Journal of Hazardous Materials 172 (2009) 240–246

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The effects of cooking oil fume condensates (COFCs) on the vegetative growth of *Salvinia natans* (L.) All.

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ARTICLE INFO

Article history:
Received 22 December 2008
Received in revised form 1 July 2009
Accepted 1 July 2009
Available online 8 July 2009

Keywords:
Cooking oil fume (COF)
Cooking oil fume condensates (COFCs)
Salvinia natans
Relative growth rate (RGR)
Floating leaf
Vegetative growth
Aquatic environment

ABSTRACT

Cooking oil fumes (COF) and their condensates (COFCs), which are suspected of causing human lung cancers, are hazardous materials to environments. The effects of COFCs on the vegetative growth of *Salvinia natans* (L.) All., a free-floating aquatic fern, are discussed in this paper. The results showed that there were no differences of the number of floating leaves and the mean numbers of new leaves of *S. natans* in all groups, but these indices in experimental groups were influenced obviously at the late stage. COFCs also influenced stem length and number of buds of *S. natans*. COFCs could cause the floating leaves to turn yellow and individuals to die quickly. All these effects were correlated with the concentration of COFCs and the time. When the concentration of COFCs was ≥0.18 g/l, above 80% individuals would die in a short time. COFCs had significant impacts on the decrease in photosynthetic areas of *S. natans* by making the floating leaves turn yellow faster and accelerating the decomposition. There were some components in minute amount benefiting to the growth of *S. natans*. *S. natans* was sensitive to COFCs and could be a potential indicator for monitoring COFCs pollution in aquatic environments.

1. Introduction

Wok cooking is very common in Chinese families, historically and presently. One of the major characteristics of this cooking style is that cooking oils are often heated in a wok at high temperatures ranging from 190 °C to 280 °C [1,2], so a great amount of cooking oil fume (COF) is produced during cooking. A part of the COF will emanate directly into the atmosphere. Most of them will be collected by kitchen ventilators and become cooking oil fume condensates (COFCs) in Chinese families presently. Emissions from cooking oil heated are hazardous to the atmosphere [3]. They are also harmful to human health. A human exposed to COF will develop lung cancer [1,4–8]. There are over 100 components in COF, such as polycyclic aromatic hydrocarbons [9–11], hexanal and 2-heptenal [2,12], aromatic amines [13], and alkyl, alkene, aldehyde [14], which are harmful to human health and the atmosphere [15]. The components in COFCs and their concentrations are related closely to the temperature of heating oils [16]. Some studies showed that the cellular immune function of experimental animals would be influenced when they were kept under COF conditions for a long time [17]; the mutation of anti-oncogene *p*53 or FHIT, or modification of DNA bases might play an important role in lung cancers caused by COF [18,19]. COFCs collected from restaurants often contain 1,3-Butadiene, benzene, acrolein, formaldehyde [1]. Oil and grease pollution will influence the ecological functions of terrestrial and aquatic ecosystems [20–22]. These ecological effects would be sustained for a long time [23–25]. As a byproduct of oil and grease, COFCs may have some ecological effects on environments. Along with urbanization in China, the amount of COFCs accumulated will increase. It is easy to speculate that the production of COFCs will be high in the areas of high-density populations. So the way to deal with COFCs suitably is not only an ecological issue but also a social–economic concern. There is, however, no official standard or approach to dispose off COFCs presently. The harmfulness of COFCs to environments, especially to terrestrial and aquatic ecosystems, is obviously underestimated. Most of the COFCs are currently treated with municipal soil wastes. Parts of COFCs are occasionally dumped directly into aquatic ecosystems from the municipal sewage. Despite their serious harmfulness to human health, little is known about the ecological effects of COFCs on plants, especially aquatic plants until now. If COFCs are disposed into aquatic ecosystems, they will form an oleaginous cover on the water surface. Is there any influence of this oleaginous film on the growth and population dynamics of aquatic plants? The answer to this question will be useful not only to evaluate the influence of COFCs on the environment and monitor the aquatic environmental situation, but also to discover the plants that have the ability to remove COFCs from the environments.

Salvinia natans (L.) All. is a free-floating fern distributed mainly in tropical and temperate regions in Eurasia and New Zealand [26,27].

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doi:10.1016/j.jhazmat.2009.07.001

It is considered to be a wide-spreading and invasive plant in temperate regions and plays a vital role in the structure of aquatic ecosystems [28,29]. However, it is also an endangered species listed in *The Red Data Book* in Japan for its decreasing populations in recent years, and very susceptible to herbicides, such as bensulfuron methyl [30]. *Salvinia* biomass has strong metal-complexation capacity [31], many species show the ability of removing heavy metals in aquatic environments [32,33]. As a biomass sorbent for oil removal, *Salvinia* biomass shows a great potential and value [34]. In China, *S. natans* is used traditionally as an important feed for livestock for its great biomass production and nutrients. In this paper, we will discuss about the effects of COFCs on the vegetative growth of *S. natans* based on experimental data.

