MICROALGAE GROWTH INHIBITION ANALYSIS IN THE NITROGEN AND PHOSPHORUS ADSORBENT (NPA)-TREATED WATER

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(Received 5th Jan 2024; accepted 13th Mar 2024)

Abstract. This study aimed to reduce the occurrence of algal blooms by decreasing nitrogen and phosphorus elution leaked from livestock manure sprayed on farmland in dams and river basins. Excessive algal growth affects water availability by disturbing the aquatic ecosystem, clogging filters of the water intake tower, generating toxins, and causing social and economic losses. Nutrients discharged from nonpoint pollution sources (farmlands and barns) often flow into rivers without being purified, thus contaminating nearby river basins. An analysis of algal growth characteristics by dividing rainfall runoff into raw solution, nitrogen and phosphorus adsorbent (NPA) compost, and general compost affected growth rate and microalgae biomass production as well as nitrogen and phosphorus removal rates. Blue–green algae (*Microcystis aeruginosa*) and green algae (*Scenedesmus acutus*) were diluted, and the effect of reduction of microalgae growth was studied in all experiments. Results of water quality analysis reveal that eco-friendly compost has the effect of reducing nutrient salts in rainfall runoff by 30%–40% compared to general compost. Furthermore, the growth of blue–green algae and green algae can be reduced if the effluent of eco-friendly compost flows into the river and the nutrient concentration is lowered.
Keywords: *blue–green algae, convert nutrients, green algae, livestock wastewater, nitrogen and phosphorus adsorbent (NPA), rainfall runoff*

Introduction

In Korea, meat consumption has increased rapidly since the 1970s due to rapid economic growth and improved national incomes; moreover, the size of livestock farms has also increased rapidly due to national growth policies. Factory livestock farming, which began in some full-time and corporate farms in the 1980s, grew into a dominant form in rural livestock farming in the 1990s and became more sophisticated in the 2000s (Song, 2013). A characteristic of modern industrial agriculture is that it is performed in a non-natural way, and factory farming is also an application of industrial agriculture in the livestock sector (Kim, 2014).

Due to the increase in the amount of livestock manure and discharge of high-concentration pollutant loads following the spread of factory farming, the water quality and health of the aquatic ecosystem have been severely damaged in several areas, and collective civil complaints have been registered due to the odor of livestock manure. Additionally, although algae occurrence in major rivers and lakes is repeated annually, it is difficult to develop a reasonable solution because interest in various fields is

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_27492762

intertwined (Sharpley and Wang, 2014; Kim et al., 2021). In 2004, the Korean government established measures for managing and using livestock manure (Ministry of Agriculture and Forestry, Ministry of Environment, 2004), 1) by establishing a resource-recycling eco-friendly livestock foundation for expanding the use of livestock manure and 2) by improving river water quality through the appropriate treatment of livestock manure step 1 to improve step 2 (Kim, 2020).

Microalgae are rapidly growing photosynthetic microorganisms that live in harsh conditions and have unicellular or simple multicellular structures (Mata et al., 2010; Elisabeth et al., 2021). They provide a way for the removal of contaminants (nitrogen, phosphorus, and carbon) from wastewater while producing biomass that could be used to produce high-value chemicals (algal metabolites) and/or biogas through anaerobic digestion (Muñoz and Guieysse, 2006; Nguyen et al., 2022). Thus, microalgae have attracted much attention in recent years as an alternative system for the treatment of biological wastewater, with several applications in wastewater treatment (Tredici et al., 1992; Kaya and Picard, 1996; Craggs et al., 1997; Kong et al., 2010; Su et al., 2011; Nguyen et al., 2022). Furthermore, microalgae can diminish the harmful effects of sewage effluent and reduce eutrophication in aquatic environments (Abdel-Raouf et al., 2012; Abdelfattah et al., 2023). Wang et al. (2010) reported a decrease in nitrogen (83% N as NH_4^+) and phosphorus (90% P as PO_4^{3-}) in municipal wastewater by *Chlorella* sp. They found that the nutrient removal rate was independent of the optimal N/P ratio; however, the concentration of these nutrients was important for algae growth systems. In another study, algae growth systems clearly showed the high potential of using an isolated algal strain of an artificial freshwater pond, *Desmodesmus communis*, to eliminate pollutants from primary wastewater (Samori et al., 2013). These photosynthetic microorganisms may provide oxygen to heterotrophic aerobic bacteria, which could biodegrade organic pollutants from municipal wastewater and release carbon dioxide that microalgae use in the presence of light. Microalgae are known to exhibit many types of metabolism (e.g., autotrophic, heterotrophic, mixotrophic, and photoheterotophic), and are capable of a metabolic shift in response to changes in environmental conditions (Delgadillo-Mirquez et al., 2016; Abdelfattah et al., 2023); therefore, they can compete with heterotrophic bacteria (Subashchandrabose et al., 2011; Delgadillo-Mirquez et al., 2016) under specific conditions. Net pollutant removal in these systems is an additive effect of algal assimilation (Su et al., 2011; Perez-Garci et al., 2011), biological processes (nitrification/denitrification), and stripping phenomena such as ammonia volatilization and phosphorus precipitation. The latter is likely caused by the high pH levels induced through photosynthetic microalgal growth (Muñoz and Guieysse, 2006; Wang et al., 2010; Lee et al., 2015; Abdelfattah et al., 2023). The algal nutrient uptake efficiency is affected by the bioavailability of nutrients and the complex interactions between physicochemical factors (e.g., pH, light intensity, photoperiod, and temperature) and biological factors (Delgadillo-Mirquez et al., 2016; Abdelfattah et al., 2023).

