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Application of barley straw to dammed river for algal control

B.J. Lim[a], J.H. Park[a], J.W. Jung[a,b], K.S. Hwang[a], M.S. Son[a], C.H. Lim[a], J.E. Na[a], S.G. Kim[a], H.M. Chai[a], K.A. Seo[a], J.H. Han[a], S.S. Park[c], J.K. Park[d,*]

[a]*Yeongsan River Environment Research Center, Cheomdan Road 208-5, Buk-gu, Gwangju, South Korea, emails: limnolim@korea.kr (B.J. Lim), thanks@korea.kr (J.H. Park), sustainwater07@gmail.com (J.W. Jung), hks55@korea.kr (K.S. Hwang), miza03@korea.kr (M.S. Son), chaehong@korea.kr (C.H. Lim), naje1033@korea.kr (J.E. Na), kskvision@korea.kr (S.G. Kim), hanjoke@korea.kr (H.M. Chai), poporiya@korea.kr (K.A. Seo), sl650515@korea.kr (J.H. Han)*

[b]*Department of Rural and Biosystems Engineering, Chonnam National University, Gwangju 500-757, Republic of Korea*

[c]*Department of Environmental Science and Engineering, University of Ewha Womans, 11-1 Daehyun-dong Seodaemun-gu, Seoul, South Korea, email: ssp@ewha.ac.kr*

[d]*Department of Civil and Environmental Engineering, University of Wisconsin-Madison, 1415 Engineering Drive, Madison, WI 53706, USA, email: jkpark@wisc.edu*

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ABSTRACT

In 1970s, barley straw has been known to be capable of controlling algal growth. *In vitro* and *in situ* experiments were performed to investigate the effects of barley straw on algal growth inhibition substances. In addition, its ecotoxicity and the algal growth inhibition substances extracted from barley straw were examined. In the *in vitro* experiment, barley straw and retinaspora leaf treatment groups caused significant decreases (p value < 0.05) in the number of cells in *Anabaena affinis*, *Microcystis aeruginosa*, and *Scenedesmus quadricauda* compared with the control group. However, the treatment group, which applied 0.2 g of barley straw, showed a tendency of increasing the number of cells in *S. quadricauda* compared with the control group. It reveals that types of algae and proper applications of barley straw are important to inhibit algal growth. In addition, the barley straw showed almost no ecotoxicities in ecotoxicity experiments while the retinaspora leaf showed a certain degree of ecotoxicity (Toxicity unit >36). In the *in situ* experiment, algal growth was inhibited by barley straw. In particular, diatoms exhibited a tendency of decreasing the number of algal cells compared with blue-green algae. It shows that the barley straw does not inhibit the growth of all algae and shows different effects according to types of algae. The algal growth inhibition substances were extracted from the barley straw and tested. It was found that a ferulic acid in various phenol compounds affected the algal growth most.

Keywords: Algal growth; Barley straw; Ecotoxicity; Inhibition; *In vitro*

*Corresponding author.

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1. Introduction

In Korea, there is a growing concern with the algal bloom due to the recent construction of dams as part of the Four Major Rivers Restoration project. Algal blooms cause various problems: toxicities [1,2], taste-and-odor, filter clogging in water treatment plants [3,4], and destruction of aquatic ecosystems due to dissolved oxygen depletion [5]. Thus, it is necessary to establish measures for preventing, controlling, and mitigating algal blooms. However, most causes of algal blooms cannot be clearly identified due to various factors interacting together or independently and thus, it is not easy to propose a simple and effective strategy for combating algal blooms. In addition, although nutrient removal from point and non-point sources, increased discharge, and chemical suppression may be used for control of algal blooms, these methods are not always easy to implement due to costs, logistics, or ecological side effects.

It is important to select a proper algal mitigation method that has a low-ecological impact and selectively eliminates algae while minimizing aesthetic views and recreational activities. Thus, it is necessary to develop a technology of controlling algae employing the concept of an allelopathic interaction, which is a natural mutual response among organisms.

In late 1970s, it has been found that barley straw were able to inhibit algal growth in the United Kingdom. Also, reports on inhibiting such algal growth using barley straw in freshwater ecosystems have been continuously presented [6–11]. In particular, [9] reported that algae in lakes decreased by applying barley straw for a long period of time. A study was conducted to develop the method of inhibiting algal growth using various plants in Lake Paldang, Han River in Korea [12,13].

