Ultrasonic treatment for quantifying bioavailable phosphorus in soil

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Abstract: Bioavailable phosphorus (BAP) has been reported to represent the severity of eutrophication, but current methods that can quantify BAP accurately and rapidly are limited. Here, we investigated an extraction using 0.1 M NaOH solution in combination with ultrasonic treatment to estimate the potential BAP from agricultural soils and suspended sediments. The BAP was evaluated to the growth of P-starved *Microcystis aeruginosa*. The extraction process is less time-consuming than alternative conventional methods allowing for a greater number of sample analyses. However, analyses in bioassays using *M. aeruginosa* observed the growth of algae in cultures using soils after extraction as a sole phosphorus (P) source, which is assumed to be non-BAP. Further study should clarify the storage of P in *M. aeruginosa* cells from the P associated with soil particles to confirm the P uptake for cell growth.

Keywords: bioavailable phosphorus, NaOH extraction, particulate phosphorus, ultrasonic treatment

Introduction

Excessive P is a major cause of harmful eutrophication in an aquatic environment. Total P (TP) has been used to evaluate the severity of eutrophication, but BAP provides a better indication than TP in understanding the impacts of P on algal growth (Ellison et al., 2006). TP in the environment includes dissolved P (DP) and particulate P (PP). DP is generally readily available for algal uptake, whereas PP is only partially bioavailable (Ellison et al., 2006). Additionally, PP bound to suspended sediment and soil particles comprises the majority of the P in surface runoff into water bodies (Inoue et al., 1991). Therefore, quantification of BAP in PP bound to soils and suspended sediments is important to understand the eutrophication process.

Although several methods have been suggested for measuring BAP (Sharpley, 1993, Pacini et al. 1999, Okubo et al., 2014), they are too time-consuming, thereby somewhat limiting for a large number of samples. Here, we are investigating a method for more accurately determining BAP in particulate forms that requires less time than conventional methods. Recently, Ultrasound has been widely-used for the extraction of various elements from food and environmental samples and takes less time and delivers a higher yield than previous methods. Despite its potentiality, its application to quantitative studies of BAP has been limited. The objective of this study was to use extraction by 0.1 M NaOH solution in combination with ultrasonic treatment to quantify BAP from soils. The bioavailability of PP in soil was evaluated by bioassays in which extracted soils were used as sole P source.

Material and Methods

Collected soil samples (*Fig. 1*) were air-dried at 40°C for 3 days, then sieved through a 0.149 mm-screen, and stored at 4°C prior to extraction.

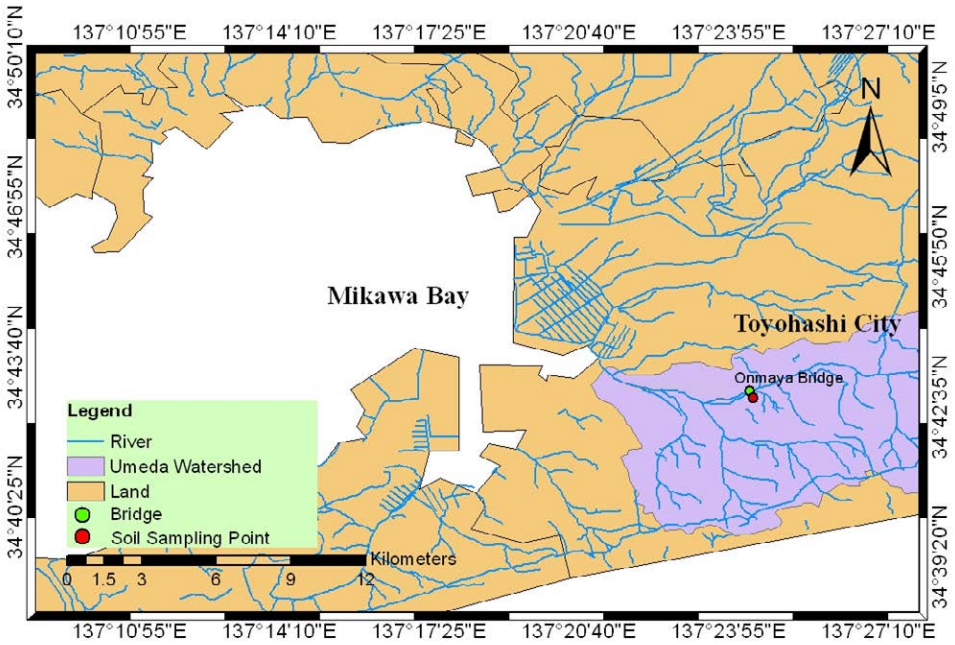

Figure 1 Location of the sampling sites. Soil samples (red point) were collected on 26 January 2011, from a Chinese cabbage field in Umeda River basin, Toyohashi City, Aichi Prefecture, central Japan. Umeda River flows into Mikawa Bay which is one of Japan's most serious nutrient pollutionlocations. The collected soils were chosen to be representative of the surface soil in the study area.