2. Methods and materials

2.1. Material collection and cultivation

S. natans is a common aquatic plant distributed widely in the east and south China [27]. Summer and autumn are the best seasons for *S. natans* growth in the east of China. All plant materials for the experiment were collected from wetlands near Yangzhou City of Jiangsu Province, China from July to September of 2008 and May to June of 2009 respectively. The materials were transferred to plastic pots and cultivated for accommodation in a greenhouse (the temperature was $30 \pm 2\,^{\circ}C$, the light provided by metal halid bulbs for 12 h/d) for a week before treatment. The nutrient medium of Hogland was added periodically into the plastic pots during the whole experiment.

2.2. Preparation of COFCs

COFCs were collected from a kitchen ventilator from a household that often used a certain type of refined cooking oil commonly sold in supermarkets in Yangzhou, China. The peroxide value and acid value of the COFCs used in the experiment were 13.11 ± 2.84 mg/100 g and 7.409 ± 0.130 mg KOH/g respectively ($n = 7$), much larger than that of edible vegetable oil ($\leq$0.25 mg/100 g and $\leq$3 mg KOH/g respectively). COFCs were diluted in distilled water to prepare cultivation medium before experiment. COFCs were added into the water and stirred to make them mixed with water equably. The concentration gradients of COFCs were prepared as follows: 0 g/l (Control), 0.02 g/l (Group 1), 0.04 g/l (Group 2), 0.08 g/l (Group 3), 0.18 g/l (Group 4), 0.28 g/l (Group 5), 0.38 g/l (Group 6), 0.48 g/l (Group 7), 0.58 g/l (Group 8), 0.68 g/l (Group 9), and 0.78 g/l (Group 10).

2.3. Treatment and data analysis

Similar size and healthy individuals of *S. natans* were selected as materials for treatment. Each individual was dissected into two parts, the one with apical bud and 8 leaves left was used for experiment. The experiment materials were divided randomly into 11 groups, each group included 20 individuals of *S. natans*. And their fresh weights were measured (W_0) before treatment. All materials were cultivated in plastic pots (with diameter of 12 cm and height of 10 cm) containing cultivation mediums with different concentrations of COFCs. Each pot had only one individual of *S. natans* for eliminating the effect of density.

The numbers of leaves and buds, pH values and the length of stem of each experimental material were recorded every day from the second day after treatment. The fresh weight (W_1) and dry weights of each individual were measured at the end of the treatment.

The following equation was used to calculate the relative growth rate (RGR) of each individual:

$$\mathrm{RGR} = \frac{\ln W_1 - \ln W_0}{\Delta t}$$

where W_0 and W_1 are fresh weights (g) of each individual at the beginning and end of the experiment, Δt is time span (day) of experiment [30,35]. Because after the day 9, the growth of some individuals in Groups 5–10 stopped entirely, we only calculated the RGRs of *S. natans* in Control, Groups 1–4 when $\Delta t = 16$.

One-way analysis of variance (ANOVA) was used to determine the differences among different treatments with SPSS 11.5. The differences were statistically significant when $p < 0.05$.

3. Results and analysis

3.1. Effects of COFCs on the death of S. natans

During the experiment, there was no individual of *S. natans* died only in Control, Groups 1 and 2. Other groups appeared died individuals more or less at different times. There was no died individual in Group 3 until the day 15 after treatment with COFCs. From Groups 5 to 10, some individuals began to die after the day 3, and all individuals died at a certain time (Table 1). This result suggested that if the concentration of COFCs was $\leq$0.04 g/l, it would not cause *S. natans* die, but when the concentration of COFCs was above 0.08 g/l, some individuals of *S. natans* would die, especially when the concentration of COFCs was $\geq$0.28 g/l, all individuals would die. The higher the concentration of COFCs was, the earlier this effect was.