The mechanism of controlling algae using barley straw has been asserted based on the fact that lignin and tannin generated from barley straw excrete phenolic compounds and quinones while these substances are dissolved by microorganisms [11,14]. In particular, Pillinger et al. [15] claimed that the phenolic compounds and quinones extracted from barley straw by aerobic microorganisms and the hydrogen peroxide generated by light energy in an auto oxidation process of phenolic compounds appeared to inhibit algae. Iredale et al. [16] reported that live activities in microorganisms affected the secretion of the growth inhibition substances in barley straw and decreased the secretion as catalase is applied to algae even though the secretion is faster than that of chopping barley straw into small pieces and projecting ultra violet. In addition, Murray et al. [11] reported that previously decomposed barley straw shows better efficiency in inhibiting algal growth than normal barley straw because the previously decomposed barley straw secrets the algal growth inhibiting substances better than the normal barley straw. However, there are few studies on the effects of using barley straw on algal growth in the large man-made lakes even though such different studies on the effects of using barley straw on algal growth in various regions have been conducted.

In this study, the effects of barley straw on algal growth have investigated through an empirical manner in order to preemptively respond to algal blooms periodically occurring in the lakes and rivers of Korea annually. Then, the effects of barley straw on algal growth and substances secreted from barley straw were examined by *in situ* experiments after verifying the ecological safety of such barley straw through ecotoxicity tests.

2. Materials and methods

2.1. Selection and culturing of algal strains

In vitro experiments were conducted to investigate whether barley straw and retinaspora leaf inhibit algal growth. *Anabaena affinis* and *Microcystis aeruginosa* (blue-green algae) and *Scenedesmus quadricauda* (green algae) were obtained from the Korea Research Institute of Bioscience and Biotechnology and used as a subcultural manner. A 10× concentrated solution using BG11 produced by Biosesang Inc. (Seoul, Korea) was used as a medium after dilution.

2.2. In vitro *algal inhibition experiments using barley straw*

For verifying the algal inhibition implemented using barley straw and retinaspora leaf, the treatment group consisted of a control, 0.1 g of retinaspora leaf, and 0.2, 0.3, and 0.4 g of barley straw. The grinded barley straw was mixed with *A. affinis* and *S. quadricauda* with a concentration of 10^4 cells/mL for 200 mL mediums. Then, it was cultivated for three weeks and the number of algal cells was counted.

2.3. Ecotoxicity experiments

A series of experiments was conducted to evaluate the effect of barley straw and retinaspora leaf on acute mobilization toxicities for daphnia following the Standard Methods for the Examination of Water Pollution issued by the Korean Ministry of Environment (2010),

based on the OECD guideline (2004). The daphnia used in the experiments were neonate less than 24 h old and a total of 20 daphnia were used for four different test concentration levels with five daphnia for each test. Light (16 h) and dark (8 h) conditions were created repeatedly and oxygen and feed were not supplied during the experiments. Finally, a 50% median effective concentration (EC_{50}) that induces a response halfway between the baseline and maximum was calculated using the TSK (Trimmed Spearman Kareor) statistical method by the United States Environmental Protection Agency and the EC_{50} was applied to Eq. (1) to obtain ecotoxicity values.

$$\text{TU}\left(\text{Toxicity unit}\right) = 100/\text{EC}_{50}\ (\%) \quad (1)$$

2.4. In situ *algal inhibition experiments using barley straw*

In situ algal inhibition experiments using barley straw were implemented at the Seungchon weir (N.L. 35° 03′55″, E.L. 126° 45′59″) in the Yeongsan River located in the southwest part of the Korean Peninsula. The average river width is 300 m, sectional area is 300,000 m^2, and water storage volume is about 1,500,000 m^3 (Fig. 1). The control group was determined at the Seochang bridge, which is about 7 km upstream of the Seungchon weir, in order to examine algal inhibition effects, according to applications of barley straw where the barley straw was applied to the pier of the Seungyoung bridge and the section with a width of 1 km between the Seungchon weir and the Seungyoung bridge using artificial structures as shown in Figs. 2 and 3, respectively. In the first application, the barley straw, 800 kg, was applied to the pier of the Seungyoung bridge from 8 March to 2 April 2012, and removed in 2 August 2012. The second and third applications, 3,000 kg and 4,500 kg, were applied during 2–7 September and 8–19 October, respectively. In order to investigate the effects of barley straw application on algae growth, Chl-*a* concentrations in the Seungyoung and Seochang (a control group) bridges were analyzed and compared. Chl-*a* was measured according to the Standard Methods for the Examination of Water Pollution issued by the Korean Ministry of Environment (2010).

