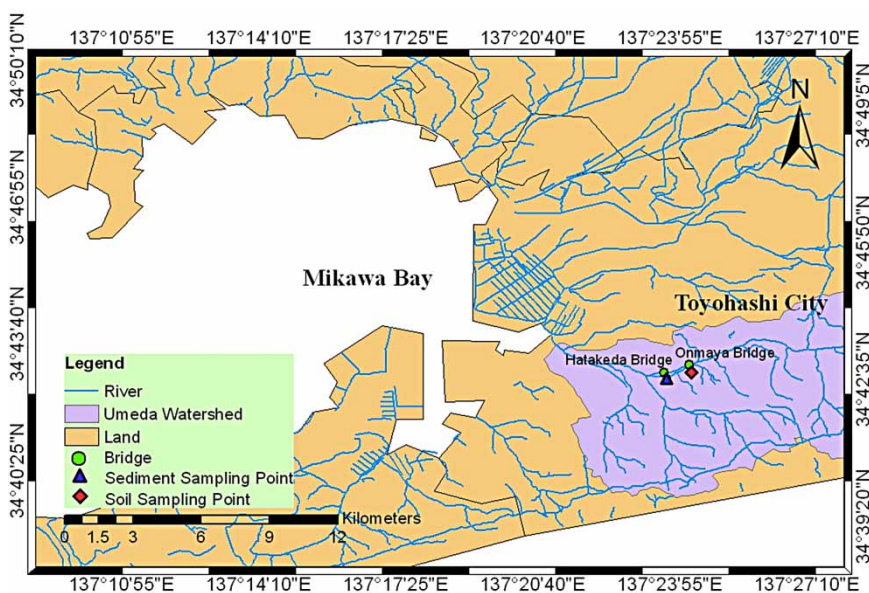


Figure 1 | Location of the sampling sites.

applied to fields that are cultivated during the autumn to replace the P depletion. That has led to widespread eutrophication of adjacent bodies of water and has exacerbated nutrient pollution in Mikawa Bay (Rasul *et al.* 2013).

On 26 January 2011, soil samples ($n = 3$) were obtained (after removing all surface litter) to a depth of 50 mm from a fertilized Chinese cabbage field located approximately 200 m southwest of Onmaya Bridge, approximately 10.6 km downstream from the river's headwaters (Figure 1). The locations in the cabbage field were chosen randomly. The collected soils were combined to produce a single homogeneous sample to be representative of the surface soil in the study area. River water samples were collected manually for suspended sediment immediately after storm events on 5 September (Sed1) and 11 September (Sed2) 2014 and 6 October (Sed3) 2014 when the suspended sediment was well mixed. River water was obtained by dipping a bucket into the river from Hataketa Bridge, near the water quality monitoring system, about 12 km downstream from the river's headwaters (Figure 1). The collected samples were representative of the suspended sediment load in the study river system in the autumn.

Immediately after collection, all samples were transported to the laboratory. To minimize changes in the P concentration during storage prior to application of the imposed treatments (water or chemical extraction, with or without the ultrasonic treatment), the sediment samples were concentrated by means of continuous-flow centrifugation at an average of $20,000 \times g$ for 250 mL/min (Hitachi Koki, Tokyo, Japan; Himac CR22G high-speed refrigerated centrifuge; R18C continuous rotor). This centrifuge intensity was applied to separate suspended sediment from river water. Therefore, it was not sufficient to separate colloidal particles from the supernatant. The soil samples and the concentrated sediment samples were then air-dried at 40°C for three days. All samples were sieved through a 0.149-mm-mesh screen to remove large particles such as plant fibers and colloids of decayed humic matter which were then stored under refrigeration at 4°C .

Extraction procedure

To extract the potential BAP, we used the conventional mechanical shaking method suggested by Dorich *et al.*

(1985). This method was shown to effectively estimate the BAP in agricultural runoff from a field in Oklahoma, USA, at a ratio of soil (or suspended sediment) to extractant of 2 mg mL^{-1} using 0.1 molL^{-1} NaOH as the extractant (Sharpley *et al.* 1991). The P extracted by 0.1 molL^{-1} NaOH was proved to be similar to BAP determined by means of a bioassay (Sharpley *et al.* 1991). We placed 100 mg of soil or sediment in a 50-mL centrifuge tube that contained 50 mL of 0.1 molL^{-1} NaOH, then shook the solution for 17 h on a multi shaker (EYELA World, Tokyo, Japan; MMS-210 orbital shaker) at room temperature.

