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Screening of Atrazine Tolerant Aquatic Plant and Roles of Plant Growth Regulators on Plant Growth and Atrazine Tolerance

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ABSTRACT

The extensive use of atrazine to control weeds in agricultural areas has contaminated atrazine in surface water and groundwater. Atrazine contamination in water resources causes human health concerns. Thus, this study investigated the possible use of aquatic plants for removing atrazine from contaminated water. The experiment was performed under plant nursery conditions and divided into two parts: (1) the atrazine-tolerant plants were screened, and (2) the most atrazine-tolerant plant was used for atrazine phytoremediation stimulated by plant growth regulators. The results showed that atrazine was toxic to all aquatic plants, as the dry weight of the plants was significantly decreased when exposed to 20 mg/L of atrazine (P<0.05). Based on five aquatic plants grown under 2.5–20 mg/L atrazine-contaminated water, *Azolla microphylla* Kaulf. was the most tolerant aquatic plant and was more suitable for use in atrazine phytoremediation than the other aquatic plants (*Ceratophyllum demersum* L., *Eichhornia crassipes* (Mart.) Solms, *Hydrilla verticillata* (L. f.) Royle, and *Salvinia cucullata* Roxb. ex Bory). The total chlorophyll, carotenoid, and proline contents in the biomass of *A. microphylla* cultured in 2.5–20 mg/L of atrazine did not significantly differ between the atrazine concentrations (P>0.05). Meanwhile, the proline contents in the other four aquatic plants increased with increasing atrazine concentrations, and the chlorophyll content significantly decreased with an increase in the atrazine concentration. However, *A. microphylla* could not remove atrazine from contaminated water, and the application of plant growth regulators (6-benzyladenine, gibberellic acid, indole-3-butyric acid, and salicylic acid) did not improve the atrazine removal from water. Atrazine in the water was around 21–26 mg/L on

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day five of *A. microphylla* cultivation compared to the initial concentration (25 mg/L). Using a plant growth regulator was ineffective for stimulating growth and atrazine removal by *A. microphylla*. Future research should explore other potential mechanisms for enhancing atrazine removal by *A. microphylla*.

Keywords: Atrazine, *Azolla*, herbicide, phytoremediation, plant growth regulator

INTRODUCTION

Atrazine is a widely used herbicide to control broadleaf weeds and annual grasses in field crops, such as corn and sugarcane (Steffens et al., 2022). Global use of atrazine is around 70,000–90,000 tons annually (H. He et al., 2019). In Thailand, atrazine is one of the top five imported herbicides (Aungudornpukdee, 2019) because it is inexpensive and efficiently controls weeds (H. He et al., 2019). The amount of atrazine used in sugarcane fields was 480–640 g/m^2 in Thailand (Ratchawang et al., 2022). The extensive use of atrazine for a long time and its chemical structure's stability makes it a ubiquitous contaminant in the environment (H. He et al., 2019; Ratchawang et al., 2022). Contamination by atrazine has been reported in surface water, sediment, and soil in many countries, including Thailand (Phewnil et al., 2012), China (Sun et al., 2017), and Iran (Almasi et al., 2020). For example, the average concentrations of atrazine in the topsoil and subsoil in the Huay Kapo Watershed, Nam Nao District, Phetchabun Province, Thailand, were 133.59 and 183.23 µg/kg, respectively (Phewnil et al., 2010). The contaminations of atrazine in the water and sediment in the agricultural catchment at Nong Bua reservoir, Wiang Sa District, Nan Province, Thailand, were 0.00016 µg/L and 0.00023 µg/kg, respectively (Thitiphuree et al., 2013). The mean concentration of atrazine in agricultural soils around the Yangtze River Delta, China, was 5.7 µg/kg (Sun et al., 2017). The concentration of atrazine in the water of the Shadegan wetland, Iran, ranged between 0 and 2,175.8 µg/L (Almasi et al., 2020). Atrazine applied to the soil leaches into water reservoirs (Rostami et al., 2021), and contamination by atrazine in water resources increases the risk of atrazine in drinking water. According to the United States Environmental Protection Agency and the European Community guidelines, the maximum concentration of atrazine in drinking water should not surpass 3.0 and 0.1 µg/L, respectively (H. He et al., 2019; Marecik et al., 2012). Using atrazine-contaminated water as a source of human drinking water is a public health concern because atrazine is an endocrine disruptor (Rostami et al., 2021; Steffens et al., 2022), and long-term human exposure to atrazine causes damage to the endocrine system (H. He et al., 2019). Moreover, preterm birth was reported in people who have consumed atrazine-contaminated water (Almberg et al., 2018).

Phytoremediation uses plants to decontaminate organic and inorganic pollutants from contaminated sites (Rostami et al., 2021). Phytoremediation of atrazine

by aquatic plants is interesting as a means to remove atrazine from contaminated water (Marecik et al., 2012). The possible mechanisms for the plant to decontaminate atrazine from the polluted water are phytodegradation: the organic contaminant degradation in plant tissue by plant enzymes, rhizodegradation: the exudation of root exudates from plant roots to stimulate the organic contaminant degradation around the root zone, and phytoaccumulation: the accumulation of organic contaminants into plant biomass (Ansari et al., 2020; Kooh et al., 2018; Q. Wang et al., 2012). Several plant species have been reported to remove atrazine from contaminated water, including sweet flag (*Acorus talamus* L.) (Marecik et al., 2012), *Iris pseudacorus* L., *Lythrum salicaria* L., and *Acorus calamus* L. (Q. Wang et al., 2012). Suitable characteristics for plants used in phytoremediation are high biomass, rapid growth under several environmental conditions, and tolerance to toxic contaminants (Sood et al., 2012). However, the sensitivity of aquatic plants to atrazine contamination is a limiting factor in the success of phytoremediation. The toxicity of atrazine has been reported in several aquatic plants, including broadleaf cattail (*Typha latifolia* L.) and narrow-leaf cattail (*Typha angustifolia* L.) (Marecik et al., 2012). Atrazine may inhibit photosynthesis and chlorosis and reduce plant biomass as a response of the plants to the toxicity of atrazine (Rostami et al., 2021; Sánchez et al., 2017).