Microalgae cultures are often grown in nitrogen and phosphorus adsorbents (NPA) to treat municipal, industrial, and/or agricultural wastewater (Kim et al., 2013; Kim, 2020; Goh et al., 2022) and that demonstrated high removal efficiencies for COD (86%), total nitrogen, (93%) and total phosphorus (83%) from untreated municipal wastewater in high-rate algal ponds (Delgadillo-Mirquez et al., 2016; Abdelfattah et al., 2023). Understanding the effects of environmental parameters (e.g., temperature, light, pH, Nitrogen, Phosphorus.) on biotic and abiotic phenomena is a way to improve and optimize the performance of NPAs (Delgadillo-Mirquez et al., 2016; Abdelfattah et al.,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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2023). In this preliminary study, the effect of both temperature and photoperiod on microalgae biomass production and pollutant removal (soluble nitrogen and phosphorus) from municipal wastewater was investigated. In the study, to test the effect of reducing algae-causing substances (P) and improving river water quality using eco-friendly compost compared to general compost, the water quality of soil runoff during rainfall is compared and analyzed to assess improvements in water quality.

Materials and methods

Sample collection

An empirical test was conducted in which granular NPA and granular general compost were directly sprayed on caw sheds at Naso-Farm located in Nasori, 621, Nongam-ro, Waryong-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea (36°64’54.10”N, 128°81’13.78”E). The spraying method is to spray directly on the cow sheds while they are stocked and have cow manure. Cow manure was collected 30 days after the cows were stocked and were prepared for analysis by putting them in gunny bags. Component analysis results of the general compost and the eco-friendly compost (*Table 1*). After that, soil runoff collected from plum, radish, peony, and apple farmland fertilized with general compost and eco-friendly compost was used as a medium to reduce microalgae, which frequently appear in dams and river basins. For water quality improvement and green algae reduction effect analysis, Rainfall runoff was collected from cultivated fields for each of the following crops: plum, radish, peony, and apple. The sample collection period indicated an average rainfall of 32.6 mm about 5 weeks from July 24 to September 3, 2020. NPA compost and general compost-water samples were collected from the soil initial runoff of rainwater collection from the cultivated fields for each of the crops. The culture solution used untreated water (runoff from farmland fertilized with general compost) and treated water (runoff water from farmland fertilized with eco-friendly compost, NPA treatment) collected from July 24 to September 3, 2020. To accurately analyze the soil initial runoff of the soil during rainfall, a representative point was selected for each farmland and a rainwater collection device was installed for each test zone to collect rainfall runoff from the soil. Among the initial soil runoff of rainwater collection from the cultivated fields for each of the crops, four rainwater collections (radish: 36°64’68.82”N, 128°83’09.57”E, 863-1, Gakeuri-gil, Waryong-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea; plum: 26°60’36.29”N, 128°84’06.68”E, 914-1, Dogok-gil, Waryong-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea; apple: 36°69’01.57”N, 128°92’01.77”E, 692, In-gye-ri, Yean-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea; peony: 36°69’55.61”N, 128°91’15.55”E, 382, In-gye-ri, Yean-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea) from around Andong Lake were investigated (*Fig. 1*). The water samples were collected in September 2020. Samples were collected by 2 L per region and the collected samples were stored in a cooler box and transferred to the laboratory.

Sample isolation

Microalgae were collected and separated from the Andong Dam. The water was filtered with a mesh diameter of 25 μm and collected in 250 ml sample bottles (Hong et al., 2012). The algae (*Scenedesmus acutus*) and the blue–green algae (*Microcystis aeruginosa*) were grown in BG-11(+) medium containing nitrate for approximately 30

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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days with 250 μg/ml cycloheximide (Sigma, St. Louis, MO, USA) (Chang et al., 2013; Hong et al., 2016). The flasks were incubated on an orbital shaker (Vision Scientific, Bucheon, Korea) at 160 rpm and 15°C under cool fluorescent lighting (approximately 70 μmol photons m^2/s), until growth was evident. An aliquot of brown-colored biomass was pipetted and sonicated for approximately 3 to 5 s with an ultrasonic cell disruptor (Model 550; Fisher Scientific, Pittsburgh, PA, USA). The filaments were then transferred to a BG-11(+) agar plate with 100 g/ml meropenem (Yuhan Pharmaceuticals, Ochang, Korea) and were incubated in the dark for 24 h to eliminate the contamination bacteria (Cho et al., 2016). The culture was then inoculated with fresh BG-11(+) agar plates and incubated for 14 days under a light–dark cycle (16:8 h) at 15°C. This experiment was performed with minor changes compared to previous studies (Kim et al., 2019). Live cells were harvested by centrifugation at 4,000 rpm for 10 min, washed in sterile distilled water, and visualized at 400× using a Zeiss Axioskop 2 light microscope (Carl Zeiss, Standort Gottingen, Vertrieb, Germany) equipped with differential interference contrast optics.

