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Distribution and Potential Effects of Novel Antifouling Herbicide Diuron on Coral Reefs

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1. Introduction

1.1 Characteristics of diuron

N'-(3,4-dichlorophenyl)-N, N-dimethylurea (diuron) is a herbicide belonging to the phenylamide family and the subclass of phenylurea (Fig. 1). It is a colourless crystalline compound in its pure form, non-ionic, with a moderate water solubility of 42 mg /L at 20 ºC. It remains a solid at ambient temperature (25 ºC) with a melting point of 158–159 ºC. Its vapour pressure is 0.009 mPa at 25 ºC and has a calculated Henry's law constant of 0.000051 Pam^3 /mol suggesting that diuron is not volatile from water or soil (Giacomazzi and Cochet, 2004).
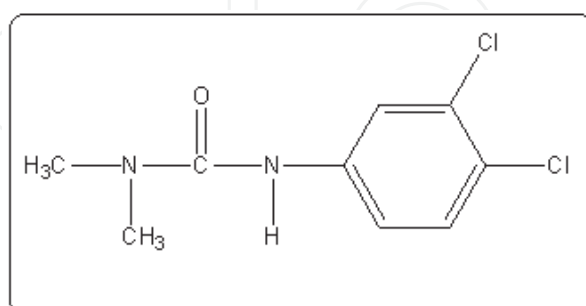

Fig. 1. Chemical structure of diuron.

Diuron has been used to control weeds on hard surfaces such as roads, railway tracks, and paths. It is also used to control weeds in crops such as pear and apple trees, forests, ornamental trees and shrubs, pineapples, sugar cane, cotton, alfalfa and wheat. Furthermore, diuron is widely used as a marine antifouling compound.

1.2 Contamination status and potential effects of diuron to coral reefs

Coral reefs are widely distributed in tropical and subtropical shallow waters (Smith and Kinsey, 1978; Suzuki and Kawahata, 2003; Inoue et al., 2005). They are characterized as highly productive carbon systems for both organic and inorganic carbon (Smith and Kinsey, 1978). Photosynthesis and calcification are the main biogeochemical processes in the coral reef ecosystems (Smith 1973; Suzuki and Kawahata, 2003).

In recent decades, coral reefs have begun to face many threats caused by both natural and anthropogenic sources. The sustainability of these ecosystems have been thrown into doubt by a number of challenges including: marine pollution, global environmental changes, and outbreaks of the crown-of-thorns starfish and coral disease. It has been estimated that 27% of the world's coral reefs have already been lost and 31% have been projected to be degraded by 2030 (Wilkinson, 2000). More integrated efforts and new conservation approaches are necessary to minimize further catastrophe in the future of coral reef ecosystems.

Diuron is considered a priority hazardous substance by the European Commission (Malato et al., 2002). Countries including the UK, Sweden, Denmark and France have restricted the use of diuron in antifouling paints (Konstantinou and Albanis, 2004; Giacomazzi and Cochet, 2004).

Diuron inhibits photosynthesis in plants by binding site of photosystem II (PSII), which limits the electron transfer (Vandermeulen, et al., 1972). Eco-toxicological studies have shown that diuron induces significant impacts on corals (Jones, 2005), as shown by the reduction of ^{14}C incorporation in *Madracis mirabilis* (Owen et al., 2002), the reduction of ΔF/Fm' in *Stylophora pistillata*, *Seriotopora hystrix* and *Acropora formosa* (Jones, 2005), the loss of symbiotic algae in *Montipora digitata* and *S. hystrix* (Jones, 2004), and the detachment of soft tissue in *Acropora tenuis* juveniles (Watanabe et al., 2006). In addition, the herbicide has been associated with serious impacts on other marine ecosystems such as mangrove diebacks (Bell and Duke, 2005).

Diuron is very persistent in the environment and can remain from one month to up to one year in a given ecosystem (Giacomazzi and Cochet, 2004). Diuron has been detected in marine environments from various regions such as western Japan (Okamura et al, 2003), the UK (Boxall et al., 2000), Spain (Ferrer and Barcelo, 1999; Martinez et al., 2000), The Nertherlands (Lamoree et al., 2002), and Sweden (Dahl and Blanck, 1996). Diuron can undergo abiotic degradation such as hydrolysis, photodegradation, as well as biotic degradation (Giacomazzi and Cochet, 2004).