To shorten the extraction time and improve the extraction efficiency, we tested a new method based on the use of an ultrasonic horn (Branson, Connecticut, USA; Ultrasonic Disrupter Sonifier II, W-450). The horn has a fixed operating frequency of 20 kHz and can generate intensities ranging from 0 to 400 W. During treatment, the horn was placed 1 cm from the bottom of the container without contacting the walls of the centrifuge vial that contained the soil or river sediment sample in the extractant (distilled water or 0.1 molL^{-1} NaOH), and which was fully immersed in an ice-water bath to prevent a temperature increase. The extraction conditions were varied to evaluate the optimal conditions for the following parameters: the ultrasonic intensity, ratio of soil or sediment to solution volume, and exposure time. First, we held the values of ratio of soil or sediment to extractant and extraction time constant at 2 mg mL^{-1} (Sharpley *et al.* 1991) and 5 min (Turner 2008), and varied ultrasonic intensity respectively 10, 15, 20, 30, and 40 W to select the optimal value. Next, we fixed the values of ultrasonic intensity constant at the aforementioned optimal value, extraction time constant at 5 min, and varied the ratio of soil or sediment to extractant respectively 0.02, 0.10, 0.30, 0.50, 0.70, 1.0, and 2.0 mg mL^{-1} (Fuhrman *et al.* 2005). Finally, we set the values of ultrasonic intensity and ratio of soil or sediment to extractant constant at the optimal values, and varied the exposure times respectively 1, 2, 5, and 15 min (Beizhen *et al.* 2008). Due to extraction being slower with distilled water (Zhou *et al.* 2001), we also included a 30-min duration for the distilled water extraction.

After extraction, the samples were centrifuged at $750 \times g$ (Kubota, Tokyo, Japan; model 5100 table-top centrifuge with an RS-4 universal swing rotor) for 30 min. The clear supernatant in each sample vial was then collected to determine

the P content in each extract, and the residue was washed twice with 50 mL of distilled water for 10 min each on the orbital shaker. The clear supernatants from these washes were collected by means of centrifugation ($750 \times g$, 30 min). They were then pooled and analyzed to prevent underestimation of the extracted P as a result of secondary adsorption of the liberated P by solid surfaces in the residue (Ruttenberg 1992; Pacini & Gachter 1999). To prepare samples for the P analysis, the extracts were neutralized with 5 molL^{-1} HCl (NaOH extracts) or with either 1 molL^{-1} HCl or 1 molL^{-1} NaOH (all samples) and diluted (NaOH extracts, to 50% of the original concentration), and kept at 4°C in the dark until analysis. All treatments were performed for three independent samples (i.e. $n = 3$).

To evaluate the effect of the separation method on the extracted P, the extracts were collected by filtration through syringe filters (Millipore, Massachusetts, USA; Millex syringe-driven filter, $0.45 \mu\text{m}$) instead of centrifugation. The filtrate from each extract was then collected to measure the P content, which would be compared with the values obtained from the aforementioned centrifugation. Three independent samples were evaluated for each treatment.

Phosphorus analysis

The concentration of P transferred into the solution phase as a result of the extraction was measured colorimetrically for the neutralized extracts by means of the molybdenum blue method (Murphy & Riley 1962). This is a standard method for determining the concentration of extracted P and is based on the reaction of $\text{PO}_4\text{-P}$ with molybdate to form a blue compound. The intensity of the color corresponds to the P concentration in the solution, and can be measured by a spectrophotometer at 882 nm using the Bran and Luebe (Norderstedt, Germany TRAACS 800 Autoanalyzer). The analysis measured the amount of soluble reactive phosphorus (SRP) in the raw extracts from each sample, whereas the analysis of the digested extracts measured the amount of TP extracted from each sample. The digestion was performed with 4% w/w potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) in an autoclave (Sanyo, Osaka, Japan; MLS-3750) at 120°C for 30 min, and converted all forms of phosphorus into SRP that could be detected by the Autoanalyzer. The quantity of non-reactive phosphorus in each extract was defined as the difference

between TP and SRP. All measurements were the average of two independent replicates per treatment.

Statistics

A two-way analysis of variance (ANOVA) test was conducted to compare the effect of extraction methods (ultrasonic treatment versus conventional extraction, using distilled water versus 0.1 molL^{-1} NaOH) on the concentration of P extracted from four samples (soil versus sediments). A Pearson correlation coefficient was measured to analyze the similarity between P extraction with the ultrasonic treatment and the conventional extraction methods at certain conditions (i.e. same ultrasonic intensity, ratio of soil or sediment to extractant, or exposure time).

