Environmental Technology & Innovation 18 (2020) 100688

Contents lists available at ScienceDirect

Environmental Technology & Innovation

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

Phytotoxicity of Class B aqueous firefighting formulations, Tridol S 3 and 6% to *Lemna minor*

Panneerselvan Logeshwaran [a], Anithadevi Kenday Sivaram [a], Meena Yadav [b], Sreenivasulu Chadalavada [c], Ravi Naidu [c], Mallavarapu Megharaj [a,*]

[a] *Global Centre for Environmental Remediation, The University of Newcastle, Callaghan, New South Wales, Australia*
[b] *Allwater, Adelaide, Australia*
[c] *Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, The University of Newcastle, Callaghan, New South Wales, Australia*

ARTICLE INFO

Article history:
Received 26 December 2019
Received in revised form 14 February 2020
Accepted 14 February 2020
Available online 19 February 2020

Keywords:
Firefighting foam
AFFF
Tridol
Lemna
Phytotoxicity
Growth inhibition

ABSTRACT

Phytotoxicity of Class B aqueous firefighting concentrates, Tridol-S 3%, and Tridol-S 6% to *Lemna minor* were studied using the parameters such as the frond number, biomass production in terms of dry weight, chlorophyll content and proline accumulation. Decrease in fresh weight, dry weight, and chlorophyll pigments; increase in proline content suggested that both the firefighting concentrates are potentially toxic to *L. minor*. Relative growth rate (RGR) also showed a similar pattern of toxicity with the corresponding increase in test concentrations of both the compounds. The EC_{50} values show Tridol-S 3% was more toxic than Tridol-S 6% in terms of frond number and dry weight. From our findings, it is clear that *L. minor* is highly sensitive to the exposure of firefighting foams, and is suitable for its use as an indicator organism for assessing the aquatic toxicity of aqueous firefighting foams. This study clearly suggests that the migration of Tridol AFFF into aquatic environments is likely to have detrimental effects on the aquatic flora. To the best of our knowledge, this study constitutes the first report on the phytotoxicity of firefighting concentrates, Tridol-S 3% and Tridol-S 6% to *Lemna minor* L.

1. Introduction

Aqueous film-forming foam (AFFF) concentrates, Tridol-S 3%, and Tridol-S 6% are used for extinguishing and securing flammable hydrocarbon liquid fires. Tridol-S 3% and 6% are intended for their use at 3% (3 parts concentrate to 97 parts water) and 6% (6 parts concentrate to 94 parts water), respectively. Vapour-sealing aqueous film produced by these compounds spread rapidly over the fuel source, which provides rapid control and extinguishment. Commercial firefighting products contain a proprietary mixture of various constituents such as glycols and surfactants for their efficiency (Giesy and Kannan, 2002; Moody and Field, 2000). Fluorinated firefighting products also contain hydrocarbons and fluorochemical surface-active agents (fluorosurfactants). Fluorosurfactants used in firefighting products possess extreme thermal, biological, and chemical stability. In addition to actual fire management scenarios, firefighting exercises around the world use a significant quantity of AFFF, i.e., 1200–3200 L of AFFF at 3.0–6.0% dilution with water (Moody and Field, 2000). The wastewater containing AFFFs generated from fire training sites are often discharged into wastewater facilities

* Corresponding author.
E-mail address: megh.mallavarapu@newcastle.edu.au (M. Megharaj).

https://doi.org/10.1016/j.eti.2020.100688
2352-1864/

and or to wastewater holding pools adjacent to the training facilities which contributes to the per- and poly-fluoroalkyl substances (PFAS) contamination of aquatic, and terrestrial ecosystems and also biota (de Solla et al., 2012; Schultz et al., 2004; Houtz et al., 2013; Bräunig et al., 2019).

Also, the accidental release of perfluorinated firefighting compounds has been reported causing long-term environmental effects, including accumulation in aquatic biota (Moody et al., 2002; Awad et al., 2011). Fish species thriving downstream of a firefighting training facility were found to possess high levels of perfluoroalkyl acids (PFAAs) (Gewurtz et al., 2014). Also, high levels of PFAAs were documented in surface water, plasma samples of snapping turtle, and amphipods in Lake Niapenco downstream of an airport with firefighting training area (de Solla et al., 2012). Munoz et al. (2017) reported that the deployment of fluorinated AFFF to contain crude oil fire has resulted in the occurrence of PFAAs in water and sediment from Lake Mégantic and Chaudière river; and PFASs in benthic white suckerfish (*Catostomus commersonii*). Similarly, Ojemaye and Petrik (2019) reported the accumulation of PFAS (perfluorodecanoic acid, perfluorononanoic acid, and perfluoroheptanoic acid) in fish tissue samples obtained from Kalk Bay harbour, Cape Town. In the UK and Sweden, groundwater associated with AFFF contamination and fire training sites showed the presence of high concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Atkinson et al., 2008; Filipovic et al., 2015). Xiao (2017), published an excellent comprehensive overview on the presence of poly and perfluorinated compounds in aquatic environments. Several studies reported the detection of PFAS in aquatic organisms. This suggests that, the use of fluorosurfactants containing firefighting products tend to transport PFAS via, water from the actual site of application/training areas to non-targeted locations. Therefore, aquatic systems are regarded as one of the significant repositories/sinks and critical media for the transport of perfluorinated compounds in the environment (Nakata et al., 2006; Pistocchi and Loos, 2009).

