http://dx.doi.org/10.5935/0103-5053.20130289

J. Braz. Chem. Soc., Vol. 25, No. 2, 380-388, 2014.
Printed in Brazil - ©2014 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Short Report

First Appraisal of Water Contamination by Antifouling Booster Biocide of 3rd Generation at Itaqui Harbor (São Luiz - Maranhão - Brazil)

Lia Gracy R. Diniz,[a] Marcelle S. Jesus,[a] Luis Alberto E. Dominguez,[b] Gilberto Fillmann,[b] Eny M. Vieira[c] and Teresa Cristina R. S. Franco[,a]*

[a]*Departamento de Tecnologia Química, Universidade Federal do Maranhão, 65085-580 São Luís-MA, Brazil*

[b]*Instituto de Oceanografia, Universidade Federal do Rio Grande, 96201-900 Rio Grande-RS, Brazil*

[c]*Instituto de Química de São Carlos, Universidade de São Paulo, 13560-970 São Carlos-SP, Brazil*

Diuron e Irgarol são anti-incrustantes que, a despeito das evidências de suas toxicidades para várias espécies marinhas, ainda são utilizados em pinturas de embarcações e estruturas marítimas. No caso do Irgarol, já existe restrição de uso em vários países. Neste estudo, que é o primeiro registro dos biocidas na região caracterizada como sendo uma das maiores extensões contínuas de manguezal em todo o mundo, a presença dos biocidas foi investigada durante dois anos, em um porto de intenso tráfego marítimo (Porto do Itaqui, nordeste do Brasil). Com intuito de selecionar o método analítico com sensibilidade e seletividade apropriadas, cromatografia líquida com detecção por conjunto de diodos (HPLC-DAD) e cromatografia líquida com detecção por espectrometria de massas em interface electrospray (LC-ESI-MS/MS) foram devidamente validados e comparados. Em ambos os métodos as amostras foram previamente concentradas em cartuchos de extração em fase sólida. LC-ESI-MS/MS foi o método selecionado em razão da melhor sensibilidade. Diuron e Irgarol foram identificados, respectivamente, em treze e dezenove das vinte e quatro amostras coletadas, em concentrações que variaram de 0,05 a 7,80 μg L^{-1} e de 0,01 a 4,80 μg L^{-1}, respectivamente. Tais concentrações são ambientalmente relevantes e potencialmente prejudiciais aos organismos aquáticos do ambiente em estudo.

Diuron and Irgarol are antifouling booster that, despite evidences of their toxicities to various marine species, are still being used in paints of boats and marine structures. Irgarol has already been restricted in some countries though. In the present study, which is the first record of the antifouling biocides in this region characterized as one of the largest continuous mangrove area worldwide, the presence of both biocides was investigated during two years in surface water of a Port with intense maritime traffic (Itaqui Harbor, Northeastern Brazil). In order to select the analytical method with suitable sensitivity and selectivity, liquid chromatography with diode array detection (HPLC-DAD) and liquid chromatography with electrospray interface tandem mass spectrometry (LC-ESI-MS/MS) were properly validated and compared. For both methods, samples were previously concentrated into solid phase extraction cartridges. LC-ESI-MS/MS was the selected method due to better sensibility. Diuron and Irgarol were identified, respectively, on thirteen and nineteen of the twenty-four samples and concentrations ranged from 0.05 to 7.80 μg L^{-1} and from 0.01 to 4.80 μg L^{-1}, respectively. Such concentrations are environmentally relevant and might cause potential harm to aquatic organisms of the studied environment.

Keywords: booster biocides, brazilian harbor, antifouling paints, diuron, irgarol.