RESULTS AND DISCUSSION

Several trials were carried out to clarify the optimal conditions for the ultrasonic treatment in which the similar P values in the conventional mechanical shaking method would be obtained. We investigated the effects of ultrasonic intensity, ratio of soil or sediment to solution, exposure time, and extractant type (distilled water versus NaOH) on the concentration of extracted P.

First, in order to optimize the ultrasonic intensity, we fixed the ratio of the solid phase (soil or sediment) to the extractant (distilled water or 0.1 molL^{-1} NaOH) at 2.0 mg mL^{-1} , and varied the intensity from 10 to 40 W. The results showed that with 5 min of exposure, increasing the ultrasonic intensity only increased the amount of extracted phosphorus slightly but not significantly (Figure 2). In both extraction treatments (using distilled water or 0.1 molL^{-1} NaOH), increasing the intensity above 30 W had only a small but not significant effect on the amount of extracted P. We did not test a higher intensity than 40 W to avoid excessive noise (which could damage the ears of laboratory workers) and to minimize the risk of degradation of the ultrasonic horn. In the water extraction of all soil and sediment samples (Figure 2), the concentration of extracted P rose marginally. In the NaOH extraction of Sed₁ (Figure 2(b)) and Sed₂ (Figure 2(c)), the P concentration fluctuated between 15 and 30 W of ultrasonic intensity. The large error bars in Figure 2(c) imply that our

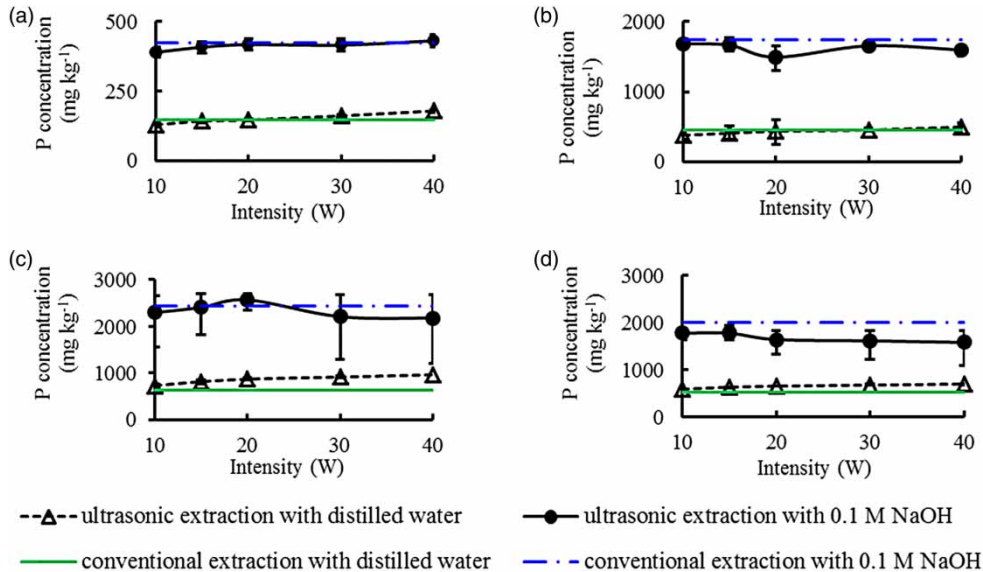


Figure 2 | Effect of ultrasonic intensity (at 5 min of exposure) on the amount of extracted P. (a) Soil sample collected on 26 January 2011. (b) Suspended sediment samples collected on 5 September 2014, (c) on 11 September 2014, and (d) on 6 October 2014. Each value represents the mean \pm SD of three measurements.

data at these points were probably underestimated. In the NaOH extraction of Sed₅ (Figure 2(d)), the P concentration gently fell when increasing the ultrasonic intensity from 15 to 20 W. However, in most cases, the concentration of P obtained by the analysis at 30 W was the most similar ($r = 0.92$) to the values extracted by the conventional mechanical shaking method. Thus, we chose 30 W as the optimal ultrasonic intensity.

Next, we examined the effect of seven samples of soil or sediments to extractant ratios with the optimal ultrasonic intensity of 30 W and 5 min of exposure. P concentration was generally the highest at the smallest ratio (0.02 mg mL⁻¹) (Figure 3). Additionally, at the small values of soil or sediment to extractant ratio (less than 0.50 mg mL⁻¹), the P concentration fluctuated. This may have resulted from the greater volume of solution into which the P could diffuse.