Previously, acute toxicity of fire retardants and foams have been reported in Chinook salmon (*Oncorhynchus tshawytscha*), fathead minnow (*Pimephales promelas*), rainbow trout (*O. mykiss*), algae (*Selenastrum capricornutum*), zebrafish (*Brachydanio rerio*) and aquatic invertebrates (*Daphnia*) (Gaikowski et al., 1996a,b; McDonald et al., 1996; Buhl and Hamilton, 1998, 2000; Zhang et al., 2018). The toxicity of PFAS has shown to impact the upper trophic level organisms that rely on the aquatic organisms as a food source. De Vries et al. (2017) reported that the reduction in the abundance of flamingo in Bonaire island is associated with the toxicological effects of PFASs on plenty of prey. Furthermore, the continuous reformulation of commercial firefighting products has led to uncertainty on their toxicological effects in terrestrial and aquatic ecosystems. Due to environmental issues, the use of PFOS in AFFFs is currently being restricted in many parts of the world, and finding PFOS surrogates/alternatives are also being primarily explored. However, 6:2 fluorotelomer sulfonamide alkylbetaine (6:2 FTAB), currently used globally in AFFFs as a PFOS alternative, has shown to induce developmental toxicity in zebrafish embryos (Shi et al., 2018). Consequently, research on the toxicity and fate associated with commercial firefighting products to aquatic systems is still required to ascertain the extent of risks posed by these chemicals.

Therefore in this study, the toxicity of commercial Class B firefighting aqueous foam products, Tridol-S 3% and Tridol-S 6% to free-floating aquatic plant, *Lemna minor* L. (duckweed) was performed. These foam products were selected for this study due to their extensive usage in Rapid Intervention Vehicles (RIVs) at major international airports and military bases worldwide for the management of hydrocarbon associated fires. Being relatively smaller in size with a high multiplication rate, vegetative mode of reproduction, and higher genetic uniformity, *Lemna minor* L. is considered as an ideal test organism for aquatic toxicity testing of pollutants (Naumann et al., 2007). The objective of the present study was to determine the effect of Class B firefighting foam concentrates, Tridol-S 3%, and Tridol-S 6% to the growth and development of *L. minor* L.

2. Materials and methods

2.1. Chemicals

Tridol-S 3% AFFF and Tridol-S 6% AFFF was purchased from Fire & Rescue Australia, South Australia. All the reagents and solvents used in the study were of analytical grade purchased from Sigma-Aldrich, Australia. The composition of the firefighting products is given in Table 1. The stock solutions of Tridol-S 3% AFFF and Tridol-S 6% AFFF was prepared using sterile deionized water (Milli-Q, 18 Ω cm^{-1}, ELGA Lab Water, UK) and stored at 4 °C in the dark. Hexavalent chromium (10 mg L^{-1}) was used as a positive control.

2.2. Cultivation and growth of L. minor

Wild type *L. minor* L. were obtained from the culture maintained in Global Centre for Environmental Remediation (GCER), The University of Newcastle. The modified Steinberg medium used for the growth of *L. minor* was prepared according to Organization for Economic Co-Operation and Development (OECD) protocol for toxicological testing (OECD 221 guidelines, 2006). The medium (pH 5.5) was composed of KNO_3 (3.46 mmol L^{-1}), $Ca(NO_3)_2{\cdot}4H_2O$ (1.25 mmol L^{-1}), KH_2PO_4 (0.66 mmol L^{-1}), K_2HPO_4 (0.072 mmol L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.41 mmol L^{-1}), H_3BO_3 (1.94 μmol L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.63 μmol L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.18 μmol L^{-1}), $MnCl_2.\ 4H_2O$ (0.91 μmol L^{-1}), $FeCl_3{\cdot}6H_2O$ (2.81 μmol L^{-1}), and EDTA Disodium-dihydrate (4.03 μmol L^{-1}).

Table 1
Compositions of firefighting concentrates.

Firefighting foam concentrate	Field application rate	Ingredients	CAS number	Content (%)
Tridol-S 3%	3% v/v with water	Diethylene glycol monobutyl ether	112-34-5	15–25
		Alkyl dimethylamine oxides	NA	5–10
		Magnesium sulphate	7489-88-9	1–5
		Fluorosurfactants	NA	5–10
		Water	7732-18-5	Balance
Tridol-S 6%	6% v/v with water	Diethylene glycol monobutyl ether	112-24-5	10–30
		Alkyl dimethylamine oxides	NA	5–10
		Fluorosurfactants	NA	1–5
		Fluorosurfactants	142-31-4	0.5–1.5
		Magnesium sulphate	7487-88-9	0.1–1.0
		Water	7732-18-5	Balance

2.3. Toxicity tests

L. minor fronds were surface sterilized using 0.5% (v/v) sodium hypochlorite solution for 1 min and then rinsed with sterile deionized water. Surface sterilized fronds were grown for 14 days in Steinberg medium for acclimatization. Three to four frond colonies (5 colonies per treatment) (Naumann et al., 2007) were randomly selected and transferred to the test medium containing firefighting compounds at concentrations ranging from 0 to 0.05%. The test containers were incubated at 24 ± 2 °C in continuous cool white fluorescent lighting (6500–10 000 lux) for an exposure duration of 7 days (OECD, 2006). All the experiments were performed in triplicate. As a positive control, hexavalent chromium Cr (VI) was used at a concentration of 10 mg L^{-1} in Steinberg medium, and Steinberg medium without chemicals served as a negative control.

