Short-Term Effects of Three Herbicides on the Maximum Quantum Yield and Electron Transport Rate of Tropical Seagrass *Thalassodendron ciliatum*

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ABSTRACT
This article describes the laboratory findings of the short-term effects of three herbicides on the tropical seagrass *Thalassodendron ciliatum*. For three days *T. ciliatum* was exposed to Diuron, Fusilade (Forte) and 2, 4-D amine, either individually or in combination. The toxic effects were investigated by measuring the effective quantum yield through rapid light curves and the maximum quantum yield (Fv/Fm), before and after exposure at intervals of 2, 4, 6, 8, 24, 48 and 72 hrs. During the recovery phase both Fv/Fm and effective quantum yield were measured after 2, 4, 6, 8, 24, 48 and 72 hrs. Results revealed an inhibition of both ETRmax and Fv/Fm after diuron exposure. No effect on ETRmax and Fv/Fm were observed when *T. ciliatum* was exposed to Fusilade Forte and 2, 4-D amine. Any combination of the herbicides that involved Diuron showed inhibition both in ETRmax and Fv/Fm. Exposure to a combination of Fusilade Forte and 2,4-D amine had no effect on both ETRmax and Fv/Fm of *T. ciliatum*. It is concluded that, diuron is toxic to *T. ciliatum* at a concentration which can be found in a polluted environment while the other herbicides did not show inhibition of the parameters measured.

KEYWORDS: *Thalassodendron ciliatum*, herbicides, toxicity, Fusilade, 2,4-D amine

INTRODUCTION
Seagrasses are among the most productive ecosystems in the shallow water marine environment. It provides habitat to a number of flora and fauna species and stabilises the sediments. Its survival is both of ecological and economic-al importance. The decline of seagrass in the past decade has mainly been attributed to anthropogenic activities. Excessive input of nutrients in the seawater has lead to an increase in epiphytic load on the seagrass that ultimately reduces light (Walker et al., 1999). Other factors in some areas may also contribute to the fast decline of seagrass. One of these can be the impact of the agricultural activities that involve utilisation of large amounts of herbicides that can leak into the seawater through runoff water or ground water seepage. For example, in Northern Queensland diuron herbicide was detected in seagrass tissue and sediments (Hynes, et al., 1998). A serious risk posed to the marine environment is the fast growing developmental activities along the coastal regions in most developing countries bordering the Western Indian Ocean. Also agricultural activities along...
the coast are among the factors that are threatening the seagrass ecosystem and other marine ecosystems due to the application of chemicals, such as: pesticides and fertilizers (Hynes et al., 1998; Hall et al., 1999). Herbicides application in agricultural fields through runoff seepage and rainfall can easily find its way into the marine environment. The problem of mishandling pesticides in developing countries i.e. the presence of large stockpiles of obsolete pesticides or the improper application of pesticides could have serious effects on the marine ecosystems in the Western Indian Ocean. For example, in Chesapeake Bay, herbicides have been identified as the main cause for the decline of seagrass in the area (Hall et al., 1999); and various types of herbicides have been reported to affect marine plants (Ralph 2000; McMahon et al., 2005; Guo et al., 2011).

In Mauritius, sugarcane fields cover more than 40% of the island and represent more than 90% of cultivated land. The remaining agriculture consists mainly of vegetables. The sugarcane fields are found in a large part along the coastline and some of the vegetables also grow near the sea, mainly in sandy soil. These agricultural activities require a high use of herbicides, which can easily flow into the seawater during heavy rainfall. In addition, there are numerous points in the lagoons surrounding Mauritius where there is ground water seepage, causing the leakage of pesticides into seawater. Examples of herbicides that are commonly used in Mauritius and other Western Indian Ocean countries are: diuron, Fusilade and 2,4-D amine.