In Japan, diuron has been extensively used in antifouling paints in shipping and agricultural activities (Okamura et al., 2003). In 2004 alone, ~11 tons of diuron was used for sugar cane crops in the Okinawa Prefecture, which was the highest amount of diuron used in Japan outside of the Tokyo metropolitan. In addition, the urban areas of Okinawa mainland, apply a significant amount of diuron as a weed control (Kitada, 2007).

Despite the extensive usage of diuron in Ryukyu Archipelago, and the associated toxicological implications in coral reefs, very little is known about the baseline levels and potential physiological effects of diuron in coral reef waters around the Ryukyu Archipelago. So far, only one study has reported the diuron contents in river sediments

around mainland Okinawa (Kitada, 2007). Yet, the risks posed by pollution of coral reef waters with the soluble fraction of toxic chemicals need to be given priority.

Therefore, this study provides a combination of the results of a systematic monitoring and behavior of diuron in coral reef ecosystems around the Ryukyu Archipelago as well as the ecotoxicological impacts of the herbicide diuron on coral reefs.

2. Materials and methods

2.1 Study area

The study was conducted around the Shiraho coral reefs, adjacent areas and main Naha Bay located in the Ryukyu Archipelago, South-western Japan. The Ryukyu Archipelago is located between 24 and 30 °N, constituting the southern part of the Nansei Islands (Fig. 2). The Archipelago is a chain of more than 100 Islands lying in between Kyushu (mainland Japan) and Taiwan, separating the East China Sea from the Pacific Ocean.
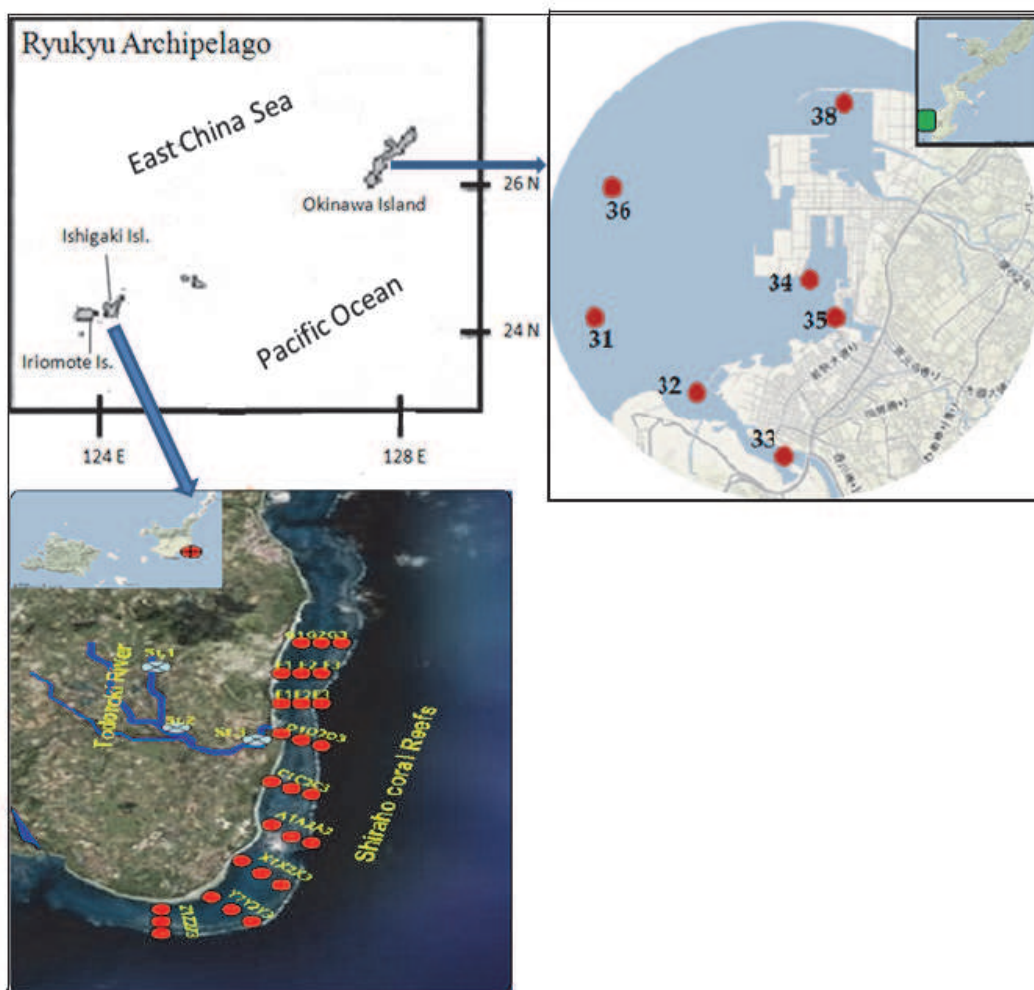

Fig. 2. Sampling locations.