Using an exogenous plant growth regulator is one way to reduce the toxic effects of contaminants on plants, and they can promote the growth of plants under abiotic stress conditions (Rahman et al., 2023; Y. He et al., 2022). Many plant growth regulators, including indole butyric acid, gibberellin, salicylic acid, and 6-benzyladenine, have been used to mitigate the toxic effects of abiotic stress on plants, including heavy metal stress (Rostami et al., 2021), drought stress (Li et al., 2018), and waterlogging stress (J. Wang et al., 2021). Probable mechanisms for plant growth regulators to alleviate toxic effects in plants grown under abiotic stress involve mediating the antioxidant defense systems and eliminating reactive oxygen species (Emamverdian et al., 2020; J. Wang et al., 2021; Li et al., 2018). For example, indole-3-butyric acid promotes the growth of adventitious roots by controlling antioxidant defense systems in mung bean (*Vigna radiata* (L.) Wilczek) for seedlings grown under cadmium and drought stresses (Li et al., 2018). Gibberellin relieved the toxicity of arsenic in rice (*Oryza sativa* L.) seedlings by reducing the arsenic accumulation in the root (Y. He et al., 2022). Salicylic acid also increases the tolerance of plants grown under heavy metal stress by stimulating antioxidant enzyme synthesis (Emamverdian et al., 2020). Reducing ethylene formation by salicylic acid application has been reported in rice grown under arsenic contamination (Khan et al., 2013, 2021). Exogenous 6-benzyladenine helped improve *Zea mays* L. tolerance to water logging by mitigating the reactive oxygen species produced under

waterlogging stress (J. Wang et al., 2021). However, no reports have investigated the effects of plant growth regulators on aquatic plant growth and the removal of atrazine under atrazine stress. Even though plant growth regulators may improve the growth of plants under atrazine stress to a similar trend as abiotic stress, described above, this study was performed to select the atrazine tolerant plants from five aquatic plant species (*A. microphylla*, *C. demersum*, *E. crassipes*, *H. verticillata*, and *S. cucullata*) because it is the first step of phytoremediation process. Then, the effect of exogenous plant growth regulators (indole butyric acid, gibberellic acid, salicylic acid, and 6-benzyladenine) on plant growth and atrazine remediation by the most tolerant aquatic plants was also determined.

MATERIALS AND METHODS

Preparation of Atrazine-contaminated Water

The atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine 80% w/w) was purchased from an agrochemical shop under the trade name Weethong (V. C. S. Agro Chem Company Limited, Thailand). The atrazine-contaminated water used in the phytotoxicity testing experiment was prepared by dissolving atrazine powder in tap water to give final concentrations of 0, 2.5, 5, 10, and 20 mg/L concentrations. The concentration of atrazine in the phytoremediation experiment was prepared as described previously to give a final 25 mg/L concentration.

Plant Preparation

The aquatic plants, *C. demersum*, *E. crassipes*, *H. verticillate*, and *S. cucullate* were purchased from a plant shop in Maha Sarakham Province, Thailand, and *A. microphylla* was purchased from a plant shop in Khonkaen Province, Thailand. All aquatic plants were gently rinsed with tap water and kept in a plant nursery before use. The environmental conditions in the plant nursery were natural sunlight and actual air temperature. The *A. microphylla* used in the phytoremediation experiment was purchased from the plant shop simultaneously. The plant sample was gently rinsed and mixed in a plastic basin before the experiment. The plant sample was weighed and divided into treatments.

Atrazine Phytotoxicity Testing

The experiment was performed under plant nursery conditions with natural sunlight and actual air temperature in November 2022 in Thailand. The atrazine phytotoxicity testing on the five aquatic plants was performed under a completely randomized design with one factor: atrazine concentration (0, 2.5, 5, 10, and 20 mg/L). The fresh weights of *A. microphylla*, *C. demersum*, *E. crassipes*, *H. verticillata*, and *S. cucullata* at the beginning of the experiment were 7, 30, 20, 30, and 40 g, respectively. Then, each aquatic plant was cultured in a plastic cup containing 500 ml of water contaminated with each concentration of atrazine for five days. The experiment was performed with five replicates. The growth of each plant was observed at the end

of the experiment, including fresh weight, dry weight, chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid, and proline contents. The relative growth rate (RGR) was calculated on a fresh weight basis, as described in Equation 1 (Riaz et al., 2017). The most tolerant aquatic plant was chosen for the atrazine phytoremediation in the next experiment.

$$\text{Relative growth rate (RGR)} = [\ln (W_2) - \ln (W_1)]/t_2 - t_1 \quad [1]$$

where, W_1 = plant weight at the beginning of the experiment; W_2 = plant weight at the last day of the experiment; t_1 = time at the beginning of the experiment; t_2 = time at the last day of the experiment.

Atrazine Phytoremediation Experiment

The experiment was performed in Thailand under plant nursery conditions with natural sunlight and actual air temperature in January 2023. The atrazine phytoremediation experiment was performed under a factorial completely random design (CRD) with 2 x 3 factors. The first factor was atrazine concentration (0 and 25 mg/L), and the second factor was the application of different plant growth regulators, indole butyric acid, gibberellic acid, salicylic acid, and 6-benzyladenine, at concentrations of 0, 1, and 10 mg/L. Atrazine-contaminated water was prepared by dissolving atrazine powder in tap water to give the final concentration of atrazine of 25 mg/L, and non-contaminated water served as a control. Indole butyric acid (Fluka, China), gibberellic acid (Sigma-Aldrich, USA), salicylic acid (Sigma-Aldrich, USA), and 6-benzyladenine (HiMedia Laboratories Pvt Ltd, India) were added separately to atrazine-contaminated water and non-contaminated water to give final concentrations for each plant growth regulator of 0, 1, and 10 mg/L. Then, 7 g of *A. microphylla* was cultured in atrazine-contaminated and non-contaminated water for five days. The experiment was performed with six replicates. The growth of *A. microphylla* and atrazine remaining in the water was observed. The growth of *A. microphylla* was observed by fresh weight, dry weight, chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid, proline, phenolic compound, and flavonoid compound contents. The RGR was also calculated on a fresh weight basis, as in Equation 1.

Atrazine Extraction and Analysis

The atrazine remaining in the water from each treatment was determined by sending it for analysis at the Central Laboratory (Thailand) Co. Ltd. (Khon Kaen branch), and each treatment was analyzed in triplicate. Atrazine was extracted and analyzed using the EPA508 method (Munch, 1995). Briefly, 500 ml of the water sample was mixed with 100 ml of dichloromethane (RCI Labscan Ltd., Thailand). The mixture was shaken in a separation funnel for 2 min and left for 10 min to separate into two layers. The dichloromethane phase was filtered through sodium sulfate (J. T. Baker, USA), and the water phase was further extracted two more times with dichloromethane using

the previously described method. Then, the dichloromethane phase of each extraction was combined, and the dichloromethane extract was dried with a rotary evaporator (EYELA, model EVC2000, Japan). The extract was redissolved with sodium acetate solution (RCI Labscan Ltd., Thailand) and mixed for 15 s with a vortex mixer. The extract of 1 ml was transferred into the vial, and the atrazine in the extract was analyzed.