Organism and growth conditions

Blue–green algae and green algae were incubated until the optical density (OD) of the seed culture at 680 nm reached 0.3–0.5 of microalgae. A milliliter of this seed culture was then inoculated into 100 ml of BG-11(+) medium in triplicate and incubated in a light–dark cycle of 16:8 h for 30 days. The aliquots of microalgae cells were maintained at 25°C, with 3-day intervals of values ranging from 12–21 was examined to determine optimum growth conditions. Subsequently, cell density was evaluated by measuring the OD of the culture at 680 nm using an Optimizer 2120 UV spectrophotometer (Mecasys Co. Ltd). Based on these experiments, blue–green algae and green algae were identified in the sample, and the cell number and chlorophyll-a content was measured. Cell numbers were counted to confirm the growth of blue–green algae and green algae. For cell counting, 1 ml of the sample fixed with Lugol's solution was placed in a Sedgewick–Rafter chamber and counted under a 200-fold optical microscope. All samples were counted three times to obtain an average value. The dry weights of the microalgal cultures were measured to analyze the biomass productivity in the stationary phase. Dry weight was calculated by filtering 10 ml of culture through a preweighed GF/C Whatman filter (47 mm, nominal pore size 0.7 μm), which was subsequently dried at 105℃ for 24 h. The dried filters were cooled to room temperature and reweighed (Yun et al., 2021).

Microalgae cultivation using NPA

Culture experiments were conducted to verify how general compost and eco-friendly compost influence the growth of blue–green algae and green algae. The culture solution used untreated water (runoff from farmland fertilized with general compost) and treated water (runoff water from farmland fertilized with eco-friendly compost, NPA treatment) collected from July 24 to September 3, 2020. Two culture experiments were carried out, the first using raw water without dilution of untreated water and treated water. In the second experiment, the culture experiment was carried out after adjusting the concentration of nutrients by dilution of untreated water and treated water 10, 50, and 100 times, respectively. In all experiments, blue–green algae and green algae were inoculated in a concentration of approximately 5,000 cells/ml within a culture medium

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
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with a total capacity of 100 ml. The culture experiment was conducted in a culture room where temperatures of 25°C, darkness cycle of light 16:8, and light intensity 50 μmol/m^2 s were maintained, and air was injected to prevent growth inhibition caused by carbon (CO_2) depletion. The culture start date was set to day 1 for 30 days, and 1 ml was taken every 3 days and fixed with Lugol solution to count the number of cells.

Table 1. *Component analysis results of cow general and NPA compost effluent*

Analysis items	Sample type	
	General compost	NPA compost
pH	8.8	7.9
Moisture Content (%)	74.6	75.1
Total Nitrogen (T-N) (mg/L)	102.4	41.6
Ammonia Nitrogen (NH_4-N) (mg/L)	31.42	9.56
Total Phosphorus (T-P) (mg/L)	18.19	8.18
Phosphate Phosphorus (PO_4-P) (mg/L)	14.60	5.12
Calcium (Ca) (mg/L)	7.01	7.13
Magnesium (Mg) (mg/L)	18.89	139.37
Ferric Ion (Fe) (mg/L)	0.52	0.53
Potassium (K) (mg/L)	121.18	195.21