3.2. Effects of COFCs on the floating leaves of S. natans

Floating leaves are the most important organs of photosynthesis for *S. natans*. They were direct contact with the oleaginous cover formed by COFCs on the water surface during the treatment. The floating leaf number of *S. natans* at different concentrations of COFCs showed an increasing trend along with cultivation times (Fig. 1). But there were no differences among the mean numbers of floating leaves of *S. natans* in different groups before the day 9 ($df = 87$, $F = 1.358$, $p = 0.216$). Form the day 9 to the end of the experiment (the day 17), all individuals in some groups with higher COFCs concentrations were died. At the end of the experiment, there were only 5 groups (including Control, Groups 1–4) had living individuals (Table 1). The mean number of floating leaves in these 5 groups increased along with the treatment time. However, the mean num-

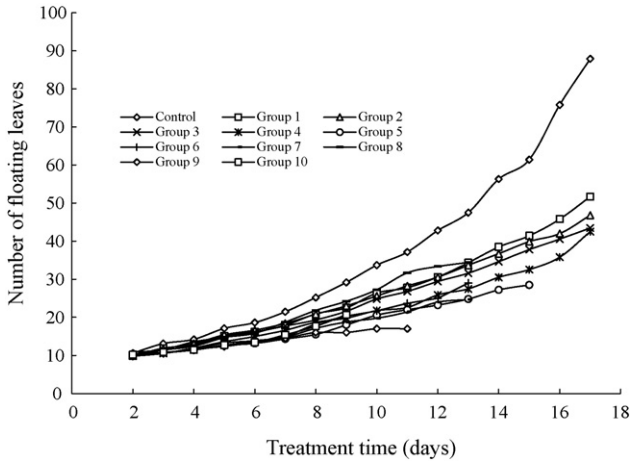

Fig. 1. The changes of the numbers of floating leaves of *Salvinia natans* in different groups along with treatment time.

Table 1
The death rate of individuals of *S. natans* in different groups along with the treatment time.

	Treatment time																
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Group 3	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (5%)	2 (10%)	2 (10%)
Group 4	0	0	1 (5%)	1 (5%)	2 (10%)	2 (10%)	2 (10%)	3 (15%)	3 (15%)	5 (25%)	5 (25%)	8 (40%)	8 (40%)	11 (55%)	16 (80%)	16 (80%)
Group 5	0	5 (25%)	6 (30%)	6 (30%)	7 (35%)	12 (60%)	12 (60%)	13 (65%)	13 (65%)	14 (70%)	15 (75%)	15 (75%)	16 (80%)	20 (100%)		
Group 6	0	2 (10%)	8 (40%)	9 (45%)	11 (55%)	15 (75%)	15 (75%)	15 (75%)	15 (75%)	15 (75%)	17 (85%)	20 (100%)				
Group 7	0	3 (15%)	6 (30%)	6 (30%)	7 (35%)	11 (55%)	12 (60%)	12 (60%)	13 (65%)	17 (85%)	17 (85%)	20 (100%)				
Group 8	0	2 (10%)	4 (20%)	4 (20%)	6 (30%)	10 (50%)	10 (50%)	12 (60%)	14 (70%)	14 (70%)	15 (75%)	20 (100%)				
Group 9	0	6 (30%)	9 (45%)	9 (45%)	12 (60%)	13 (65%)	15 (75%)	16 (80%)	18 (90%)	20 (100%)						
Group 10	0	4 (20%)	6 (30%)	6 (30%)	7 (35%)	14 (70%)	17 (85%)	20 (100%)								

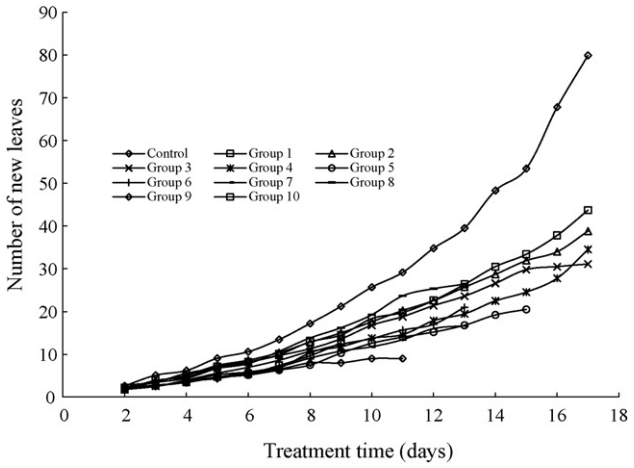

Fig. 2. The number of new leaves of *S. natans* in different groups along with the treatment time.

ber of floating leaves in Control was higher significantly than that of Groups 1–4 (df = 39, $F = 6.697$, $p < 0.01$), but there were no differences in the mean numbers of floating leaves among Groups 1–4 (df = 31, $F = 1.346$, $p = 0.280$).

The mean numbers of new leaves in the treatment groups were equal to the numbers in Control before the day 9 (df = 87, $F = 1.380$, $p = 0.206$) (Fig. 2). After the day 9, the mean accumulated numbers of new leaves in Control were significantly higher than that in Groups 1–4 (df = 39, $F = 6.961$, $p < 0.001$), but there were no differences in the mean accumulated numbers of new leaves among Groups 1–4 (df = 31, $F = 1.482$, $p = 0.241$). The results suggested that the presence of COFCs had no effects on the number of new leaves of *S. natans* at the early stage (before the day 9), but would have a significant influence on the appearance of new leaves at the late stage.