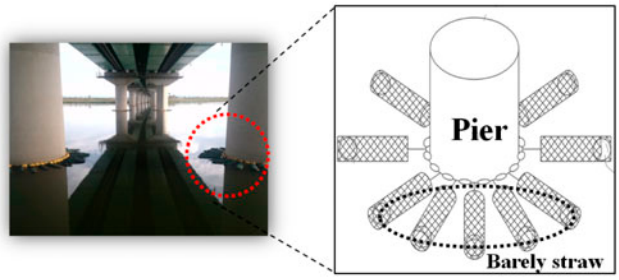

Fig. 2. Bridge pier equipped with barley straw nets.

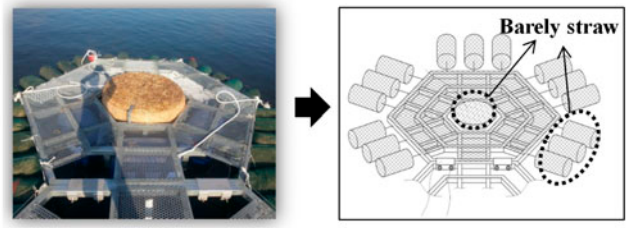

Fig. 3. Artificial equipment for installing barley.

2.5. *Analysis of algal growth inhibition substances*

In order to analyze algal growth inhibition substances, 20 g of the barley straw chopped by 2 cm and 1 L distilled water was placed in a flask. The extract

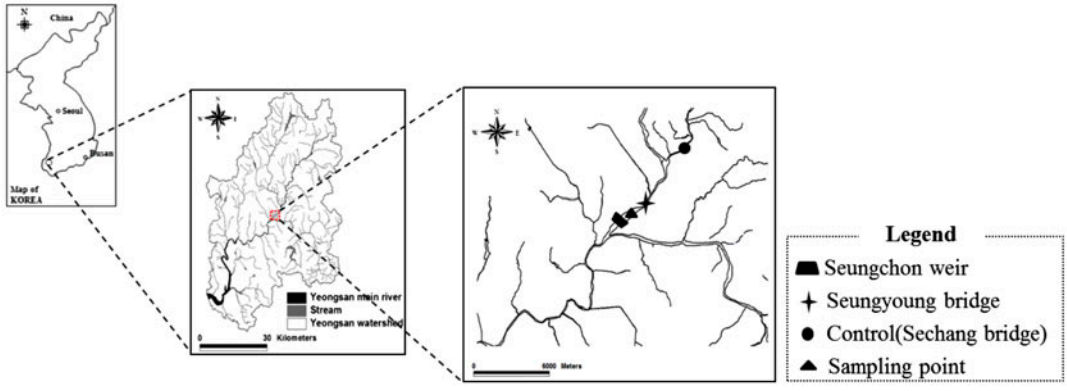

Fig. 1. Locations of control and sampling points.

was obtained after 90 d for *in vitro* experiments. Then, the extract and 800 kg of barley straw was applied to the test site and phenolic compounds of the sample were analyzed regularly. Since, the lignin in plants releases various types of phenolic compounds under the aerobic condition [17], the samples were analyzed for seven different types of the phenolic compounds: vanillic acid, syringic acid, p-coumaric acid, gallic acid, ferulic acid, caffeic acid, and sinapic acid. A derivatization reagent (BSTFA + TMCS, 99:1 (Supelco, St. Louis, MO, USA) was used for analysis. Then, it was analyzed using GC/MS (6890 N GC, 5975 MS, by Agilent). Also, the DB-1MS capillary column (30 m × 250 μm × 0.25 μm) by J&W (Folson, CA, USA) was used as a GC column in which helium gas was used as a carrier gas and applied as a rate of 1 mL/min. The temperature of the oven used in the experiments was maintained at 80˚C for 1 min and increased up to 200˚C with a rate of 10˚C/min. Then, it was increased up to 310˚C with a rate of 5˚C/min and maintained for 5 min. An aliquot of 1 μL was injected to GC through the inlet with a temperature of 270˚C using a splitless method. Then, the extraction was carried out using the MS-SIM method.

