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Original Research Paper

Effects of Main Chemical Compounds in Cooking Oil Fume Condensates (COFCs) on Growth of *Salvinia natans* (L.) All.: II. Hexadecane

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ABSTRACT

Hexadecane is one of the main chemical compounds in Cooking Oil Fume Condensates (COFCs), which has been proved to influence the vegetative growth of *Salvinia natans* (L.) All. significantly. Its effects on the growth of *S. natans* were studied by using static toxicity testing method in this paper. The results showed that relative growth rate (RGR), leaves, buds and stems of *S. natans* were inhibited significantly when exposed to higher concentrations. Hexadecane could accelerate the leaves of *S. natans* becoming yellow or decomposed. Biomass went down with the increase of hexadecane concentrations. The LC_{50} on day 4 and day 12 after treatment were 275 mg/L and 244 mg/L, respectively. Hexadecane had no effect on the pH value and conductivity of the cultivation medium. It can be concluded that hexadecane would significantly affect the vegetative growth of *S. natans*., and *S. natans* is sensitive to hexadecane that might be useful as an indicator of hexadecane pollution in freshwater.

INTRODUCTION

Water pollution, especially of surface water, is becoming a serious environmental problem worldwide (Lemly 2004, Li et al. 2007, Oberholster 2008, Yoshiaki 2008). There are many different pollutants contaminating water environments (Goel 2006) such as heavy metals (Dixit & Tiwari 2008, Kar et al. 2008), industrial and agricultural wastes (Ramos et al. 2006), etc. Water pollution can impact economic development (Reddy & Behera 2006), living standards (Dwight et al. 2005, Li et al. 2007), public health (Ake & Bo 2005), etc. Among the pollutants, oil and grease from food manufacturing industries (such as dairies and slaughter houses) and domestic activities is a serious one (Omil et al. 2003, Khan et al. 2004, Cammarota & Freire 2006). It has many ecological effects (Salanitro 1997, Jiang et al. 2009). Cooking oil fume condensates (COFCs), a kind of waste discharged from restaurants and domestic cooking, are one of the sources of oil and grease pollutants (Ko et al. 2000, Metayer et al. 2002, Miao et al. 2005, Jiang et al. 2009). It is obvious that the amount of COFCs will increase with urbanization. It is reported that 4600 restaurants in Guangyuan city will collect at least 460 kg/d COFCs (Yan et al. 2003). There is no effective method for treating COFCs presently. Much of it is discharged directly as rubbish. COFCs have a significant effect on the vegetative growth of *Salvinia natans*, a floating-leaves aquatic plant (Jiang et al. 2009). There are over 100 components in COFCs, such as polycyclic aromatic hydrocarbons, hexanal and 2-heptenal, aromatic amines, alkyl, alkene, and aldehyde (Leson & Winer 1991, Chiang et al. 1999, Zhu & Wang 2003, Kawai et al. 2006). However, we have little knowledge on what components in COFCs contribute to its ecological effects. Hydrocarbons are very important chemical components in COFCs because of their great amount and diversity (Liu et al. 2002). In our earlier paper, we have reported that dodecane, one of hydrocarbons in COFCs, has significant influences on the vegetative growth of *S. natans* (Wu et al. 2011). We discuss the effects of another hydrocarbon, hexadecane, on the growth of *S. natans*, and compare the influences on growth of floating aquatic plants between dodecane and hexadecane in this paper.

MATERIALS AND METHODS

Collection of *S. natans* and treatment by hexadecane: The collection and adaptation of culture of *S. natans* are conducted as described in our earlier paper (Wu et al. 2011).

Static toxicity testing method has been used. Hexadecane was diluted in distilled water to prepare test solutions with the following concentration gradient: 0 mg/L (Control), 10 mg/L (C1), 20 mg/L (C2), 40 mg/L (C3), 80 mg/L (C4), 160 mg/L (C5), 240 mg/L (C6), and 320 mg/L (C7).