The leaves of *S. natans* in Control began turning yellow or decomposed from the day 5 (Table 2). The mean number of yellow leaves was 0.45 (±1.15) on the day 5, lower significantly than that of Groups 2–10 ($p < 0.01$). The individuals of *S. natans* in the treatment groups appeared yellow or decomposed leaves earlier than Control. Furthermore, it was earlier when leaves began becoming yellow in the groups with higher concentrations of COFCs (Groups 4–10) than that in the groups with lower concentrations of COFCs (Groups 1–3) (Table 2). The numbers of yellow or decomposed leaves of *S. natans* under the treatments of COFCs increased approximately with the treatment time and COFCs concentration (Table 2 and Table 3). Except Group 1, the average number of yellow or decomposed leaves of *S. natans* in other experimental groups was significantly higher than that in Control on the day 3 (Table 2). 100% of individuals appeared yellow leaves in Groups 7–10 on the day 3. On the day 4, all individuals in Groups 4–6 appeared yellow leaves. Each individual in Control and Group 1 did not appear yellow leaves until the day 16 (Table 3). It indicated that COFCs might cause and accelerate the floating leaves of *S. natans* to turn yellow, and these effects occurred earlier in the groups with higher concentrations of COFCs than that in the groups with lower concentrations of COFCs. Furthermore these effects also correlated closely to the treatment time. New leaves in Control appeared yellow from the day 16, but some new leaves in Groups 7 and 9 began to turn yellow from the day 3, and in Groups 8 and 10 from the day 4. On the day 9, the numbers of new leaves turning yellow in Groups 4–10 were higher significantly than that in Control. All individuals in Groups 8–10 had yellow new leaves (Table 4). This result also suggested that COFCs could cause not only the mature leaves, but also the new leaves of *S. natans* to turn yellow, especially higher concentrations of COFCs.

Table 2
The mean numbers of yellow leaves of *S. natans* at different concentration of COFCs ($n = 20$).

	Treatment time															
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17
Control	0	0	0	0.45 ± 1.15	0.60 ± 1.27	0.55 ± 1.32	0.70 ± 1.53	0.85 ± 1.46	0.90 ± 1.55	0.95 ± 1.64	1.20 ± 1.85	2.63 ± 1.86	4.10 ± 2.19	4.50 ± 2.66	6.65 ± 3.82	7.40 ± 3.20
Group 1	0	0.20 ± 0.52	0.25 ± 0.72	0.55 ± 0.89	0.75 ± 1.07	0.80 ± 1.10	1.00 ± 1.12	1.35 ± 1.34	1.35 ± 1.53	1.80 ± 1.32	2.15 ± 1.76	3.95 ± 2.50*	5.20 ± 2.44	6.55 ± 3.07*	8.80 ± 3.07	9.70 ± 3.44*
Group 2	0	2.10 ± 1.89**	2.45 ± 1.73**	3.60 ± 2.28**	5.21 ± 2.20**	5.60 ± 1.93**	6.50 ± 2.16**	6.20 ± 1.96**	6.70 ± 1.95**	6.90 ± 1.65**	6.85 ± 1.50**	7.70 ± 0.92**	8.70 ± 1.72**	9.55 ± 2.87**	13.65 ± 4.56**	16.45 ± 6.04**
Group 3	0	3.15 ± 1.89**	3.80 ± 2.26**	4.55 ± 2.46**	5.75 ± 2.22**	6.05 ± 2.21**	6.20 ± 2.68**	6.75 ± 2.10**	7.35 ± 2.25**	7.50 ± 2.26**	8.00 ± 1.45**	9.15 ± 1.46**	9.80 ± 2.07**	11.40 ± 3.90**	13.61 ± 5.63**	14.17 ± 7.16**
Group 4	0.20 ± 0.52	3.55 ± 2.35**	5.60 ± 2.20**	6.79 ± 2.04**	7.68 ± 1.45**	7.39 ± 1.50**	8.39 ± 1.79**	8.39 ± 1.41**	8.59 ± 1.54**	8.94 ± 2.01**	9.73 ± 2.25**	11.87 ± 3.29**	12.17 ± 2.59**	13.33 ± 3.23**	17.22 ± 6.12**	18.00 ± 5.89**
Group 5	0.55 ± 0.95*	5.15 ± 2.72**	5.80 ± 2.04**	7.36 ± 1.40**	8.36 ± 1.91**	8.54 ± 2.18**	8.50 ± 1.69**	8.75 ± 1.83**	9.29 ± 2.63**	9.29 ± 2.50**	9.17 ± 2.04**	11.00 ± 2.00**	11.80 ± 1.79**	13.00 ± 1.15**		
Group 6	0.60 ± 0.94*	6.65 ± 2.73**	8.06 ± 2.31**	7.92 ± 2.31**	8.18 ± 3.43**	9.70 ± 3.13**	9.00 ± 4.00**	9.83 ± 3.37**	10.67 ± 3.01**	11.00 ± 2.76**	11.67 ± 2.87**	19.00 ± 11.17**				
Group 7	1.30 ± 1.49**	6.85 ± 2.30**	7.71 ± 2.37**	8.43 ± 2.74**	9.00 ± 2.91**	10.00 ± 1.63**	10.89 ± 2.03**	10.00 ± 1.07**	11.38 ± 1.77**	12.00 ± 3.65**	11.33 ± 1.15**	14.67 ± 1.53**				
Group 8	1.90 ± 2.00**	6.30 ± 2.54**	7.72 ± 2.95**	8.75 ± 2.11**	9.63 ± 1.86**	9.50 ± 3.39**	10.10 ± 0.99**	10.80 ± 1.03**	11.00 ± 2.62**	11.67 ± 2.94**	16.33 ± 6.25**	16.60 ± 2.97**				
Group 9	2.20 ± 1.70**	7.30 ± 2.18**	8.36 ± 2.02**	9.64 ± 1.29**	9.73 ± 1.35**	11.25 ± 2.38**	11.00 ± 1.00**	11.60 ± 0.89**	13.00 ± 2.00**	15.00 ± 1.41**						
Group 10	2.50 ± 1.15**	6.85 ± 1.42**	8.44 ± 1.41**	9.43 ± 1.87**	9.93 ± 2.16**	10.31 ± 1.97**	11.33 ± 1.63**	12.67 ± 3.06**								

