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〈Original article〉

The Identification of Limiting Nutrients Using Algal Bioassay Experiments (ABEs) in Boryeong Reservoir after the Construction of Water Tunnel

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Abstract - The objective of the study was to determine nutrition regime and limitation in the Boryeng Reservoir where there's a water tunnel between Geum River and the reservoir. Evaluation was conducted through *in situ* algal bioassay experiments (*in situ* ABEs) using the cubitainer setting in the reservoirs. For in situ ABEs, we compared and analyzed variations in chlorophyll-*a* (CHL-*a*) and phosphorus concentrations in Boryeong Reservoir before and after the water tunnel construction. We then analyzed the nutrient effects on the reservoir. Analysis for nitrogen and phosphorus was done in the three locations of the reservoir and two locations of the ABEs. The *in situ* ABEs results showed that phosphorous and Nitrogen, the primary limiting nutrient regulating the algal biomass was not limited in the system. The treatments of phosphorus or simultaneous treatments of N + P showed greater algal growth than in the control of nitrate-treatments, indicating a phosphorus deficiency on the phytoplankton growth in the system. The water from the Geum River had 5 times higher total phosphorus (TP) than the water in the reservoir. Efficient management is required as pumping of the river water from Geum River may accelerate the eutrophication of the reservoir.

Keywords : phosphorus limitation, chlorophyll-a, Boryeng Reservoir, water tunnel

INTRODUCTION

Water storage levels in Boryeong Reservoir dropped down frequently due to small watershed and low rainfall. In 2015, severe shortage of drinking water occurred in Boyrung Reservoir which supplies the water to the west-northern region within the Geum River watershed. The Korean government decided to construct a water tunnel between the downstream of Geum River and Boyrung Reservoir to solve the problems of minimum water stage of year 2015 in the reservoir. This was mainly due to regional severe drought and reduced rainfall in 2015 in the watershed. The construction of water tunnel, thus, occurred in September 2015 to supply the river water to the reservoir in and then completed in February 2016 (Lee 2006).

Generally, water tunnel connected between two water bodies or watersheds is frequently constructed for water supplies of drinking or irrigation purposes when water shortages occur. The constructions of a water tunnel between a reservoir and reservoir (or natural lake) or between a river and reservoir were in San Antonio Lake in California, Lake Hodges in San Diego (Piek 2006) and tunnel of Guldam Dam in Turkey (Basarir *et al*. 2005). Similar constructions

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are shown in water tunnels of Yimha Reservoir-Yeoungcheon Dam and Andong Reservoir-Imha Reservoir in Korea. All these were constructed for frequent water shortages in the watersheds.

The water tunnel connections between two water bodies may influence changes in water quality and aquatic ecology. Especially, water chemistry may be largely influenced by inflowing waters when the two water bodies have large differences in the trophic conditions of nutrients, ionic contents, and suspended solids (Lindeman 1942; Hynes 1970; Minshell 1988; Wetzel 2001). These water quality difference, in turn, resulted in changes of nutrient compositions (N:P ratios), sediment deposition and algal growth in the receiving water. In other words, both the nitrogen and phosphorus concentrations, acted as limiting elements of phytoplankton in water bodies, are higher in rivers or streams than in lakes. Furthermore, the river and lake are completely different (Wetzel 2001). Thus, this results the negative impact on lake water such as eutrophication or change on biological community.

The water tunnel construction between Geum River and Boryeong Reservoir may help the water volume increases in Boryeong Reservoir, but may have side effects on the reservoir water due to higher nutrient contents (N, P) and organic matters (BOD, COD) of inflowing waters, when we analyze the water quality of inflowing waters of Geum River. For this reason, it is necessary to evaluate ambient waters of the two locations as the previous researches were suggested under the circumstances (Hynes 1970; Wetzel 1990). Under this construction, we believe that the physicochemical change could bring about an increase of biologic standing crop of phytoplankton and the change of biotic species composition in the receiving water.