2.4. Growth parameters

Following OECD guidelines for whole organism measurement of plant health, (a) frond number was recorded before exposure and at the end of the exposure; (b) dry weight: Colonies were collected and briefly rinsed with distilled water and the excess moisture was removed using blotting paper before being dried at 60 °C till constant weight was observed; (c) Frond Relative Growth Rate (RGR) was calculated according to the standard OECD formula (OECD 221 guidelines, 2006) (Eq. (1)); (d) inhibition of growth on the basis of frond number and dry weight was calculated according to OECD guidelines (Eq. (2)).

$$\mu_{i-j} = \frac{\ln(Nj) - \ln(Ni)}{t_j - t_i} \tag{1}$$

where:
μ_{i-j}: average specific growth rate from the time i to j
Ni: measurement variable in the test or control vessel at the time i
Nj: measurement variable in the test or control vessel at time j
t: time period from i to j

$$\%I_r = \frac{\mu_c - \mu_t}{\mu_c} \times 100 \tag{2}$$

where:
$\%I_r$: per cent inhibition in average specific growth rate
μ_c: mean value for μ in the control
μ_t: mean value for μ in the treatment group

2.5. Estimation of chlorophyll content

At the end of the experimental duration (7 days), the fronds were removed from the exposure vessels and rinsed with deionized water and dried with soft paper and weighed in pre-weighed microcentrifuge tubes on ice. Fresh tissue (125 mg) was homogenized with 80% (w/v) ice-cold acetone, homogenized manually with a glass homogenizer, centrifuged at 5000 g for 10 min and the absorbance of the clear extract was measured with spectrophotometer at 663, 646 and 470 nm in spectrophotometer (Synergy™ HT, Bio-Tek equipped with KC4 software). The concentration of chlorophyll *a*, *b* (mg g^{-1} fresh weight) was calculated from the equation (Harmut and Lichtenthaler, 1987).

2.6. Estimation of free proline content

Free proline content in the fronds was measured as described by Bates et al. (1973). Fresh tissue (125 mg) was manually homogenized in 3% (w/v) sulfosalicylic acid and centrifuged at 1000 g for 3 min. Ninhydrin reagent was added to the supernatant and heated at 100 °C for 1 h in a water bath and cooled in ice. The chromophore obtained was extracted from the liquid phase with toluene, and the absorbance of the organic layer was read at 520 nm in a spectrophotometer (Synergy^{TM} HT, Bio-Tek equipped with KC4 software). Proline concentration was determined from the calibration curve using L-proline as standard and expressed as μmol g^{-1} fresh weight.

2.7. Analytical methods

The concentration of the test products in all the treatments was measured using HPLC-MS before the inoculation of *L. minor* fronds. The HPLC-MS was carried out in the Agilent 1100 series, and the analysis was done using Agilent Poroshell 120 (EC-C18 2.7 μm, 4.6 $\times$ 50 mm) column at 35 °C. Gradient elution was done with solvents consisting of ammonium acetate (20 mM) water-based (Solvent A), water (Solvent B), and acetonitrile (Solvent D). The gradient solutions were filtered through a 0.45 μm filter before use. The elution gradient was 10% A, 85% B and 5% D (0–5 min), 10% A and 90% D (5–27 min) and 10% A, 85% B and 5% D (27–30 min) at a flow rate of 0.6 ml min^{-1}. The eluent was introduced into the AP-ES source, and negative ions were selected and detected by MS, operating in SIM mode. Quantitation was done using the ChemStation software included with the instrument by extracting specific ion in the product. The specific ion for both the test compounds was 586 *mz*. The QA/QC procedures were carried out by injecting a solvent blank and known standard after every ten samples in order to check the sensitivity of the instrument and also to make sure that the column was clean without traces of the compounds that are carried over between samples. Around 98% of the spiked test compounds were recovered as measured by quantification.

2.8. Statistical analysis

All the statistical analyses were carried out using Minitab 17.0 Statistical Software package.

3. Results

3.1. Effect on frond growth

The firefighting formulations, Tridol-S 3% and Tridol-S 6% were found to have a significant effect on the number of fronds produced (Fig. 1a and b). Among the two formulations, Tridol-S 3% has resulted in the reduction of almost half the number of fronds produced in *L. minor* when exposed to a concentration of 0.0075% when compared to Tridol-S 6% at 0.015%. At the end of the experimental period (7 days), complete inhibition in frond production was observed at 0.015, and 0.04% of Tridol-S 3% and Tridol-S 6%, respectively, whereas unexposed *L. minor* (control) showed higher growth and normal development by producing healthy and replicating fronds. At 7 days of incubation, positive control (Hexavalent chromium), showed inhibition of *L. minor* growth by $<$25%. Relative growth rates (RGR) based on the number of fronds showed that both the firefighting products influenced the growth of *L. minor*. Tridol-S 3% showed a significant reduction in RGR from 2 days of exposure at a concentration of 0.015%. In contrast, Tridol-S 6% did not show any significant effect on the growth of *L. minor* at any concentration tested, during the 2 days. At 5, 6, and 7 days of the experimental period, a higher concentration of Tridol-S 3% resulted in negative RGR (Fig. 2a). On the other hand, plants exposed to Tridol-S 6% did not produce any significant effect during the initial 2 days of exposure. However, with an increase in the experimental duration, there was a significant effect on RGR at 0.035% concentration (5 days exposure). Further exposure resulted in a gradual increase in the adverse impact on the growth even at a low concentration of Tridol-S 6% (0.015% at 7 days). Negative RGR was observed at a higher concentration of Tridol-S 6% during 6 and 7 days of the experiment (Fig. 2b).