Asterisks indicate the level of significant differences between Control and treatment groups.
* $p < 0.05$.
** $p < 0.01$.

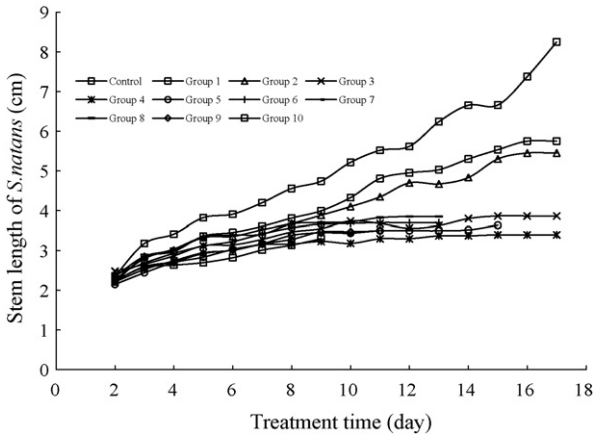

Fig. 3. The changes of stem lengths of *S. natans* in different groups along with the treatment time.

3.3. Effects of COFCs on the stem length and bud occurrence of S. natans

The stem length is another indicator which may be used to reflect the vegetative growth of *S. natans*. The average stem lengths of *S. natans* at different COFCs concentrations went up during treatment. The stem length showed the same trend as in Control (Fig. 3). However, the mean length of stem of *S. natans* in Control was higher significantly than that of treatment groups before the day 9 (df = 87, $F = 2.698$, $p = 0.007$). After the day 9, the stem growth of *S. natans* in Control was faster significantly than that in Groups 1–4 (df = 39, $F = 38.504$, $p < 0.001$). The stem lengths in Groups 1 and 2 were higher significantly than that of Groups 3 and 4 after the day 9 (df = 31, $F = 46.197$, $p < 0.001$). This result indicated that COFCs had obvious effects on the stem length of *S. natans*, and there were correlations between the COFCs effects on the stem length of *S. natans* and its concentration.

Buds play an important role in the vegetative propagation and morphological characteristics of *S. natans*. The accumulated number of buds in Control increased steadily with the time. It was lower significantly on the day 9 than that on the end of the experiment (i.e. the day 17) (df = 39, $F = 43.172$, $p < 0.01$). The accumulated numbers of the treatment groups were not very regular. But at the end of the experiment, the accumulated number of buds in Control was higher significantly than that in Groups 1–4 (Fig. 4). The results suggested that COFCs had obvious effects on the bud occurrences of *S. natans*.