Introduction

Antifouling paints have long been used on boat hulls to prevent the growth of bacteria, macroalgae, mussels, barnacles and other invertebrates that compromise the mobility of the vessels, thus increasing fuel consumption.[1,2] Firstly based only on copper and zinc oxides, paint manufacturers have developed formulations containing organic biocides as booster agents for a broad spectrum of activity. In the early 60's, triphenyltin (TPT), tributyltin (TBT) and other

*e-mail: teresant@ufma.br

organotin derivatives were added to improve efficiency, but these second-generation of antifouling paints[3,4] were highly toxic and were internationally banned since January of 2008.[5] Because of the restrictions on organotin compounds, the antifouling paint industry sought new alternatives and created a third generation of biocides, including 20 substances, such as non-metallic organic compounds (Irgarol, Diuron, Sea-nine 211, Dichlofluanid, Chlorothalonil, Thiram, Busan, Densil, Pyridine-triphenylborane, Capsaicin, Econea, Medetomidine and Tolylfluanid), organometallic compounds (Copper pyrithione, Copper naphthenate, Zinc pyrithione, Zineb and Maneb) and inorganic substances (Oxides and Copper thiocyanate).[1,6-8]

Current antifouling compounds are often used in mixtures of up to five components with copper and zinc in combination of, at least, one booster biocides of third generation. Among the used biocides are Diuron and Irgarol, although Dichlofluanid and Chlorothalonil have been also currently cited, while Sea-nine 211, Zinc pyrithione and Zineb are applied, but to a lesser extent.[7-11] However, precise information about quantities and types of the most used biocides around the world or even at national territory are scarce. Several countries from Europe and the United States have even restricted the use of some of these biocides in antifouling paints.[4,12] Thus, it is important to obtain data on the occurrence of the biocides on natural environments.

Irgarol 1051 (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine) was first used in antifouling paints in late 1980s.[5] It is a s-triazine compound that inhibits efficiently the electron transport in the photosynthetic system (PSII), thus causing oxidative stress of chlorophyll and consequent cell necrosis.[9,12] Irgarol is more toxic than some triazines that act in a similar manner, such as atrazine and simazine.[5,6,13,14] The biocide acts more effectively against freshwater and seawater algae and less effectively against animal organisms. Even though Irgarol presents higher toxicity for various microalgae (EC_{50} ranging from 0.45-2.12 μg L^{-1}) significant toxicity is also observed for other aquatic organisms, for example, crustaceans ($LC_{50\text{-}96h}$ = 0.4 mg L^{-1} for mysid shrimp) and fishes (LC_{50} = 0.4 mg L^{-1} for rainbow trout).[14] As showed at Table 1, Irgarol presents relatively low water solubility and high organic carbon partition coefficient, which suggests higher tendency to be retained in soils and sediments with high organic content, such as mangroves.[5,14] Despite this, the biocide has been mainly detected in surface waters from marinas and harbors areas.[1,6,7,11,12] Irgarol is also not easily biodegradable and half-lives between 1 to 3 months have been reported in fresh or salt waters.[5,7,15,16] Several studies on aquatic plant communities have demonstrated Irgarol acute toxicity and bioaccumulation in macrophytes, phytoplankton and periphyton.[5,6,13,14,16] Bao *et al.* observed that Irgarol was even more toxic than TBT on the growth of autotrophic species.[13] In a study carried out by Dyer *et al.* with the green alga *Tetraselmis suecica* at sub-lethal concentrations, bioconcentration factors (BCF) between 15,000 and 80,000 were observed, thus suggesting a possible pathway for the Irgarol uptake into marine webs.[16]

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a substituted-urea herbicide used since 1950's which inhibits photosynthesis and limits the production of high-energy compounds such as adenosine triphosphate (ATP).[17] The herbicide is cited as one of the most used antifouling paint booster, however it also has agricultural and non-agricultural applications, so it is difficult to estimate its contribution mainly in freshwater and seawater from ports and marinas located near to estuarine areas.[12] The biocide is relatively persistent in seawater, with half-lives ranging from 43 to 2,180 days at pH 7.0 and 25 ºC (Table 1).[17] Under aerobic conditions, it degrades through N-demethylation to form DCPMU (3-(3,4-dichlorophenyl)-3-methylurea), DCPU (3,4-dichlorophenylurea) and DCA (3,4-dichloroaniline).[15,17,18] Diuron has relatively low octanol-water and organic carbon partition coefficients (log K_{ow} = 2.87 and log K_{oc} = 2.75), which indicates a low tendency for retention in either soil or sediment and high water mobility, consequently with high potential for contamination of marine organisms.[12,16,19] The Diuron toxicity for photosynthetic aquatic biota has been widely demonstrated.[1,12,19] However, the biocide has also been proven to be toxic for the crustacean *Daphnia magna* and for fish species on their different stages of life.[12]