The atrazine concentrations in the extracts and standards were measured using a gas chromatography-mass spectrometric detector (Model 6890 Network GC System, Agilent Technologies, China), and the separation was achieved using an HP-5MS column (0.25 nm x 250 µm x 30 m, Agilent J&W, China). The sample volume injected into the column was 2 µl under splitless conditions. The oven temperature was 80°C, followed by a linear increase of 10°C per min to 200°C and held for 2 min. The temperature was increased from 200 to 230ºC at 10°C per minute and held for 5 min. The internal quality control for atrazine analysis was reported as the percentage of atrazine recovery. It was performed by spiking 10 µg/L of atrazine into the clean water and extracting it with the same procedure as for the atrazine extraction of the samples. The percentage of atrazine recovery was 100%. The relative percent difference RPD was calculated from duplicates of the sample, with each being 10% of the sample, and the percentage of RPD was less than 20%. The calibration curve was generated from 3–5 points of known atrazine concentration. The reagent blank was analyzed for atrazine, and atrazine was not detected. The limit of detection (LOD) and the limit of quantitation (LOQ) were also included. The detection limit was 10 µg/L, and the quantification limit was 100 µg/L.

Proline Content

The proline content in the plant biomass was determined according to the methods described by Ábrahám et al. (2010) and Bates et al. (1973). For this, 300 mg of fresh plant material was grounded in 5 ml of 3% (w/v) sulphosalicylic acid (Loba Chemie Pvt Ltd, India) in liquid nitrogen, and the sample was centrifuged at 5,120 x *g* for 15 min. Next, 2 ml of the supernatant was transferred and mixed with 2 ml of glacial acetic acid (QRëc, New Zealand) and 2 ml of acid ninhydrin (Kemaus, Australia). Then, the mixture was incubated at 100ºC for 1 hr. The reaction in an ice bath was terminated for 10 min. Afterwards, 4 ml of toluene (Fisher Chemical, United Kingdom) was added and mixed with a vortex mixer for 20 min. The solution was left to separate into two layers before the toluene phase with a red color was transferred to determine the absorbance with a spectrophotometer (EMCLAB, Germany) at a wavelength of 520 nm. The amount of proline was calculated using a reference standard curve of L-proline (Sigma-Aldrich, USA) solution.

Chlorophyll and Carotenoid Contents

Chlorophyll and carotenoid contents were determined according to the methods described in Lichtenthaler (1987) as well

as Sardoei and Rahbarian (2014). For this, 200 mg of fresh plant material was ground in acetone (AnaPure, New Zealand). Then, the mixture was centrifuged at 1,280 x *g* for 5 min. The supernatant was then transferred into a new tube, and the volume with acetone was adjusted to 15 ml. The absorbance was determined with a spectrophotometer (EMCLAB, Germany) at 662, 647, and 470 nm wavelengths. Then, the chlorophyll *a* [2], chlorophyll *b* [3], total chlorophyll [4], and carotenoid [5] contents were calculated using these equations:

Chlorophyll *a* = (12.25 x A662) - (2.79 x A647) [2]

Chlorophyll *b* = (21.50 x A647) - (5.10 x A662) [3]

Total chlorophyll = Chlorophyll *a* + Chlorophyll *b* [4]

Carotenoids = (1000 x A470) - (1.82 x Chlorophyll *a*) - (85.02 x Chlorophyll *b*)/198 [5]

Phenolic and Flavonoid Contents

The crude extract was prepared using methods adapted from Kumari and Pandey-Rai (2018). For this, 100 mg of the dry plant material was grounded to a fine powder and then extracted with 30 ml of 90% ethanol (QRëc, New Zealand) and shaken at 150 rpm for 24 hr. The plant's fine powder was macerated for six days and shaken occasionally daily. The fine powder was filtered through filter paper (Whatman No. 1) to give the crude extract, and the fine powder was repeatedly extracted according to the method described previously one further time. Then, the crude extracts were combined, and the volume was reduced with a rotary evaporator (Buchi Syncore, Switzerland).

The total phenolic content was investigated using the Folin-Ciocalteu method, as described in Lertcanawanichakul et al. (2019). For this, 50 µl of crude extract was mixed with 25 µl of 10% (v/v) Folin-Ciocalteu reagent (Merck, USA), 50 µl of 7.5% sodium carbonate (Ajax FineChem Pyt Ltd, New Zealand), and 50 µl of reverse osmosis water, mixed thoroughly and was allowed to react at 45°C for 45 min. The absorbance was determined with a microplate reader (BMG LABTECH, SPECTROstar®Nano, Germany) at the wavelength of 765 nm. The total phenolic compounds in the sample were calculated using a gallic acid (Sigma-Aldrich, China) standard curve.

The total flavonoid content was investigated using the aluminum chloride colorimetric assay described in Phonprapai and Oontawee (2019). For this, 80 µl of the crude extract was mixed with 50 µl of 2% (w/v) aluminum chloride (Ajax FineChem Pyt Ltd, New Zealand) and 100 µl of 10% (v/v) ethanol (QRëc, New Zealand) and allowed to react under dark conditions for 30 min. The absorbance was determined with a microplate reader (BMG LABTECH, SPECTROstar®Nano, Germany) at a wavelength of 425 nm. The total flavonoid compound in the sample was calculated using a quercetin (Sigma-Aldrich, China) standard curve.

Statistical Analysis

One-way and two-way analysis of variance (ANOVA) were used to analyze the phytotoxicity and plant growth regulator experiments via Microsoft Excel 2019, respectively. The least square difference (LSD) was used for pairwise comparison.