Large dam reservoirs in Korea have high abundance of phytoplankton biomass and the dominance of bluegreen algae on the surface waters due to high water temperatures and high nutrients (N, P). These resulted in ecological problems as well as limitation of water resource use (Lee *et al.* 2006; Jeong *et al.* 2011). For these reasons, various researches such as analysis of reservoir trophic state, nutrient loading of N and P (Rast and Lee 1978; Volluenweider and Krerekes 1980) and N : P ratios on the algal growth (Rhee 1978; Smith 1983) were conducted along with a *in situ* application of Algal Bioassay Experiments (ABEs) on phytoplankton growth. In late 20 century's studies, amongst various approaches, ABEs were frequently applied to the lakes or reservoirs of other countries (Lean and Pick 1981; Robert *et al.* 1985). This experiment by adding and analyzing ambient nutrients is one of the good key tools for a diagnosis of nutrients or light limitations (Elser and Kimmel 1986; Camacho 2003; Park and An 2012). In order to evaluate the main cause of algae growth, such technique is useful for predicting and judging the changing of algae as consider potential limiting concentrations and format of nutrients that flow into lake water (Box 1983; Lopez and Davalos-Lind 1998). In the nutrient bioassays, various methods were used to set up cubitainers with polyethylene (Elser and Kimmel 1986) at site or incubate water drawn indoor (Sakamoto 1971) or conduct whole lake experiment (Schindler 1971).

Previous numerous studies on nutrient bioassays in the reservoirs or lake showed that algae growth was frequently limited by single phosphorus addition (Elser and Kimmel 1986; An 2003), nitrogen addition (Camacho 2003), a modification of N : P ratios (Robert and Gary 2001) or simultaneous additions of N and P (Robert and Gary 2001), and that the growth is varied depending on the seasons (Elser 1988). Similar researches on the in situ algal bioassays were conducted in large Korean reservoirs or wetland ecosystem (Oh *et al.* 1998; Joo *et al.* 2002; Park and An 2012; Jeong and An 2013).

Also, various empirical models and trophic state determinations were analyzed to diagnose the eutrophication of the reservoirs (Dillon and Rigler 1974; Cloern *et al.* 1995). The empirical models of nutrients-chlorophyll or chlorophyll-transparency were frequently used in Korean reservoirs (An and Park 2002; Park and An 2007) as well as the temperate lakes and reservoirs of North America and Europe (Dillon and Rigler 1974; Schindler 1978; Vollenweider 1990; Watson *et al.* 1997) to determine the key nutrients regulating the phytoplankton growth. Little, however, is known about how the nutrient controls the algal growth especially in the connected ecosystems between a river and a reservoir.

The key objective of this study was to determine nutrient limitation in the Boryeng Reservoir after the connection of the water tunnel. For the evaluations, ABEs were conducted using the cubitainer setting in the reservoirs. For this ABEs, we compared and analysed variations of chlorophyll-*a* and phosphorus concentrations in Boryeong Reservoir before and after water tunnel construction, and then analyzed the nutrient effects on the reservoir.

MATERIALS AND METHODS

1. Description of Sampling Sites and Algal Bioassays

One lentic system of Boryeong Reservoir and two lotic systems of Bangyo Stream and Woongcheon Stream were selected for the chemical and biological analysis. The dam of Boryeong Reservoir was constructed in 1991 for the multi-purpose uses of drinking water, industrial and irrigation. The water volume capacity and the elevation are 116.9×10^6 m^3, and 50 m, respectively, and the water quality is generally oligotrophic-mesotrophic state, except for some seasons.