Table 1. Physical-chemical properties and toxicity of antifouling biocides

Anti fouling	Water solubility / (mg L^{-1})	Log K_{ow}	Log K_{oc}	H / (atm m^3 mol^{-1})	$t_{1/2}$ / days	Acute toxicity on marine microorganism[c] $EC_{50\text{-}96h}$ / (μg L^{-1})
Diuron	36.4	2.81-2.87	2.62-2.75	5.1×10^{-10}	372-995[a] 43-2180[b]	5.9-27
Irgarol	7.0	4.1	3.14	5.9×10^{-9}	502-956[a] 30-90[b]	0.6-1.1

[a]half-life in soil; [b]half-life in water; [c]obtained for the green algae *Dunaliella tertiolecta* and the diatom *Navicula forciopata*.

Mangroves are coastal forest typical from tropical and subtropical regions.[20] They act as natural nurseries for many species, producing and purifying large amounts of organic matter in these environments.[21] Mangrove ecosystem is also important for coastal food chain due to its high primary production. Regarding to their ecological relevance, mangroves must be preserved and monitored since any impact on these environments will be reflected throughout the marine food chain.[20,21] Nevertheless, mangrove ecosystem has been exploited by several coastal human activities such as harbor and marinas. In these environments, antifouling paints could represent a serious impact. For instance, the toxicity of Irgarol and Diuron on macrophytes and other relevant species related to the primary production on marine ecosystems have been demonstrated at concentrations close to that found in monitoring studies.[4,10,12,19]

Due to relevant toxicities for aquatic marine organisms, Diuron and Irgarol have been mainly determined on water samples.[6,8,12,15,18,22] High performance liquid chromatography with diode array detection (HPLC-DAD) has suitably been employed to determine both biocides.[15,22,23] Gatidou *et al.* had developed and validated a robust method for Diuron, Irgarol and their metabolites by using solid phase extraction (SPE) followed by HPLC-DAD.[18] They obtained satisfactory analytical conditions to determine such biocides and their metabolites into sea water samples collected from the Greek marine environments.[18] Biocides concentrations as low as 0.03 μg L^{-1} could be observed, with limits of detection of 0.007 μg L^{-1} and 0.011 μg L^{-1} for Diuron and Irgarol, respectively, considering a concentration factor of 500 and injection volume of 100 μL. Recently, Rodriguez *et al.* also determined Diuron and Irgarol on harbors of the Gran Canaria Island, Spain, in concentration ranging from 0.044 to 0.1 μg L^{-1} and 0.033 to 0.046 μg L^{-1}, respectively, by using SPE- HPLC-DAD.[23] The limits of detection of the applied method, considering the concentration factor of 1,000 and a volume of injection of 20 μL, were 0.038 and 0.031 μg L^{-1}, respectively, by using an injection volume of 30 μL of the concentrated extract.[23] When using on-line extraction procedure, all the extract was directly detected and enhanced sensitivity was obtained.[18,23]

Nevertheless, most of the recent works have applied SPE followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) for both determination of Diuron as Irgarol in seawater samples collected from ports and marinas around the world.[24-26] By using this method, high concentrations for Diuron were observed in harbors and marinas of the Gulf of Napoli (0.173 μg L^{-1}) while for Irgarol, the highest concentration was found in East Anglia, UK (2.43 μg L^{-1}).[24] Biocides were concentrated using C-18 SPE cartridges and, with concentration factors close to 1,000 times, the limits of detection for Diuron and Irgarol were 0.008 μg L^{-1} and 0.001 μg L^{-1}, respectively.[10]