During the main sampling event, the Shiraho reef was divided into nine transects, where three sampling points were established in each transect. Ten sampling points were selected

along the Todoroki River. An extensive survey of diuron in the waters around the Shiraho coral reefs and inflow from the Todoroki River was carried out during various seasons in 2007, 2008 and 2009. A total of 22 and 191 water samples were analyzed for the Todoroki River and Shiraho coral reefs, respectively. In Naha Bay, 42 samples were collected between Sept., 2007 and Feb., 2008).

At each location, a 1 L sample of water was collected in an acetone-washed amber bottle. Samples were returned to the laboratory and stored at < 4ºC in a cold dark room and extracted within 10 days.

2.1.2 Sample pre-treatment

Diuron in water was analyzed following the solid phase extraction LC/MS method of analysis of pesticides in drinking water as recommended by the Ministry of Health, Labor and Welfare, Japan (Okinawa Prefectural Enterprise Bureau, 2006). Water samples were pre-concentrated in the solid phase extraction cartridges (PLS-3, GL sciences, Japan). Prior to the extraction of the diuron, the columns were conditioned with 10 mL of acetonitrile, followed by methanol and milli-Q water, respectively. 10 mL of 0.2 M EDTA was added to 1 L of the water sample and pH was kept at 3.5. 1 mL of 1mg/L diuron D-6 ($C_9H_4Cl_2D_6N_2O$) was spiked as a surrogate standard in order to monitor the recovery of diuron. Water samples were eluted using an automatic solid phase extraction controller (Shimadzu, Japan) at a flow rate of 20 mL/min. PLS-3 cartridges were then dried under nitrogen gas for 5 min. Diuron was eluted from the column using 5 mL of acetonitrile. Finally, acetonitrile was evaporated to 0.2 mL with pure nitrogen gas.

2.1.3 Instrumental analysis

The analysis of diuron was achieved using LC-MS (Agilent 1100LC/MSD SL System) under the following operating conditions: Column; Agilent ZORBAX Eclipse XDB-C18 4.6 × 30 mm,1.8 μm, Column temperature,   40 ºC. Mobile phase; A, HCO_2 H/Water (0.1%), B, acetonitrile, Gradient 95% A-(liner gradient 5min)-80 % B (7 min). Flow rate; 0.5 mL min^{-1}. Injection volume 10 μl. MS conditions; Ionization mode, negative ion-ESI SIM/Scan mix mode, Desolvation gas, nitrogen 12 L min^{-1}, Desolvation temperature, 350 ºC, Capillary voltage, 2.5 kV, SIM monitor ion; m/z 231 and 237. The detection limit was 0.02 μg/L. The recovery of Diuron D-6 was > 90 % in the spiked samples. Data for the environmental samples were corrected for recovery values.