3.2. Effect on the dry weight

The dry weight of *L. minor* was found to be significantly lower in plants exposed to both the firefighting formulations. When compared to control, a significant difference in the dry weights was recorded in all the experimental concentrations of Tridol-S 3%. At 0.0025% of Tridol-S 3%, a 50% reduction in the biomass was observed with an almost complete reduction in the dry weight observed at 0.0075% (Fig. 3a). Tridol-S 6% also caused a significant decrease in the biomass production of *L. minor* when compared to control. Production of biomass was reduced up to 50% (0.015%), followed by complete reduction at higher concentrations (Fig. 3b). The decrease in the dry weight was in direct correlation with the decrease in frond number.

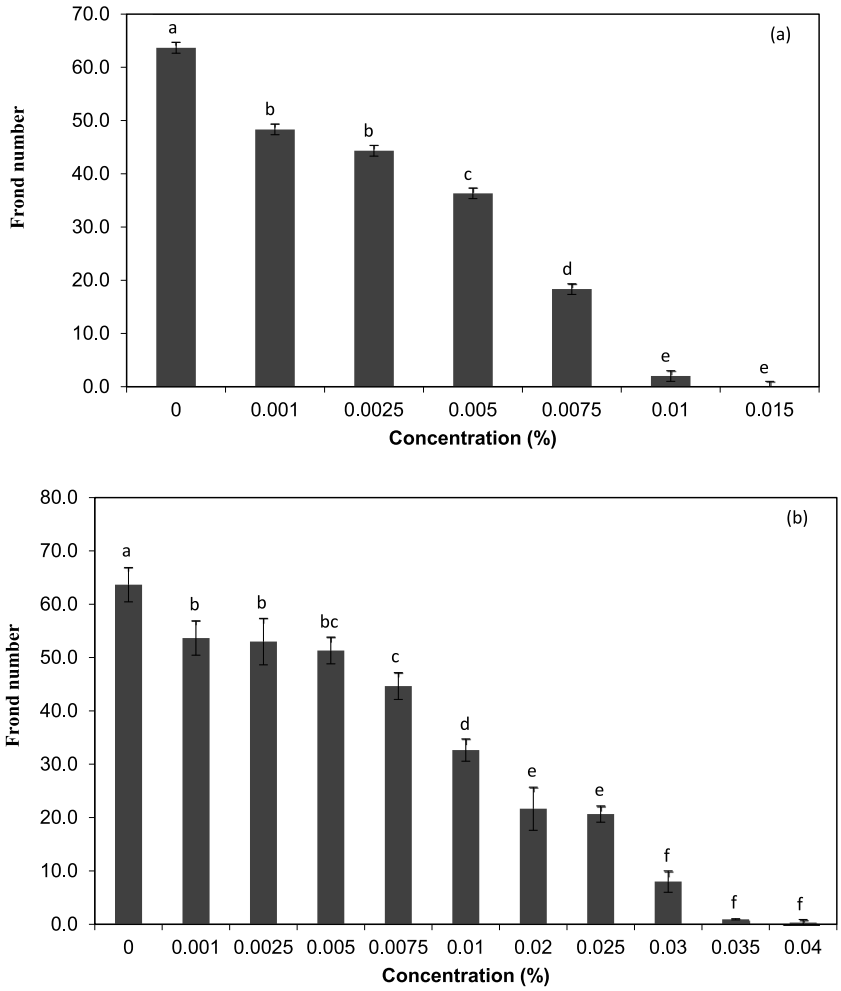

Fig. 1. *L. minor* growth (frond number) after exposure to firefighting concentrates. (a) Tridol-S 3% (7 days); (b) Tridol-S 6% (7 days). Bars denote standard error ($n = 3$). The different letter indicates a significant difference (1-way ANOVA followed by post hoc Tukey's test, $p < 0.05$; means sharing the same letters in the superscript are not significantly different).

Table 2
EC_{50} values of firefighting concentrates, Tridol-S 3% and Tridol-S6% on the basis of growth responses (dry weight and number of fronds) in *L. minor*.

Firefighting concentrates	EC50 value (%)	
	Dry weight	Frond number
Tridol-S 3%	0.00098 ± 0.0002	0.0058 ± 0.001
Tridol-S 6%	0.0074 ± 0.0012	0.016 ± 0.007

3.3. Acute toxicity

A range-finding test with various concentrations of Tridol-S 3% and Tridol-S 6% was conducted before the start of the actual experiment to select the suitable test concentration range. *L. minor* growth inhibition due to the exposure of firefighting formulations was calculated based on the mean values of the number of fronds produced and the dry weight. Growth inhibition of *L. minor* occurred in all the experimental concentrations of Tridol-S 3% and Tridol-S 6%. Among the factors measured (dry weight and number of fronds), inhibition of growth based on dry weight is higher and had a marked difference when compared to the number of fronds produced at the end of 7 days. Tridol-S 3% at 0.01% and above caused 100% inhibition in growth (Fig. 4a). Inhibition of growth based on dry weight showed a similar pattern in treatment containing Tridol-S 6%. At and above 0.035%, Tridol-S 6% produced complete inhibition in the growth of *L. minor* (Fig. 4b). The concentration of firefighting formulations that caused the half-maximal effect (EC_{50}) in *L. minor* was calculated. EC_{50} value was found to be lower for Tridol-S 3%, both for dry weight and frond numbers than Tridol-S 6% (Table 2). In both the

