Ecological Indicators 76 (2017) 304–316

Contents lists available at ScienceDirect

Ecological Indicators

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

Comparative assessment of single and joint effects of diuron and Irgarol 1051 on Arctic and temperate microalgae using chlorophyll *a* fluorescence imaging

Sreejith Kottuparambil[a,b], Murray T. Brown[c], Jihae Park[d,e], Soyeon Choi[d], Hojun Lee[f], Han-Gu Choi[g], Stephen Depuydt[e], Taejun Han[f,h,*]

[a] *Red Sea Research Center, Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia*
[b] *Institute of Green Environmental Research Center, 169, Gaetbeol-ro, Yeonsu-gu, Incheon 21999, Republic of Korea*
[c] *School of Biological and Marine Sciences, Plymouth University, Drake Circus, Plymouth PL4 8AA, United Kingdom*
[d] *Division of Life Science, Incheon National University, 119, Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea*
[e] *Lab of Plant Growth Analysis, Ghent University Global Campus, Songomunhwa-Ro, 119, Yeonsu-gu, Incheon 21985, Republic of Korea*
[f] *Department of Marine Science, Incheon National University, 119, Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea*
[g] *Korea Polar Research Institute, 26, Songdomirae-ro, Yeonsu-gu, Incheon 21990, Republic of Korea*
[h] *Ghent University Global Campus, Songomunhwa-Ro, 119, Yeonsu-gu, Incheon 21985, Republic of Korea*

ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form 21 January 2017
Accepted 22 January 2017
Available online 6 February 2017

Keywords:
Chlamydomonas
Chlorella
Diuron
Irgarol 1051
Toxicity
Polar
Temperate

ABSTRACT

Ship groundings and ice-breakers can cause pollution of the polar environment with antifouling biocides such as diuron and Irgarol 1051. The present study used pulse amplitude modulated fluorometry to compare single and joint toxicities of diuron and Irgarol 1051 on two freshwater taxa of microalgae (*Chlorella* and *Chlamydomonas*) originating from Arctic and temperate regions. 30 min acute toxicity tests using chlorophyll *a* (Chl *a*) fluorescence revealed that Arctic strains of microalgae were more sensitive to herbicides than their temperate counterparts. Diuron and Irgarol 1051 had equal toxicities in the Arctic species, while Irgarol 1051 was more toxic (EC_{50} = 5.55–14.70 μg L^{-1}) than diuron (EC_{50} = 12.90–>40 μg L^{-1}) in the temperate species. Toxicity assessment of various mixtures of diuron and Irgarol 1051 revealed antagonistic, additive, and synergistic effects. Our data suggest that herbicides can adversely affect photosynthesis in Arctic microalgae at relatively low levels, and their impact can increase under complex mixture conditions.

1. Introduction

Arctic ecosystems represent some of the most extreme environmental conditions on the planet. Nevertheless, they contribute significantly to global primary production and represent over 11% of the world's organic matter pool (Callaghan and Maxwell, 1995). The primary production rates per day measured in some Arctic algal blooms (carbon at >5 g M^{-2}) rank among the highest values recorded anywhere (Sakshaug, 2003).

Arctic ecosystems may be much more susceptible to biological damage at low levels of pollutants than higher-energy ecosystems in temperate latitudes (CARC, 1990). Even though current levels of pollution are considerably lower than in most urban and industrialized areas in the mid-latitudes, trans-boundary pollution appears to have been increasing over the past few decades (ACIA, 2005). Recently, organochlorine pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers, and heavy metals have been detected in the Arctic environment (Moiseenko, 1999; Garbarino et al., 2002; Hung et al., 2010), and trace amounts (<4 ng L^{-1}) of dimethyl tetrachloroterephthalate (DCPA), a selective pre-emergent herbicide, have been detected in northwest Alaskan Arctic estuaries (Garbarino et al., 2002).