2.6. Data analysis

In the *in vitro* experiments, the differences in algal growth between the control group and the barley straw treatment group were analyzed using ANOVA as the data satisfied its normality. If the normality was not satisfied, the Kruskal–Wallis tests were implemented. In addition, in the *in situ* experiments, the differences in algal growth according to applications of barley straw were analyzed through paired *t*-tests in which the statistical significant level was considered below 0.05.

3. Results and discussion

3.1. Characteristics of inhibiting algal growth by types of algae and treatment groups in in vitro experiments

Fig. 4 shows the results of the *in vitro* experiments. All treatment groups with 0.1 g of retinaspora leaf and 0.2, 0.3, and 0.4 g of barley straw exhibited decreases in the number of cells in *A. affinis* during the experiment period, about three weeks (p value < 0.05). In the case of the treatment group with 0.3 g of barley straw, the number of cells for *A. affinis* decreased at the initial stage but increased after two weeks from that stage. In the case of *S. quadriqauda*, although the number of cells in *S. quadriqauda* decreased in the treatment groups with 0.1 g of retinaspora leaf and 0.3 and 0.4 g of barley straw (Fig. 3, $p < 0.05$), the treatment group with 0.2 g of barley straw shows an increase in the number of cells in *S. quadriqauda* (Fig. 3, $p < 0.05$). In addition, the number of cells in *M. aeruginosa* is observed as a very low level in all treatment groups with 0.2, 0.3, and 0.4 g of barley straw. The treatment with 0.1 g of retinaspora leaf and 0.4 g of barley straw showed the highest growth inhibition (>90%) for *A. affinis*, *S. quadriqauda*, and *M. aeruginosa*. It can be said that a proper amount of applying barley straw is very important to effectively control algal growth [18,19].

3.2. Analysis of the ecotoxicity in barley straw and retinaspora leaf

The daphnia's ecotoxicity was evaluated using the extracts from the mixtures of 1 g of retinaspora leaf and 10, 20, and 30 g of barley straw with 1 L of distilled water over 90 d and the results are presented in Table 1. The EC_{50} values of the treatment groups with 10, 20, and 30 g of barley straw were >10,000, >20,000, and >30,000 mg/L respectively. The values of TU were all zero. It reveals that barley straw shows no toxicities. In the case of retinaspora leaf, however, the value of TU is >36, demonstrating that retinaspora leaf has greater toxicity than barley straw. Since retinaspora leaf showed higher algal growth inhibition than barley straw in the *in vitro* experiments, the application should be more carefully evaluated and monitored.

3.3. Characteristics of inhibiting algal growth in applying barley straw to sites

The Chl-*a* concentrations at the Seochang bridge were used as a control and compared with those at the test site (Seungyoung bridge). Fig. 5 shows the experimental results. Before applying barley straw, the Chl-*a* concentrations at the Seochang bridge were lower than the Seungyoung bridge (paired *t*-test, $p < 0.05$), However, after applying barley straw, the control (Seochang bridge) had higher Chl-*a* concentrations than the test site (paired *t*-test, $p < 0.05$). It took almost two months for the test site (Seungyoung bridge) to reach lower Chl-*a* concentrations than the control.

Changes in the number of algal cells before (2011) and after (2012) applying barley straw to the Seungyoung bridge and Seungchon weir are presented in Fig. 6. Diatoms in March 2012 were >40,000 cells/mL, before the first application of barley straw represent higher levels than the cells in 2011 and the diatoms are presented as a dominant species during the period of March–August 2012 in which the number of algal