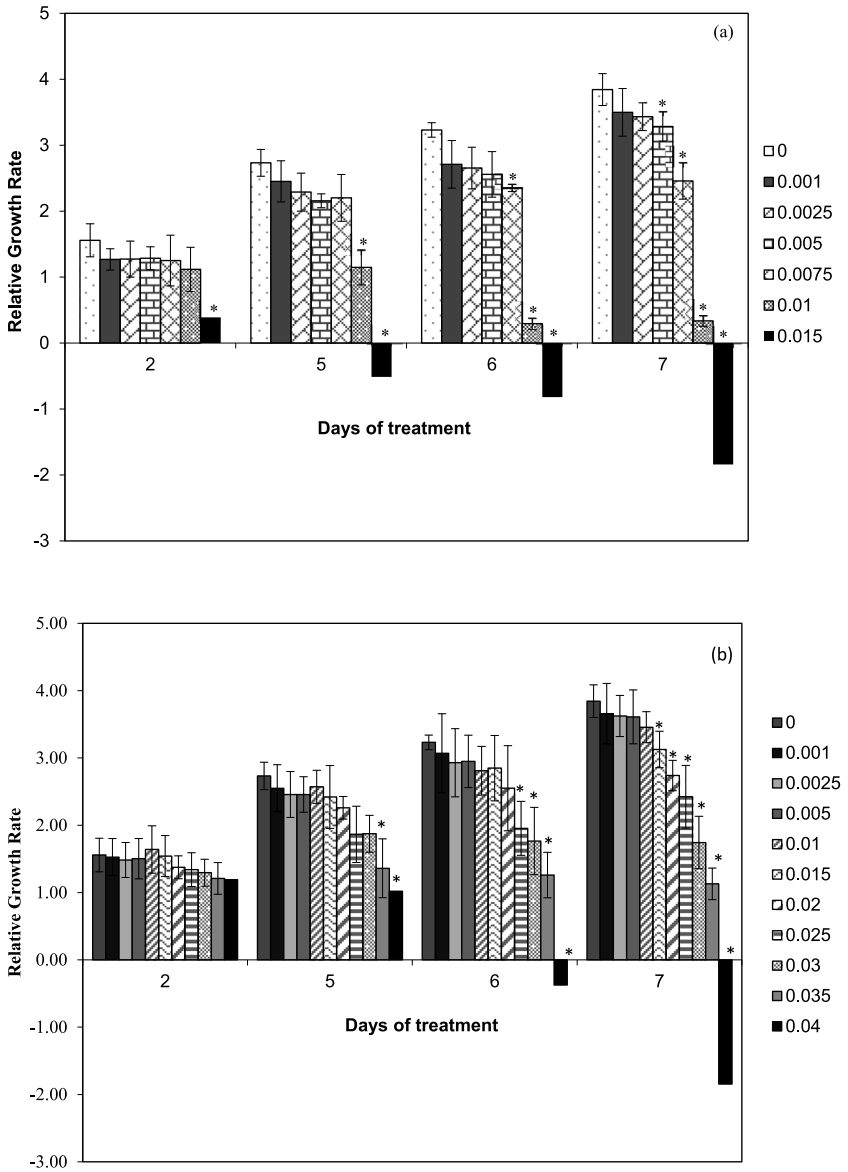

Fig. 2. *L. minor* growth (relative growth rate) after exposure to firefighting concentrates. (a) Tridol-S 3% (for 7 days) and (b) Tridol-S 6% (for 7 days). Bars denote standard error ($n = 3$). Asterisks indicate a significant difference between treatment and control (1-way ANOVA followed by post hoc Tukey's test, $p < 0.001$).

firefighting concentrates, dry weight was more influenced by the exposure to firefighting concentrates when compared to the number of fronds. From the EC_{50} values, it was more evident that Tridol-S 3% is more toxic when compared to Tridol-S 6%.

3.4. Effect on chlorophyll and proline

Fronds of *L. minor* showed signs of chlorosis and necrosis (dead and white fronds) (Fig. 5) as a result of exposure to higher concentrations of Tridol-S 3% and Tridol-S 6% with a corresponding reduction in chlorophyll *a* and *b* contents (Tables 3 and 4). Significant decrease in chlorophyll *a* and *b* were recorded above 0.005% and 0.01% of Tridol-S 3% and Tridol-S 6%, respectively. On the other hand, proline a stress-induced amino acid was found to be significantly increased with the increase in the concentration of both the firefighting formulations (Tables 3 and 4). Among the concentrates, Tridol-S 3% (0.0075%) was responsible for inducing more stress on *L. minor* plants with the increased proline content (13.9%) over control. Tridol-S 6% (0.02%) also caused an increase in the proline content up to 7.6% over control. Chl *a*, *b*