Regular water samplings were conducted from one site (R1) in the reservoir (Fig. 1). We sampled water at R1 before and after the construction of water tunnel in 2015. Furthermore, the sample from R1 were analyzed to check the water parameters (TP and CHL). Also, the *in situ* ABEs (A1) were conducted to determine the key limiting nutrients for phytoplankton growth in the reservoir site of A2. The specific sampling sites are as follows:

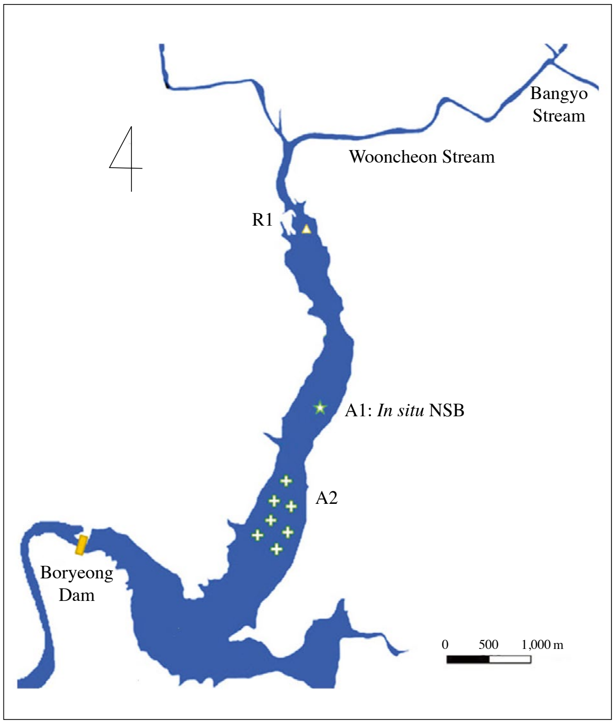


Fig. 1. The sites of *in situ* ABEs (A1–A2) and the sampling sites for chemical analysis at the Boryeong Reservoir (R1).

I) Nutrient-spiking experimental sites for *in situ* ABEs
A1: Punggye-ri, Misan-myeon, Boryeong-si, Chungcheongnam-do
A2: Yongsu-ri, Misan-myeon, Boryeong-si, Chungcheongnam-do

II) Sampling sites of Boryeong Reservoir
R1: Punggye-ri, Misan-myeon, Boryeong-si, Chungcheonnam-do

For *in situ* ABEs, epilimnetic waters were sampled from the reservoir, and the *in situ* mesocosm for the experiments were constructed near the edge of the reservoir. We set the equipments of *in situ* ABEs were set during 10th to 16th August 2016 and analyzed chlorophyll-*a* and nutrients using samples obtained from the cubitainers. However, during the ABEs were conducted, the water tunnel was not in use. In this study, water parameters from R1 at Boryeong Reservoir were analyzed before and after the construction of the water channel in 2015.

2. *In situ* Algal Bioassays Experiments (ABEs)

In situ Algal Bioassays Experiments (ABEs) were conducted to determine a primary limiting nutrient for algal growth in the reservoir. These bioassays were conducted for the tests the influence of the nutrient-rich water on the chlorophyll-*a* of the reservoir after the water tunnel construction between Geum River and the reservoir. In the bioassays, nutrients of nitrogen and phosphorus were added to the *in situ* 10 L cubitainers at Boryeong Reservoir during 10th–16th August, 2016 and analyzed the samples of nutrients and chlorophyll-*a*. One control and five treatments of nitrogen addition (ammonia-N, nitrate-N), phosphorus addition (phosphate-P) and simultaneous addition of N and P were used for the *in situ* experiments as shown in other regions (Kilham 1976; Schindler 1977).