One hundred and sixty healthy individuals of *S. natans* with similar size (four pairs of the latest floating leaves-old leaves, similar lengths and fresh weights) were assigned randomly to 8 groups (one control and seven treatments) and treated by test solutions according to the method in our earlier reports (Jiang et al. 2009, Wu et al. 2011). Each individual of *S. natans* had only one apical bud and no branch at the beginning of the experiment. A plastic pot (Ø12 cm and 10 cm high) contained one individual only and the pots were

kept in a greenhouse (at temperature 30±2°C, the light was provided by metal halide bulbs for 12 h/d).

The following parameters were recorded on day 2, 4, 6, 9 and 12: mortality, number of leaves, number of buds, stem length, number of leaves turning yellow and conductivity of the test solution. The pH value was also measured on day 0, 2, 4, 6, 9 and 12. The leaf area, fresh weight and dry weight of each individual were measured at the end of the experiment.

The analysis of data: The relative growth rate (*RGR*) and percent inhibition of growth rate (*% I*) were determined based on the methods suggested by OECD (2006), the two equations have been introduced in our earlier papers (Jiang et al. 2009, Wu et al. 2011). In this paper, Δt =12 days.

One-way analysis of variance (ANOVA) was used to determine the differences among different treatments, and linear regression was used for analysis of correlation between the parameters of the vegetative growth of *S. natans* and the concentration of hexadecane with SPSS 16.0. All variables were tested for normality and homogeneity of variances. The differences were statistically significant at $p<0.05$.

RESULTS

Effects of hexadecane on *RGR* and *% I* of *S. natans*: The *RGR* in different groups are shown in Fig. 1. The *RGR* was much higher in control than in C4-C7 (df = 68, F = 6.421, $p<0.001$), but there was no significant difference between control and C1-C3 (df = 77, F = 0.577, p = 0.632). There was a significant linear correlation between % *I* and the concentration of hexadecane (R^2 = 0.949, F = 93.501, $p<0.001$) (Fig. 2). The results indicated that hexadecane limited the *RGR* of *S. natans* significantly, and its effect was correlated positively to its concentration, especially when > 40mg/L.

Effects of hexadecane on the leaves of *S. natans*: Although the leaf number of *S. natans* in all experimental groups increased stably during the experiment, C5-C7 increased more slowly than other groups after day 4 (Table 1). As the same as the variation of leaf number, the total leaf area of control was significantly higher than that of C5-C7 (df = 49, F = 33.181, $p<0.001$). When the concentration of hexadecane was up to 80mg/L, it had no significant effect on the leaf area of *S. natans* (df = 96, F = 0.465, p = 0.761) (Fig. 3).

Hexadecane could accelerate the leaves of *S. natans* turning yellow, and its effect was correlated closely with its concentration and the exposure time (Fig. 4). The number of old leaves turning yellow in C1-C7 was significantly higher than that in control after day 6 (df = 23, F = 21.158, $p<0.001$). The old leaves in C6-C7 completely turned yellow after day 9 (Fig. 4). However, the effect of hexadecane on the new leaves was not as acute as the old leaves. There were no new leaves turning yellow in control during the experiment, but in all of the treatment groups until day 12. There were significant differences between control and C4-C7 while no difference between control and C1-C3 from day 4. The influence of hexadecane on the old leaves of *S. natans* was more significant than the new leaves.

Effects of hexadecane on the stem and the bud of *S. natans*: The stem length of *S. natans* in all experimental groups went up during the experiment (Fig. 5). The stem length in control was significantly higher than that in C6-C7 (df = 14, F = 8.855, p = 0.004). However, there was no significant difference between control and C1-C5 (df = 29, F = 0.37, p = 0.864). The result indicated that hexadecane would significantly limit the growth of the stems of *S. natans* when its concentration was more than 160mg/L.

