Effects of Diuron, Terbuthylazine and Isoproturon on Photochemical and Non-Photochemical Quenching of Ectocarpus siliculosus

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Abstract. The influence of anthropogenic discharges on the environment is an increasing concern among environmental toxicologists. This investigation set out to analyse the impacts of selected herbicides; diuron, terbuthylazine and isoproturon on two strains of Ectocarpus siliculosus with different pollution histories, LIA4 and Es524. Evaluation of their effects on photochemical quenching (qP) and non-photochemical quenching (qN) indicators have demonstrated negative impacts of all herbicides on both strains. From the results, diuron shows to exert significant negative effect at concentration as low as 5 µg L⁻¹ followed by terbuthylazine at 10 µg L⁻¹ (qP) and 5 µg L⁻¹ (qN), and isoproturon at 100 µg L⁻¹ (qP) and 50 µg L⁻¹ (qN). Non-photochemical quenching (qN) indicator was found to exhibit greater sensitivity to the herbicides compared to photochemical quenching (qP). In both strains of E. siliculosus, the three herbicides were ranked in order of toxicity: diuron > terbuthylazine > isoproturon. This investigation provides new information on ecotoxicology of herbicides towards brown algae.

Keywords: Diuron, Terbuthylazine, Isoproturon, Ectocarpus siliculosus, photochemical quenching, non-photochemical quenching

1. Introduction

Herbicides, such as diuron, terbuthylazine and isoproturon are used extensively for weed control in agriculture. Not limited to just agricultural applications, these herbicides were also being incorporated in antifouling paints to be applied on various floating structures. Excess-usage of this herbicide may lead to its accumulation in ecosystem and cause detrimental impact to the environment.

Thus, in order to monitor the environmental status, the need for sensitive biomarkers are necessary so that the impacts of anthropogenic discharges in the environment can be address sustainably. “Biomarkers of exposure are defined as any functional measure of exposure that is characterized at a sub-organism level of biological organization” [1]. Biochemical, physiological, or ecological structures or processes that have been correlated or causally connected to biological impacts evaluated at one or more levels of biological organisation are called biomarkers of effect (bioindicators) [2]. In this study, we have evaluated the effects of selected herbicides on photochemical and non-photochemical quenching indicators of Ectocarpus siliculosus strains with different pollution histories.
2. Materials and Methods

2.1. Culture conditions of Ectocarpus siliculosus
Stock cultures of two strains of *E. siliculosus* (LIA4 and Es524; Culture Collection of Algae and Protozoa (CCAP) accession numbers 1310/339 and 1310/333, respectively), originating from a pristine area in Scotland (Lon Liath) and a copper-polluted site in Chile (Caleta Palito), respectively, were cultured in acid washed, 10 L polycarbonate carboys containing filtered and autoclaved natural seawater enriched with Provasoli medium [3] in a controlled environment chamber (CEC, Sanyo MLR-350/HT) at 15 ± 1 °C. Lighting was provided by cool white fluorescence tubes (Philips) with a photon fluence rate (PFR) of 40 μmol m\(^{-2}\) s\(^{-1}\) on a 14:10 h light:dark cycle. To avoid CO\(_2\) depletion and keep the seaweed in suspension, cultures were bubbled with filtered (0.22 µm) compressed air [4]. The culture medium was changed every two weeks.

2.2. Test chemicals and solutions
Stock solutions of the herbicides were prepared according to Manzo et al., 2008 [5] with dimethyl sulfoxide (DMSO, ≥99.5%), due to their moderate/low solubility in water (diuron= 42 mg L\(^{-1}\) [6]; terbutylazine= 8.5 mg L\(^{-1}\) [7]; isoproturon= 65 mg L\(^{-1}\) [8]). The final concentration of DMSO in the exposure media did not exceed 0.005% (v/v). The three herbicides (diuron (CAS No: 330-54-1), terbutylazine (CAS No: 5915-41-3) and isoproturon (CAS No: 34123-59-6) were added to filtered, enriched autoclaved natural seawater (taken from the south side of Mount Batten Pier, coordinates: 50° 21′ 34″ N, 4° 07′ 47″ W) to yield different nominal concentrations of 1, 5, 10, 50, 100 and 500 µg L\(^{-1}\). The pH, salinity and temperature of the test solutions were 7.4 ± 0.1, 33 ± 1.0 ppt and 15 ± 1 °C respectively.

2.3. Chlorophyll fluorescence
Photochemical quenching (qP) and non-photochemical quenching (qN) parameters were evaluated based on the chlorophyll fluorescence measurements using Mini-PAM, Walz, Germany. “The photochemical (qP = (Fm’- Fs) / (Fm’- Fo’)) and non-photochemical (qN = 1- ((Fm’-Fo’) / (Fm - Fo)) quenching were determined as in Schreiber et al.,1986” [9].

2.4. Statistical analysis
Statistical analyses were carried out using STATGRAPHICS Centurion (Version XVI, Statpoint Technologies, Inc., USA).
3. Results and Discussion

Figure 1 The effects of diuron (1st row), terbutylazine (2nd row) and isoproturon (3rd row) on photochemical quenching (qP) and non-photochemical quenching (qN) of *Ectocarpus siliculosus*. Values with an asterisk are significantly different at $P < 0.05$ compared to control carrier. Data sets which are significantly different from each other are represented by different letters ($P < 0.05$).
Macroalgae are the principal producers in near-shore ecosystems, and brown seaweeds (Phaeophyceae) are particularly significant bio-engineer organisms [10], providing shelter, food, and habitat for a variety of other marine biota [11]. In this investigation, two different strains of brown seaweed, *E. siliculosus*; LIA4 and Es524 were used in order to assess the effects of herbicides exposure.