Herbicide diuron targets the photosynthetic pathways by blocking the electron transfer from QA to QB (Sandmann and Bölger, 1986). Diuron is used mainly as a post-emergence pesticide to control all weeds, without affecting the not yet germinating seeds. Besides being used as herbicide, diuron is also found in a number of anti-fouling paints used for boat hulls (Thomas et al., 2000). Diuron is used worldwide in the marine environment, as an antifouling agent, and in the terrestrial environment, as a herbicide to control weeds (Boxell et al., 2000; Thomas et al., 2000). Diuron is reported to be highly toxic for some non-targeted organisms when applied to the field (Teisseire et al., 1999). Its potential toxicity at cellular and sub-cellular level has also been demonstrated (Chauhan et al., 1998). 3,4-dichloroaniline (DCA) is a degradation product not only of diuron, but of some other herbicides, such as propanil, and shows toxicological properties greater than its parent compounds (Taxier et al., 2000). Diuron has been shown to inhibit photosynthesis in seagrasses, at concentrations as low as 0.1 μg/L (Jones and Kerswell, 2003); however little is known of the effects of diuron on the common tropical seagrass, T. ciliatum.

Fusilade is another commonly herbicide used in the Western Indian Ocean region. Its active compounds (fluazifop-p-butyl) form part of the aryloxyphenoxypropionates group of herbicides. They inhibit the carboxylase enzyme, acetyl CoA, in the carbon cycle during the dark reaction in photosynthesis. 2,4-D amine are systemic herbicides selective on broad leaf weeds. They are lipid synthesis inhibitors and are classified in the phenoxy compound group. Though having a small half-life (less than one week) in the aquatic environment, they can have a negative impact. In case of heavy rainfall, large amounts can be washed off into the lagoons. In Mauritius, these herbicides are used in combination, to be more effective in controlling weeds. Runoff water and groundwater seepage from agricultural fields, therefore, can contain a range of different herbicides that can affect non-targeted plants, such as seagrass. The combination of these pollutants may have a synergistic, additive or antagonistic effect. Hence for this study, it was of interest to investigate both the effects of the individual herbicides (diuron, Fusilade Forte and 2,4-D amine) and their combination on the tropical seagrass, T. ciliatum.

MATERIALS AND METHODS
Chemicals
Diuron (3, (3,4-dichlorophenyl)1-1- dimethyurea; > 99% purity) was purchased from Sigma-Aldrich UK; Fusilade forte and 2-D amine were purchased in formulated form ready for application as herbicides.
Experimental Set-Up

Samples of the seagrass *Thalassodendron ciliatum*, were collected from the western Indian Ocean coast, Palmar Beach, on the East coast of Mauritius. Whole plants were placed in aerated water tanks and left for 14 days to acclimatise prior to use. The samples were washed with seawater to remove sediments and epiphytes, and then transported to the laboratory for culturing at the Botany Department of Stockholm University. Whole plants were placed in aerated watertanks and acclimatised to tropical conditions for 14 days prior to use. Plants were cultured in natural seawater collected from the Swedish West Coast with a salinity of 33%. The temperature of the seawater was maintained at 25°C. Exposure to light was about 120 μmol photons m⁻² s⁻¹.

The seagrass was exposed to three different concentrations of each herbicide: Diuron at 1, 10 and 100 μg/l; Fusilade Forte and 2, 4-D amine at 10, 100 and 1000 μg/l. The design also included controls with no addition of herbicides. Combinations of either two or three herbicides were also tested. Combined concentrations for herbicides were: diuron + fusilade and diuron + 2,4-D amine at 1+10, 10+100, 100+1000 μg/l. Fusilade+2,4-D amine at 10:10, 100:100 and 1000:1000 μg/l. There were three replicates containers for each treatment and for controls. Before adding the herbicides there was an acclimatisation of 24 hrs. The plants were exposed to herbicides for three days then washed thoroughly with seawater and allowed to recover in fresh seawater with no herbicides for three days. Plant performance was monitored after 7 days and replicate blocks re-randomised on the culturing hood twice a week.