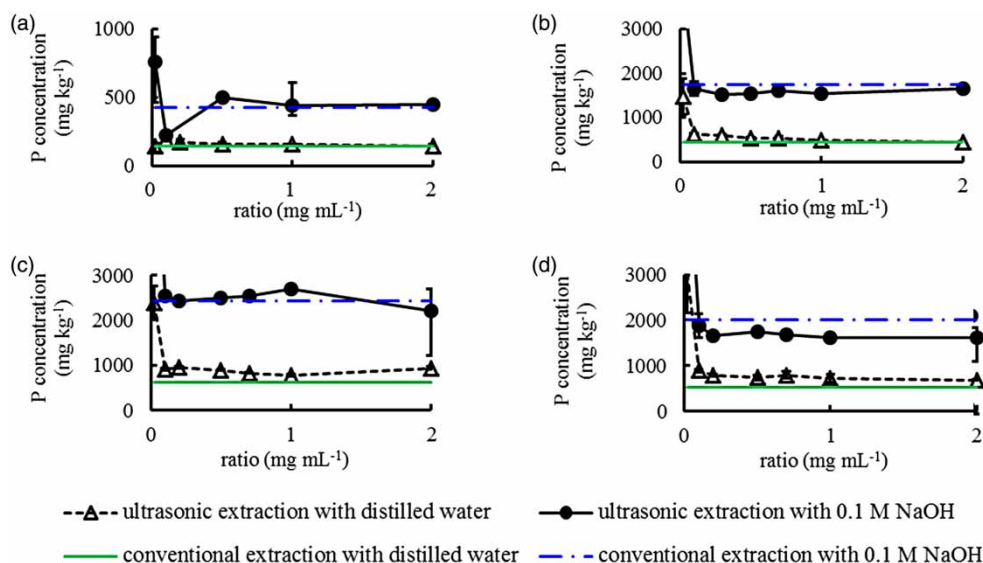


Figure 3 | Effect of ratio of the solid phase (soil or suspended sediment) to the extractant volume on the extracted P. (a) Soil sample collected on 26 January 2011. (b) Suspended sediment samples collected on 5 September 2014, (c) on 11 September 2014, and (d) on 6 October 2014. Each value represents the mean \pm SD of three measurements.

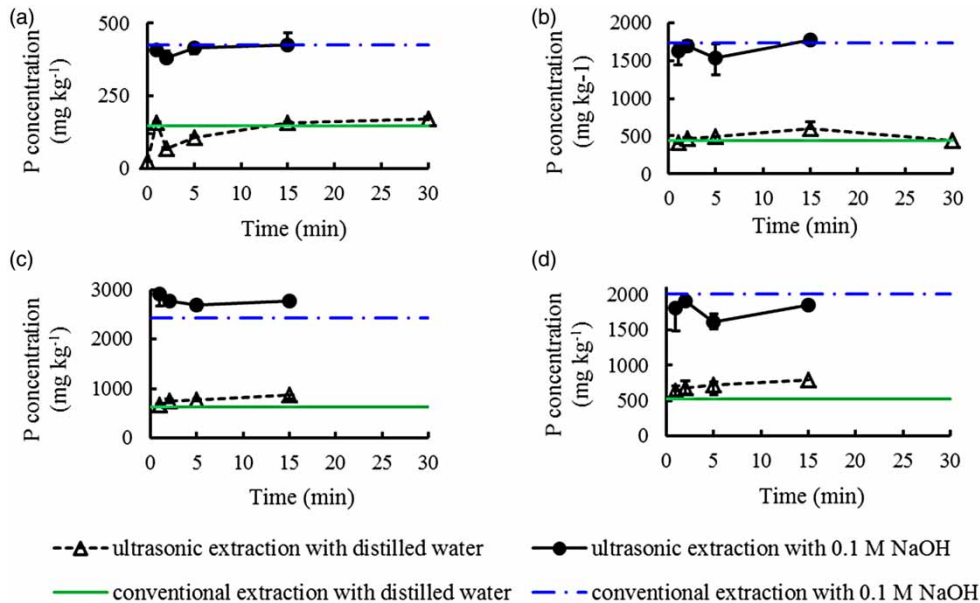


Figure 4 | Effect of exposure time on the extracted P. (a) Soil sample collected on 26 January 2011. (b) Suspended sediment samples collected on 5 September 2014, (c) on 11 September 2014, and (d) on 6 October 2014. Each value represents the mean \pm SD of three measurements.

To avoid this problem, we chose the optimal ratio as 1.0 mg mL^{-1} , because the extracted P at this point in most cases was the most similar ($r = 0.97$) to the values obtained using the conventional mechanical shaking method. This contrasts with the advice of Sharpley *et al.* (1991), who proposed that the ratio of 2.0 mg mL^{-1} was the most effective ratio for chemical extractions.