To our knowledge, there is no record about the presence of Diuron and Irgarol in Brazilian ports and marinas located on mangrove areas. Thus, the objective of the present work is to provide information, for the first time, on the presence of the antifouling booster biocides Diuron and Irgarol in an area of intense maritime traffic located in the northeastern Brazil. Seawater samples were chosen because, once identified the presence of such biocides, it would be possible to compare with a more representative number of records of the compounds in various parts of the world. Furthermore, since the booster biocides are considerably persistent in water and Diuron has been mainly found in the dissolved phase, we chose to investigate the presence of both biocides in surface seawaters. Before the environmental study, a survey was made on the analytical methods suitable for determining the biocides in seawater samples. High performance liquid chromatography with diode array detection (HPLC-DAD) was compared with liquid chromatography with electrospray interface tandem mass spectrometry (LC-ESI-MS/MS). On both methods the pre-concentration of the water samples were made by using solid phase extraction cartridges. The methods were validated and compared in order to decide which was more suitable for the proposed study.

Experimental

Chemicals and reagents

Analytical standards of Irgarol 1051 and Diuron (purity > 99%) were purchased from Riedel-de-Häen (Germany) and Sigma Aldrich (USA), respectively. Stock solutions containing 100 μg mL^{-1} were separately prepared in methanol and stored at –18 °C until use. Working solutions were monthly prepared while new analytical curves were prepared for each new batch of samples to be analyzed. HPLC-grade organic solvents (methanol and acetonitrile) and water were obtained from Merck (Germany). Formic acid (purity 98-100%) was also obtained from Merck (Germany) and synthetic sea salt from Red Sea Salt (Germany). The SPE cartridges (500 mg of C-18, 6 mL of volume capacity) were purchased from Phenomenex (USA).

Description of study area

The Port Complex of São Luis (Figure 1) consists of the Port of Itaqui and two terminals of private companies. It is situated in the northeastern coast of Brazil and is

one of the most extensive in the country, with a 1,936 m long wharf and depths between 9.5 and 19 m according to the tide, which provides maneuverability to deep draft vessels. This is the closest harbor from the European and American markets, trading several raw materials and manufactured products. Due to enhancements made by state-owned and private terminals, the maritime traffic in the port complex has grown considerably in recent years, reaching an average of 1,295 ships/year.[21] In addition, the harbor is known for being located in a large mangrove forest of São Luís Island, which constitutes, along with the northeastern coast of Pará, the world largest continuous mangrove system. This system is known as the Macro-Tides Mangrove Coast of the Amazon (*Costa de Manguezais de Macromaré* da Amazônia - CMMA).[20] The macrotidal regime is semi-diurnal with amplitudes ranging between 4 and 7 meters. The climate is tropical, warm and humid, with mean air temperature of approximately 26 °C. Annual mean precipitation is about 2,300 mm, however there is a well-defined dry season, which occurs between July and December, when monthly mean precipitation is typically less than 50 mm.[20,21]

Sample collection and solid phase extraction procedure

About 2.5 L of subsurface seawater samples were taken during high tide, from six locations along the Port of Itaqui (São Luís, Maranhão, Northeastern Brazil). Samples were collected twice at rainy (April 2010 and 2011) and twice at dry season (November 2010 and 2011) (Figure 1). Five sites were selected as representative areas of intensive maritime traffic, while one was located at an internal harbor area, near to the mangrove in an internal area of the harbor mangrove area with no vessels in the vicinity. During sampling, temperature, salinity and pH of seawater were measured by using a multiparameter instrument (Hanna, HI 9828). The samples were collected in pre-cleaned amber glass bottles, kept at 4 °C and transported to the laboratory where they were immediately filtered using a glass fiber filters (pore size 0.45 µm, Millipore, USA). Solid phase extraction was performed as previously described in Gatidou *et al.*[18] with minor modifications. SPE cartridges of C-18 were conditioned with 10 mL of methanol and 10 mL of ultrapure water. For HPLC-DAD analysis, aliquots of 1 L of seawater samples were loaded through the cartridges (4 mL min^{-1}) and, after that, the analytes were eluted with 6 mL of methanol. The eluates were concentrated by rotary evaporator (35 °C), dried under a gentle stream of nitrogen gas (purity > 99.999%, White Martins, Brazil) and reconstituted with 1 mL of methanol. For LC-MS/MS, 250 mL of seawater samples were passed through the SPE cartridges (10 mL min^{-1}), followed by clean-up with 4 × 2.5 mL of ultrapure water and dried with air flow for 10 min. After that, the analytes were eluted with 3 × 2 mL of methanol; the volume was reduced with a gentle stream of nitrogen gas and reconstituted to 1 mL of methanol.