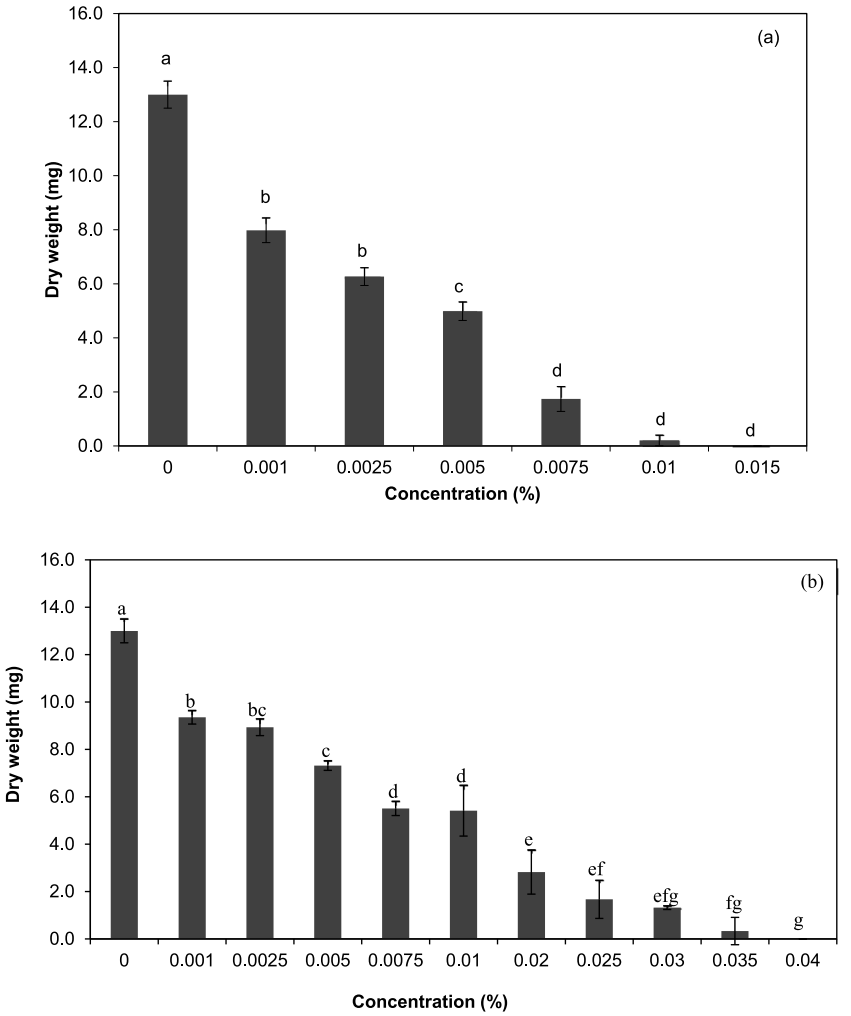

Fig. 3. *L. minor* growth (dry weight) after exposure to firefighting concentrates. (a) Tridol-S 3% (for 7 days); (b) Tridol-S 6% (for 7 days). Bars denote standard error ($n = 3$). Different letters indicate a significant difference (1-way ANOVA followed by post hoc Tukey's test, $p < 0.05$; means sharing the same letter are not significantly different).

Table 3
Effect of Tridol-S 3% AFFF on chlorophyll a and b (mg g^{-1} FW) and proline (μM g^{-1} FW) in *L. minor*.

Concentration (%)	Chlorophyll *a*	Chlorophyll *b*	Proline
C	0.62 $\pm$0.04^{a}	0.25 $\pm$0.02^{a}	4.83 $\pm$0.01^{a}
0.001	0.57 $\pm$0.02^{a}	0.18 $\pm$0.01^{a}	4.86 $\pm$0.01^{a}
0.0025	0.32 $\pm$0.02^{a,b}	0.09 $\pm$0.02^{a,b}	5.04 $\pm$0.03^{b}
0.005	0.31 $\pm$0.06^{b}	0.08 $\pm$0.01^{b}	5.58 $\pm$0.01^{c}
0.0075	0.29 $\pm$0.02^{b}	0.05 $\pm$0.01^{b}	5.61 $\pm$0.05^{c}

Values are means of six replicates $\pm$ SD. Means in each column with different letters (a–c) in the superscript indicate a significant difference ($p < 0.05$).

and proline content for Tridol-S 3% and Tridol-S 6% at concentrations above 0.0075% and 0.02%, respectively were not available since the samples above these concentrations were not suitable for performing the assays as the fronds were completely bleached at the end of the experimental duration.