Whole water of 140 L were mixed using each 20 L water samples from seven locations near the A2 site and were spilt into twelve cubitaners of duplicate one control and five treatments (T1, T2, T3, T4, and T5). Based on all the data of

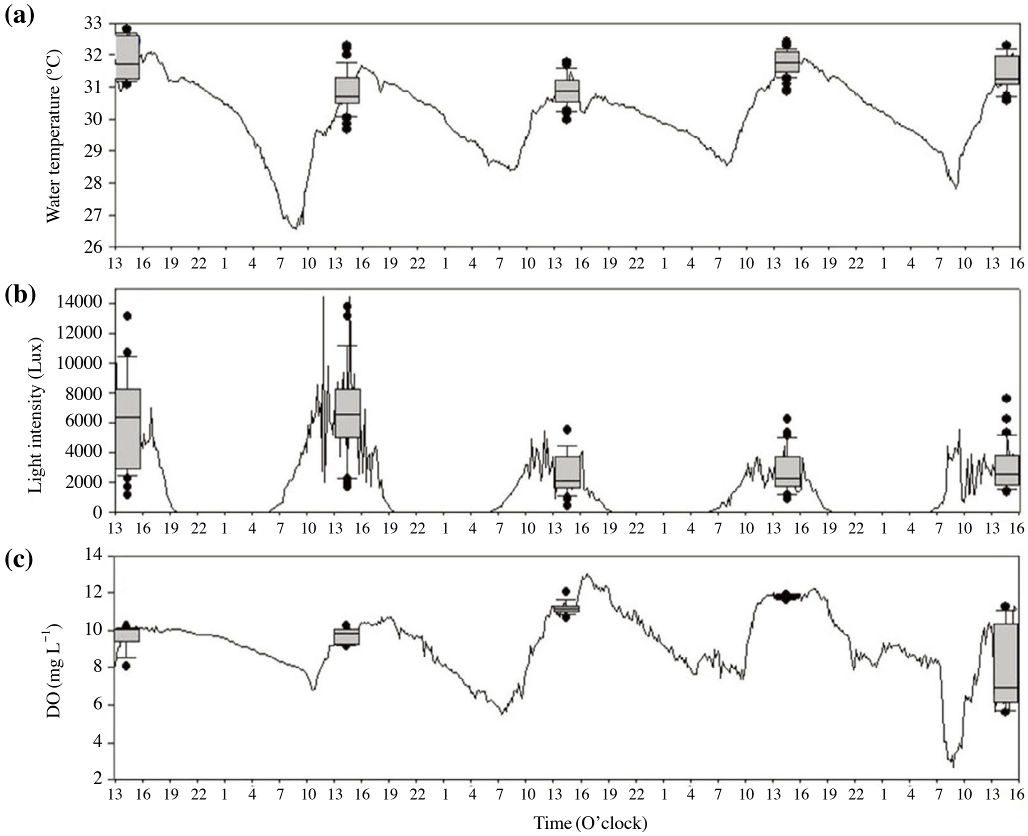


Fig. 2. The diel variations of water temperature, dissolved oxygen (DO), and light intensity measured by HOBO (Onset) during the periods of cubitainer incubation (from Day 0 to Day 6) in ABEs in Boryeong Reservoir.

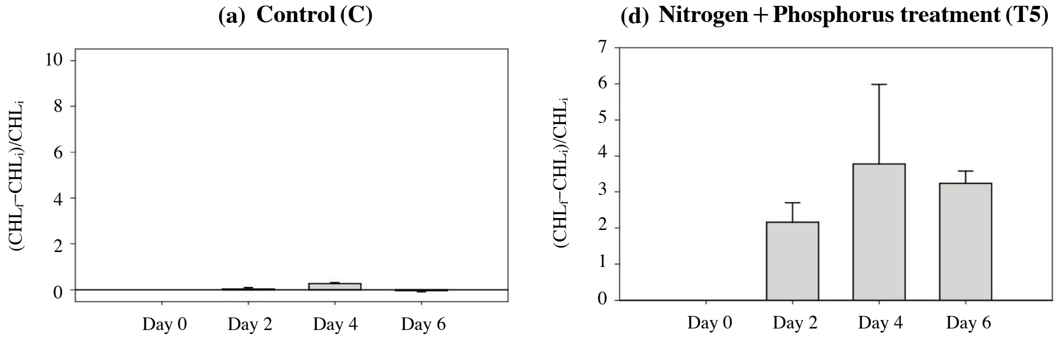
total phosphorus, nitrate-nitrogen, ammonium-nitrogen provided by Ministery of Environment in Korea, the following concentrations of the treatment to be spiked were suggested. The control was the cubitainer with no-nutrient additions. Treatment 1 (T1), and T2 were added 15 μg L^{-1}, and 30 μg L^{-1} P of KH_2PO_4 stock solution to whole water, as P, and 2P in the phosphorus concentration, respectively. Also, treatments of T3 were added 1.3 mg L^{-1} NO_3-N of KNO_3 being NO_3-N to whole water, T4 was added 50 μg L^{-1} NH_4-N of NH_4Cl stock solution to whole water as the concentrations of NH_4-N and NH_4-N, and treatments of T5 were simultaneously added 15 μg L^{-1} P of KH_2PO_4 stock solution and 2.0 mg L^{-1} N of KNO_3 stock solution as a treatments of P+NO_3-N. All the experiments were conducted as duplicate controls and treatments.

