TaJONAS
Tanzania Journal of Natural & Applied Sciences

ONLINE ISSN 1821-7249
2012: Volume 3, Issue 1, 458-466

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

Shalena F. Wahedally[1], Florence A. Mamboya[2*], Thomas J. Lyimo[3], Mitrasen Bhikajee[4], and Mats Björk[5]

[1] *Faculty of Science, University of Mauritius, Reduit, Mauritius*
[2] *Department of Science and Laboratory Technology, Dar es Salaam Institute of Technology, Dar es Salaam, Tanzania.*
[3] *Department of Molecular Biology and Biotechnology, University of Dar es Salaam, Dar es Salaam, Tanzania.*
[4] *Mauritius Oceanography Institute, France Centre, Victoria Avenue, Quatre-Bornes, Mauritius*
[5] *Botany Department Stockholm University, Stockholm, Sweden*

Received: 28 February 2012 *Accepted: 28 March 2012* *Published: 23 July 2012*

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, Fusillade (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: *Thalossodendron ciliatum,* herbicides, toxicity, Fucilade, 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

* Corresponding author
Email: mamboya@dit.ac.tz

© 2012, *St. John's University of Tanzania*

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 causefor the decline of seagrass in the area (Hall *et al.*, 1999); andvarious types of herbicides have been reported to affect marine plants (Ralph 2000; McMahon *et al.*, 2005; Gao *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, causingthe 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 Q_A to Q_B (Sandmann and Bölger, 1986). Diuron is used mainly as a post-emergence pesticide to control all weeds, without affecting the not yet geminating seeds. Besides being used as herbicide, diuron is also found in a number of antifouling 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- dimethylurea; > 99% purity) was purchased from Sigma-Aldrich UK; Fusilade forte and 2-D amine were purchased in formulated form ready for application as herbicides.

© 2012, *St. John's University of Tanzania*

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 ug/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 (Gent*y 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 (EC_{50}) of *T.ciliatum* for 72 hrs of exposure as presented in Table 1.

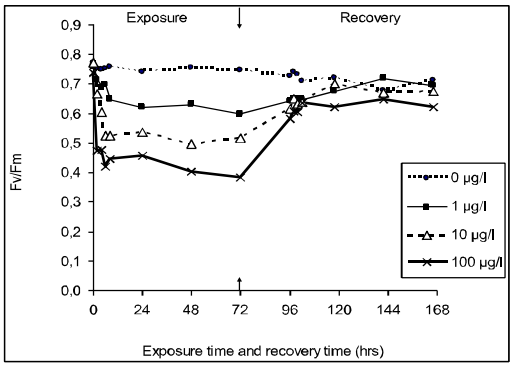


FIGURE 1. Maximum quantum yield of Thalassodendron exposed to different concentration of diuron.

© 2012, *St. John's University of Tanzania*

TABLE 1: EC_{50} of diuron exposure to Thalassodendron ciliatum for 72hrs of exposure.

Exposure duration	EC_{50} (µg/L)	NOECvalue (µg/L)
72 hrs	7.9	0.48
48 hrs	8.5	0.56
24 hrs	12.6	0.79

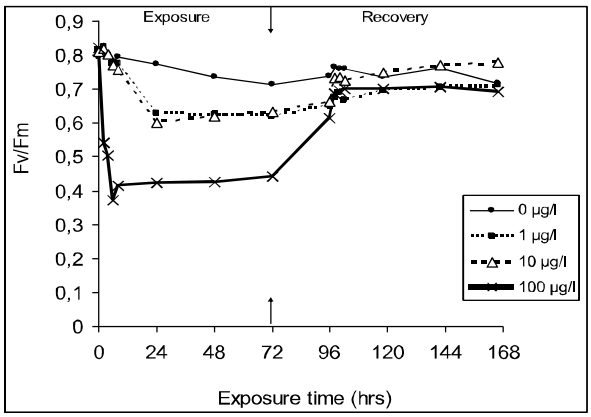


FIGURE 2: *Maximum quantum yield of T. ciliatum exposed to different concentration of herbicides DCMU and 2,4-D amine.*

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 sea-water. The effect as a result of combination of diuron and other herbicides showed no addition--al effect, meaning that the effect was largely influenced by diuron and not the other herbici-des.