Measurement of Maximum Quantum Yield (Fv/Fm)

To access the herbicides’ toxicity in *T. ciliatum*, Fv/Fm was measured using Diving PAM (Walz, Germany), before and after 2, 4, 6, 8, 24, 48 and 72 hours exposure to herbicides. After the last measurement of exposure the plants were rinsed with fresh seawater and allowed to grow in seawater free from herbicides. During the recovery phase, Fv/Fm was measured after 2, 4, 6, 8, 24, 48 and 72 hrs.

Measurement of Electron Transport Rate (ETR)

Likewise rapid light curves were made at the same intervals as Fv/Fm. From light curves ETRs were estimated using the formula: photosynthetic ETR was calculated from incident PFD and the efficiency of PSII in the light (ΔF/Fm'), where ETR=ΔF/Fm' x PFD (Photon flux density) x 0.6 x0.5, equal distribution of absorbed light between PSII (Photo-system II) and PSI (Genty et al., 1989; Beer and Björk, 2000). ETR were plotted against PFD. ETR max represent maximum ETR reached before *T. ciliatum* became light-saturated and it was determined from ETR and PFD values using computer software programme.

RESULTS

Maximum Quantum Yield (Fv/Fm)

The Maximum Quantum Yield (Fv/Fm) of *T. ciliatum* was significantly lower (p<0.05) after two hours of exposure to diuron (Figure 1). Fv/Fm was decreasing with increase in exposure time in all diuron exposure concentrations (1, 10 and 100μg/l, figure 1). The higher the diuron exposure concentration, the higher was the inhibition in Fv/Fm of *T. ciliatum*. However, there was a remarkable recovery immediately after washing and changing the growth media free from diuron (Figure 1). After 2 hrs exposure to diuron, significant differences in the Fv/Fm of the *T. ciliatum* were recorded between controls and diuron exposed seagrass (Figure 1). Effective concentration of diuron caused an effect of 50% when compared to the control (EC₅₀) of *T.ciliatum* for 72 hrs of exposure as presented in Table 1.

![FIGURE 1. Maximum quantum yield of Thalassodendron exposed to different concentration of diuron.](image-url)
TABLE 1: EC$_{50}$ of diuron exposure to Thalassodendron ciliatum for 72hrs of exposure.

<table>
<thead>
<tr>
<th>Exposure duration</th>
<th>EC$_{50}$ (µg/L)</th>
<th>NOEC value (µg/L)</th>
</tr>
</thead>
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<tr>
<td>72 hrs</td>
<td>7.9</td>
<td>0.48</td>
</tr>
<tr>
<td>48 hrs</td>
<td>8.5</td>
<td>0.56</td>
</tr>
<tr>
<td>24 hrs</td>
<td>12.6</td>
<td>0.79</td>
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</table>

FIGURE 2: Maximum quantum yield of *T. ciliatum* exposed to different concentration of herbicides DCMU and 2,4-D amine.

Exposure to Fusilade Forte and D-amine showed no effects on the Fv/Fm of *T. ciliatum* (figures are not shown here). No significant differences between Fv/Fm recorded during exposure and recovery from Fusilade Forte and 2, 4-D amine exposure. In addition, combination of 2, 4-D amine, and Fusilade showed no effect on Fv/Fm. However, all combinations that involved herbicide diuron, showed a significant reduction in Fv/Fm (Fig. 2, 3 and 4) when compared to a control followed by a significant recovery after washing and re-culturing in uncontaminated seawater. The effect as a result of combination of diuron and other herbicides showed no additional effect, meaning that the effect was largely influenced by diuron and not the other herbicides.

FIGURE 3: Graph showing the maximum quantum yield of the *T. ciliatum* exposed to different concentrations of the combination of herbicides diuron and fusilade, and their recovery.

FIGURE 4: Maximum quantum yield of *T. ciliatum* exposed to different concentration of DCMU, Fusilade and 2,4-D amine salt.