RESULTS AND DISCUSSION

Growth of Aquatic Plants under Various Concentrations of Atrazine

Aquatic plants are a suitable choice to be used as atrazine phytoremediators in aquatic environments because aquatic plants naturally grow in the water, have contact with the contaminant directly, and can adapt to aquatic environmental conditions (Sood et al., 2012). The results in this study revealed that atrazine concentrations ranging from 2.5–20 mg/L exerted toxicity to all aquatic plants used in this study when considering the dry weight of the aquatic plants. The dry weights of *A. microphylla*, *C. demersum*, *E. crassipes*, *H. verticillate*, and *S. cucullata* decreased to 56.9–90.5% of the plants in non-contaminated water when atrazine concentration increased to 20 mg/L, the most toxic concentration (Table 1). In addition, the relative growth rate of all plants decreased significantly with increasing concentrations of atrazine ($P<0.05$) (Table 1). The reduction in the dry weight of the five aquatic plants was related to the decrease in the total chlorophyll content in the biomass of the aquatic plants (Table 1). The chlorophyll contents of *A. microphylla*, *C. demersum*, *E. crassipes*, *H. verticillata*, and *S. cucullata* grown in non-contaminated water were higher than atrazine-contaminated water (Table 1). The chlorophyll contents were decreased in *A. microphylla*, *H. verticillate*, and *S. cucullata* when the atrazine concentration was over 2.5 mg/L and decreases in the chlorophyll contents were observed in *C. demersum* and *E. crassipes* when the atrazine concentration was over 5 mg/L (Table 1). In general, the total chlorophyll content usually decreased in proportion to the increase in the atrazine concentration (Phewnil et al., 2012), which was also observed in all aquatic plants used in this study. The chlorophyll content in *A. microphylla* did not decrease further when the atrazine concentration increased from 5 to 20 mg/L. The total chlorophyll content in *A. microphylla* grown under different concentrations of atrazine did not significantly differ from each other ($P>0.05$). The reductions in the chlorophyll content are a sign of atrazine toxicity because the toxic effect of atrazine was to inhibit photosystem II in plants (Salem & El-Sobki, 2021; Yang & Zhang, 2020). If protein and photosynthetic pigment in the plant photosystem are destroyed, the ability to fix carbon and plant growth will decrease (Yang & Zhang, 2020). Thus, the loss of chlorophyll content from atrazine toxicity results in photosynthesis inhibition, which can decrease plant biomass (Phewnil et al., 2012; Yang & Zhang, 2020). Phytotoxic effects from atrazine have been previously reported; for example, 2.5 mg/L of atrazine inhibited growth, decreased the fresh weight and dry weight, and decreased the chlorophyll content in *Lemna perpusilla* Torr. after seven days of cultivation (Phewnil

et al., 2012). Gao et al. (2011) also reported that 10 µg/L of atrazine decreased the fresh weight and chlorophyll content in *Zostera marina* L., and 86.67% of plants died after exposure to 100 µg/L of atrazine. Decreasing weight and transpiration rate of *T. latifolia* were observed when plants were exposed to 20 µg/L of atrazine (Pérez et al., 2022). Exposure to atrazine at 2 nmol/L for two days decreased the chlorophyll content of *Phaeodactylum tricornutum* Pt-1 to only 37.5% compared to the control without atrazine exposure (Yang & Zhang, 2020). Yang et al. (2019) also reported that the genes encoding for proteins in photosystem II (*PsbO*, *PsbP*, *PsbU*, *PsbQ*, and *Psb27*) and genes encoding for electron transport in *Phaeodactylum tricornutum* Pt-1 were repressed under atrazine exposure (Yang et al., 2019). Another impact of atrazine on plants is tissue necrosis (Phewnil et al., 2012), but it was not observed in *A. microphylla*, *C. demersum*, *E. crassipes*, *H. verticillata*, and *S. cucullata* in this work. None of the aquatic plants showed any phytotoxic symptoms, and the plants looked green when observed by the naked eye.

The carotenoid contents in plant biomass of *A. microphylla* and *S. cucullata* grown in atrazine-contaminated water did not significantly differ from that grown in non-contaminated water (P>0.05) (Table 1). It indicated that atrazine was not toxic to both plants. Meanwhile, a decrease in the carotenoid content was detected in *C. demersum* and *E. crassipes* after atrazine exposure (Table 1). The fluctuation in carotenoid content is a sign of plant response to abiotic stress because carotenoids can act as an antioxidant molecule to neutralize the free radicals produced from photosynthetic reactions in plants (Kopsell et al., 2009). Thus, increasing the carotenoid content is a plant response mechanism to abiotic stress found in *H. verticillata* (Table 1). The decrease in the carotenoid content in *C. demersum* L. and *E. crassipes* may be because these plants used carotenoid molecules to neutralize the toxic effect of the free radicals produced during plant growth under atrazine contamination. Oxidative stress is a sign of toxicity in plants grown under atrazine exposure (Singh et al., 2018). Plants usually overcome oxidative stress by enzymatic and non-enzymatic mechanisms (Singh et al., 2018), and the production of carotenoids is a non-enzymatic mechanism plants use to detoxify the free radicals (Kumari & Pandey-Rai, 2018; Pérez-Gálve et al., 2020). The atrazine tolerance in *A. microphylla* and the proline content in aquatic plants was confirmed again because the proline content in *A. microphylla* was constant between plants grown under atrazine contamination and non-contamination conditions. However, the proline content in *C. demersum*, *E. crassipes*, *H. verticillata*, and *S. cucullata* increased when the atrazine concentration was increased. Increasing the proline content in the plant is another plant response mechanism to oxidative stress (Bibi et al., 2019) because proline can also act as an antioxidant molecule in plants (Din et al., 2020). Increased proline content has been reported in maize seedlings exposed to atrazine at 500 and 1,000 mg/L (Bibi et al., 2019).

Table 1

Relative growth rate (RGR), dry weight, chlorophyll content, carotenoid content, and proline content in five aquatic plants grown in atrazine-contaminated water for five days (data shown as mean ± SE)