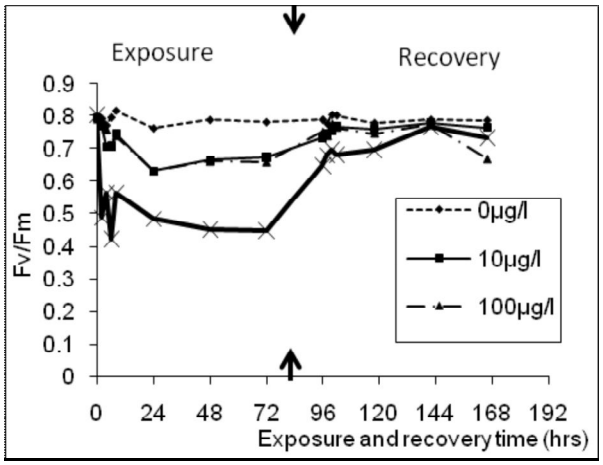


FIGURE 3: *Graph showing the maximum quantum yield of the T. ciliatum exposed to different concentrations of the combination of herbicides diuron and fusillade, and their recovery.*

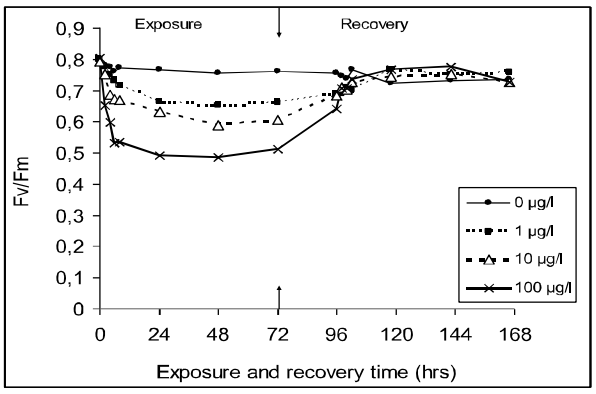


FIGURE 4: *Maximum quantum yield of T. ciliatum exposed to different concentration of DCMU, Fusilade and 2,4-D amine salt.*

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 ETRmax was. The effect occurred immediately after 2 hrs of exposure. Figure 5, shows results for ETRmax of *T. ciliatum,* exposed to diuron, while Tables2 and 3 show statistical results. As with Fv/Fm, there were recoveries of ETRmax 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 ETRmax (Figs. 6, 7 and 8). Recovery was as well observed in all the

© 2012, *St. John's University of Tanzania*

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.

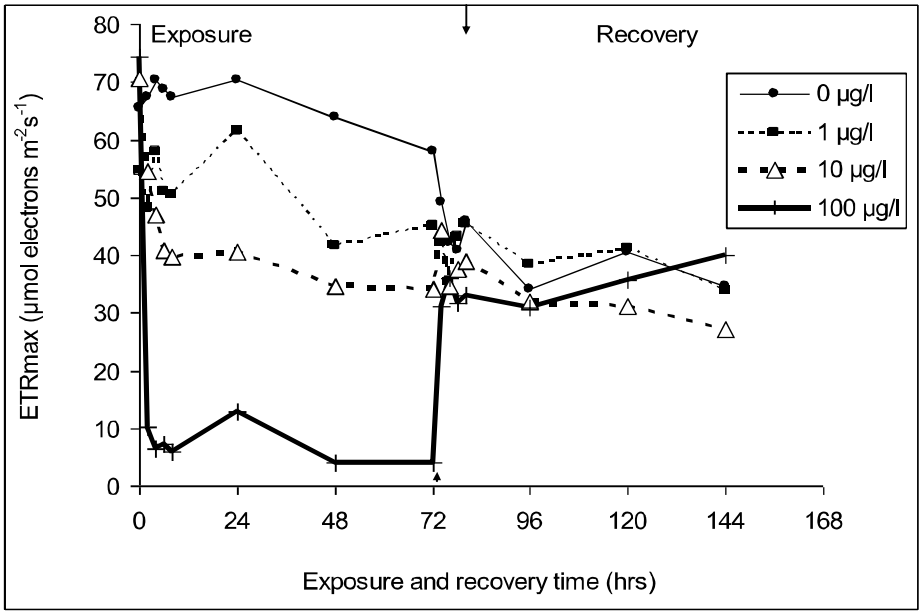


FIGURE 5*: ETRmax of T. ciliatum exposed to different concentration of diuron at different exposure and recovery time.*