2.2 Laboratory incubation experiment

2.2.1 Coral sample preparation

A colony of coral *Galaxea fascicularis*(Fig.3) was collected from the shallow zones in front of the University of the Ryukyus Tropical Biosphere Research Center (TBRC), Sesoko Island (127º25'E26º39'N) with permission from the Okinawa Prefectural Government (# 17-04). The colony was then transported in a bucket with approximately 5 L of seawater and then transferred immediately into an open-circuit, fresh seawater aquaria exposed to sunlight through a black mesh roof. The coral colony was tagged and cut into 1.5-2.0 cm pieces that were anchored in PVC tubes on acryl resin screws. The fragments were acclimatized for ~4 weeks in the aquarium before being brought to the laboratory for the experiment.
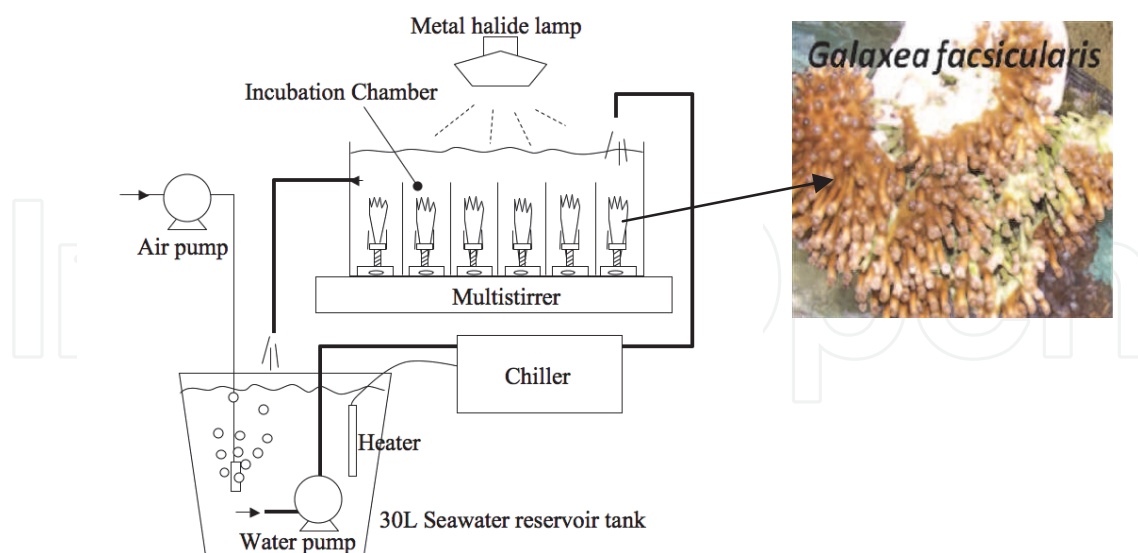
Fig. 3. Schematized diagram showing the experimental set-up (seawater circulation system).

The study was conducted in a continuous-flow seawater aquarium (15 x 30 x 20 cm^3) (Fig 3.). Seawater temperature (27 ^{o}C) was carefully controlled by Chiller (G x C-200, China) while light intensity was controlled by a metal halide lamp (Neo Beam Light 24W, KAMIHATA, Japan) which provided illumination at the coral surface at a Photosynthetic Available Radiation (PAR) of 300 µmol m^{-2} s^{-1} during a 12:12-h light/dark cycle , respectively.

Stock solutions of diuron (Sigma-Aldric, Germany) were prepared in filtered seawater using acetone (PCB and pesticide analysis grade) to improve dissolution. Another stock solution containing only acetone in filtered seawater was prepared for control treatment.

2.2.2 Exposure experiment

Corals were exposed to various treatments (0 ng L^{-1} (control), 1000 ng L^{-1}, and 10,000 ng L^{-1}) of diuron for 96 h. Six replicates of the coral *Galaxea fascicularis* were used for each treatment. Each coral were incubated in an acryl chamber (0.18 L) for a duration of 2 h. Water circulation was stopped during the incubation period. The seawater samples were collected at the beginning and at the end of incubation. The pH of seawater was recorded *in-situ* using pH meter (Orion 290 A+Thermo, USA). The total alkalinity (A$_T$) was measured using an Auto Titration System (TIM 860 Radiometer, France) within 10 days after sampling.

Changes in inorganic carbon production (IP) and organic carbon production (OP) were then determined by the alkalinity and total inorganic carbon depletion method (Smith 1973; Smith and Kinsey 1978; Fujimura et al., 2001) as follows:

$$IP = -0.5 \cdot \Delta A_T \cdot \rho \cdot V/\Delta t \cdot A \qquad (1)$$

$$OP = \Delta C_T \cdot \rho \cdot V/\Delta t \cdot A - IP \qquad (2)$$

IP= inorganic production, OP=organic production, ΔA_T= Change of total alkalinity, ρ= density of seawater, Δt= Change of incubation time, A=surface area of coral, V=volume of sea water used for coral incubation, ΔC_T=Change of total inorganic carbon. C_T was obtained from pH and alkalinity according to carbonate equilibrium as described by Fujimura et al., (2001).

2.3 Statistical analysis

Statistical analyses were performed using SPSS 16 to test for significant differences between the results of the individual treatments. Differences between doses (treatments) were tested for significance using a one-way analysis of variance (ANOVA), with an α of 0.05.