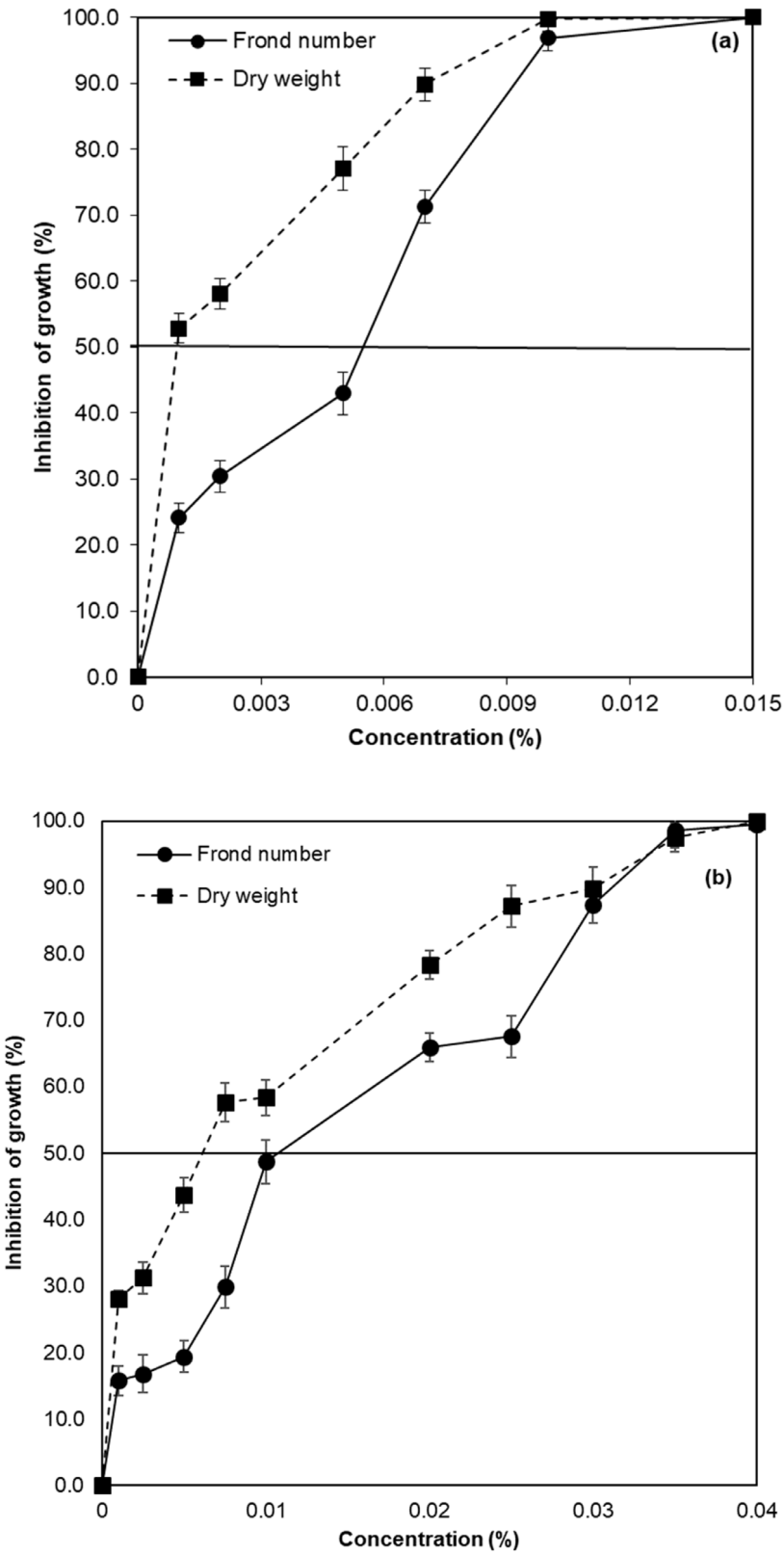

Fig. 4. Growth inhibition in *L. minor* (based on dry weight and frond number) after exposure to firefighting concentrates. (a) Tridol-S 3% (for 7 days) and Tridol-S 6% (for 7 days).

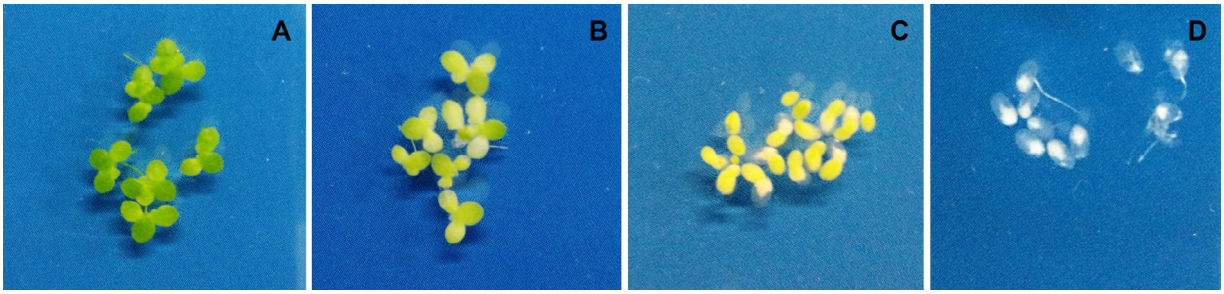

Fig. 5. Effects of firefighting concentrates, Tridol-S 3% and Tridol-S 6% to *Lemna minor*. (A) Control; (B) Chlorosis; (C) Single chlorotic fronds; (D) Dead white fronds.

Table 4
Effect of Tridol-S 6% AFFF on chlorophyll a and b (mg g^{-1} FW) and proline (μM g^{-1} FW) in *L. minor*.

Concentration (%)	Chlorophyll *a*	Chlorophyll *b*	Proline
C	0.61 ±0.04[a]	0.19 ±0.01[a]	4.85 ±0.01[a]
0.001	0.73 ±0.06[b]	0.13 ±0.02[b]	4.84 ±0.01[a]
0.005	0.53 ±0.02[c]	0.11 ±0.01[c]	4.93 ±0.04[b]
0.01	0.33 ±0.01[d]	0.08 ±0.01[d]	5.12 ±0.01[c]
0.02	0.30 ±0.01[d]	0.05 ±0.01[d]	5.25 ±0.01[d]

Values are means of three replicates ± SD. Mean in each column with different letters (a–d) in the superscript indicate a significant difference ($p < 0.05$).