The accumulated number of buds in control and C1-C4 increased stably during the experiment (Table 2). At the end of the experiment, the accumulated number of buds in control was significantly higher than that in C3 and C5-C7. Hexadecane could influence development of the buds of *S. natans*. The higher the concentration is, the more significant effect on the growth of buds would be.

Effects of hexadecane on the root of *S. natans*: The submerged leaves of *S. natans* have the function of absorbing nutrients from water like 'root'. The plants produced fewer 'roots' per frond when exposed to hexadecane, and the length of 'roots' was also affected significantly by hexadecane in C3 and C5-C7 during the experiment (Fig. 6).

Effects of hexadecane on the biomass of *S. natans*: The dry weights of different organs of *S. natans* at the end of the experiment are showed in Fig. 7. The dry weight of floating leaves (df = 126, F = 5.107, $p<0.001$), stems (df = 126, F = 7.335, $p<0.001$) and roots (df = 126, F = 5.541, $p<0.001$) in control were significantly higher than those in the treatment groups. Except the root, the dry weight of floating leaves, stems and whole plants were correlated negatively with the concentrations of hexadecane (Table 3). The results showed that hexadecane would influence the accumulation of biomass of *S. natans*. The higher the concentration of hexadecane is, the lower biomass of *S. natans* would be.

Effects of hexadecane on the mortality of *S. natans*: Only in control and C2, no dead individuals of *S. natans* have been observed during the experiment. At the end of the experiment, there were only 7 individuals (35%) survived in both C6 and C7. In these two groups, the dead individuals were observed on day 2 firstly (Table 4). Thirty three individuals died during the experiment totally, and 24 (72.7%) died until day 4. There was significantly positive linear cor-

Table 1: The number of leaves of *S. natans* in different experimental groups during the experiment (m ± SE, n = 20).

Experimental group	Day 2	Day 4	Day 6	Day 9	Day 12
Control	11.0±0.3	16.1±0.7	24.9±0.9	38.7±1.9	54.6±3.2
C1	11.2±0.3^{ns}	16.7±0.6^{ns}	24.4±1.1^{ns}	36.6±2.1^{ns}	51.2±2.4^{ns}
C2	11.1±0.2^{ns}	16.1±0.5^{ns}	22.3±1.0^{ns}	33.0±1.7*	48.4±2.7^{ns}
C3	11.2±0.3^{ns}	16.6±0.7^{ns}	23.7±1.2^{ns}	35.5±2.2^{ns}	45.4±2.8*
C4	11.8±0.3^{ns}	17.8±0.8*	26.2±1.5^{ns}	38.2±2.0^{ns}	48.5±3.0^{ns}
C5	10.5±0.2^{ns}	13.8±0.5*	18.1±0.9**	23.4±1.7**	27.8±2.2**
C6	10.2±0.3^{ns}	13.1±0.7**	15.3±1.3**	19.4±2.4**	21.7±3.4**
C7	10.3±0.3^{ns}	13.2±0.7*	15.6±0.7**	17.1±0.9**	18.9±1.1**

'ns' indicates that there are no differences between the control and the experimental group ($p>0.05$); * indicates that there are differences between the control and the experimental group ($p<0.05$); ** indicates that there are significant differences between the control and the experimental group ($p < 0.01$).

Table 2: Mean number of buds of *S. natans* in different experimental groups during the experiment (m ± SE, n = 20).