Based on these results, we studied the effect of sonication time on the amount of extracted P using 30 W and a ratio of 1.0 mg mL^{-1} of soils or sediments to extractant. There was no clear relationship between extracted P and the duration of ultrasonic treatment, although there was some evidence that the P concentration increased with extending extraction duration (Figure 4).

Extending the extraction time beyond 15 min did not increase the efficiency of extraction, thus using a shorter exposure time would improve the efficiency of the analysis. Figure 4 shows that the quantity of P extracted in 1 min was similar to the values obtained using other durations, and the values at this duration were generally close to those obtained by the conventional mechanical shaking treatment ($r = 0.98$). Therefore, the optimal conditions for extraction with 0.1 mol L^{-1} NaOH appear to be 30 W of ultrasonic intensity for the duration of extraction, a ratio of 1.0 mg soil or sediment per mL of extractant, and a duration of 1 min.

Table 1 summarizes the concentrations of P obtained by the different extraction methods. On average, the concentrations of P obtained using distilled water extraction

Table 1 | Mean values of extracted P concentration obtained using the different methods. Sed₁, Sed₂, Sed₃ were the sediment samples respectively collected on 5 September 2014, 11 September 2014, and 6 October 2014. Soil sample was collected on 26 January 2011

P conc. (mg/kg)	TP	Water extraction				0.1 mol L ⁻¹ NaOH extraction			
		Shake 17 h	Shake 1 min*	Ultra. 5 min	Ultra. 1 min	Shake 17 h	Shake 1 min*	Ultra. 5 min	Ultra. 1 min
Soil	$1,124 \pm 155$	148 ± 4		108 ± 5	157 ± 5	425 ± 15		416 ± 9	409 ± 21
sed1	$2,819 \pm 128$	444 ± 19	130	535 ± 28	417 ± 58	$1,736 \pm 186$	1,080	$1,536 \pm 176$	$1,632 \pm 30$
sed2	$3,413 \pm 44$	627 ± 47	140	883 ± 34	680 ± 46	$2,435 \pm 760$	2,010	$2,497 \pm 878$	$2,908 \pm 123$
sed3	$2,580 \pm 49$	525 ± 52	130	736 ± 10	639 ± 19	$2,000 \pm 41$	1,180	$1,748 \pm 317$	$1,803 \pm 53$

under the optimal conditions for the ultrasonic method ranged from 14.0 to 24.8% of total P, versus 13.2 to 20.3% for the conventional method (Table 1). In the 0.1 molL⁻¹ NaOH extraction, P values ranged from 36.4 to 85.3% of TP, versus 38.4 to 77.5% for the conventional method. Our results were in close agreement with those of previous studies which proved percent BAP of TP was 32.0 to 83.0% in runoff (Sharpley 1993; Fabre *et al.* 1996). In addition, the P concentrations extracted using 0.1 molL⁻¹ NaOH plus ultrasonic treatment were notably ($F=99.0$, $P < 0.0001$) higher than those extracted by distilled water. Even after only 1 min, much of the total P was extracted by both the mechanical shaking and ultrasonic treatment methods (Table 1). There are two likely explanations. First, NaOH is a stronger solvent than distilled water, thus it is able to extract more P. Second, NaOH extraction may lead to selective extraction of iron-bound P that would not be extracted by water (Holtan *et al.* 1988). The sediment samples were yellow (Sed₂ and Sed₃) and yellowish grey (Sed₁), possibly due to the presence of iron components. Therefore, the P concentrations obtained by extracting these sediment samples with NaOH would be higher than those obtained from the soil samples which were dark grey and therefore probably contained less iron.

CONCLUSION

In this study, we examined the suitability of an ultrasonic treatment for the extraction of BAP from an agricultural soil and suspended sediments. We determined the optimal conditions for the ultrasonic treatment, with distilled water and NaOH as extractants; the NaOH extracted a higher proportion of TP. The technically simple procedure we proposed in this study allows an extraction time of only 1 min, compared to 17 h for BAP extraction using the conventional mechanical shaking method. The ratio of soil to extractant in the new technique was also lower, at 1 mg mL⁻¹. The BAP values obtained using the ultrasonic treatment with 0.1 molL⁻¹ NaOH were similar to ($r = 0.97$) those obtained using the conventional method. These findings suggest that the new method provides a promising alternative for quantifying BAP in soils and river sediments. Future research should be done to test the suitability of the new method for

analyses of soil and sediment samples that possess significantly different characteristics.

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