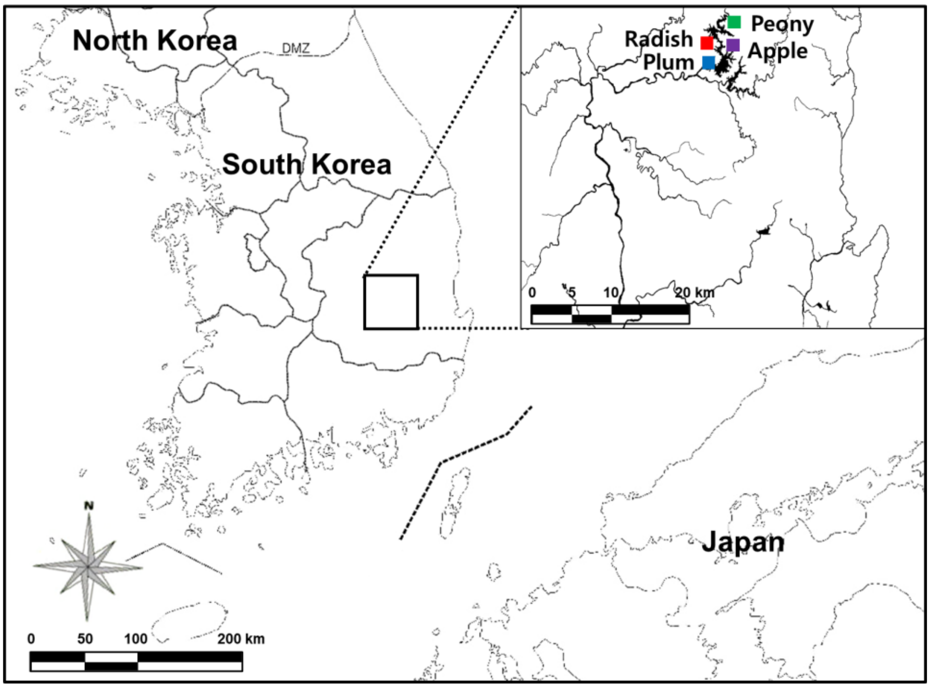

Figure 1. *Location of sampling sites in four places of Andong Lake (black box) in South Korea. (A) Radish (red box): 36°64'68.82''N, 128°83'09.57''E, 863-1, Gakeuri-gil, Waryong-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea. (B) Plum (blue box): 26°60'36.29''N, 128°84'06.68''E, 914-1, Dogok-gil, Waryong-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea. (C) Apple (purple box): 36°69'01.57''N, 128°92'01.77''E, 692, In-gye-ri, Yean-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea. (D) Peony (green box): 36°69'55.61''N, 128°91'15.55''E, 382, In-gye-ri, Yean-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea, marked with small boxes*

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Measurement of chlorophyll-a content

For chlorophyll content assays, strains were harvested from 100 ml cultures ($OD_{630-750}$). Whole cultivated cells were harvested from a 10 ml culture, with whole pellets resuspended in 90% methanol, and measured using the method used by Halimatul et al. (2014) with some modifications. The absorption spectra of the cells were determined by harvesting extracted chlorophyll-a samples in the presence and absence of NPA treatment. In order to explain in more detail, chlorophyll-a assays were performed by scanning from 260 to 800 nm using a spectrophotometer (Infinite M200 Pro microplate reader, Tecan). For the estimation of chlorophyll-a in blue–green and green algae, microalgae were sampled from cells grown in the presence and absence of NPA treatment for approximately 2–4 weeks. All tests had three biological replicates. The total content of chlorophyll were calculated using extraction coefficients and equations previously reported (Şükran et al., 1998; Kim et al., 2018).

Results and discussion

Rainfall runoff water quality analysis

Agricultural nonpoint source (NPS) pollution from crop paddy fields can be effectively managed when an appropriate drainage water management practice is imposed (Kim et al., 2012; Goh et al., 2022). The water-borne pollution loads from agricultural NPS pollution are expected to increase due to ongoing precipitation. Therefore, it is essential to develop a best management practice (BMP) that is suitable for agricultural environments in Korea. This study aimed to develop an environmentally friendly BMP to reduce the NPS pollution load caused by agricultural activities. In this study, a small eco-friendly drainage pond was proposed to avoid direct drainage of agricultural runoff and eventually reduce the amount of pollutants discharged into the surrounding water environment (Kim et al., 2013; Cho et al., 2016; Goh et al., 2022). The blue–green algae used in the experiment is the main cause of the algae boom in summer and is monitored cyclically by producing microcystin, a liver toxin. Furthermore, in the case of green algae, which is a common species in domestic water systems, it was judged to be an appropriate species for confirming NPA's effect and then was used in the experiment. A field experiment was conducted for approximately 15 months to identify the water quality characteristics of rainfall runoff after the application of NPA compost. From June–July 2020, site visits and experimental sites were selected (*Fig. 2A*), while water collection devices (*Fig. 2B*) were installed in mid-July. Rainfall runoff before composting was collected once to September 3, 2020 (*Fig. 2C*), and this was used as a background value. Field experiments were conducted by installing NPA and general compost test plots on the same lot by minimizing rainfall runoff variables such as soil quality and slope. *Figure 1* shows the rainfall event in the Andong region and the progress of the field experiment.

Analysis of the growth patterns using NPA

Blue–green algae and green algae have been shown to grow optimally with no significant differences in their NPA and maximum productivity (*Fig. 2D*). Analyses of growth patterns revealed that blue–green algae and green algae, as these strains would require supplementation (four types; plum, radish, peony, and apple), reused the treated water. This suggests that the green algae strains had more efficiency of NPA a rainfall

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runoff (four types; plum, radish, peony, and apple) water than blue–green algae *(Figs. 3* and *4*). Furthermore, *Microcystis aeruginosa* showed better growth patterns in rainfall runoffs of radish, peony, and apple than rainfall runoff of plum under various NPA conditions, particularly in rainfall runoff of all four (four types; plum, radish, peony, and apple) (*Fig. 3*), reaching the stationary phase within 30 days of inoculation. Furthermore, *Microcystis aeruginosa* strains grew weekly in plum rainfall runoff than at other three rainfall runoff (three types; radish, peony, and apple), while normal and NPA conditions have been shown to grow optimally with no significant differences under rainfall runoff of plum, radish, peony, and apple (*Fig. 3*). Our results revealed that the growth rates of rainfall runoff of plum were higher in the week than rainfall runoff of radish, peony, and apple under rainfall runoff (four types; plum, radish, peony, and apple) conditions. The fertilizer potential of *Chlorella vulgaris* and *Scenedesmus obliquus* is known to influence microalgal growth and, indirectly, biomass productivity (Alvarenga et al., 2023; Delgadillo-Mirquez et al., 2016). Furthermore, each microalgal species has a unique optimum for light intensity and temperature; therefore, selecting a microalgae species suitable for the biomass production environment is one of the strategies to increase biomass productivity (Kim et al., 2023; Roleda et al., 2013; Lee et al., 2015; Abdelfattah et al., 2023).