Experimental group	Day 2	Day 4	Day 6	Day 9	Day 12
Control	1.9±0.2	3.7±0.3	4.6±0.3	5.4±0.4	6.5±0.5
C1	2.4±0.3^{ns}	3.3±0.3^{ns}	3.8±0.3*	4.5±0.4^{ns}	6.0±0.5^{ns}
C2	1.7±0.2^{ns}	3.4±0.3^{ns}	3.6±0.3*	4.9±0.4^{ns}	6.2±0.5^{ns}
C3	2.4±0.2^{ns}	2.6±0.3**	3.2±0.3**	3.7±0.3**	4.7±0.4**
C4	1.9±0.2^{ns}	2.8±0.2*	3.6±0.3**	4.6±0.5^{ns}	5.5±0.5^{ns}
C5	1.8±0.2^{ns}	2.3±0.2**	2.5±0.2**	3.1±0.3**	3.1±0.3**
C6	1.1±0.2*	1.7±0.2**	1.8±0.2**	1.7±0.2**	1.2±0.2**
C7	1.1±0.2*	1.9±0.1**	1.9±0.1**	1.9±0.1**	2.0±0**

'ns' indicates that there are no differences between the control and the experimental group ($p>0.05$); * indicates that there are differences between the control and the experimental group ($p<0.05$); ** indicates that there are significant differences between the control and the experimental group ($p < 0.01$).

Table 3: The regression equations between the dry weight of different organs (y) and the concentration of hexadecane (x).

Organ	Regression equation	R^2	p
Floating leaves	y = -0.000021x + 0.028	0.605	0.023
Stems	y = -0.0000078x + 0.005	0.654	0.015
Roots	y = -0.0000065x + 0.007	0.289	0.17
Whole plants	y = -0.000035x + 0.04	0.608	0.023

Table 4: Mortality of *S. natans* in different experimental groups from day 2 to day 12.

Experimental group	Day 2	Day 4	Day 6	Day 9	Day 12
Control	0%	0%	0%	0%	0%
C1	0%	0%	0%	5%	5%
C2	0%	0%	0%	0%	0%
C3	0%	0%	0%	5%	5%
C4	0%	0%	0%	0%	5%
C5	0%	10%	10%	15%	20%
C6	25%	55%	60%	65%	65%
C7	15%	55%	60%	65%	65%

relation between the mortality and the concentration of hexadecane (Fig. 8). LC_{50} of hexadecane for *S. natans* based on probit analysis was 275 mg/L on day 4 and 244 mg/L on day 12, respectively.

The pH value and conductivity of the cultivation media during the experiment: The pH value and conductivity of the cultivation media in different experimental groups had similar trends with the treatment time (Fig. 9). There was no difference of pH values (df = 47, F = 0.029, p = 1.000) and conductivities (df = 47, F = 1.831, p = 0.108) among the experimental groups during the experiment. This result suggested that the presence of hexadecane had no effect on the pH value and conductivity of the water environment.

DISCUSSION

Hydrocarbons are considered as the most threatening hazardous pollutants from oils due to their persistence in environments as well as their mutagenic and carcinogenic properties (Lemiere et al. 2005). Although hexadecane and dodecane both belong to hydrocarbons, they show a little different influence on the growth of *S. natans*. LC_{50} of hexadecane (275 mg/L) to *S. natans* on day 4 after treatment is between that of COFCs (801 mg/L) and dodecane (190 mg/L) (Jiang et al. 2009, Wu et al. 2011). This means that hexadecane is another important component in COFCs, which contributes greatly to the ecological effects of COFCs. It can limit the bud, stem and leaf growth of *S. natans*. It also can accelerate the leaves becoming yellow, then influ-

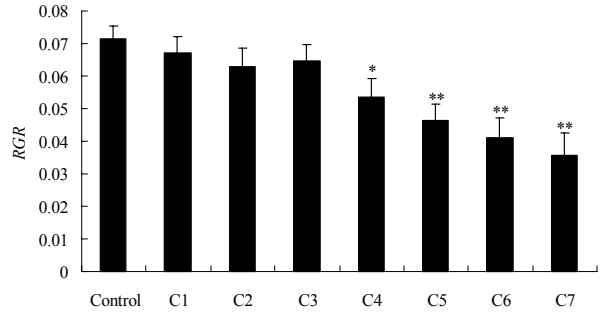

Fig. 1: The *RGR* (mean ± SE, n = 20) of *S. natans* in different experimental groups. * indicates that there are differences between the control and the experimental group ($p<0.05$). ** indicates that there are significant differences between the control and the experimental group ($p<0.01$).