3. Results

3.1 Distribution of diuron

The levels of diuron in the samples were ranged between ND (not detected)-753.8 ng L^{-1}. The maximum concentration (753 ng L^{-1}) of diuron was found in the portion of the Todoroki river draining to Shiraho coral reefs. The level of contamination of diuron in the waters around the Ryukyu Archipelago is not as high in comparison to western Japan and other bodies of water around the world. For example, concentrations of up to 3,050 ng L^{-1} were detected in the Seto Inland Sea, (Okamura et al., 2003); 42,000 ng L^{-1}in lagoon water in Italy (Gennaro et al., 1995); 768 ng L^{-1} in the marina UK, (Boxall et al., 2000); 6,742 ng L^{-1} estuaries UK, (Thomas et al., 2001), 2,000 ng L^{-1} in Mediterranean coast, Spain, (Martinez et al., 2000), and 1,130 ng L^{-1} in Marinas Netherlands, (Lamoree et al., 2002). However, the maximum level detected in this study has exceeded the permitted maximum levels of 430 ng L^{-1} as set by the Dutch National Institute of Public Health and the Environment (Giacomazzi and Cochet, 2004). Thus, diuron could pose significant risks and general ecological health concerns.

Diuron residues showed significant spatial variations in the sampling areas (Fig. 4, Table 1). Based on the results, it is evident that the distribution reflects anthropogenic activities and possible diuron sources. The detection frequency of diuron at Naha Bay was comparable to the Shiraho reef. In addition, diuron was frequently detected at station 35 (Fig. 4), indicating an active source of diuron in Naha Bay. The frequency of diuron detection of diuron in Okinawa mainland is comparative to the coastal waters in southern California (93%) (Sapozhnikova et al., 2007).

The concentration of diuron in the Todoroki River was relatively high at upstream, ST1, 753. 8 ng L^{-1}, compared to the rest of the points towards the lagoon (Table 1). Also, relatively high concentrations of diuron were found at transect F and E at the Todoroki River mouth (Table 1). These results suggest that the Todoroki River could be a potential source of diuron from farms to the Shiraho coral reefs. In Ishigaki Island, diuron is extensively used for agricultural activities.

This research also showed seasonal variations. The concentrations of diuron in the Shiraho coral reefs were relatively higher during November (Winter) as opposed to August-September (Summer) and Spring (May-June), 2007, 2008 and 2009 (Table 1). Thus, there is a possibility that diuron is retained in the soils in summer during the maximum usage

	Spring (May 2007)	Summer (Aug, 2007)	Winter (Nov., 2007)	Spring (May, 2008)	Winter (Nov, 2008)	Winter (Jan, 2009)
A1	ND	0.62	2.07	0.23	0.26	ND
A2	ND	0.9	ND	0.36	5.43	ND
A3	ND	ND	2.18	0.31	5.8	ND
C1	ND	ND	ND	1.45	ND	ND
C2	ND	ND	ND	ND	ND	ND
C3	ND	ND	ND	0.83	19.3	ND
D1	0.88	0.87	2.25	ND	7.64	ND
D2	0.7	ND	ND	ND	ND	ND
D3	ND	ND	ND	ND	7.34	ND
E1	6.27	1.15	12.9	6.21	15.9	6.4
E2	ND	ND	0.23	ND	ND	ND
E3	ND	0.59	3.25	ND	10.2	ND
F1	1.28	ND	0.73	9.23	0.18	12.9
F2	1.61	ND	ND	ND	0.82	ND
F3	1.24	ND	15.61	ND	ND	ND
G1	0.92	0.93	ND	9.83	ND	ND
G2	0.78	1.5	ND	0.06	10.8	ND
G3	0.67	ND	ND	2.97	0.06	ND
X1	ND	ND	ND	ND	ND	ND
X2	ND	1.34	ND	1.61	ND	ND
X3	ND	0.72	ND	ND	ND	ND
Y1	ND	ND	24.2	3.97	2.81	ND
Y2	ND	ND	ND	ND	ND	ND
Y3	ND	ND	**90.02**	ND	0.92	ND
Z1	ND	ND	39.07	ND	ND	ND
Z2	ND	ND	8.48	1.48	ND	ND
Z3	ND	ND	ND	ND	7..22	ND
	Todoroki River					
ST1	12.5	NS	NS	68.6	**753.8**	NS
ST2	1.2	NS	NS	3.06	37.6	NS
ST3	1.7	NS	NS	7.64	50.1	NS
ST4	NS	NS	NS	NS	42.8	NS
ST5	NS	NS	NS	NS	11.2	NS
ST6	NS	NS	NS	NS	25.2	NS
ST7	NS	NS	NS	NS	21	NS
ST8	NS	NS	NS	NS	10.3	NS
ST9	NS	NS	NS	NS	77.7	NS
ST10	NS	NS	NS	NS	67.8	NS

NS means No Sample;   ND means Not Detected

Table 1. Spatial and temporal variation of diuron around Shiraho coral reefs (ng L^{-1}).