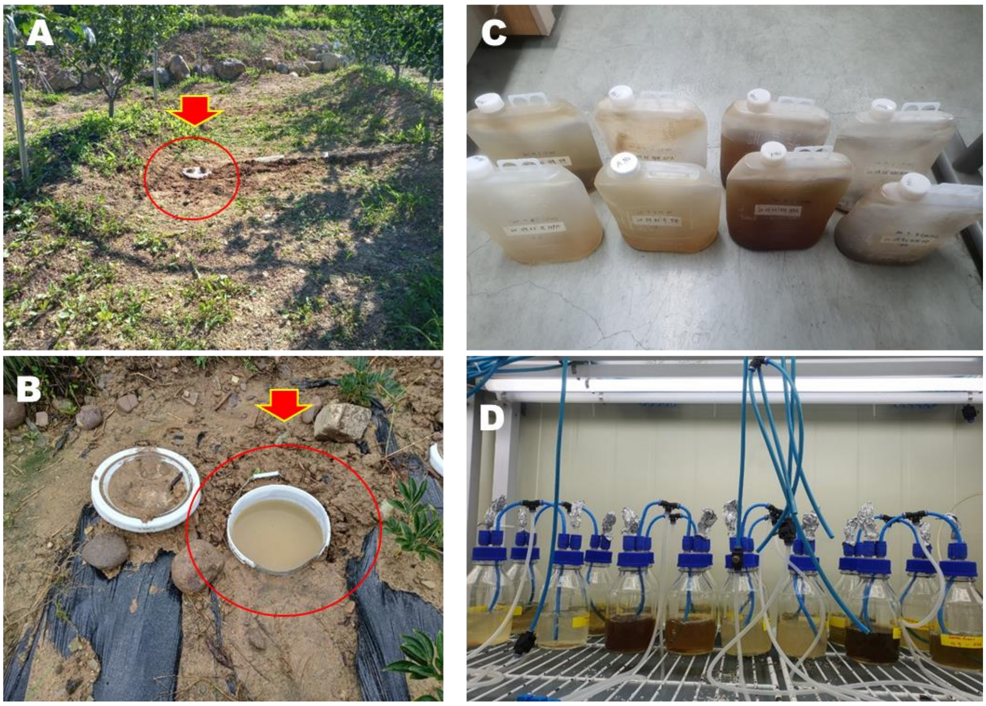

Figure 2. *To accurately analyze the initial soil runoff during rainfall, a representative point was selected for each farmland and a rainwater runoff collection device was installed for each test plot to collect rainfall runoff from the soil. (A) Field experiment site, (B) stormwater runoff collection and sampling, and (C) soil runoff was collected from cultivated fields for each of the four selected crops. The samples were collected by 2 L per region in NPA-untreated water (normal) and NPA-treated water. (D) In all experiments, blue–green algae (Microcystis aeruginosa) and green algae (Scenedesmus acutus) were inoculated into a culture medium with a total volume of 500 ml at a concentration of approximately 5,000 cells/ml. The culture experiment was carried out in a culture room maintained at a temperature of 25℃, light: dark cycles 16:8, and light intensity of 50 μmol/m^2 s, and air was injected to prevent growth inhibition due to carbon (CO_2) depletion*