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

	Df	MS		
	Effect	Effect	F	p-level
Exposure time	7	6705.6	5.6	<0.0005***
Exposure concentration	3	65152.9	188.6	<0.05*
Time: Concentration	21	2452.7	7.1	<0.0005***

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

Main effect: concentration of diuron				
	Control	**1 µg/l**	**10 µg/l**	**100 µg/l**
Mean ETRmax	66.6	51.6	45.5	15.5
Control		<0.005*	<0.0005***	<0.0005***
1 ug/l			0.031*	<0.0005***
10 ug/l				<0.0005***
100 ug/l				

© 2012, *St. John's University of Tanzania*

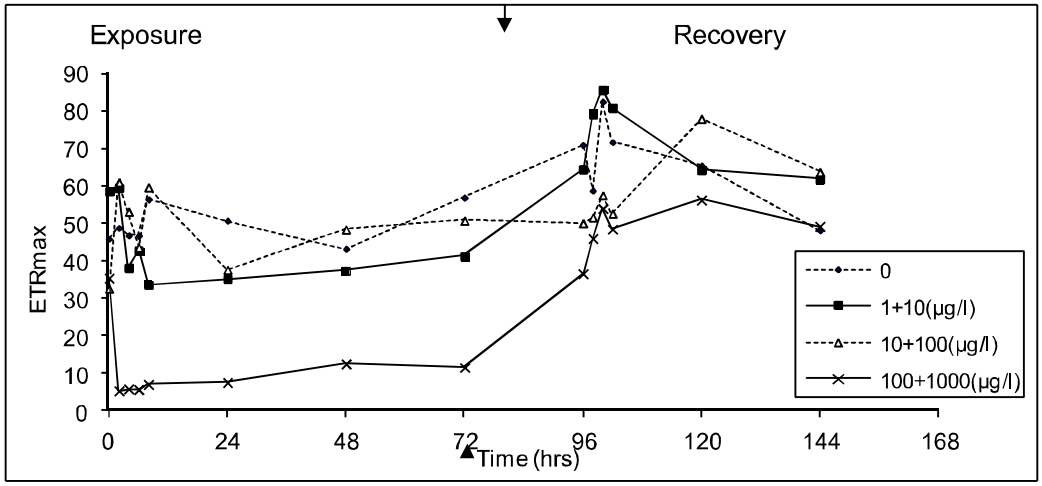


FIGURE 6: *Graph showing the ETRmax of the T.ciliatum exposed to different concentrations of a combination of herbicides diuron and fusillade, and their recovery.*

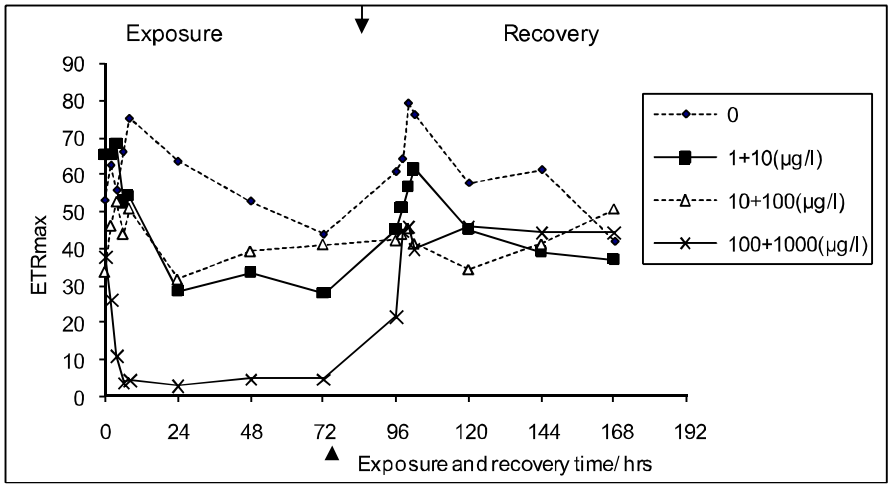


FIGURE 7: *Graph showing the ETRmax of the T.ciliatum exposed to different concentrations of a combination of herbicides diuron and 2,4 D amine salt, and their recovery.*