(summer) in the region and released to the aquatic systems during high rainfall during December (Winter). The retention of diuron in the soils is may due to the fact that it has a long half-life 43-2180 days (Sapozhnikova et al., 2007). In Naha Bay, relatively high levels were detected in September compared to other months (Fig. 4). This season coincides with the high boating season in Okinawa Island, thus suggesting that the main source of diuron is from antifouling paints released from ships. Okamura et al., (2003) found considerable levels of diuron inside the fishing ports in Western Japan.
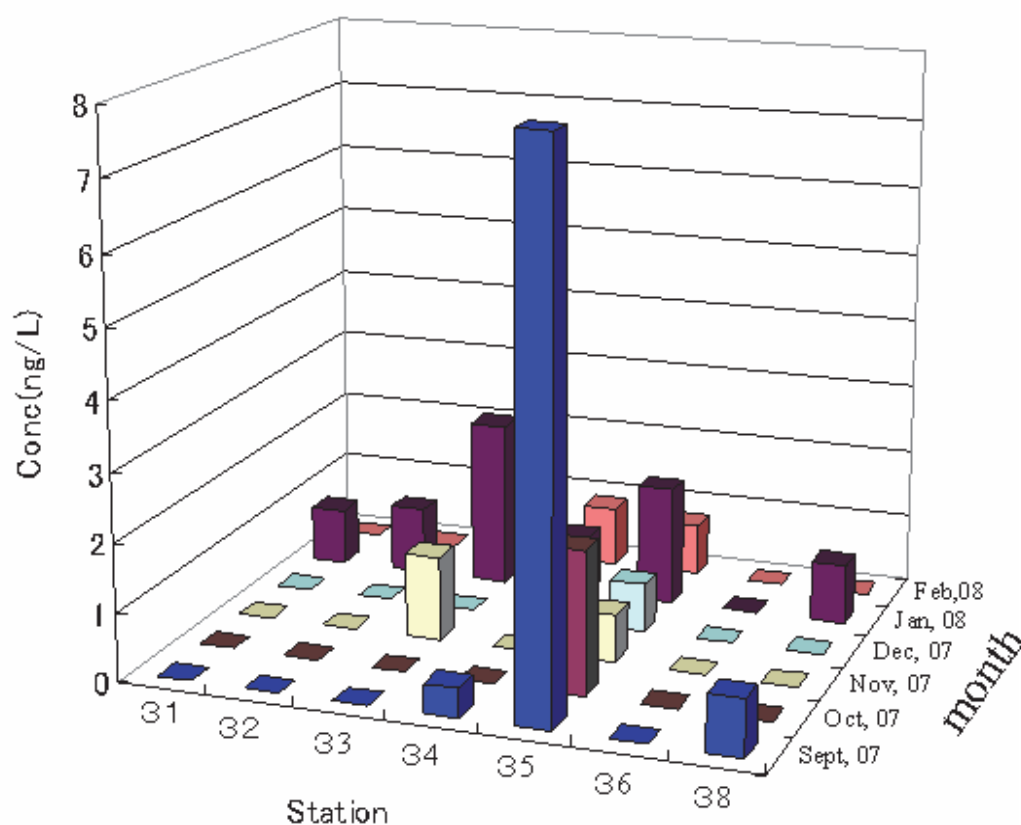
Fig. 4. Spatial and temporal distribution of diuron in Naha Bay (Sep. 2007-Feb. 2008).

3.2 Effect of diuron on coral metabolism

3.2.1 Effects of diuron on photosynthesis rate

Photosynthesis was significantly reduced (ANOVA $p < 0.05$) when the coral was exposed to 10,000 ng L^{-1}. Diuron concentrations of 10,000 ng L^{-1} caused rapid decrease in photosynthesis after 96 h exposure (Fig. 5). The results show that 1,000 ng L^{-1} reduced photosynthesis 6.5 % relative to the control but was not significant (ANOVA $p > 0.05$). The photosynthesis was gradually decreased from control, 1000 ng L^{-1} to 10,000 ng L^{-1} (Fig. 5).