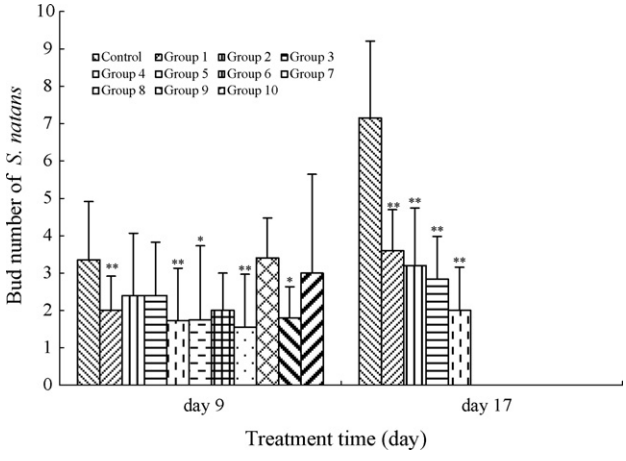

Fig. 4. The number of buds of *S. natans* in different groups on the days 9 and 17. Asterisks indicate the level of significant differences between Control and treatment groups (**$p < 0.01$; *$p < 0.05$).

Table 3
The percentage of individuals appearing yellow leaves in different groups ($n = 20$).

	Treatment time														
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16
Control	0	0	0	15%	20%	20%	20%	30%	30%	30%	35%	75%	85%	85%	100%
Group 1	0	20%	20%	40%	45%	45%	50%	60%	60%	75%	75%	90%	95%	95%	100%
Group 2	0	65%	80%	90%	90%	95%	95%	95%	100%						
Group 3	0	90%	95%	95%	95%	95%	95%	95%	100%						
Group 4	15%	95%	100%												
Group 5	30%	95%	100%												
Group 6	30%	95%	100%												
Group 7	60%	100%													
Group 8	65%	100%													
Group 9	75%	100%													
Group 10	95%	100%													

Table 4
The mean numbers of new leaves turning yellow and the percentage of individuals bearing yellow new leaves of *S. natans* ($n = 20$).

Treatment	Day 9		Day 17	
	Number of new leaves turning yellow	Percentage of individuals	Number of new leaves turning yellow	Percentage of individuals
Control	0	0	0.8 ± 1.91	20%
Group 1	0	0	2.15 ± 2.94	50%
Group 2	0	0	$8.65 \pm 5.70^{**}$	85%
Group 3	0	0	$8.15 \pm 5.71^{**}$	95%
Group 4	$0.68 \pm 0.94^{**}$	36.8%	$7.42 \pm 5.31^{**}$	95%
Group 5	$1.95 \pm 1.58^{**}$	73.7%		
Group 6	$2.30 \pm 1.72^{**}$	90%		
Group 7	$2.35 \pm 1.63^{**}$	90%		
Group 8	$3.45 \pm 1.57^{**}$	100%		
Group 9	$2.95 \pm 1.10^{**}$	100%		
Group 10	$3.10 \pm 1.77^{**}$	100%		

Asterisks indicate the level of significant differences between Control and treatment groups $^{**}p < 0.01$.

3.4. Effects of COFCs on RGR and dry weights of S. natans

During the first 5 days, there were no differences of RGR among Control, Groups 3–6 (df = 99, $F = 0.748$, $p > 0.05$). However, RGRs of *S. natans* in Groups 7–10 were higher significantly than that of Control (df = 99, $F = 3.355$, $p < 0.05$) (Fig. 5). At the end of the experiment, there were only 5 groups which had living individuals. The results indicated that low concentration of COFCs ($\leq$0.38 g/l) had no obvious effect on RGR of *S. natans*, but high concentrations of COFCs (0.48–0.78 g/l) could benefit to RGR of *S. natans* treated by COFCs in a short time. Along with the treatment time, low concentration of COFCs also showed an obvious influence on RGR. Fig. 6 showed RGR of *S. natans* in Control and 4 treatment groups at the end of the experiment. The RGR showed a negative correlation with the concentrations of COFCs. There were significant differences of RGR among Control, Groups 1–4 (df = 85, $F = 87.700$, $p < 0.001$). Some components from COFCs may act as nutrition to the growth of *S. natans*. So at the early stage of treatment, they could help the growth of *S. natans*; but at the late stage, COFCs would limit the growth of *S. natans* significantly.

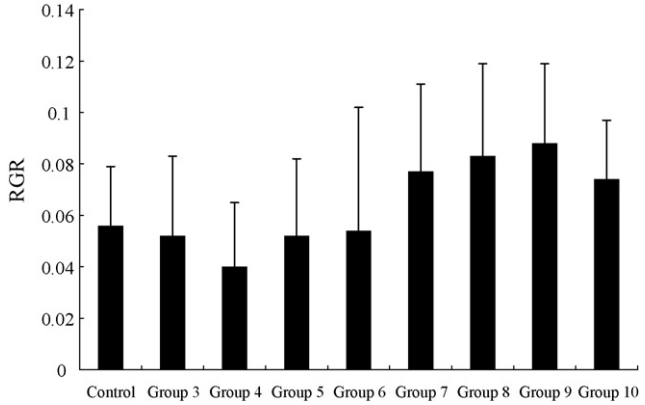

Fig. 5. The RGR of *S. natans* in different groups on the day 5.