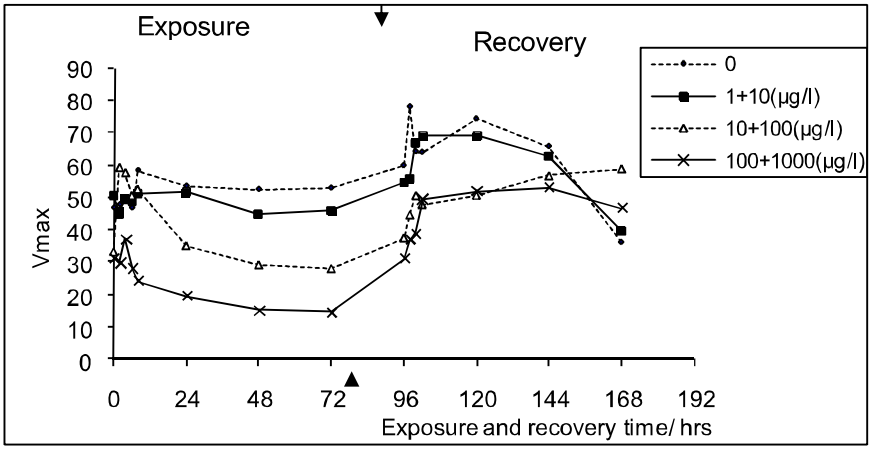


FIGURE 8: *Graph showing the ETRmax of the T.ciliatum exposed to different concentrations of a combination of herbicides diuron, fusilade and 2,4 -D amine salt, and their recovery.*

© 2012, *St. John's University of Tanzania*

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 1μg/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 ug/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 PSII by displacing the electron from Q_B niche in D1 protein and blocking Q_b 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 Q_B, 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 fusillade 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. These two 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.

© 2012, *St. John's University of Tanzania*

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. ciliatum*is probably adopted to have ability to resists 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.

ACKNOWLEDGEMENTS

We would like to thank SIDA/SAREC and WIOMSA through the seagrass MASMA project, for sponsoring this study. The Botany Department at Stockholm University is acknowledged with thanks for hosting this study.

REFERENCES

Beer S. and Björk M. (2000). 'Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated' (PAM) fluorometry. *Aquatic Botany*, 66: 69-76.

Boxall, A.B.A., Comber, S.D., Conrad, A.U., Howcroft, J., Zaman, N., (2000). 'Inputs, monitoring and fate modelling of antifoulingbiocides in U.K. estuaries'. *Mar. Pollut. Bull.*, 40: 898–905.

Chauhan S., Barbieri P. and Wood T.K. (1998). 'Oxidation of trichloroethylene, n1, 1-dichloroethylene, and chloroform by toluene/*o*-monooxygenasefrom *Pseudomonas stutzeri* OX11'. *Appl Environ Microbiol.*,64: 3023–3024.

Fahl G.M., Kreft L., Altenburger R., Faust M., Boedecker W. and Grimme L.H. (1995). 'pH dependent sorption, bio-concentration and algal toxicity of sulfonylurea herbicides'. *Aquatic Toxicology,* 31: 175-187.

Gao Y., Fang J., Zhang J., Ren L., Mao Y., Li B., Zhang M., Liu D. and Du M. (2011). 'The impact of the herbicide atrazine on growth and photosynthesis of seagrass, *Zostera marina* (L.), seedlings'. *Marine Pollution Bulletin*, 62 (8): 1628–1631.

Genty B., Briantais J. and Baker N.R. (1989). 'The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence'. *Biochim. Biophys. Acta,* 990*:* 87–92.