3.2.2 Effects of diuron on calcification rate

Diuron had a significant impact on calcification rate only at the highest concentration of diuron (10,000 ng L^{-1}) (ANOVA $p < 0.05$), the calcification rate dropped to 67.3 % less than the control (Fig. 5).
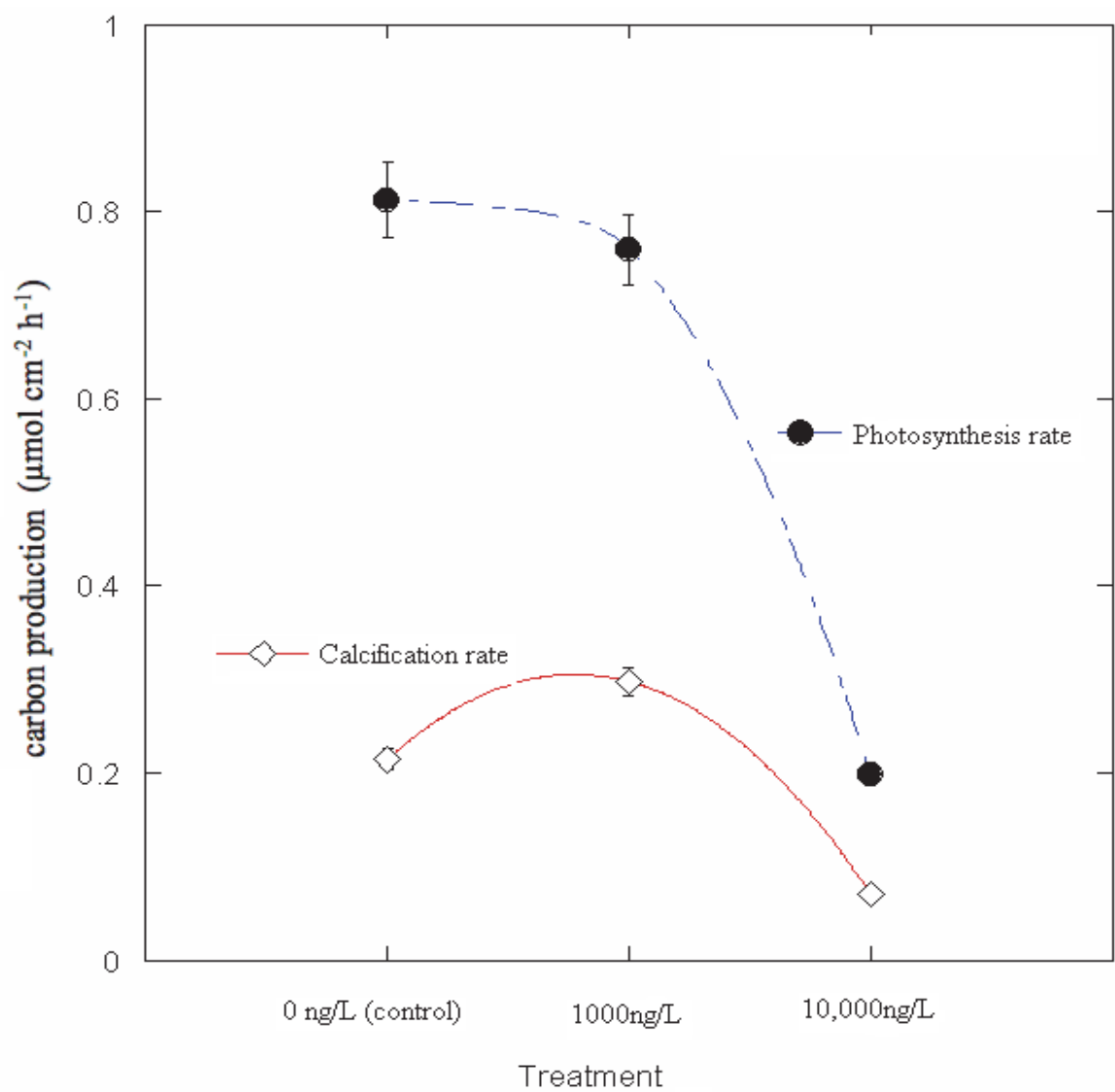
Fig. 5. Effects of diuron on calcification and photosynthesis.

Diuron (DCMU) inhibits photosynthesis by blocking electron transport in phosystem II (PSII), eventually causing immediate disruption in the symbiosis between zooxanthellae and host coral (Råberg et al., 2003). When temporarily bound, they can disrupt photosynthetic electron flow, and can eventually lead to a loss of excitation energy at photosystem reaction center (Jones and Kerswell, 2003).

Our results clearly show that the photosynthesis rate gradually decreased when corals were exposed to treatments of 1,000 and 10,000 ng L^{-1} of diuron. These results are supported by previous studies by Jones & Heyward (2003) and Owen et al. (2002) which also showed that diuron inhibit the photosynthesis of corals by blocking the conversion of excitation energy into chemical energy (Jones, 2005).