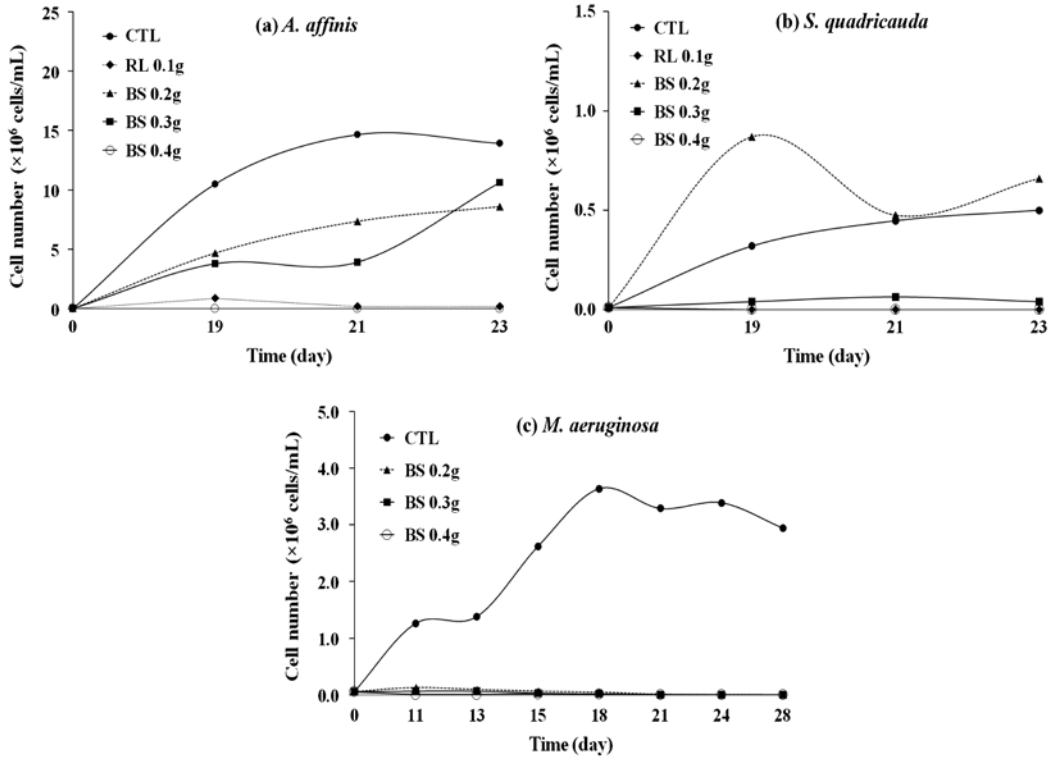

Fig. 4. Growth inhibitions of *A. affinis*, *S. quadriqauda*, and *M. aeruginosa* using barley straw and retinaspora leaf *in vitro* tests for three weeks.

Table 1
Ecological toxicities in barley straw and retinaspora

Items	Barley straw			Retinaspora
	10 g/L	20 g/L	30 g/L	1 g/L
EC_{50} (mg/L)	>10,000	>20,000	>30,000	<139
TU	0	0	0	>36

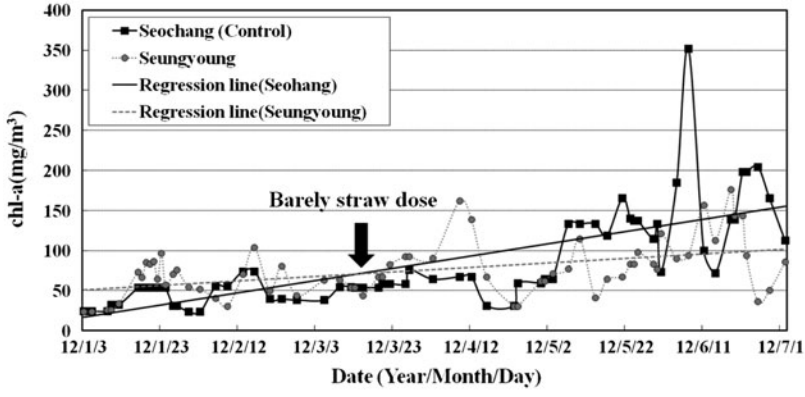

Fig. 5. Changes in the Chl-*a* concentrations after barley straw application in the Seochang and Seungyoung bridges.

