別紙４－１（課程博士（英文））

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# Abstract (Doctor)

| Title of Thesis | **Ultrasonic Extraction for Estimating Bioavailability of Phosphorus in Particulate Forms**<br>(懸濁物質中の生物利用可能性リンの超音波抽出法による定量) |
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Approx. 800 words


Phosphorus (P) is an essential nutrient for plant growth and required to maintain profitable crop and livestock production in the prevalent intensive agricultural plans. However, its excessive use has caused undesirable growth of algae and cyanobacteria in aquatic environment, leading to the harmful eutrophication which is blamed for mortality of aquatic culture in closed water areas and threats to human health, particularly during the summer period. In order to control human-caused eutrophication, acceptable levels of total phosphorus (TP) were limited in the water quality standards in many countries but it includes all of P species. Some of them are complex forms that do not either feed algae or contribute to eutrophication. Hence, the term of bioavailable P (BAP) appeared to define the P fraction which is readily available for algal uptake and really contribute to eutrophication. Regardless of the difference in chemical forms, in environment P can be separated by centrifugation or filtration into two states: dissolved phosphorus (DP) and particulate phosphorus (PP). DP is generally considered readily available for algal uptake, whereas PP is partially bioavailable. P has a strong affinity to particulate matter, thus PP bound to sediment and soil particles comprises the majority of P in surface runoffs flowing either into drains or overland into aquatic environments. Therefore, an understanding of the BAP fraction in PP bound to soils and suspended sediments, especially related to agricultural sources, is necessary for better management of eutrophication in a watershed. Recent reports have also alleged the involvement of bioavailable phosphorus (BAP) in particulate forms in the severity of eutrophication. However, current methods that can estimate particulate BAP are too time-consuming and meticulous. They require longer than 14 days for incubation in bioassays, 4 days for the sequential extraction scheme or 17 hours for single step-extraction using mechanical shaking, respectively. Thus, the amounts of analyzed samples are very limited. It leads to the demand of a new method which require less time for determining accurate BAP concentration in PP. A possible solution is using ultrasonic treatment to accelerate the transformation of P into extracts. Recently, ultrasonic treatment has been studied as an efficient extraction technique that takes less working times as well as improves yields and quality of the extracts from food and environmental samples. In this study, we investigated an extraction using 0.1 M NaOH solution in combination with an ultrasonic treatment to quantify the potential BAP in particulate forms, especially in soil and suspended sediment from river water related to agricultural sources.

The first study used Sharpley's extraction with the mechanical shaking in 17h as the conventional method to examine the BAP concentrations in our target samples of soil and suspended sediment. Next, we evaluated our proposed ultrasonic method by comparing with Sharpley's method. The most optimal working conditions of the ultrasonic treatment were

defined at which, the BAP extracted by ultrasonic treatment was similar to those obtained by the conventional extraction. The investigated working conditions of ultrasonic treatment included intensity, extraction time and ratio of sample to extractant. Finally, we evaluated the bioavailability of P obtained from the extractions by bioassays for the growth of P-starved *Microcystis aeruginosa*.

We quantified BAP concentrations in soil and suspended sediment samples collected from a representative agricultural field in Umeda River basin. Umeda River was selected because it flows into Mikawa Bay which has been reported one of the most eutrophic regions in Japan's main island. Our study was compatible with the previous study about the proportion of BAP in PP in agricultural streams. It confirmed the potential risk of P pollution in Umeda River basin and Mikawa Bay come from agricultural sources.

The most optimal conditions for ultrasonic treatment were identified. The proposed method allowed for an extraction time of only 1 min whereas the mechanical shaking method requires 17 hours for BAP extraction. The extraction process is less time-consuming than alternative conventional methods and permits analyses of a greater number of samples.

The growth of algae in the media containing samples after the ultrasonic extraction was at an identically similar level with those in the media containing samples after conventional extraction. It reinforced the notion that ultrasonic treatment could provide a similar quantification for BAP in soil and suspended sediment samples when using conventional extraction. The high correlation between the amount of extracted BAP and algal growth at the stationary phase of incubation suggested that the BAP fraction could be entirely obtained in a single extraction. Additionally, The limiting effect of P on the algal growth was confirmed. Although bioassays observed the growth of algae in cultures using samples after extraction as the sole phosphorus (P) source, we confirmed that the remained P was able to be in algal cells. We suggested that when nutrients, especially P, were deficient, the algae could utilize cellular nutrients for their growth. Thus, using *M.aeruginosa* would properly be inappropriate for accurate AGP tests to evaluate the bioavailability in P depleted environments.