We hanged the cubitainers of the controls and treatments at the epilimnetic depth (about 0.6 m) at the site A1 of the reservoir. This is in the same lake eco system as the other 7 spots we had sampled the water (A2). Water temperature, light intensity, and dissolved oxygen were observed using the apparatus of HOBO every 10 minutes during the *in situ* algal bioassay (Fig. 2). Water samples were collected from the controls and five treatments every two days from the sites (Day 0, Day 2, Day 4, and Day 6) and then were transported in the laboratory at that day. Concentrations of total phosphorus (TP), soluble reactive phosphorus (SRP), and total dissolved phosphorus (TDP) were determined by the ascorbic acid method (APHA 2005). Total nitrogen (TN), and nitrate nitrogen (NO_3-N) were determined by cadmium reduction method (Henrikson and Selmer-Olsen 1970; APHA 2005). Chlorophyll-*a* concentrations were determined by the analytical approach of Sartory and Grobbelaar (1984).

RESULTS AND DISCUSSION

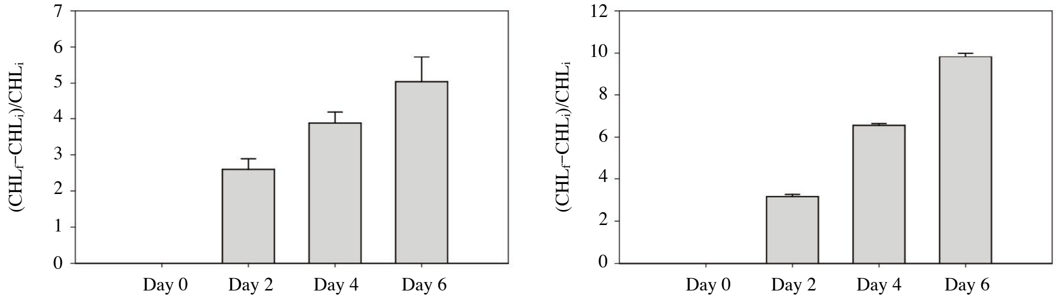
1. *In situ* Algal Bioassay Experiments (ABEs)

During the experiments of *in situ* ABEs, water tempera-


(a) Control (C)

(d) Nitrogen + Phosphorus treatment (T5)

(b) Phosphorus treatment (T1, T2)



(c) Nitrogen treatment (T3, T4)