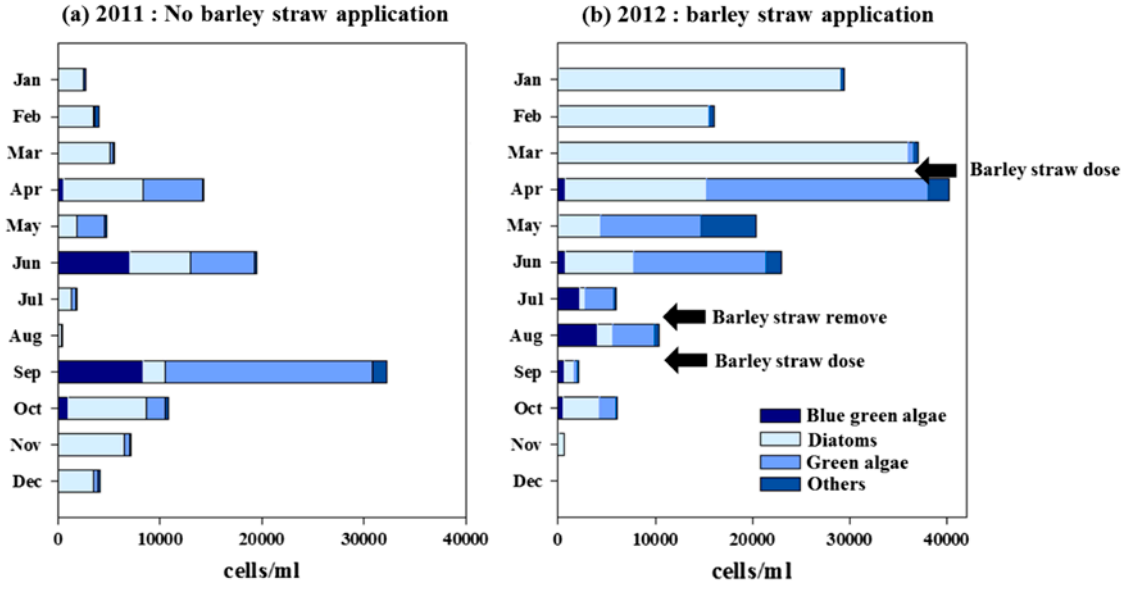

Fig. 6. Changes in the algal population before and after barley straw application in 2011 and 2012.

cells was higher in 2011. After the first application of barley straw in April 2012, the number of algal cells decreased. After removing barley straw in August 2012, the number of algal cells increased from ~6,000 to 10,000 cells/mL. Once barley straw was applied again in September 2012, the number of algal cells decreased to 1,000 cells/mL. This is much lower than 30,000 cells/mL in September 2011. In the case of the blue-green algae, the number of algal cells decreased significantly after applying barley straw. However, the diatoms shows a trend of decreasing the number of algal cells as a gradual manner compared to that of the blue-green algae. Considering that the nutrient (total nitrogen and total phosphorus) and water temperature showed similar trend in 2011 and 2012 (Fig. 7), it could be said that barley straw dose inhibit algal growth in the study site.

3.4. Characteristics of inhibiting algal growth for types of algae in applying barley straw to sites

Fig. 8 shows the results of the phenolic compounds extracted from the laboratory and site. The concentrations of the phenolic compounds from the laboratory and test site samples were 293.9 and 17.1 μg/L, respectively. The laboratory sample had much greater phenolic compounds than the site sample. Interestingly, the compositions of the phenolic compounds from both samples were different. In the site sample, gallic acid, p-coumaric acid, and syringic acid were detected. The laboratory sample that extracts pure barley straw only had high concentrations of syringic acid, ferulic acid, p-coumaric acid, and vanillic acid. In particular, ferulic acid concentrations were 21% in the laboratory sample but only 1% in the site sample. Thus, the major phenolic compound secreted from the barley straw is thought to be ferulic acid. In order to confirm this result, we conducted additional experiments where 0.2 and 0.4 g of barely straw are applied to 200 mL of the *M. aeruginosa* nutrient solution. The experiments showed that algal growth inhibition was observed after 35-d. Although the proportions of p-coumaric acid of the total phenolic compounds in the treatment groups with 0.2 and 0.4 g of barley straw were about 50% at the experiments initial stage, it decreased to 10% after 35-d. However, the proportions of the ferulic acid of the total phenolic compounds in the treatment groups with 0.2 and 0.4 g of barley straw were 30 and 40%, respectively, at the experiment's initial stage while the proportions increased up to 52 and 66%, respectively, after 35-d (Fig. 9). In addition, the proportion of ferulic acid increased as the dose rates of barley straw increased. In summary, it is verified that ferulic acid is a major phenolic compound extracted from barley straw. The amount of barley straw and extraction time were the major factors affecting the concentration of ferulic acid.