Photosynthetic Electron Transport Rate (ETR)

Diuron showed a significant reduction of the electron transport rate compared to the controls. The higher the diuron concentration, the higher the inhibition in ETR$_{\text{max}}$ was. The effect occurred immediately after 2 hrs of exposure. Figure 5, shows results for ETR$_{\text{max}}$ of *T. ciliatum*, exposed to diuron, while Tables 2 and 3 show statistical results. As with Fv/Fm, there were recoveries of ETR$_{\text{max}}$ of *T. ciliatum* exposed to different concentrations of diuron. In addition, as it was observed in Fv/Fm, all other combinations that involved diuron showed a similar effect in ETR$_{\text{max}}$ (Figs. 6, 7 and 8). Recovery was as well observed in all the
combinations that involved diuron and other herbicides, but no full recovery was observed. In addition, no effect in ETRmax was observed when *T. ciliatum* was exposed to neither Fusilade forte nor 2, 4-D amine or the combination of the two.

![Graph](https://via.placeholder.com/150)

**Figure 5:** ETRmax of *T. ciliatum* exposed to different concentration of diuron at different exposure and recovery time.

**Table 2:** Two-way ANOVA results for exposure to diuron.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>MS</th>
<th>F</th>
<th>p-level</th>
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<tr>
<td></td>
<td></td>
<td>Effect</td>
<td></td>
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<tr>
<td>Exposure time</td>
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<td>6705.6</td>
<td>5.6</td>
<td>&lt;0.0005***</td>
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<tr>
<td>Exposure concentration</td>
<td>3</td>
<td>65152.9</td>
<td>188.6</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Time: Concentration</td>
<td>21</td>
<td>2452.7</td>
<td>7.1</td>
<td>&lt;0.0005***</td>
</tr>
</tbody>
</table>

**Table 3:** Tukey HSD test statistical results (diuron exposure). Post Hoc Tests.

<table>
<thead>
<tr>
<th>Mean effect: concentration of diuron</th>
<th>1 μg/l</th>
<th>10 μg/l</th>
<th>100 μg/l</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<tr>
<td>ETRmax</td>
<td>66.6</td>
<td>51.6</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>&lt;0.005*</td>
<td>&lt;0.0005***</td>
<td>&lt;0.0005***</td>
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<tr>
<td>1 μg/l</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
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<tr>
<td>10 μg/l</td>
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<tr>
<td>100 μg/l</td>
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FIGURE 6: Graph showing the ETRmax of the T.ciliatum exposed to different concentrations of a combination of herbicides diuron and fusillade, and their recovery.

FIGURE 7: Graph showing the ETRmax of the T.ciliatum exposed to different concentrations of a combination of herbicides diuron and 2,4-D amine salt, and their recovery.

FIGURE 8: Graph showing the ETRmax of the T.ciliatum exposed to different concentrations of a combination of herbicides diuron, fusillade and 2,4-D amine salt, and their recovery.
DISCUSSION

Diuron, as 50% of other commercial herbicides, is known to inhibit photosynthetic pathways in marine primary producers (Ralph, 2000; Jones and Kerswell 2003; Jones et al., 2003; Owen et al., 2003). Results observed in this study clearly indicate how Fv/Fm and ETRmax in T. ciliatum is affected by diuron, even at as low a concentration as 1 μg/L. Such concentrations are very likely to be found in the environment following rain-runoff or water bodies downstream. Exposure to a concentration of 100 μg/L of diuron, was more effective in inhibiting both ETRmax and Fv/Fm. Concentrations of 100 μg/L of diuron are not likely to be found in the environment, unless there is an accidental spillage, direct application to water ways or misuse and mishandling of herbicides. Incidents mishandling of pesticides, misapplication and poor storage in developing countries are common, and pose a serious environmental threat. Diuron of a concentration of less than 1 μg/L has been shown to affect other species of seagrass (Haynes et al., 2000; Ralph 2000). About 0.3 μg/L of diuron has been reported to cause an effect in symbiotic dinoflagellates in corals. Different responses might occur in different species, because it is possible that the uptake of diuron differs depending on the type of membranes, before it reaches the chloroplast target sites (Fahl et al., 1995).