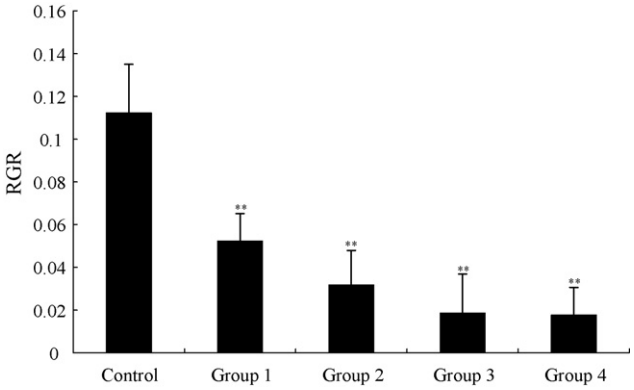

Fig. 6. The RGR of *S. natans* in different treatment groups at the end of the experiment. Asterisks indicate the level of significant differences between Control and treatment groups ($^{**}p < 0.01$; $^{*}p < 0.05$).

The dry weights of different parts of *S. natans* at the end of the experiment were showed in Fig. 7. The dry weights of floating leaves (df = 97, $F = 95.645$, $p < 0.001$), stems (df = 97, $F = 21.833$, $p < 0.001$), and submerged leaves (df = 97, $F = 3.583$, $p = 0.009$) in Control were significantly higher than that of Groups 1–4. The dry weights of different parts of *S. natans* were correlated negatively with the

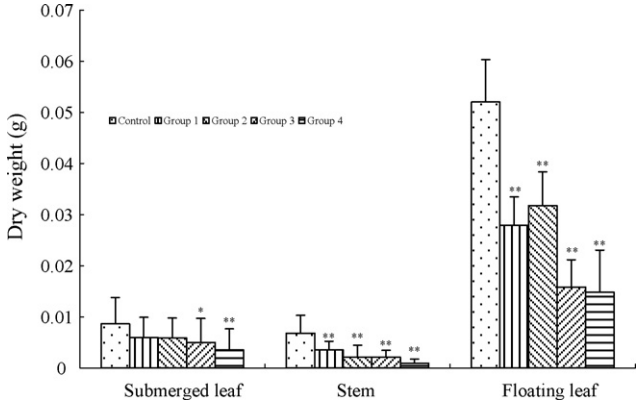

Fig. 7. The dry weights of different parts of *S. natans* at the end of the experiment. Asterisks indicate the level of significant differences between Control and treatment groups ($^{**}p < 0.01$; $^{*}p < 0.05$).

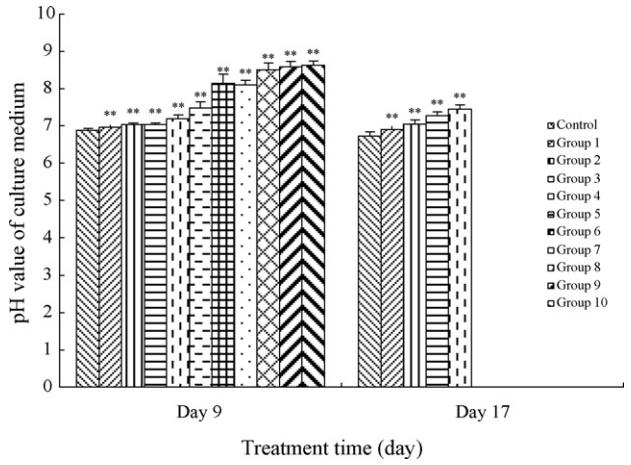

Fig. 8. The pH values of culture mediums in different groups on the days 9 and 17. Asterisks indicate the level of significant differences between Control and treatment groups ($^{**}p < 0.01$; $^{*}p < 0.05$).

concentrations of COFCs. The results showed that COFCs would influence the accumulation of biomass of *S. natans*. The higher the concentration of COFCs was, the lower the biomass of *S. natans* was.

3.5. Changes of the pH values of cultivation mediums during the experiment

During the experiment, there were significant differences of the pH values in Control and treatment groups along with the treatment time. The pH values were higher significantly in the treatment groups than that in Control (on the day 9: df = 143, $F = 478.210$, $p < 0.001$; on the day 17: df = 83, $F = 104.855$, $p < 0.001$) (Fig. 8). Additionally, the higher the concentration of COFCs was, the higher the pH value in cultivation medium was. COFCs might influence the growth of *S. natans* by increasing the pH values of cultivation mediums.