For investigating the effects of barley straw on algal growth inhibition, *in vitro* and *in situ* experiments are implemented. In the *in vitro* experiments, the number of cells in algae in the treatment groups with 0.2, 0.3, and 0.4 g BS exhibited significant

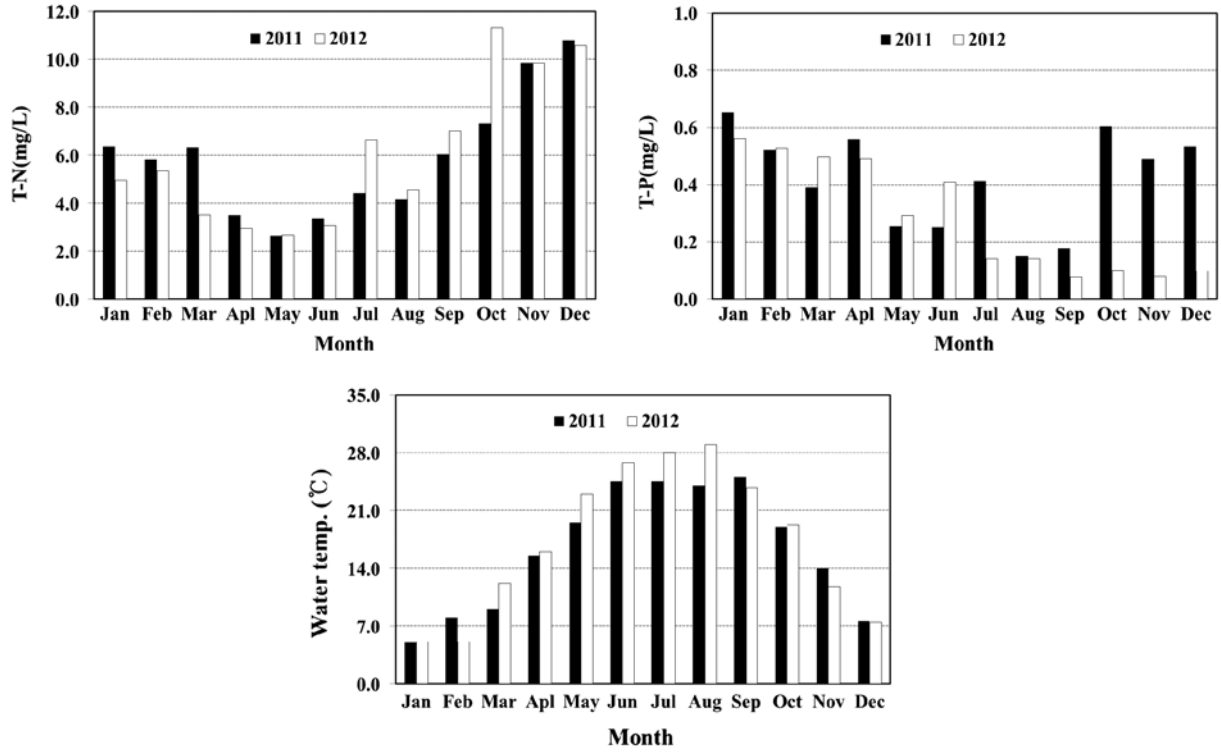

Fig. 7. Monthly changes in T-N, T-P, and water temperature in the study site.