The calcification of corals is controlled by a mutualistic relationship with zooxanthellae. The zooxanthellae utilize the waste products of a host such as CO_2 from respiration (Liat et al., 2005). Our results demonstrate that a 10,000 ng L^{-1} treatment of diuron caused a sharp decrease in the calcification of *Galaxea fascicularis* after 96 h exposure. This may be caused by

the disruption of symbiosis between zooxanthellae and the host coral. Diuron might also block the energy which may be needed to trigger calcium uptake from the seawater coeleteron of coral *Galaxea fascicularis* (Al-Horani et al., 2003). These findings suggest that the deterioration of calcification rate might be caused by the blockage of energy transfer by the PSII compounds. This is attributed to a study conducted by Chalker and Taylor (1975), which showed that energy is needed for the calcification process to transport ions and for the formation of organic matrix.

4. Potential eco-toxicological effects of diuron on coral reefs

The average levels of diuron detected around the Ryukyu Islands including the Shiraho coral reef waters were more than three-fold less than the lowest observable effects concentration (LOEC) to corals as shown by laboratory eco-toxicological studies for example Reduction of $\Delta F/F`$ in *Stylophora pistillata* 250 μg L^{-1} (Jones & Heyward., 2003); Loss of algae in *Pocillopora damicornis* 30,000 ng L^{-1} (Negri et al., 2004); reduction of respiration in *Porites cylindrica* 10,000 ng L^{-1} (Råberg et al., 2003); Loss of algae in *Seriotopora hystrix* 10,000 ng L^{-1} (Jones , 2004).

These results suggest that the present contamination of diuron in the coastal waters around the coral reefs and adjacent areas in the Ryukyu Archipelago are not at an alarming stage, and does not seem to pose a serious threat to the health of corals during acute exposure. However, it is important to highlight that the maximum value of diuron concentration (753 ng L^{-1}) detected has already approached the threshold limit, and thus could have a significant risk on corals in the future, solely or synergistically. It remains uncertain in the field of marine science how chronic exposure to the environmentally relevant concentrations of diuron coupled with other environmental stressors such as acidification, nutrients, temperature and sedimentation will affect corals.

5. Conclusions

Based on the findings of our investigation, we may conclude the following;

5.1 The results show that, presently, low background levels of diuron contamination (<1, 000 ng L^{-1}) have no detectable impacts on the survival of coral reefs at present. However, in order to maintain the coral health, the risk studies for hazardous chemicals, including diuron, should remain a matter of concern.

5.2 Agricultural activities, such as those involved with sugarcane plantations, significantly contribute to diuron contamination in coral reefs around this region rather than antifouling paints from ships.

5.3 The concentration of diuron of 1,000 ng L^{-1} effectively reduces photosynthesis rate of coral under short-term exposure but does not seem to affect calcification rate.

5.4 According to environmental concentrations of diuron reported in elsewhere, it is possible that the rate of photosynthesis in corals will be inhibited in the current study sites and alike.

5.5 Further scientific investigations regarding for long-term exposure to novel antifouling chemicals, including diuron, to corals are still needed in order to implement appropriate monitoring and guideline levels of these in coastal uses.

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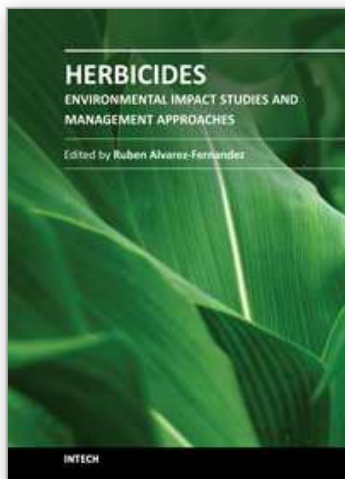
Herbicides - Environmental Impact Studies and Management Approaches

Edited by Dr. Ruben Alvarez-Fernandez

Weeds severely affect crop quality and yield. Therefore, successful farming relies on their control by coordinated management approaches. Among these, chemical herbicides are of key importance. Their development and commercialization began in the 1940's and they allowed for a qualitative increase in crop yield and quality when it was most needed. This book blends review chapters with scientific studies, creating an overview of some the current trends in the field of herbicides. Included are environmental studies on their toxicity and impact on natural populations, methods to reduce herbicide inputs and therefore overall non-target toxicity, and the use of bioherbicides as natural alternatives.

How to reference

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