4. Discussion

4.1. The effects of COFCs on the growth of S. natans

S. natans has two types of leaves, floating leaves and submerged leaves, located in nodes of the stems which are floating. Each node has two floating leaves and one rootlike submerged leaf. Floating leaves are one of the main vegetative organs for *S. natans*, which are responsible for providing carbohydrates and energy for the whole plant, while submerged leaves are responsible for producing sporocarps and absorbing nutrients from environments [27]. At the early stage (before the day 9), there are no differences of the number of floating leaves and the mean numbers of new leaves of *S. natans* in all groups, but these indices in experimental groups were influenced obviously at the late stage. Stem length and number of buds did also show the same trend. COFCs could increase the numbers of yellow or decomposed leaves, especially high concentrations of COFCs. When the concentration of COFCs was ≥0.08 g/l, it could also cause the individuals die. The death rate correlated positively with the concentrations of COFCs at the same time. Appearance of yellow or decomposed leaves signifies the decreasing of effective photosynthetic area of the plant. So COFCs may influence the vegetative growth of *S. natans* by decreasing its photosynthetic effectiveness.

COFCs are the products of edible oils under high temperatures. Besides some harmful components, they may contain components benefiting the growth of aquatic plants. In this experiment, it is found that RGRs of *S. natans* under the treatments of higher COFCs concentrations are much higher than that in other groups. It is easy to speculate that these beneficial components to growth of aquatic plants are in a minute amount.

According to *Integrated wastewater discharge standard* of China (GB 8978-1996), the concentration of animal or plant oil should be equal or under 20 mg/l (classes I and II) or 100 mg/l (class III). In our experiment, the concentrations of COFCs ranged from 0.02 g/l to 0.78 g/l. At least class III of wastewater may impact the vegetative growth of *S. natans*.

4.2. The possible mechanism of COFCs affecting the vegetative growth of S. natans

Based on the discussion above, we can conclude that COFCs may affect the vegetative growth of *S. natans* by making floating leaves turn yellow faster, or making them decomposed more rapidly. Floating leaves of *S. natans* are the main organs responsible for its assimilation. When they turn yellow or decomposed, photosynthesis will be influenced. COFCs used in the experiment show high peroxide value and acid value. High peroxide value and acid value can cause leaves turning yellow [36,37]. Non-polar components, such as lipophilic substances, can enter the cell through the lipid bilayer of the plasma membrane and alter structure and functions of organelles [38]. So lipophilic substances in COFCs may have influences on the photosynthesis of *S. natans* by damaging its chloroplasts. In water bodies, COFCs will form an oleaginous cover on the water surface. The floating leaves of *S. natans* will be surrounded by COFCs, which makes floating leaves submerged and completely covered by the film of COFCs, especially at higher concentrations of COFCs. This oleaginous film may influence air exchange of leaves through stomata with the outside. Fernández found that an obvious decrease in net photosynthetic rate and stomatal conductance of *Pouteria orinocoensis* was shown in emerged leaves, this phenomenon was related to the increases in both relative stomatal and non-stomatal limitation [39]. The net photosynthesis of *Mnium undulatum* could be strongly inhibited when their leaves under the hypoxia condition [40]. Aqueous components from crude oil had significant disturbances on stomatal behavior [41], or caused a reduction of chlorophyll contents in leaves [42]. It is possible that some components in COFCs may impact chlorophyll contents in leaves, making the leaves turn yellow. Tsuchiya reported that periphyton accumulation on leaf surface in water was analogous to herbivory reducing leaf life span [43]. Oleaginous film formed by COFCs may have the similar effect of herbivory and periphyton accumulation on the life span of leaves. So there may be multiple ways in which COFCs cause the floating leaves turning yellow.

5. Conclusion

Because COFCs will form an oleaginous film on the water surface when they enter aquatic systems, so the floating-leaved plants will contact COFCs directly. Although COFCs do not have obvious effects on emergences of new leaves and new buds of *S. natans* during a short time treatment, they can cause floating leaves to turn yellow or make them decomposed. Yellow or decomposed leaves mean the decrease in photosynthetic areas. Attention should be paid to the question of which component of COFCs contribute to turn floating leaves of *S. natans* yellow and whether *S. natans* has the ability of removing COFCs in future studies.

S. natans can be taken as a potential indicator for monitoring COFCs pollution. Even at 0.08 g/l, it will cause some individuals die. When the concentration of COFCs is ≥0.28 g/l, all individuals will die during a short term. Furthermore, when COFCs exist, the floating leaves of *S. natans* will turn yellow very quickly. So *S. natans* can be used to monitor COFCs pollutions in aquatic environments.

Acknowledgements

Mr. Weirong Bai, Ms. Wen Wen and Yeqiao Luo participated in a part of the experiment. The authors are indebted to Dr. Ziwei Li from the Montreal Neurological Institute, McGill University, Canada for help in improving the writing. The authors also thank the two anonymous reviewers' valuable comments to this paper.

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