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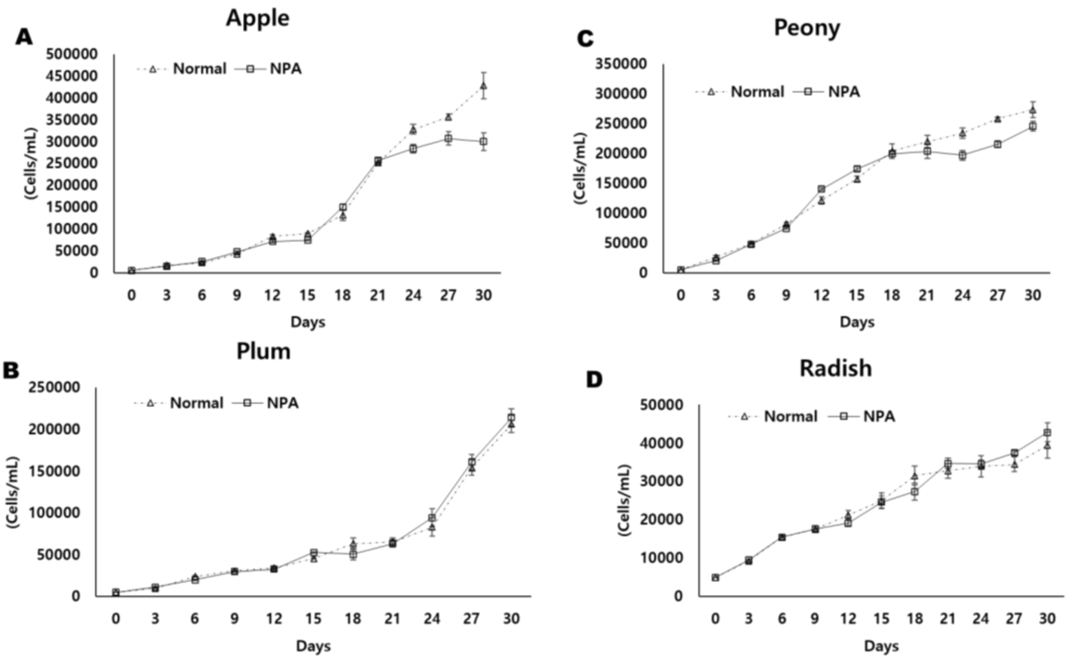

Figure 3. *Cell number counting was recorded in each culture condition during the culture process. A culture experiment was conducted to verify the effect of general and NPA composts on the growth of blue–green algae (Microcystis aeruginosa). The culture medium was tested using untreated water (effluent from farmland fertilized with general compost) and treated water (effluent from farmland fertilized with eco-friendly compost, NPA treatment) collected on September 3, 2020. (A) Apple, (B) plum, (C) peony, and (D) radish. The culture was started for 30 days, and 10 ml was taken every 3 days, fixed with Lugol's solution, and the number of cells was counted. Blue–green algae and green algae were incubated until the optical density (OD) of the seed culture at 680 nm reached 0.3–0.5 of the initial cell concentration*

Growth results showed the characteristics of green algae for the conditions of rainfall runoff (four types; plum, radish, peony, and apple). Under growth conditions, rainfall runoff of plum showed higher growth rates than rainfall runoff radish, peony, and appl). Conversely, rainfall runoff of radish, peony, and apple showed a wide range of growth patterns compared to rainfall runoff of plum. Furthermore, the growth of *Microcystis aeruginosa* strains was not inhibited under rainfall runoff (one type; plum), while *Scenedesmus acutus* was significantly inhibited (*Figs. 3* and *4*). This is thought to be the result of a higher concentration of phosphorus nitrogen than rainfall runoff (three types; radish, peony, and apple) conditions. Furthermore, *Scenedesmus acutus* showed better growth patterns in rainfall runoff of radish, peony, and apple than rainfall runoff plum, particularly in rainfall runoff (four types; plum, radish, peony, and apple under various NPA conditions) (*Fig. 4*), reaching the stationary phase within 30 days of inoculation. Moreover, *Scenedesmus acutus* strains weak-grew at plum rainfall runoff than at rainfall runoff (3 types; radish, peony, apple), while NPA and normal conditions have been shown to grow optimally with some differences under rainfall runoff (four types; plum, radish, peony, and apple) (*Fig. 4*). Our results revealed that the monthly growth rates of green algae were greater than blue–green algae under the four rainfall-runoff (four types; plum, radish, peony, and apple) conditions. Therefore, we conclude that green algae strains with a high growth rate among the tested microalgae strains are more suitable biological sources than blue–green algae.

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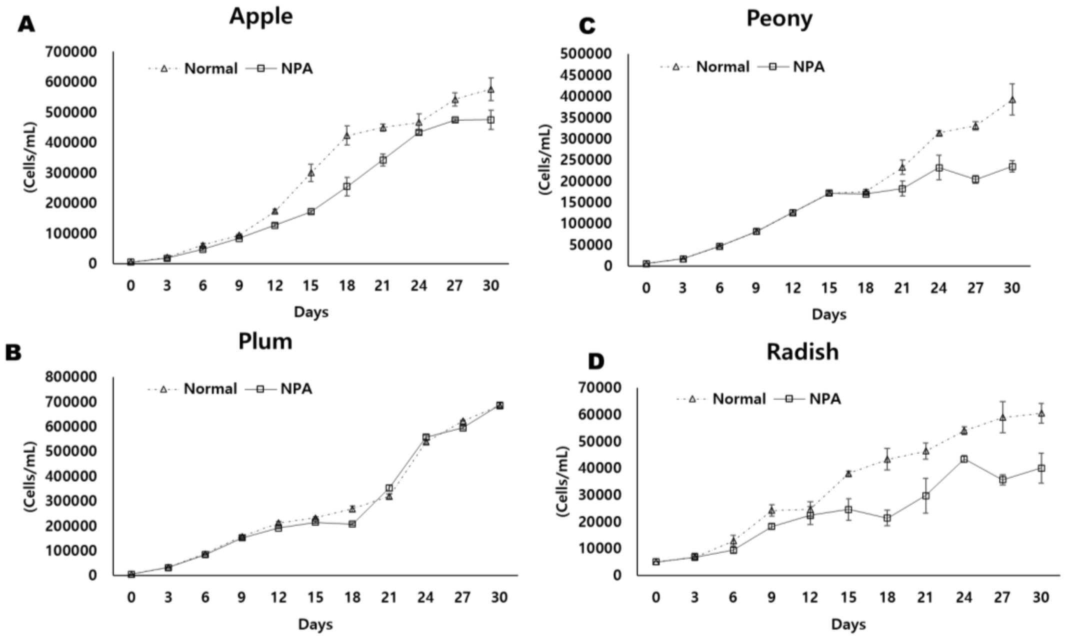