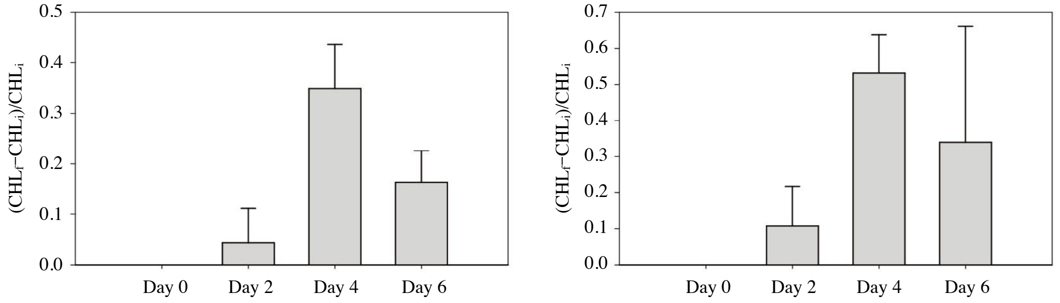


Fig. 3. The *in situ* ABEs of Day 0 to Day 6 in the control (C), phosphorus treatments (T1, T2), nitrogen treatments (T3, T4) and simultaneous treatments of nitrogen and phosphorus (T5). The algal response was expressed as the ratio of ($CHL_f - CHL_i$) to CHL_i [CHL_f = final chlorophyll-*a* concentration; CHL_i = initial chlorophyll-a concentration].

tures incubated, available light, and dissolved oxygen concentrations were maintained well for the algal growth. Mean water temperature near the cubitainers was 30.2°C and ranged from 27 to 33°C (1–4 PM), depending on the exposure time of the sun light. The light intensities were minimum (0 Lux) in the night vs. maximum (23,422 Lux) in the mid-day and declined rapidly from 23,422 Lux at 1:00 PM to 463 Lux at 4:00 PM. Mean concentrations of dissolve oxygen (DO) was 9.1 mg L^{-1} and varied from 9.6 to 12.1 mg L^{-1}, depending on the intensity of the algal photosynthesis (Fig. 2).

The *in situ* ABEs showed that algal response of P-treatments was more than 4 fold compared to that of the controls, but the treatments added only nitrogen (N) had no significant differences ($p = 0.142$) with the control. On day 0, as an initial ABEs, initial concentration of CHL was 5.28 μg L^{-1} in the control and this CHL concentrations had no significant differences ($p = 0.126$) in the Kruskal-Wallis tests on the treatments of N and P.

In the ABEs on Day 0, values of CHL averaged 5.28 μg L^{-1} in the control and all types of treatments of nitrogen and phosphorus and showed slight differences between the