Häder D.P and Figueroa F.L. (1997). 'Photoecophysiology of marine macroalgae'. *Photochem. Photobiol*, 66: 1–14.

Hall L. W. J., Anderson R. D., Kilian J. and Tierney D.P. (1999). 'Concurrent exposure assessments of atrazine and metolachlor in the mainstream, major tributaries and small streams of the Chesapeake Bay Watershed: Indicators of ecological risk'. *Environmental Monitoring and Assessment.* 59: 155-190.

Hall, L.W., Giddings, J.M., Solomon, K.R., Balcomb, R., (1999). 'An ecological risk assessment for the use of Irgarol 1051 asan

© 2012, *St. John's University of Tanzania*

algaecide for antifoulant paints'. *Crit. Rev. Toxicol.,* 29: 4.

Haynes, D., Muller, J., Carter, J., (2000b). 'Pesticide and herbicide residues in sediments and seagrasses from the Great Barrier Reef World Heritage Area and Queensland coast'. *Mar. Pollut.Bull.,* 41: 279–281.

Haynes, D., Ralph, P., Prange, J., Dennison, B., (2000a). 'The impact of the herbicide Diuron on photosynthesis in three species of tropical seagrass'. *Mar. Pollut. Bull.,* 41: 288–293.

Hill G.D., McGahen J.W., Baker H.M., Finnerty D.W. and Bingeman C.W. (1955). 'The fate of substituted urea herbicides in agricultural soils'. *Agron. J.*, 47: 93-104.

Jansen, M.A.K., Mattoo, A.K., Malkin, S., Edelman, M., (1993). 'Direct demonstration of binding-site competition betweenphotosystem II inhibitors at the QB niche of the D1 protein'. *Pestic. Biochem. Physiol.*, 46: 78–83.

Jones R.J., Kerswell A.P. (2003). 'Phytotoxicity of Photosystem II (PSII) herbicides to coral'. *Mar. Ecol. Prog. Ser.,* 261:149–159.

Jones R.J., Muller J., Haynes D., Schreiber U., (2003). 'Effects of herbicides diuron and atrazine on corals of the Great Barrier Reef, Australia'. *Mar. Ecol. Prog. Ser.,* 251:153–167.

McMahon K., Nash S.B., Eaglesham G., Müller J. F., Duke N.C. and Winderlich S. (2005). 'Herbicide contamination and the potential impact to seagrass meadows in Hervey Bay, Queensland, Australia'. *Marine Pollution Bulletin*, 51(1-4): 325–334.

Montgomery J.H. (1993). *A grochemicals Desk Reference: Environmental Data.* Lewis Publishers, Chelsea, MI, FL, 625 pp.

Okamura, H., Aoyama, I., Takami, T., Maruyama, T., Suzuki, Y.,Matsumoto, M., Katsuyama, I., Hamada, J., Beppu, T., Tanaka,O., Maguire, R.J., Liu, D., Lau, Y.L., Pacepavicius, G.J., (2000). 'Phytotoxicity of the new antifouling compound Irgarol 1051 and a major degradation product. *Mar. Pollut. Bull.*, 40: 9.

Ralph J.P. (2000). 'Herbicide toxicity of *Halophila ovalis* assessed by chlorophyll a fluorescence'. *Aquatic. Bot.*, 66: 141-152.

Sandmann G. and Boelger P. (1986). 'Sites of herbicide action at the photosynthetic apparatus'. In: Staehelin, L.A., Arnzten, C.J. (Eds.), Encyclopedia of Plant Physiology, vol. 19. Springer, Berlin, pp. 595–602.

Teisseire, H., Couderchet, M., Vernet, G., (1999). 'Phytotoxicityof Diuron alone and in combination with copper or folpeton duckweed (*Lemna minor*)'. *Environ. Pollut.*, 106: 39–45.

Thomas K.V., Blake S.J. and Waldock M.J. (2000). 'Antifouling paint booster biocides in UK marine sediments'. *Marine Pollution* Bulletin, 40: 739-745.

© 2012, *St. John's University of Tanzania*