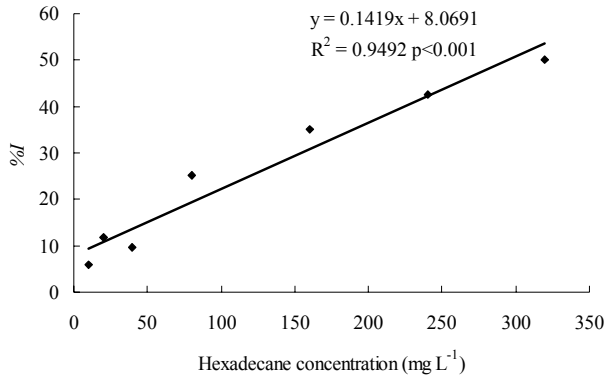

Fig. 2: The relationship between % *I* and the hexadecane concentration at the end of the experiment.

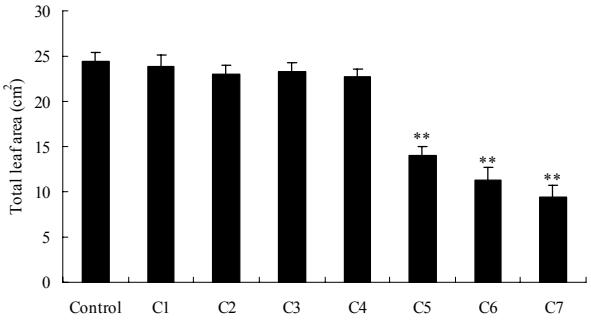

Fig. 3: The total leaf area (mean ± SE, n = 20) of *S. natans* in different experimental groups. * indicates that there are differences between the control and the experimental group ($p<0.05$). ** indicates that there are significant differences between the control and the experimental group ($p<0.01$).

ence the photosynthesis and biomass accumulation of *S. natans*. It can lead to the death of *S. natans* at the early stage. *S. natans* is more sensitive to hexadecane than to COFCs.

COFCs can influence the pH value of water when it enters a water body (Jiang et al. 2009). However, both

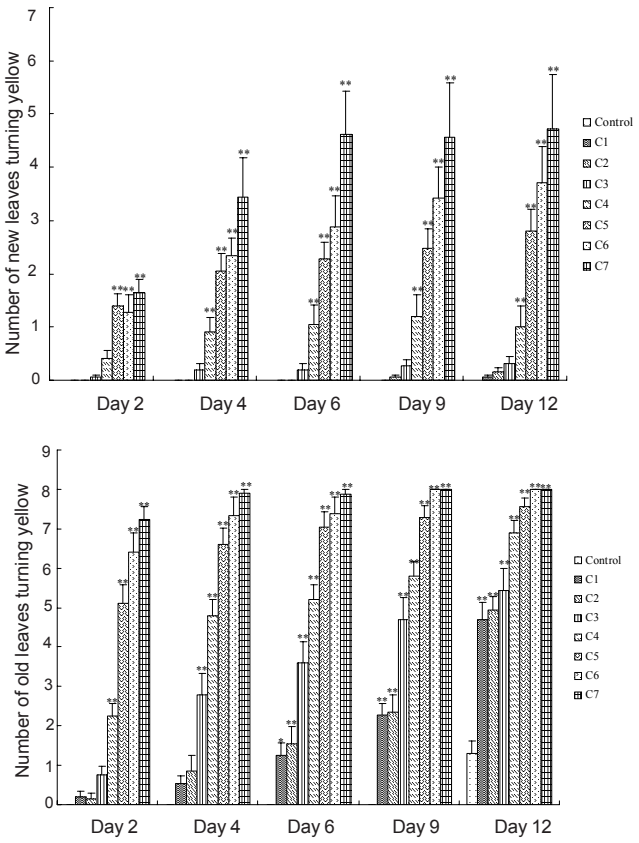

Fig. 4: The numbers of old and new leaves turning yellow (mean ± SE, n = 20) in the experimental groups on day 2, 4, 6, 9, 12. * indicates that there are differences between the control and the experimental group ($p<0.05$). ** indicates that there are significant differences between the control and the experimental group ($p < 0.01$).