Diuron is well known for its mechanism of action of affecting the PSI I by displacing the electron from Qb niche in D1 protein and blocking Qb reduction (Jansen et al., 1993; Hall et al., 1999). Increase in the concentration of the herbicide resulted in increased concentrations of diuron that block the Qb, hence reducing the photosynthetic electron transfer. The continuous blocking of electron transfer of electrons could result in inhibition of the photosynthetic process that can cause plant starvation and eventually it will die. The observed recovery of ETRmax and Fv/Fm after washing the plant, minimised or reduced the uptake of diuron, hence more electrons were allowed to be transferred for photosynthesis. However, the recovery was not full recovery. This may be due to the fact that some of the quinones were still occupied with diuron, or the medium still had some traces of diuron that were continuously inhibiting the plant.

Results also demonstrated that T. ciliatum was not inhibited when it was exposed to Fusilade forte and 2, 4-D amine. Unlike diuron these two herbicides have different modes of action. They are not specific for inhibiting the photosynthetic pathways but they do affect other metabolic activities of the plant. Probably this has been a reason why the effect of these herbicides was not detected through measuring the Fv/Fm and the ETRmax. Fusilade forte is a post emergency herbicide known to control annual and perennial weeds. The mode of action of fusilade forte is known to inhibit the Carboxylase enzymes, Acetyl CoA in the carbon cycle during the dark reaction in the photosynthesis process. 2, 4-D amine are systemic herbicides, they inhibit lipid synthesis and it is one of the phenoxy compounds. Two these herbicides might have acted on Thalassodendron but their effects were not detected by the methods used for toxicity test in this study.

Several methods have been reported for studying toxicity of herbicides to macrophytes, among them are these two methods (Fv/Fm and ETR) which have been used in this study. In this study no effect to ETRmax and Fv/Fm were detected when T. ciliatum was exposed to Fusilade Forte and 2, 4-D amine, this could be due to the fact that these two herbicides do not act directly on photosynthesis inhibition. Their effects probably were directed on other metabolic pathways other than photosynthetic pathways. The use of other methods of toxicity could have probably given negative impacts of these two herbicides. Hence the observed no effect in this study when Fusilade and D-amine were used, is not necessarily that the T. ciliatum was not inhibited. However, if it was a long-term exposure, these methods could have indicated the inhibition because any inhibition after a long time of exposure in most of the metabolic pathways in the long run might advance their effects to photosynthesis process.
Unlike other herbicides tested in this study, diuron is known to be poorly soluble in water (42 mg/L at 25°C) and it has low vapour pressure (0.14 mpa), hence potential losses due to volatilisation are insignificant after application (Montgomery, 1993; Hill et al., 1955). The poor solubility properties of diuron favours it to be more persistent in the environment compared to other herbicides tested in this study. Because of it’s poor solubility probably it might have made it more toxic compared to other herbicides because it’s active form is retained for a longer period in the media compared to other soluble herbicides. A study by Okamura et al., 2003 demonstrated that about 86% of aquatic environment water samples studied showed a 3.05 µgL⁻¹ concentration of diuron. Furthermore, a high level of more than the maximum acceptable concentration of 0.43 µgL⁻¹ was detected in the Dutch coastal and Marinas waters (Lamoree et al., 2002).

2,4-D amine and Fusillade Forte did not show an impact on T. ciliatum, this could be due to resistant mechanisms. T. ciliatumis probably adopted to have ability to resist the inhibition from 2,4-D amine and Fusillade Forte with their toxicity specific to inhibition of the lipid synthesis and acetyl CoA respectively. For lipid synthesis the direct method of investigating the effect on the lipid synthesis is measured on the chlorophyll concentration, while the method of measuring the effect directed to acetyl CoA is done by measuring the carbohydrates content or growth. In this study diuron was toxic to photosynthetic activity of T. ciliatum but not the other two herbicides tested. The other herbicides could have caused an effect on other metabolic processes other than photosynthetic pathways.

Therefore, from this short-term experiment, diuron has been found to affect the photosynthetic performance of T. ciliatum although a certain percentage of recovery occurred. It is of great importance to protect marine environment from diuron contamination. However, further studies are needed to assess the long-term effects of herbicides exposure.

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