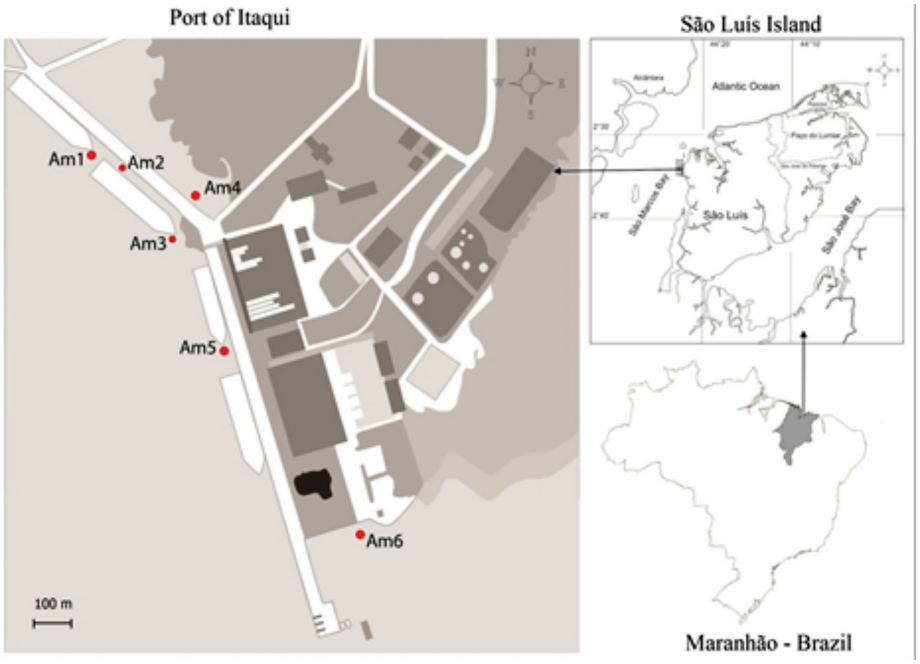

Figure 1. Sampling sites at Port of Itaqui (São Luís, Maranhão, Brazil). Six sampling sites where selected along the port, including areas with intense maritime traffic and at internal mangrove area (Am4), in which there are no boats. Subsurface seawater samples were collected at distinct seasonal periods, from 2010 to 2011.

Chromatographic methods

HPLC-DAD

For HPLC-DAD analysis, a Varian ProStar 210 (USA) equipped with a binary gradient pump, a Rheodyne (USA) 20 μL manual loop injector and a PDA detector (Varian ProStar 335, USA) was employed. Analytical C-18 column (150 × 4.6 mm, 5 μm, VertiSep GES C-18) and C-18 guard pre-column were obtained from Vertical and Phenomenex (USA), respectively. Preliminarly, different mobile phases (with methanol or acetonitrile and water) and flow rates (1.7, 1.4 and 1.0 mL min^{-1}) were tested in order to obtain suitable analytical separation and highest analytical signals for the analytes. Satisfactory results were obtained with a gradient mobile phase consisted of acetonitrile (A) and MilliQ water (B), starting with 40:60 (A:B, v/v) for 5 minutes, followed by 70:30 for 15 minutes, at a flow rate of 1.4 mL min^{-1}. The mobile phases were previously degassed for 30 minutes in an ultrasonic bath. The diode array detector allowed UV spectra in the range 190-300 nm. Based on absorbance signals observed in the DAD spectrum of the standard solutions, Irgarol and Diuron were detected and quantified at 224 nm and 244 nm, respectively.