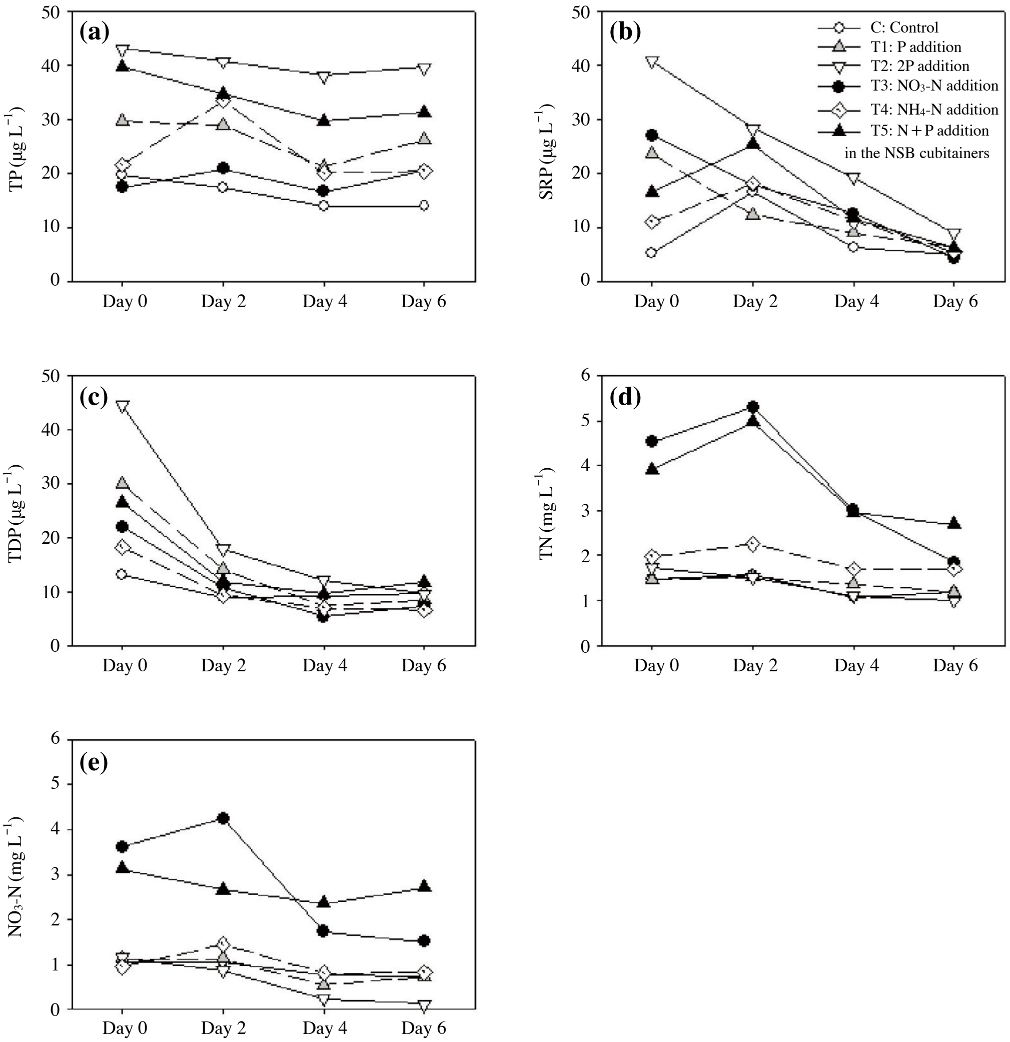


Fig. 4. The variations of phosphorus [total phosphorus (TP), soluble reactive phosphorus (SRP), and total dissolved phosphorus (TDP)] and nitrogen [total nitrogen (TN) and nitrate-N] in the cubitainers during in situ ABEs from Day 0 to Day 6.

cubitainers. The experiments on Day 2, Day 4, and Day 6 showed that the response of algal growth in the phosphorus treatments of T1, T2, and T5 were significantly greater than those in the controls (C) and nitrogen treatments (T3, T4) ($p < 0.05$). Algal response on the spiking experiments is shown as the relation of final chlorophyll-*a* values (CHL_f) on the initial values (CHL_i) on Day 0 to Day 6 and was expressed as the ratio of ($CHL_f - CHL_i$) to CHL_i. The initial values of CHL in the controls had no significant differences ($p > 0.05$) with final CHL values on each experiment, and on Day 6, even the final values of CHL (CHL_f) were lower than the initial algal content (CHL_i; Fig. 3a).

The algal response was directly determined by the magnitude of phosphorus addition in the cubitainer experiments but did not respond to nitrogen addition. The phosphorus treatments in the T1, and T2 had distinct increases of CHL values, and when the phosphorus added 2-fold to the treatments of T1 and T2, the increasing rate of CHL was 28.6% and 5.3% in the treatments of T1 and T2, respectively. In contrast, nitrogen addition in the algal bioassays had no significant differences with the controls, so the final chlorophyll-*a* values (CHL_f) did not increase or even decreased compared to the initial values (CHL_i; Fig. 3c). The final algal response in the T5 as simultaneous treatments of N + P, was similar to the initial response of T1, and the response in the single nitrogen treatments of T3, and T4 had even decreased