The extensive use of pesticides in agriculture has resulted in concentrations exceeding the buffering capacity of soil compartments causing contamination of surface and ground-water [12]. In fact, researchers have highlighted the increasing frequency of pesticide pollution in lakes, rivers, estuaries and coastal waters, with a high predominance of herbicides [13] [14]. Moreover, the use of herbicides as booster biocides in antifouling paint formulations such as diuron have gained popularity since the ban of tributyltin (TBT).

From the results (Figure 1, first row) a concentration-dependent decrease of photochemical quenching (qP) response was observed in both strains after exposure to diuron. Significant decreases as compared to the carrier control were observed at 5 µg L\(^{-1}\) and above in both strains. No significant difference was observed between the strains (S×T, P=0.05) for the qP response within the concentrations of diuron tested. Diminutions as much as 19-85 % were recorded in LIA4 between 5 to500 µg L\(^{-1}\), while 12- 89 % decreases were observed in Es524. As for non-photochemical quenching response (qN), exposure to diuron caused increments in both *E. siliculosus* strains. Compared to the carrier control, significant increments were observed between 5 to 50 µg L\(^{-1}\) in both strains. At 100 µg L\(^{-1}\) and above, the qN responses have significantly reduced, which may indicate damage to the photosynthetic apparatus. Maximum inhibitions of 85 and 89 % photochemical quenching (qP) observed in LIA4 and Es524 respectively, indicating that the redox state of the quinone pool in both strains was modified by the herbicide [15]. Moreover, the binding of diuron to the D1 protein, has increased the fluorescence and non-photochemical quenching (qN), restricting the photochemical potential of the system [16]. In fact, stimulation of qN by more than 100% may represent a disruption in the electron transport necessary for energy dissipation by non-photochemical processes (transthylakoid pH gradient formation, antenna movements, photoinhibition) [17] [18].

Exposure to terbuthylazine caused a consistent decrease in qP values in both strains (Figure 1, second row). Significant decreases as compared to carrier control were observed at 10 µg L\(^{-1}\) and above, with diminutions as much as 11-75 % and 21-84 % recorded in LIA4 and Es524 respectively. On the other hand, the increases of qN values were observed in both strains up to 50 µg L\(^{-1}\) before declined. Significant changes of qN values as compared to carrier control were observed at 5 µg L\(^{-1}\) and above in both strains. Increments as much as 31-90 % and 47-121 % were recorded between 5 to 50 µg L\(^{-1}\) in LIA4 and Es524 respectively. Interestingly, significant reductions were observed at 100 and 500 µg L\(^{-1}\), with 30 and 49 % in LIA4 respectively and 33 and 54 % in Es524. This condition could be related to the destruction of the PSII due to excessive blocking on the electron transport chain by the herbicide.

The photochemical quenching (qP) responses of LIA4 and Es524 strains to isoproturon are shown in Figure 1, third row. A slight increment was observed at 10 µg L\(^{-1}\) before a decline. Significant decrease of qP values were observed at 100 and 500 µg L\(^{-1}\), with 21 and 37 % recorded in LIA4 respectively, while 11 and 24 % in Es524. These findings were in line with previous study by [15] using *Scenedesmus obliquus* and *Scenedesmus quadricauda* which also have reported 23% inhibition of qP at 20 µg L\(^{-1}\) of isoproturon. In contrast to qP, exposure to isoproturon has induced increments of non-photochemical quenching response in both strains. Significant changes as compared to the carrier control, were observed at 50 µg L\(^{-1}\) and above with increment up to 68 and 114 % in LIA4 and Es524 respectively. Again, this finding was in agreement to the isoproturon effects on *Sc. obliquus* and *Sc. quadricauda*, where stimulation of qN up to 20% was reported [15]. Compared to diuron and terbuthylazine, exposure of isoproturon on *E. siliculosus* strains (LIA4 and Es524) had a lower toxicity impact. This toxicity ranking (diuron more toxic than isoproturon) is consistent with the study by [19] and [20] reported previously.

In conclusion, the effects of these chemicals on photochemical and non-photochemical quenching indicators of *E. siliculosus* demonstrated in this study highlight the need to examine the extent to which pollutants from terrestrial runoff and shipping activities can enhance the damaging effects at different
levels of biological organizations. Moreover, besides elucidating the harm that these chemicals exerted towards *E. siliculosus*, one can surely ascertain that these herbicides and antifouling agents can also tremendously influence other marine communities as well. In fact, the results of the present study also revealed that photochemical and non-photochemical quenching indicators (qP, qN) serve as important and sensitive indicators for assessing the impacts of diuron, terbuthylazine and isoproturon on *E. siliculosus*. Other investigations of metal toxicity demonstrated a concurrent loss in photochemistry efficiency with an increase in non-photochemical quenching when the electron transfer was adjusted, in order to reduce damage to the photosynthetic apparatus [21][22]. In contrast to qP, which is an indicator of only the active reaction centres of PSII, qN is a more sensitive indicator since it incorporates all harmful effects on the electron transport pathway [23][24][25].

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