Atrazine concentration (mg/L)	RGR (mg/g/day)	Dry weight (% of control)	Chlorophyll *a* (µg/g FW)	Chlorophyll *b* (µg/g FW)	Total Chlorophyll (µg/g FW)	Carotenoid (µg/g FW)	Proline (µg/g FW)
Salvinia cucullata							
0	0.084 ± 0.004 a	100.00 ± 0.44 a	319.28 ± 5.25 a	307.91 ± 7.09 a	627.19 ± 1.84 a	50.83 ± 3.41 a	6.97 ± 0.37 d
2.5	0.075 ± 0.002 b	94.16 ± 0.60 b	283.76 ± 1.79 b	289.14 ± 6.21 ab	572.90 ± 4.53 b	57.58 ± 2.24 a	9.26 ± 0.17 c
5	0.047 ± 0.004 c	92.31 ± 0.49 c	244.36 ± 2.81 c	273.51 ± 3.88 b	517.87 ± 5.97 c	59.22 ± 2.52 a	9.35 ± 0.09 c
10	0.024 ± 0.003 d	91.39 ± 0.54 cd	234.48 ± 5.10 c	282.71 ± 5.89 b	517.19 ± 8.45 c	54.73 ± 3.06 a	11.47 ± 0.25 b
20	0.014 ± 0.002 e	90.24 ± 0.25 d	233.88 ± 4.13 c	278.38 ± 6.84 b	512.26 ± 2.89 c	54.45 ± 2.45 a	13.68 ± 0.31 a
Eichhornia crassipes							
0	0.034 ± 0.003 a	100.00 ± 4.16 a	446.03 ± 23.64 a	212.94 ± 21.01 a	658.97 ± 3.94 a	161.66 ± 20.55 a	6.12 ± 0.29 c
2.5	0.033 ± 0.002 a	95.67 ± 5.15 a	324.23 ± 29.70 b	280.94 ± 18.82 a	605.16 ± 17.60 a	111.36 ± 15.81 ab	8.83 ± 0.31 b
5	0.028 ± 0.004 ab	73.55 ± 3.82 b	285.42 ± 2.90 b	218.28 ± 52.96 a	503.70 ± 50.61 b	79.03 ± 31.07 b	8.67 ± 0.29 b
10	0.019 ± 0.004 bc	76.90 ± 4.01 b	129.85 ± 5.32 c	88.82 ± 17.63 b	218.67 ± 18.48 c	81.12 ± 14.55 b	11.22 ± 0.29 a
20	0.017 ± 0.004 c	67.43 ± 5.39 b	104.65 ± 7.55 c	75.41 ± 19.03 b	180.06 ± 19.22 c	58.32 ± 16.42 b	10.71 ± 0.15 a
Ceratophyllum demersum							
0	0.018 ± 0.003 a	100.00 ± 0.62 a	427.68 ± 7.38 a	216.01 ± 6.37 a	643.68 ± 4.89 a	168.34 ± 11.55 a	8.24 ± 0.17 c
2.5	-0.039 ± 0.007 b	96.00 ± 0.94 b	399.84 ± 3.95 b	218.44 ± 10.93 a	618.28 ± 7.12 a	172.98 ± 16.13 a	7.90 ± 0.15 c
5	-0.068 ± 0.007 c	90.86 ± 1.20 c	148.68 ± 2.86 c	108.95 ± 8.87 b	257.62 ± 9.29 b	40.62 ± 1.67 b	9.26 ± 0.45 b
10	-0.070 ± 0.004 c	90.51 ± 1.41 c	82.09 ± 1.84 d	92.94 ± 17.98 b	175.03 ± 18.87 c	59.59 ± 11.06 b	9.69 ± 0.29 b
20	-0.086 ± 0.008 c	90.51 ± 1.81 c	63.45 ± 1.59 e	80.36 ± 17.08 b	143.81 ± 18.38 c	28.55 ± 9.62 b	11.05 ± 0.31 a
Hydrilla verticillata							
0	0.041 ± 0.004 a	100.00 ± 0.82 a	593.18 ± 26.31 a	791.32 ± 6.15 a	1384.50 ± 20.74 a	-57.61 ± 9.26 d	8.16 ± 0.29 c

Table 1 *(Continue)*

Atrazine concentration (mg/L)	RGR (mg/g/day)	Dry weight (% of control)	Chlorophyll *a* (µg/g FW)	Chlorophyll *b* (µg/g FW)	Total Chlorophyll (µg/g FW)	Carotenoid (µg/g FW)	Proline (µg/g FW)
2.5	0.030 ± 0.004 b	87.17 ± 1.65 b	459.16 ± 24.51 b	649.84 ± 3.96 b	1109.00 ± 21.40 b	13.21 ± 10.44 c	7.82 ± 0.22 d
5	-0.021 ± 0.002 c	59.47 ± 0.59 c	298.88 ± 9.35 c	474.00 ± 6.85 c	772.91 ± 6.46 c	65.68 ± 9.07 b	9.35 ± 0.09 b
10	-0.045 ± 0.004 c	55.53 ± 1.04 d	293.71 ± 7.80 c	444.66 ± 2.75 d	738.37 ± 10.55 c	72.78 ± 14.78 b	9.52 ± 0.09 b
20	-0.053 ± 0.004 c	56.93 ± 1.43 cd	147.19 ± 26.34 d	278.88 ± 4.42 e	426.07 ± 22.66 d	138.11 ± 8.49 a	10.62 ± 0.09 a
Azolla microphylla							
0	0.021 ± 0.004 a	100.00 ± 2.84 a	164.63 ± 4.63 a	93.15 ± 1.81 a	257.78 ± 5.67 a	73.03 ± 3.83 a	9.52 ± 0.52 a
2.5	-0.036 ± 0.004 b	90.07 ± 1.81 b	147.65 ± 4.90 b	61.07 ± 11.62 b	208.72 ± 9.29 b	60.44 ± 3.07 a	9.35 ± 0.68 a
5	-0.046 ± 0.007 bc	89.36 ± 2.07 b	136.94 ± 3.82 b	60.95 ± 5.76 b	197.89 ± 3.23 b	65.77 ± 6.35 a	10.62 ± 1.11 a
10	-0.046 ± 0.009 bc	83.69 ± 1.42 b	138.38 ± 2.81 b	58.83 ± 9.40 b	197.20 ± 7.00 b	59.72 ± 6.24 a	10.79 ± 0.22 a
20	-0.059 ± 0.002 c	75.89 ± 3.98 c	143.11 ± 2.31 b	55.84 ± 2.65 b	198.95 ± 1.17 b	58.06 ± 0.61 a	10.96 ± 0.29 a

Note. Different lowercase letters show significant differences ($P<0.05$) between atrazine concentrations within the same aquatic plant