Increased shipping activities in polar water bodies have contaminated the water with toxic biocides, such as the herbicides added

Abbreviations: Chl, chlorophyll; F_0, minimum fluorescence yield of dark adapted samples; F_m, maximum fluorescence yield of dark adapted samples; *F*, steady state fluorescence yield; F_v/F_m, maximum quantum yield of PSII; Y(II), Effective or operational quantum yield of PSII; NPQ, non-photochemical quenching; $rETR_{max}$, maximum relative electron transport rate; ML, measuring light; PS II, photosystem II; QA, plastoquinone A; QB, plastoquinone B; EC_{50}, 50% effect concentration; EC_{10}, 10% effect concentration; LOEC lowest observed effect concentration; NOEC, no observed effect concentration; CI, confidence interval; CV, coefficient of variation.

* Corresponding author at: Department of Marine Science, Incheon National University, 119, Academy-ro, Yeonsu-gu, Incheon 22012, Republic of Korea.
E-mail address: hanalgae@hanmail.net (T. Han).

http://dx.doi.org/10.1016/j.ecolind.2017.01.024
1470-160X/

to antifouling paints used on ship hulls to control unwanted plant growth. Ship groundings and ice-breakers have been identified as major sources of these herbicides, and the contamination is associated with the paint being abraded from vessel hulls and leading to patchy but locally intense pollution (Negri and Marshall, 2009). Diuron and Irgarol 1051 are the most commonly used herbicides in antifouling paints (Konstantinou and Albanis, 2004). Diuron is a substituted urea that is classed as a broad-spectrum general-use herbicide; it is widely used for pre- and post-emergence control of annual grasses and broad-leaved weeds in agricultural lands. Irgarol 1051, a symmetrical triazine compound, is the first booster biocide to gain prominence as an environmental contaminant (Konstantinou and Albanis, 2004). Phytotoxic effects of diuron and Irgarol 1051 have been well documented, although most aquatic studies have focused only on marine environment and organisms (Buma et al., 2009). Recent studies suggest that these biocides can also inhibit the growth and photosynthetic activity of freshwater algae and change the phytoplankton community structure (Deng et al., 2012a,b). Although the compounds are from different chemical families, they have a similar effect on photosystem (PS) II, the integral component of the photosynthetic process (Dafforn et al., 2011). Specifically, they disrupt the electron transport between PSI and PSII by replacing the plastoquinone B (Q_B) on the acceptor side, which in turn disrupts the synthesis of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP), thereby adversely affecting the growth rate (Voulvoulis et al., 1999). They can also affect periphyton productivity and species composition (Mohr et al., 2008) and accumulate inside freshwater autotrophs (Tóth et al., 1996). Irgarol 1051 and diuron have been reported to occur together in natural waters (Thomas et al., 2001), and they may have a greater negative impact together than present separately. Such joint toxic effects of multiple chemicals are an important consideration in the ecotoxicological assessment of herbicides.

Recently, pulse amplitude modulated (PAM) fluorometry and the saturation pulse technique have served as reliable analytical tools in the rapid ecotoxicological assessment of PSII herbicides for marine and freshwater microalgae (van der Heever and Grobbelaar, 1998; Magnusson et al., 2008; Kottuparambil et al., 2013). PSII-inhibiting herbicides reduce the overall photosynthetic efficiency of the target plants or algae, and this reduction can be estimated by measuring the *in vivo* Chl *a* fluorescence.

The objective of the present study was to investigate the individual and joint toxicities of diuron and Irgarol 1051 in unialgal cultures of two genera of freshwater microalgae (*Chlorella* and *Chlamydomonas* spp.), isolated from the Arctic and temperate regions. We hypothesize that the actual toxic impacts of these herbicides as a heterogeneous mixture in the freshwater ecosystems could be alarming. Despite recent advances in the taxonomy, genetics, and ecology of polar microalgae, studies addressing the effect of aquatic contaminants on their ecophysiology are lacking. The algal taxa used in this study are assumed to be adapted to their respective environments and have therefore evolved a particular genome. This will possibly restrict the relevance of the observation onto a broad spectrum of photosynthetic organisms in the Arctic and temperate regions. However, to the best of our knowledge, this is the first comparative assessment of PSII herbicides in freshwater microalgae from Arctic and temperate regions.