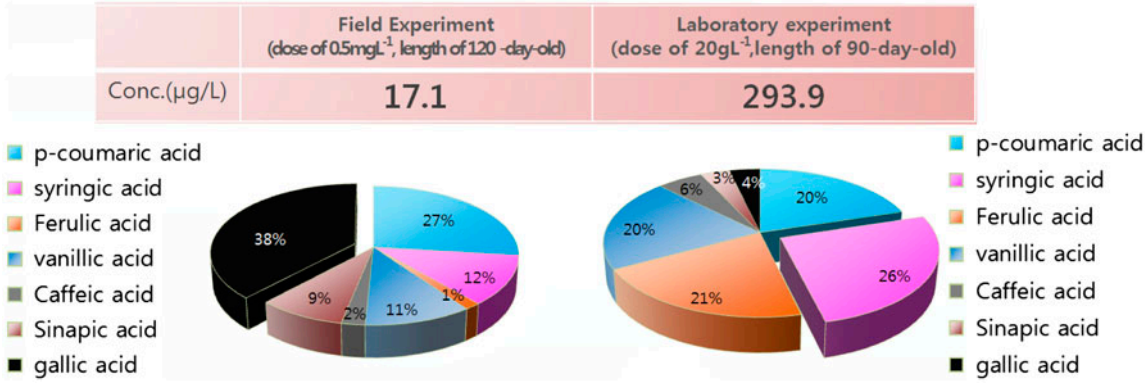

Fig. 8. Release rates of seven phenol compounds in the field and laboratory samples with the extracts of barley straw.

decreases in the cells. It agrees with the fact that barley straw inhibits algal growth argued by different researchers [11,15,16,18,20–23]. In the case of the *S. quadriqauda*, which is a green alga, however, the treatment group with 0.2 g BS showed an increase in the cells compared to that of the control group. It reveals that the type of algae and proper applications of BS are important to perform algal growth inhibition. Newman and Berrett [20] showed that applications of barley straw rather increase algal growth according to types of algae and other reports on this issue have also presented [18,19]. In addition, the *in situ* experiments showed the inhibition effects according to applications of barley straw (Figs. 5 and 6). In the case of the blue-green algae, applying barley straw showed a rapid decrease in the number of cells but diatoms represented a gradual decrease in the cells. It shows a similar tendency to the results reported by Barrett et al. [9] Ball et al. [23] and Everall and Lees [14] have reported that the effects of barley

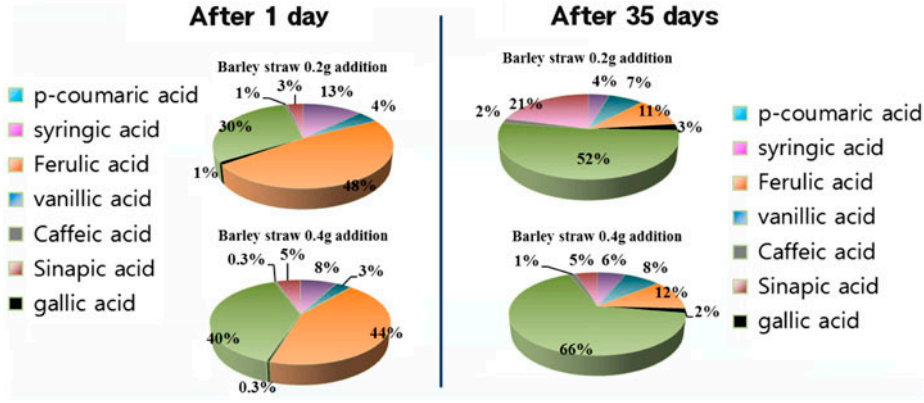

Fig. 9. Release rates of seven phenol compounds in culture media with barley straw chips after 35-d.

straw on algal growth inhibition are not presented in all algae unconditionally but a species-specific characteristic in general. Although the blue-green and green algae represent large growth inhibition effects by barley straw, diatoms represent a characteristic that does not show rapid growth inhibition effects compared to blue-green and green algae [10].

4. Conclusions

From a series of *in vitro* and field experiments were performed to evaluate the effect of barley straw on algal growth, the following conclusions can be drawn:

(1) The treatment groups with 0.2, 0.3, and 0.4 g of barley straw significantly reduced algal population except *S. quadriqauda*, a green algae where the treatment by 0.2 g of barley straw had an increase in the cells compared to that of the control group. Also, the barley straw showed no toxicities in ecotoxicity experiments.

(2) The type of algae and proper applications of barley straw were important in controlling specific types of algae.

(3) Although the phenol compounds consist of various substances including vanilic acid, syringic acid, p-coumaric acid, galic acid, ferulic acid, caffeic acid, and sinapic acid, in this study the ferulic acid is verified as a core substance in growing algae. In addition, it is verified that the concentration of this substance is largely affected by the amount of barley straw and the extraction time.

(4) The use of barley straw for algae control in dammed rivers appears to be feasible despite the relatively volume of the water.

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