Growth of *A. microphylla* under Plant Growth Regulator Application

Based on the dry weight, total chlorophyll, carotenoid, and proline contents of the tested aquatic plants described above, *A. microphylla*, the model aquatic plant, was selected for atrazine remediation in the next experiment because it is the most atrazine-tolerant plant of those tested. When observed by the naked eye, *A. microphylla* showed no sign of phytotoxicity at 20 mg/L of atrazine (Figure 1). Another suitable characteristic of *Azolla* for phytoremediation is its rapid growth. It is a free-floating plant that would be easy to manage after the phytoremediation process. Aquatic ferns have been reported to be used in the phytoremediation of various pollutants, namely heavy metals and pesticides (Sood et al., 2012). The atrazine remediation experiment was performed by growing *A. microphylla* in 25 mg/L of atrazine and using a plant growth regulator (indole butyric acid, gibberellin, salicylic acid, or 6-benzyladenine) to promote the growth of *A. microphylla* under atrazine contamination. There was a significant interaction between atrazine concentration and different types of plant growth regulators for all plant traits (Table 2). In atrazine-contaminated water, only 10 mg/L gibberellic acid, 1 mg/L indole butyric acid, and 1 mg/L 6-benzyladenine could increase the dry weight of *A. microphylla* significantly compared with plants exposed to atrazine without any plant growth regulator application. All plant growth regulators could significantly increase the relative growth rate in non-contaminated water. However, only 10 mg/L 6-benzyladenine, 1 mg/L indole butyric acid, 1–10 mg/L salicylic acid, and 10 mg/L gibberellic acids could increase the relative growth rate of *A. microphylla* in atrazine-contaminated water significantly (Table 2). Without a plant growth regulator, the results revealed that 25 mg/L atrazine decreased the total chlorophyll and carotenoid content and increased the proline content of *A. microphylla* (Table 2). The proline content of *A. microphylla* grown under atrazine contamination was higher than that grown under the non-contaminated condition. It was evident in the response of *A. microphylla* grown under 25 mg/L of atrazine, whereas proline acts as an antioxidant molecule that plants synthase in response to atrazine stress (Bibi et al., 2019). In addition, the phenolic and flavonoid contents in *A. microphylla* grown under atrazine-contaminated water without the plant growth regulator application did not significantly differ (P>0.05) from that with the plant growth regulator (6-benzyladenine, gibberellic acid, indole butyric acid, and salicylic acid) application (Table 2). Likewise, phenolic and flavonoid compounds are

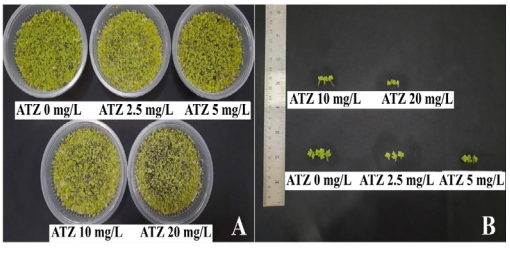

Figure 1. Growth of *Azolla microphylla* grown under atrazine-contaminated water in a concentration range from 0–20 mg/L for five days
Note. ATZ = Atrazine

secondary metabolites that protect plants from oxidative stress in plants (Kiani et al., 2021). Plant homeostasis between reactive oxygen species and phenolic or flavonoid compounds was a general mechanism of plant adaptation to abiotic stress (Kiani et al., 2021). The unchanged amount of phenolic and flavonoid compounds in *A. microphylla* may be due to the tolerant nature of the plant to atrazine. Thus, increasing the synthesis of phenolic and flavonoid compounds in response to atrazine stress was unnecessary for *A. microphylla* in this study. The exogenous plant growth regulator used did not affect the amount of both compounds; it may be due to the plant growth regulator not inducing the synthesis of phenolic and flavonoid compounds. Meanwhile, the previous research by Kumari and Pandey-Rai (2018) reported that exogenous plant growth regulators could induce the synthesis of phenolic and flavonoid compounds in plants grown under abiotic stress.

The plant growth regulators (6-benzyleadenine, indole butyric acid, salicylic acid, and gibberellic acid) used in this study did not improve *A. microphylla* growth under atrazine contamination. However, 10 mg/L of 6-benzyladenine and 10 mg/L of gibberellic acid tended to increase the dry weight, total chlorophyll content, and carotenoid content in *A. microphylla* grown under atrazine contamination to a greater extent than the other plant growth regulators (Table 2). Both plant growth regulators were used to stimulate growth, conserve chlorophyll content, and alleviate the toxic effects of abiotic stress on plants by various mechanisms. For example, gibberellic acid at 100 ppm has increased the weight of wheat grown under heat stress. However, it did not affect the level of antioxidant enzymes, lipid peroxidation, and membrane stability (Nagar et al., 2021). Pre-treatment of wheat seeds with 0.01–1.0 µM gibberellic acid alleviated Ni toxicity by increasing the chlorophyll content and decreasing the percentage of electrolyte leakage (Siddiqui et al., 2011). The 6-benzyladenine at 10 µM alleviated the abiotic stress from salt in *Solanum melongena* Mill. by increasing the chlorophyll content, decreasing the superoxide anion production, decreasing the malondialdehyde content, and increasing the antioxidant enzymes and proline content (Wu et al., 2014). Moreover, 0.5 mM of 6-benzyleadenine also promoted the shoot and root growth, reduced the superoxide anion and hydrogen peroxide accumulation, reduced the malondialdehyde content, and increased the antioxidant enzymes in a maize waterlogging sensitive strain (SY-XT1) grown under waterlogging conditions (J. Wang et al., 2021). However, promoting the growth of *A. microphylla* by salicylic acid and indole butyric acid was not observed in this study. However, both plant growth regulators have been used to stimulate the growth and tolerance of plants under heavy metal stress with a similar mechanism as other plant growth regulators (Kumari & Pandey-Rai, 2018; Šípošová et al., 2021). The concentration of the plant growth regulator also influenced the response of plants (Šípošová et al., 2021). This study found that only ten mg/L of 6-benzyladenine

Table 2

Relative growth rate (RGR), dry weight, chlorophyll, carotenoid, proline, phenolic, and flavonoid compounds in Azolla microphylla *grown under atrazine-contaminated water with various plant growth regulators used for five days (data shown as mean ± SE)*