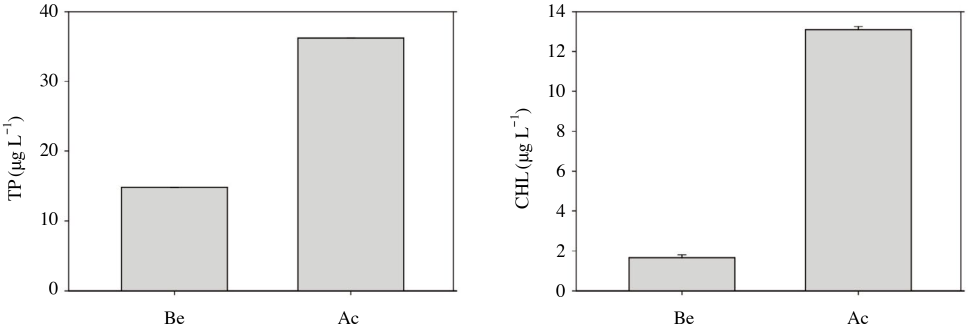


Fig. 5. Actual TP and CHL concentrations in the lake water before and after the construction of the water tunnel (Bc, before construction; Ac, after construction).

$(CHL_f - CHL_i)/CHL_i$ values, compared to the T1 (Fig. 3c).

Overall, the *in situ* spiking experiments of ABEs revealed that the algal response was determined by phosphorus and not by nitrogen, indicating that the primary limiting nutrient for algal growth in this reservoir was phosphorus and the additions of nitrate-N and ammonia-N to the cubitainers did not result in algal growth in Boryeong Reservoir. This result was consistent with lentic water bodies of lakes and reservoirs having the algal bloom with increase of phosphorus (Monrtimer and Hickling 1954; Vinberg and Liakhnovich 1965), and the algal growth *in situ* bioassays was a linear function of phosphorus concentrations added (Vinberg and Liakhnovich 1965; Lee *et al*. 2010).

During the *in situ* ABEs, the nutrients concentrations of the treatments were greater than those of the control, which decreased with time. Amount of N, and P values reduced were used for algal growth (Fig. 4). In terms of total phosphorus value, P treatments (T1, T2, and T3) had higher P values than the others were nearly 20 μg L^{-1}. Especially, T2 treatment added with 30 μg L^{-1} P were the highest figure with 43.08 μg L^{-1}. TP values decreased with time dragging (Fig. 4a).

The total dissolved phosphorus (TDP) concentration of T2, T1, and T5 were in order from the highest to lowest results, while T2, T3 and C were lower than the results above mentioned. T2 spiked with the highest P concentration had the highest figure of TDP. TDP decreased faster than TP did after 6 days. Merely, TDP value of T2 was higher than TP on Day 0, which was revealed as an experimental error (Fig. 4c). The total nitrogen concentrations of T3, and T5 were in order from the highest to lowest results, while the others were lower than the results above mentioned (Fig. 4d). The nitrate-N values of T3, and T5 were greater than the others. In theory, TN value is always greater than NO_3-N value in the same water, as TN contains NO_3-N. However, NO_3-N value of T5 was higher than TN on Day 0, which was revealed as an experimental error (Fig. 4e).

These *in situ* experiments of ABEs were supported by actual observations of nutrients and chlorophyll-*a* in Boryeong Reservoir before and after the construction of the water tunnel (Bc, Ac; Fig. 5). The analysis of actual TP and CHL in the reservoir before and after the construction of water tunnel (Bc and Ac) showed that TP values in the reservoir water increased over 2-fold after the construction of the water tunnel and also CHL values increased over 12-fold in response to the increased P (Fig. 5). This used the water data from R1 of Fig. 1. This result suggests that before the construction, TP and CHL values were low in the reservoir water, and that the inflowing of nutrient-rich water (high P) from the Geum River contributed to the high P in the reservoir water. The nutrient-rich water, in turn, increased directly CHL concentrations in the reservoir. These results suggest that influx of high P from the water tunnel to the reservoir directly increases algal growth in the future if the river water from the tunnel is supplied to the reservoir when water shortage is maximized in the reservoir.

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