Figure 4. *Cell number was recorded for each culture condition during the culture process. A culture experiment was conducted to verify the effect of general compost and NPA compost on the growth of green algae (Scenedesmus acutus). The culture medium was tested using untreated (normal) and treated water (NPA treatment) water. The four selected crops: apple (A), plum (B), peony (C), radish (D). The cells was cultured for 30 days, and 10 ml was taken every 3 days, fixed with Lugol's solution, and the number of cells was counted*

Microalgae growth rate and chlorophyll-a content according to runoff of the entire experimental group

All indicators of photosynthetic capacity were enhanced in microalgae in the presence of rainfall runoff (four types; plum, radish, peony, and apple) compared to those in the control conditions (*Fig. 5*). The main chlorophyll fluorescence parameters measured (Samorì et al., 2013; Kim et al., 2018) included maximal chlorophyll fluorescence intensity (F_m), the effective quantum yield of photochemical energy conversion in photosystem II (QY), nonphotochemical quenching (NPQ), and chlorophyll fluorescence decrease ratio (R_{Fd}) (Kim et al., 2018). These fluorescence parameters and ratios were measured in a dark-adapted state to determine the functionality of photosystem II. For all parameters, the blue–green algae strains and green algae strains showed lower levels compared to those observed under normal conditions (*Fig. 5A, C*). From the beginning of its measurement until 2–4 weeks of growth, chlorophyll values were measured in rainfall runoff (four types; plum, radish, peony, and apple) that received fertilization. Furthermore, chlorophyll-a concentrations in the *Microcystis aeruginosa* experiment showed a tendency to decrease due to the effect of NPA in the apple and peony field effluent experiments; however, in the plum and radish field effluent experiments, the chlorophyll-a concentration in the treated water tended to be similar or rather high. In the *Scenedesmus acutus* experiment, the chlorophyll concentration decreased significantly due to the effect of NPA in the plum, peony, and radish field runoff experiments, but the concentration of chlorophyll-a increased in the treated water in the apple field runoff. Thus, it was determined that the nutrients contained in the effluent were not affected by NPA because the concentration

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2203_27492762

was sufficient for the growth of blue–green algae and green algae even if NPA was treated. Additionally, the growth of peony and radish field effluents with relatively low nutrient concentrations was inhibited; thus, it was predicted that NPA treatment with reduced nutrient concentrations would be effective. To confirm the effect of NPA, a second experiment was conducted using effluent from apple and plum fields, which had relatively high nutrient concentrations and did not show differences between experiments.