4. Discussion

The possibility of firefighting chemicals entering into aquatic environments through surface runoff and leaching is very high. The growth inhibition test of Lemna is an internationally standardized method for analysing the toxicity of chemicals, pesticides, and a wide range of aqueous samples (ISO, 2001; Kokkali and van Delft, 2014; Ziegler et al., 2016). Hence, the results presented here demonstrated that both the firefighting formulations, Tridol-S 3% AFFF and Tridol-S 6% AFFF are toxic to aquatic macrophyte, *L. minor*. Growth inhibition by positive control (hexavalent chromium) yielded the expected result as reported by Pomati et al. (2004). Overall results obtained at the end of 7 days exposure suggested that the effects of firefighting compounds to *L. minor* was evident within the experimental duration (7 days) as per OECD guidelines. Similarly, *L. gibba* G3 exposed to perfluorooctane sulfonate (PFOS), an anthropogenic compound known for its extremely high thermal, biological and chemical stability, resulting in a significant reduction in frond number, fresh weight and qualitative physical appearance (Boudreau et al., 2003). On the other hand, Pietrini et al. (2019) reported there were no physiological changes in *L. minor* when exposed to PFOA. However, the accumulation of PFOA was found to be higher with the corresponding increase in PFOA concentration in the growth medium.

In the present study, plants exposed to the concentration above 0.005% of both formulations were characterized with a number of small and pale single fronds and there was a corresponding decrease in the frond number and dry weight compared to the control. The composition of the tested firefighting products as per manufacturer's data contains fluorosurfactants, which could be the reason for their phytotoxic effects. As reported, a higher number of single fronds and small colonies are considered to be indicative of environmental stress (Li and Xiong, 2004; Radić et al., 2010). At a higher concentration of the firefighting formulations, root growth was found to be decreased (data not shown). Boudreau et al. (2003) also reported that being one of the sensitive test organisms in their study, *L. gibba* showed a decrease in root length when exposed to PFOS. Relative growth rate (RGR) of *L. minor* was found to be reduced by both the firefighting compounds with an increase in their concentrations.

Practically, harmless chemicals produce an acute toxicity measure (EC_{50} or IC_{50}) with values ranging from 100 to 1000 mg L^{-1}, moderately toxic chemicals produce an acute toxicity measure with values ranging from 10 to 100 mg L^{-1}, and slightly toxic chemicals produce an acute toxicity measure with values ranging from 1 to 10 mg L^{-1} (McDonald et al., 1996). The EC_{50} values for Tridol-S 3% represent moderately to slightly toxic, but Tridol-S 6% falls under the category of moderately toxic compounds. Growth inhibition based on the dry weight in *L. minor* was concentration-dependent with the EC_{50} of 0.00 098 and 0.0074% for Tridol-S 3% and Tridol-S 6%, respectively which was more sensitive than the frond number response. A similar observation was made by Gubbins et al. (2011) where the EC_{50} value based on dry weight response for silver nanoparticles to *L. minor* was more sensitive than the frond number whereas Boudreau et al. (2003) reported EC_{50} concentration (31.1 mg L^{-1}) based on wet weight of PFOS in *L. gibba*.

Measurement of the chlorophyll levels in *L. minor* can be used as an indicator of growth inhibition (Taraldsen and Norberg-King, 1990). The decrease in chlorophyll content in *L. minor* can be associated with the inhibition of enzymes

associated with chlorophyll biosynthesis or peroxidation processes in chloroplast membrane lipids by the reactive oxygen species (Sandalio et al., 2001; Assche and Clijsters, 1990). In *L. minor,* the chlorophyll and carotenoids concentration was also reported to be affected due to the exposure to metals and herbicides (Kirby and Sheahan, 1994; Artetxe et al., 2002; Radić et al., 2010). In this study, a slight increase in the chlorophyll content in *L. minor* exposed to Tridol-S 6% (0.001%) could be due to the formation of the shade-type chloroplast. This phenomenon was recorded in *L. minor* when exposed to diuron, a weedicide (Teisseire et al., 1999; Körner et al., 2001; Park et al., 2017). In the current study, chlorophyll content was reduced with the increased concentration of both the products. These findings suggested that the firefighting products inhibited the growth of duckweed with an increase in the concentrations. Proline is an amino acid that participates in several plant physiological functions, including regulation of oxidative chemically induced abiotic stress (Szabados and Savouré, 2010). Proline has also been reported to scavenge hydroxyl radicals and singlet oxygen species thus protecting against ROS-induced cell damage (Matysik et al., 2002). In our study, the proline accumulation was found to be higher both in Tridol-S 3% and Tridol-S 6% exposed *L. minor* over non-exposed control plants. Accumulation of proline in *L. minor* during the exposure to metals has been reported previously (Bassi and Sharma, 1993; Radić et al., 2010). Nunes et al. (2014) reported that *L. minor* is very sensitive than *L. gibba* in terms of proline accumulation when exposed to paracetamol. These evidences suggests that the Class B firefighting foam products could pose a significant potential risk to the environment mainly to aquatic ecosystems.

5. Conclusions

The present study is the first report that demonstrated the toxicity of firefighting products, Tridol-S 3%, and Tridol-S 6% to *L. minor*. Though there are no reports on the ecological concentrations of aqueous firefighting compounds in aquatic ecosystems, it is evident that these compounds pose a potential threat to aquatic plants like Lemna. Therefore, further investigations are required to evaluate the ecotoxicological effects of commercial firefighting formulations on other freshwater organisms for their ecological risk assessment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Panneerselvan Logeshwaran: Investigation, Methodology, Data curation, Validation, Writing - original draft. **Anithadevi Kenday Sivaram:** Investigation, Methodology, Writing - review. **Meena Yadav:** Methodology, Formal analysis. **Sreenivasulu Chadalavada:** Project administration, Review. **Ravi Naidu:** Supervision, Resources, Writing-editing. **Mallavarapu Megharaj:** Conceptualisation, Supervision, Writing - review & editing.

Acknowledgements

This work was funded through CRC CARE and the Department of Defence, Australia.

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