LC-ESI-MS/MS

The LC-MS/MS analysis was performed in a Waters Alliance 2695 (USA) equipped with an autosampler, quaternary pump and a degasser system. Mass spectrometry was performed on a Micromass® Quattro Micro™ API with an electrospray interface (LC-ESI-MS/MS). The analytical separation was carried out in an XTerra analytical column (50 × 3 mm, 3.5 μm) (Waters, USA). The optimized conditions for the Diuron and Irgarol determination by LC-ESI-MS/MS were previously developed (reference available under request from authors). The mobile phase was acetonitrile and MilliQ water acidified with 0.1% formic acid (52:48, v/v), at constant flow of 0.5 mL min^{-1}, with injection volume of 10 μL. Control of analytical instrument, data acquisition and treatment were performed by software Masslynx version 4.1, 2005 (Waters, USA). Parameters were optimized by injection of the antifouling standard solutions at 1 ng μL^{-1}. Preferential ionization was in the positive mode for both analytes. The mass spectrometer was operated in scan, product ion scan and MRM (multiple reaction monitoring) modes. The confirmation was achieved by using the main fragmentation ions. The interface conditions were adjusted to provide maximum intensity of the precursor ions as follows: capillary voltage at 4 kV, nebulizer and desolvation gas (nitrogen) at flow rates of 550 and 50 L h^{-1}, respectively; source block and desolvation temperatures were 100 and 350 ºC, respectively; Argon was used as collision gas. The optimized conditions for Diuron and Irgarol analysis by LC-ESI-MS/MS also included: cone voltages at 28 V for Diuron and 30 V for Irgarol, collision cell energies, respectively, at 15 and 20 eV for confirmation and quantification of Diuron and at 19 and 30 eV for Irgarol. Dwell time was 0.2 s.

Analytical parameters

The performance of the HPLC-DAD and LC-ESI-MS/MS methods was established according to validation parameters by using standard solutions and spiked seawater samples. Linearity was assessed using seven different concentrations ranging from 0.05 to 1.0 mg L^{-1} for HPLC-DAD and from 0.001 to 0.5 mg L^{-1} for LC-ESI-MS/MS for each compound with three repetitions per concentration. The linear relationships were obtained by plotting the peak areas and the sample analyte concentrations. The linearity was assessed by linear regression and only determination coefficients (r) above 0.99 were accepted. The limits of detection (LOD) and quantification (LOQ) for both methods were estimated from the signal-noise ratio by the visual method considering, respectively, as three and ten times the baseline noises obtained close to the retention times of the compounds.[14,18,19] The precision was evaluated in terms of repeatability, expressed as the relative standard deviation which was obtained by carrying out the extraction and analysis of spiked artificial seawater samples at three different concentrations. For the HPLC method, the complete procedure was repeated five times and, after that, injected three times. For the LC-ESI-MS/MS, the extraction was repeated three times and each extract was injected three times. The accuracies (recoveries) of those methods were investigated by mean recoveries obtained when comparing the extracted amounts with direct injections of the corresponding antifouling standards at the beginning of the procedure.

Considering that sensitivity of the HPLC-DAD and LC-ESI-MS/MS methods could be different, the injected volumes were, respectively, 20 μL and 10 μL in such a way that quantities of antifouling compounds that were injected into the HPLC-DAD were eight times greater than the quantities injected into the LC-ESI-MS/MS.

Results and Discussion

Analytical parameters

The determination of organic contaminants in natural waters involves the pre-concentration of the samples and the most commonly used technique is solid phase extraction (SPE). Specifically for Diuron and Irgarol,

Table 2. Analytical parameters of HPLC-DAD and LC-ESI-MS/MS methods

Antifouling	Linear range / (μg L^{-1})		r		LOQ method / (μg L^{-1})	
	HPLC-DAD	LC-ESI-MS/MS	HPLC-DAD	LC-ESI-MS/MS	HPLC-DAD	LC-ESI-MS/MS
Diuron	0.1-10	0.004-2.0	0.9994	0.9986	0.10	0.004
Irgarol	0.05-10	0.02-2.0	0.9980	0.9997	0.05	0.02

C-18 bounded silica cartridges have proven to be effective with suitable recovery rates.[6,8,18,19,22] Since Diuron is thermally unstable, liquid chromatography is required for analytical separation and quantification. The detection is accomplished using diode array (LC-DAD) or mass spectrometry (LC-MS/MS) detectors. The first provides information about peak identity through spectra of each compound and the latter combines the advantages of the chromatographic separation with structural information, also being selective and sensitive.