2. Materials and methods

2.1. Algal test species and culture conditions

Arctic strains of *Chlorella* sp. (KOPRI–ArF0004) and *Chlamydomonas* sp. (KOPRI–ArF0006) were provided by the Korea Polar Research Institute (KOPRI). Temperate *Chlorella vulgaris* (KMMCC–FC0001) and *Chlamydomonas segnis* (KMMCC–FC0035) were obtained from the Korea Marine Microalgae Culture Center (KMMCC). The tested algae were grown in 250 mL Erlenmeyer pyrex glass flasks containing 100 mL of Bold's basal medium (Bischoff and Bold, 1963). All strains were maintained under standard conditions (at pH 6.8 under a photosynthetic photon flux density of 40 $\mu mol\ m^{-2}\ s^{-1}$ with a 16:8 light-dark cycle) at 5 °C and 20 °C for Arctic and temperate strains, respectively. Exponentially growing cells were harvested and resuspended in fresh medium for treatment with herbicides.

2.2. Test chemicals and solutions

Analytical grade diuron ($C_9H_{10}Cl_2N_2O$, CAS no. 330-54-1) and Irgarol 1051 ($C_{11}H_{19}N_5S$, CAS no. 28159-98-0) were purchased from Sigma Aldrich (Sigma Aldrich, USA). Stock solutions ($10\ mg\ L^{-1}$) were prepared in acetone-rinsed glassware using dimethylsulfoxide (HPLC grade) as a carrier solvent because diuron and Irgarol 1051 have relatively low water solubility. These stock solutions were tightly capped and stored in the dark at 5 °C. Concentration series of herbicides in Bold's basal medium were prepared by serial dilution immediately prior to each experiment. The concentration of carrier solvent did not exceed 0.1% v/v of the test culture volume.

For diuron and Irgarol 1051, the following sets of concentrations were used for the toxicity test solutions: 0.625, 1.25, 2.5, 5, and 10 $\mu g\ L^{-1}$ and 2.5, 5, 10, 20, and 40 $\mu g\ L^{-1}$, for the Arctic and temperate microalgae, respectively.

2.3. Measurement of Chl a fluorescence

Chl *a* fluorescence imaging of microalgae suspensions was done using the Maxi Imaging PAM Chlorophyll Fluorometer (Max–IPAM, Heinz Walz GmbH, Germany). Optimal instrument settings (ML intensity 3 and ML pulse frequency 8, corresponding to an integrated photosynthetically active radiation photon flux density of 1 $\mu mol\ m^{-2}\ s^{-1}$) were used to measure the initial minimal fluorescence yield (F_0) of the dark-adapted algal suspension and maximal fluorescence yield (F_m) on a saturation pulse followed by repetitive measurements of the Chl *a* fluorescence parameters (actinic light intensity 36 $\mu mol\ m^{-2}\ s^{-1}$), steady state fluorescence yield (F), and maximum fluorescence yield ($F_{m'}$) by saturation pulse of light (Schreiber et al., 2007). The maximal PSII quantum yield of the dark-adapted cells (F_v/F_m) was given by (F_m-F_0)/F_m. Effective or operational quantum yield (Y(II)) of PSII is given by the ratio ($F_{m'}$-F)/$F_{m'}$ (Genty et al., 1989). The non-photochemical quenching (NPQ) and relative electron transport rate (rETR) were also calculated following the formula reported by Schreiber et al. (1995).

2.4. Individual toxicity testing

Multi-well microplate toxicity tests were conducted in which microalgae suspensions were exposed for 30 min to a range of concentrations of diuron and Irgarol 1051 in black polypropylene 96-well plates (well dimension of 6.3 mm; SPL Life Sciences, Korea) and incubated under the optimal environmental conditions previously described. The strength of the cell suspension was selected as the lowest possible cell density emitting a steady state fluorescence signal between 0.08 and 0.12 units at a low gain setting in imaging PAM (Schreiber et al., 2007). An incubation time was set based on ensuring maximum toxicity for all the wells across the plate and minimizing the influence of different exposure times across the plate (Muller et al., 2008). At the end of incubation period, the plates were subjected to Chl *a* fluorescence measurements. All treatments were done in triplicate.