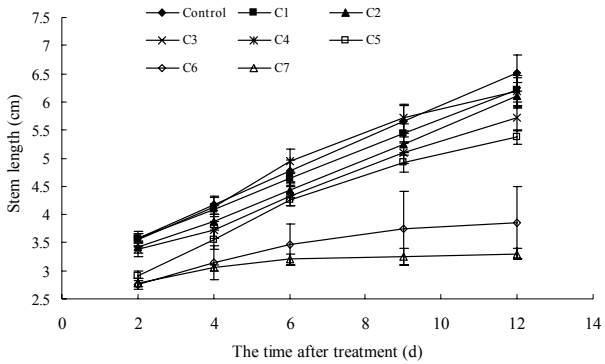

Fig. 5: The stem length of *S. natans* in different experimental groups during the treatment (mean ± SE, n = 20).

hexadecane and dodecane do not change the pH value of cultivation media significantly. Hexadecane has no effect on the conductivity of the cultivation media during the experiment. This means that hexadecane does not influence the vegetative growth of *S. natans* by changing the water envi-

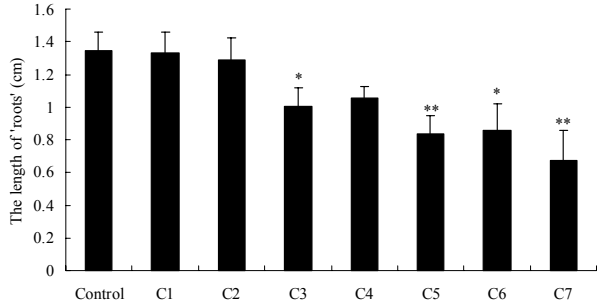

Fig. 6: The length of 'roots' (mean ± SE, n = 20) of *S. natans* in different experimental groups. * indicates that there are differences between the control and the experimental group ($p<0.05$). ** indicates that there are significant differences between the control and the experimental group ($p<0.01$).

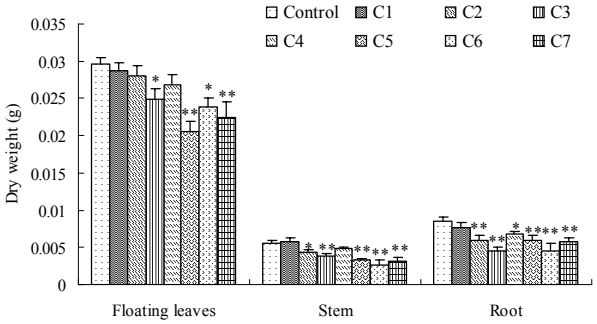

Fig. 7: The dry weights of different parts of *S. natans* (mean ± SE, n = 20) of *S. natans* in different experimental groups. * indicates that there are differences between the control and the experimental group ($p<0.05$). ** indicates that there are significant differences between the control and the experimental group ($p<0.01$).