Plant Growth Regulator	RGR (mg/g/day)	Dry weight (% of control)	Chlorophyll *a* (µg/g FW)	Chlorophyll *b* (µg/g FW)	Total chlorophyll (µg/g FW)	carotenoid (µg/g FW)	Proline (µg/g FW)	Phenolic (µg gallic acid/ mg DW)	Flavonoid (µg quercetin/ mg DW)
Non-contamination									
Indole butyric acid (1 mg/L)	0.039 ± 0.001 Aa	103.88 ± 1.32 Aab	87.21 ± 1.60 Abc	39.58 ± 2.07 Ad	126.79 ± 1.54 Ad	24.82 ± 1.19 Aab	83.65 ± 1.22 Bb	N.D.	N.D.
Indole butyric acid (10 mg/L)	0.025 ± 0.005 Aa	99.46 ± 2.09 Ab	88.59 ± 0.89 Abc	43.96 ± 2.90 Ad	132.56 ± 2.71 Ad	22.17 ± 1.77 Ab	79.18 ± 1.04 Bbc	N.D.	N.D.
Gibberellic acid (1 mg/L)	0.039 ± 0.003 Aa	104.20 ± 1.75 Aab	91.87 ± 1.32 Abc	55.56 ± 1.96 Bc	147.43 ± 3.02 Ac	23.02 ± 0.05 Aab	81.74 ± 1.81 Bbc	N.D.	N.D.
Gibberellic acid (10 mg/L)	0.039 ± 0.002 Aa	106.14 ± 1.45 Aa	113.79 ± 1.77 Aa	70.85 ± 2.28 Ab	184.63 ± 0.77 Aa	25.07 ± 1.40 Aab	69.60 ± 2.63 Bc	N.D.	N.D.
Salicylic acid (1 mg/L)	0.033 ± 0.005 Aa	101.40 ± 1.58 Ab	97.27 ± 1.48 Ab	62.47 ± 1.77 Abc	159.74 ± 1.03 Ab	27.68 ± 0.42 Aa	73.44 ± 2.18 Bc	N.D.	N.D.
Salicylic acid (10 mg/L)	0.030 ± 0.004 Aa	99.78 ± 1.84 Ab	63.86 ± 1.94 Acd	47.01 ± 2.49 Acd	110.87 ± 2.44 Ae	19.47 ± 1.57 Ab	92.59 ± 1.22 Ba	N.D.	N.D.
6-benzyladenine (1 mg/L)	0.033 ± 0.004 Aa	105.06 ± 2.43 Aab	77.77 ± 22.46 Acd	88.89 ± 10.66 Aa	166.66 ± 11.87 Ab	18.50 ± 4.36 Ab	92.59 ± 1.22 Ba	N.D.	N.D.
6-benzyladenine (10 mg/L)	0.037 ± 0.002 Aa	103.66 ± 1.73 Aab	100.67 ± 0.63 Aab	61.43 ± 0.97 Abc	162.10 ± 1.43 Ab	26.10 ± 0.74 Aab	75.35 ± 1.65 Bc	N.D.	N.D.
No plant growth regulator	0.011 ± 0.002 Ab	100.00 ± 1.75 Ab	83.75 ± 0.60 Abc	52.84 ± 1.40 Acd	136.59 ± 1.88 Ad	23.51 ± 0.49 Aab	91.95 ± 4.30 Ba	N.D.	N.D.
Atrazine-contamination									
Indole butyric acid (1 mg/L)	0.013 ± 0.003 Bb	101.40 ± 0.77 Aab	61.04 ± 1.70 Bb	38.66 ± 5.07 Ac	99.69 ± 4.43 Bc	24.89 ± 2.76 Aa	107.28 ± 1.81 Ac	11.40 ± 0.93 a	109.40 ± 13.09 a
Indole butyric acid (10 mg/L)	-0.038 ± 0.006 Bd	76.94 ± 0.94 Be	37.99 ± 1.23 Bc	33.76 ± 2.32 Ac	71.75 ± 3.53 Be	12.05 ± 0.25 Bc	130.91 ± 4.22 Aa	12.43 ± 1.38 a	121.28 ± 11.88 a

Table 2 *(Continue)*

Plant Growth Regulator	RGR (mg/g/day)	Dry weight (% of control)	Chlorophyll *a* (µg/g FW)	Chlorophyll *b* (µg/g FW)	Total chlorophyll (µg/g FW)	carotenoid (µg/g FW)	Proline (µg/g FW)	Phenolic (µg gallic acid/ mg DW)	Flavonoid (µg quercetin/ mg DW)
Gibberellic acid (1 mg/L)	-0.023 ± 0.008 Bc	75.75 ± 1.47 Be	53.41 ± 1.95 Bbc	72.34 ± 3.42 Aa	125.75 ± 1.88 Bb	9.17 ± 1.74 Bcd	104.09 ± 1.22 Ac	13.86 ± 2.40 a	173.78 ± 9.32 a
Gibberellic acid (10 mg/L)	0.024 ± 0.014 Aa	103.45 ± 1.48 Aa	93.57 ± 2.70 Ba	57.53 ± 4.64 Bb	151.09 ± 2.65 Ba	23.11 ± 1.97 Aa	88.12 ± 2.66 Ae	11.09 ± 2.55 a	116.45 ± 21.02 a
Salicylic acid (1 mg/L)	0.001 ± 0.007 Bb	84.16 ± 1.22 Bcd	44.40 ± 0.79 Bc	37.96 ± 2.33 Bc	82.35 ± 2.94 Bd	5.83 ± 0.79 Bd	114.94 ± 2.76 Ab	17.18 ± 2.97 a	110.01 ± 16.87 a
Salicylic acid (10 mg/L)	0.010 ± 0.006 Bb	81.90 ± 0.91 Bd	43.75 ± 1.52 Bc	41.28 ± 2.20 Ac	85.03 ± 2.92 Bd	3.09 ± 0.44 Bd	116.22 ± 1.65 Ab	13.64 ± 1.58 a	137.43 ± 16.91 a
6-benzyladenine (1 mg/L)	-0.012 ± 0.001 Bc	86.32 ± 1.29 Bc	63.79 ± 1.31 Ab	54.29 ± 1.51 Bb	118.08 ± 0.78 Bbc	17.58 ± 1.02 Ab	106.64 ± 1.61 Ac	13.97 ± 1.09 a	108.72 ± 21.14 a
6-benzyladenine (10 mg/L)	0.029 ± 0.004 Aa	98.92 ± 1.46 Bb	92.33 ± 1.42 Aa	63.76 ± 1.78 Aab	156.09 ± 1.80 Aa	22.53 ± 0.88 Aa	95.15 ± 2.63 Ad	11.91 ± 1.14 a	116.11 ± 15.92 a
No plant growth regulator	-0.024 ± 0.002 Bc	82.00 ± 0.97 Bd	61.49 ± 1.24 Bb	46.85 ± 2.38 Abc	108.35 ± 1.33 Bc	14.64 ± 1.97 Bbc	118.14 ± 1.22 Ab	14.76 ± 2.11 a	160.41 ± 13.04 a
Atrazine	**	**	**	**	**	**	**	-	-
Plant growth regulator	**	**	**	**	**	**	**	-	-
Atrazine x PGR	**	**	**	**	**	**	**	-	-

Note. Different lowercase letters show significant differences (P<0.05) between plant growth regulators within the same atrazine concentration; different capital letters show significant differences (P<0.05) between with and without atrazine at the same plant growth regulator (PGR); N.D. = Not determined; ** = Statistical significance (P<0.05)

and gibberellic acid stimulated the growth of *A. microphylla*. However, 1 mg/L of both plant growth regulators did not increase the dry weight, total chlorophyll content, and carotenoid content in *A. microphylla*. The findings in this study correspond with the study of Šípošová et al. (2021), who revealed that indole butyric acid at 10^{-9} M promoted the growth of maize under soil contaminated with 50 mM of cadmium nitrate, while 10^{-7} M of indole butyric acid inhibited the growth of maize under cadmium nitrate contamination (Šípošová et al., 2021).