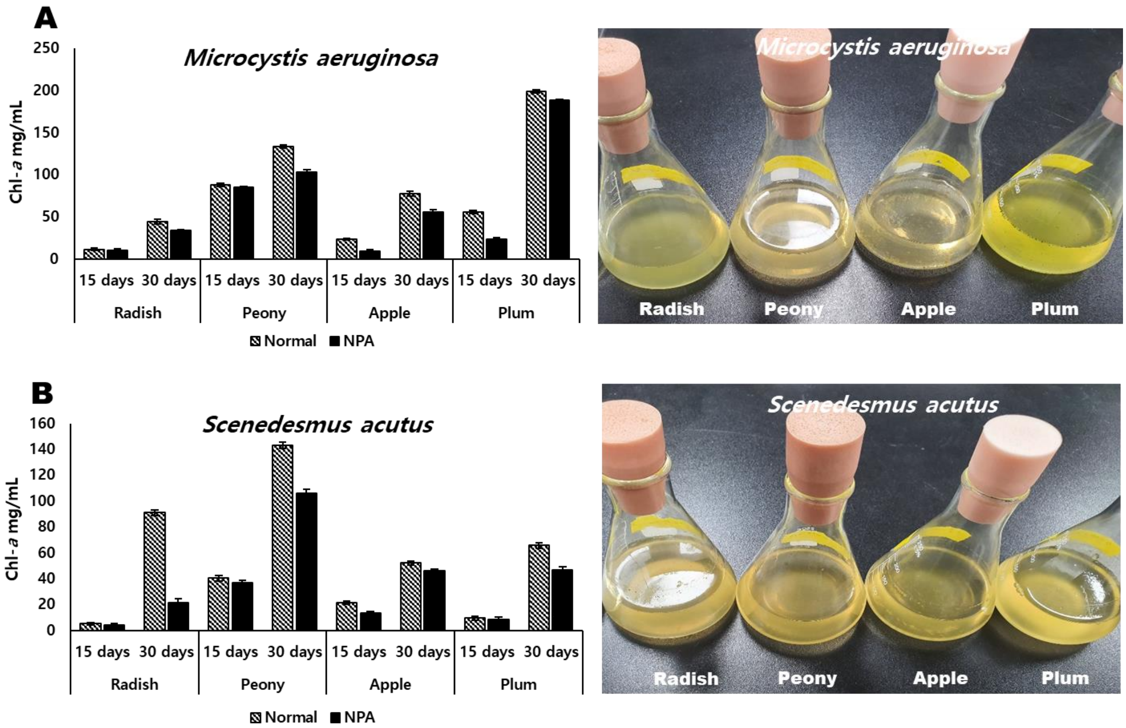

Figure 5. *Treated water (NPA: nitrogen and phosphorus adsorbent; effluent from farmland fertilized with eco-friendly compost) compost and untreated water (effluent from farmland fertilized with general compost) compost affected the growth rate and microalgae biomass production as well as nitrogen and phosphorus removal rates. A comparison of phenotypes and chlorophyll-a in (A) blue–green algae (Microcystis aeruginosa) and (B) green algae (Scenedesmus acutus) cells grown under normal and NPA treatment conditions. For the growth pattern of each strain through phenotypes and chlorophyll-a in the experiment, the concentration was measured twice at 15 and 30 days of the experiment, 10 ml of each experimental group and chlorophyll-a was extracted*

In previous studies, the effect of temperature and photoperiod on microalgal growth and biomass productivity (Martinez et al., 2010; Roleda et al., 2013; Goh et al., 2022; Abdelfattah et al., 2023). In all experiments, it showed a tendency to grow continuously until the end of the culture period for 30 days. In the case of green algae experiments in the peony and radish field runoff, there was a notable difference in growth between treated and NPA-untreated water, but there was no difference in other experiments. Instead, the average maximum cell number of NPA-treated water was higher in some cases. The average number of cells in the blue–green algae experiment in apple orchard runoff increased to a maximum of 428,000 cells/ml in NPA-untreated water (normal), which tended to be higher than that in NPA-treated water (NPA; 300,000 cells/ml). The

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DOI: http://dx.doi.org/10.15666/aeer/2203_27492762

average cell number in the green algae showed a trend similar to that of blue–green algae, and showed a higher tendency than that of treated water (NPA; 475,000 cells/ml), growing up to a maximum of 576,000 cells/ml in untreated water (normal). Our results indicated that the chlorophyll concentration and growth patterns showed a trend similar to cell number (dark green color), and the concentration decreased when NPA was treated in all experiments. The growth of all blue–green algae and green algae was confirmed to decrease in the experiment. We found that the growth of microalgae could be reduced when NPA-treated water flowed into the river and the concentration of nutrients was reduced. Additionally, it was found that it was necessary to reduce the total amount of nutrients to obtain the effect of efficient NPA. Furthermore, it was suggested if nutrients are reduced using NPA, the result could be effective or halved if a large amount of runoff flows into the river.

Conclusion

We compared and analyzed microalgae growth in soil runoff during rainfall and the reduction effect of microalgae using NPA through a culture test. For farmland runoff using NPA compost, the effect of reduction of microalgae bloom was confirmed in a laboratory-scale cultivation experiment. The average number of cells in the blue–green algae experiment in apple orchard runoff increased to a maximum in NPA-untreated water (normal), which tended to be higher than that in NPA-treated water. The average cell number in the *Scenedesmus acutus* experiment was similar to that of *Microcystis aeruginosa* and exhibited a higher trend than that of NPA-treated water, growing up to a maximum in NPA-untreated water. In the peony field runoff experiment, the results were similar to the apple field runoff results, and in all untreated water experiments, the maximum cell number was higher than that of the NPA-treated water. Furthermore, clear difference was observed in the green algae experiment compared to the blue–green algae experiment. In the plum field runoff experiment, there was a significant difference in all experiments. The cell number and growth rate (growth curve) were very similar, highlighting the effect of NPA. In the *Microcystis aeruginosa* experiment in radish field runoff, the maximum cell number was found to be higher in the experimental group treated with NPA, and similar to the plum field experiment, there was a similar effect between treated and untreated water. In our studies, the growth of *Scenedesmus acutus* and *Microcystis aeruginosa* tended to be inhibited in the experimental group of water treated with NPA. Therefore, we seem that be able to clearly demonstrate the reduction of green algae and blue–green algae in NPA treatment of crop compost. Thus, future studies should confirm the microalgae reduction effect after treatment with NPA on a wider variety crops.

Acknowledgments. We thank The Korea Water Resources Corporation (K-water) for helpful discussions and assisting with the Materials and Methods.

Funding. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2016R1A6A1A05011910), South Korea.

Conflict of interests. The authors declare that they have no conflict of interests in publishing this manuscript.

DOI: http://dx.doi.org/10.15666/aeer/2203_27492762

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