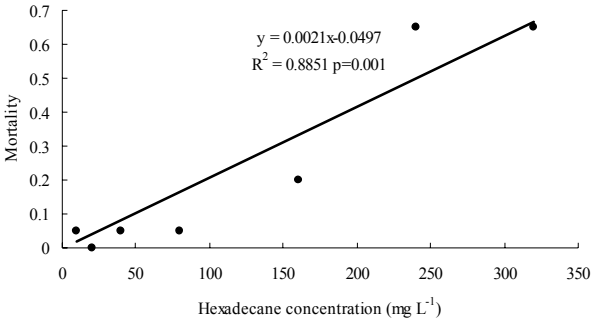

Fig. 8: The relationship between mortality and different concentrations of hexadecane.

ronment. It may directly affect the plant self. It also suggests that there are other components in COFCs which lead to change of the pH value of the water. The molecular weight of hexadecane is 226.44 and the relative density is 0.773. It is difficult to be dissolved in water and will form an 'oil'

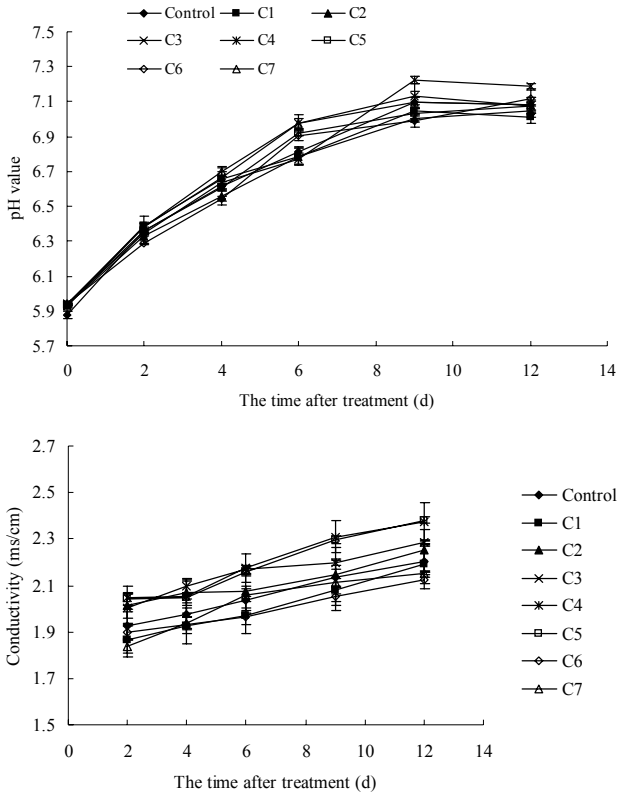

Fig. 9: pH values and conductivity of cultivation media in different experimental groups during the treatment (mean ± SE, n = 20).

film on the surface of water. This oil film will cover the surface of leaves, stems and buds of *S. natans*, then influence the photosynthesis of the plant.

For the components in oil and grease with lower relative density (<1.0), they will float on the surface of water when they enter a water body. They contact the floating plants directly, especially their leaves. This oleaginous film may influence air exchanges of leaves through stomata with the outside (Jiang et al. 2009). Many studies have reported that aqueous components from crude oil can disturb stomatal behaviour significantly (Youssef 2002), or reduce chlorophyll contents in leaves (Achuba 2006). The variation of stomatal behaviour and chlorophyll content will influence the photosynthesis of plants (Rzepka et al. 2005, Fernandez 2006). So oil and grease may have more serious influence on floating-leaves plants than other kinds of aquatic plants.

CONCLUSION

As main chemical components of COFCs and other oil and grease, both hexadecane and dodecane significantly influence the growth of leaf and bud of *S. natans* by disturbing its photosynthesis. *S. natans* can be used as an indicator of

hexadecane, as well as of dodecane. Hydrocarbons in oil and grease are important chemical components which can affect the growth of aquatic plants, especially the floating-leaves plants. It is very necessary to study the relationship between the amount of hydrocarbons in COFCs and the heated temperature of edible oil. The low amount of hydrocarbons in COFCs may reduce its ecological effects. Additionally, because hexadecane may influence floating-leaves plants directly, such as influence their photosynthesis and other physiological processes, it is also necessary to conduct experiments to test how hexadecane affects floating-leaves plants at physiological levels.

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