Quantification of extractable BAP in soils

BAP concentrations in soils were determined by a single-step extraction suggested by Dorich et al. (1985) in 0.1 M NaOH using conventional mechanical shaking for 17 hours. Contemporarily, they were also examined by combining 0.1 M NaOH with ultrasonic treatment at optimal working conditions that would provide estimates of extracted BAP similar to those obtained from a conventional mechanical shaking method. The working conditions of the ultrasonic treatment included the following parameters: an ultrasonic intensity of 30 W, ratio of soil to extractant of 1.0 mg mL^{-1}, and exposure time of 1 min. The clear supernatants from the extracts were collected by centrifugation (750 × g) for 30 min. The P content in each supernatant was determined by the standard molybdenum blue method (Murphy et al., 1962). The residues were washed twice with distilled water to remove remaining NaOH, then air-dried at 40°C prior to the bioassays. The soils without any extraction were defined as "control soil" (a); the soils extracted with ultrasonic treatment were termed asextracted soils "with ultrasonic treatment" (b); and "with mechanical shaking" (c).

Bioassays using Microcystis aeruginosa

We cultured P-starved *M.aeruginosa* (clone NIES44) in P-free CB media containing soils, equivalent to 0.1 mg-P L^{-1}, as the sole P source. Three types of soil samples (a), (b) and (c) were prepared by the aforementioned procedures. Incubation took place in a growth chamber under a cool white fluorescent light with a 12:12-h light:dark cycle at 25 °C for 30 days. The growth yield was monitored by a cell count, chlorophyll-*a* (Chl-*a*), and DP, PP. All measurements were analysed using samples taken on days 0, 10, 20 and 30, and were the average of three independent replicates per sample.

Results and conclusions

The concentrations of extracted P approximately 57.3% of TP for the ultrasonic extraction, compared to 57.4% for the conventional mechanical method. Our results closely agree with those of previous studies which proved the percentage of BAP in TP was 32.0% to 83.0% in agricultural soils and runoff (Sharpley, 1993).

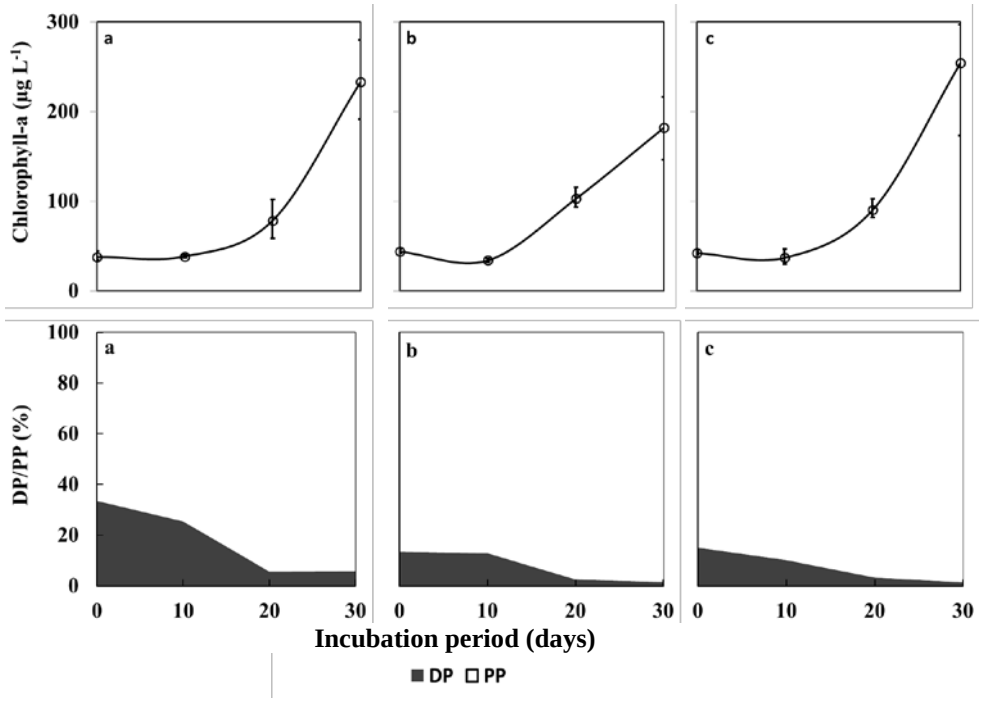

Figure 2 Chl-*a* contents (upper) and DP and PP concentration (lower) in cultures of *M. aeruginosa* incubated with (a) control soil, and with soil extracted (b) with ultrasonic treatment, (c) with mechanical shaking. Each value is the mean of triplicates.

Figure 2 illustrated *M. aeruginosa* grew in all soil types as the Chl-*a* concentrations increased. The results implied *M. aeruginosa* could use some P forms in soil after extraction, which have been considered to be non-bioavailable, for their cell growth. Although there was no clear pattern for the relationship between the cell growth (Chl-*a*) and the increase of PP, the ratio values of Chl-*a* to PP ranged between 0.6 to 1.1 in cultures incubated with all three soil types and is consistent with the study of Spears et al. (2013). It reported that if the ratio of Chl-*a* to PP was more than 0.2, P could be an important factor limiting algal growth. A technique to clarify the intracellular P concentration in *M. aeruginosa* cells from particulate matters in soil particles is necessary, thereby confirming the proportion of BAP transformed to *M. aeruginosa* cells.

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