Removal of Atrazine by *A. microphylla*

Cultivation of *A. microphylla* was unable to remove atrazine from contaminated water because the amount of atrazine remaining in the water after five days of *A. microphylla* cultivation was 22.67 mg/L, which was not significantly different from the amount of atrazine remaining in the unplanted control (25.33 mg/L). The initial concentration of tested atrazine was 25 mg/L (Table 3). The application of the plant growth regulators (6-benzyleadenine, indole butyric acid, salicylic acid, and gibberellic acid) did not improve the ability of *A. microphylla* to remove atrazine from the contaminated water (Table 3). The atrazine remaining in the water was around 21–26 mg/L when the plant growth regulator was applied, and the amount of atrazine remaining was not significantly different from the treatment without the plant growth regulator application ($P>0.05$). However, using 10 mg/L of 6-benzyladenine and 1 mg/L of indole butyric acid stimulates the removal of atrazine by *A. microphylla* compared to using both concentrations of salicylic acid. However, the amount of atrazine remaining in the water when using each type of plant growth regulator did not significantly differ from that without the plant growth regulator application ($P>0.05$). Based on our results,

Table 3
Atrazine remaining in water after cultivation of Azolla microphylla *with various plant growth regulators for five days (data shown as mean ± SE)*

Treatment	Atrazine (mg/L)
Indole butyric acid (1 mg/L)	21.50 ± 1.04 b
Indole butyric acid (10 mg/L)	26.00 ± 1.32 a
Gibberellic acid (1 mg/L)	23.92 ± 1.92 ab
Gibberellic acid (10 mg/L)	22.83 ± 0.17 ab
Salicylic acid (1 mg/L)	25.50 ± 1.04 a
Salicylic acid (10 mg/L)	25.42 ± 0.79 a
6-benzyladenine (1 mg/L)	22.17 ± 0.73 ab
6-benzyladenine (10 mg/L)	21.00 ± 0.29 b
No plant growth regulator	22.67 ± 0.17 ab
Non-cultivation of *A. microphylla*	25.33 ± 2.13 a

Note. Different lowercase letters show significant differences ($P<0.05$) between plant growth regulators within the same atrazine concentration

it was difficult to indicate the possible mechanisms of atrazine removal by *A. microphylla* because the amount of atrazine in contaminated water did not decrease significantly (P>0.05) between planting and not planting in atrazine-contaminated water. It suggests that *A. microphylla* could tolerate atrazine only. However, it could not remove atrazine from contaminated water. The findings in this study contradicted the previous studies by other researchers. For example, aquatic macrophytes, namely *I. pseudacorus*, *L. salicaria*, and *A. calamus*, could degrade atrazine by 75.6, 65.5, and 61.8%, respectively, when cultivated under hydroponic conditions for 20 days with an initial atrazine concentration of 4 mg/L (Q. Wang et al., 2012). The main mechanism of atrazine removal was the activity of plants to degrade atrazine and other mechanisms to remove atrazine, such as microbial degradation and abiotic degradation (Q. Wang et al., 2012). The reason why *A. microphylla* could not improve the atrazine removal from water in this study, possibly due to limited atrazine-degrading microorganisms found in the water used. Tap water was used to prepare the atrazine-contaminated water in this study, despite heterotrophic bacteria being a common microorganism found in tap water (Harnroongroj et al., 2012). Atrazine degradation activity was generally poor under sterilized conditions (Q. Wang et al., 2012). Moreover, the period of *A. microphylla* cultivation in this study was short, at only five days for this experiment. *Azolla microphylla* and related microorganisms might not have adapted to degrade atrazine during the experiment. The study by Q. Wang et al. (2012) reported that *I. pseudacorus*, *L. salicaria*, and *A. calamus* took 20 days for atrazine removal. However, the period for atrazine removal varied depending on the plant species. Marecik et al. (2021) reported that cultivation of *A. calamus* under hydroponic conditions for seven days could reduce atrazine by 57% (the initial concentration of atrazine was 3.5 g/L) and 97% of atrazine was removed after extending the time for *A. calamus* to 21 days. Meanwhile, *T. latifolia* took 50 days for 90% atrazine removal under hydroponic conditions (Marecik et al., 2021). Based on the cultivation of aquatic plants in other studies, it can be suggested that the cultivation period of *A. microphylla* was extended for more than five days; the atrazine may be removed.

Despite there being no atrazine removal by *A. microphylla* in this study, however, the mechanism for pollutant removal in genus *Azolla* in water is often by accumulation or phytoextraction, such as for cadmium (Rai, 2008) and methyl violet 2B dye (Kooh et al., 2018). Applying plant growth regulators in this study did not increase the plant capacity to remove atrazine. It may be due to the concentration of the plant growth regulator being suitable only to stimulate plant growth but not for increased atrazine removal. In addition, some plant growth regulators, such as cytokinin and salicylic acid, have been reported to decrease pollutant accumulation in some plants and algae. For example, cytokinin decreased Pb accumulation in

the algae cells of *Acutodesmus obliquus* (Piotrowska-Niczyporuk et al., 2018). Salicylic acid application to hemp grown in cadmium-contaminated sand decreased the Cd uptake into plants (Shi et al., 2009). If the atrazine-degrading microorganisms were insufficient, it is possible that some plant growth regulators that supported plant-microbe interactions were not working well. Thus, the use of *A. microphylla* in atrazine phytoremediation in the future should be done with the augmentation of atrazine-degrading microorganisms combined with the cultivation of *A. microphylla* in contaminated water.

CONCLUSION

Azolla microphylla was an atrazine-tolerant plant, but it could not remove atrazine when cultivated in contaminated water alone. About 22.67 mg/L of atrazine was remained in water at the end of experiment. Applying salicylic and indole butyric acid did not promote the growth and phytoremediation by *A. microphylla* grown in atrazine-contaminated water. Gibberellic acid and 6-benzyladenine were suitable for stimulating the growth of *A. microphylla* under atrazine contamination. Percentage of dry weight and total chlorophyll content of *A. microphylla* were 103 and 156.09 ug/g fresh weight when recieving 10 mg/l of giberellic acid and 6-benzyladenine, respectively. However, the suitable concentration should be determined when using both plant growth regulators. The application of *A. microphylla* and atrazine-degrading microorganisms may be interesting in aiding atrazine degradation. This assumption should be investigated in future work.

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