In order to establish the best analytical conditions for determining Diuron and Irgarol in seawater samples, the performance of HPLC-DAD and LC-ESI-MS/MS were compared considering not only sensitivity but also simplicity, cost and accessibility of the methods. The validation parameters obtained for both methods are shown in Table 2. LC-ESI-MS/MS was much more sensitive, although both methods presented an adequate linearity (within a different concentration range). The limits of detection (LOD) and quantification (LOQ) for the LC-ESI-MS/MS method were, respectively, 0.0012 and 0.004 μg L^{-1} for Diuron and 0.006 and 0.02 μg L^{-1} for Irgarol, whilst for the HPLC-DAD method the limits were, respectively, 0.030 and 0.1 μg L^{-1} for Diuron and 0.015 and 0.05 μg L^{-1} for Irgarol (Table 2). The limits of quantification confirm that LC-ESI-MS/MS presents the best sensitivity, since the LC-ESI-MS/M is twenty-five times more sensitive than by HPLC-DAD for Diuron and about twice for Irgarol. Although different volumes of samples and extracts have been used by the methods to minimize the matrix effect, the LC-MS method showed the best sensitivity (200 fold for Diuronand 20-fold for Irgarol).

The selectivity for the LC-ESI-MS/MS method was achieved by monitoring two characteristic ions for each biocide. The characteristic fragments (m/z) were 198 and 108 for Irgarol and 72 and 46 for Diuron, where the first two ions of each compound were used for quantification (i.e., 198 and 72). The best selectivity of the analysis by LC-ESI-MS/MS was already expected, considering that the HPLC-DAD presents reduced capacity to distinguish absorbance signal of the analytes. However, it should again emphasize that the matrix effect is pronounced in the analysis made by LC-ESI-MS/MS and this must be considered when using such method. These considerations demonstrate that the determination of biocides in samples of seawater is most properly performed by using LC-MS.

The precision (repeatability) was tested using synthetic spiked seawater of salinity 30 and 10 for the HPLC-DAD and LC-ESI-MS/MS methods, respectively (Table 3). Different salinities were used due to matrix effect observed for the biocides analysis when using the LC-ESI-MS/MS method. It was observed that for Diuron, the matrix effect for spiked water at salinity 25 was relatively high (65.3%) whilst for Irgarol the effect was less significant (11.2%). This was expected, since the Diuron molecule has polar groups which can interact with matrix components, thus compromising the biocide extraction.[27] In order to avoid the matrix effect, both biocides were determined by using analytical curves made on spiked seawater samples.

Accuracy (recovery) was tested by analyzing synthetic seawater samples spiked at different concentrations, chosen in accordance with the sensitivities of the methods. For each concentration, the samples were prepared five and three times, respectively, for the complete analyse by SPE-HPLC-DAD and SPE-LC-ESI-MS/MS and, after that, the sample extracts were injected three times. Relative standard deviations for the repeated injections were lower than 6.7% (results not presented). Table 3 shows the relative standard deviations obtained for the full analytical procedures. Satisfactory precisions were observed when using both methods, since recoveries higher than 96% were obtained. Relative standard deviations for the complete

Table 3. Recovery (R%) and Repeatability (expressed as relative standard deviation, RSD) of the complete analytical methods. Artificial spiked seawater at salinity 30 and 10 were used, respectively, for the analysis by SPE-HPLC-DAD and SPE-LC-ESI-MS/MS at different concentrations of the antifouling biocides. Relative standard deviations of each extract injected in triplicate were not shown (inferior to 6.7%)

Antifouling	HPLC-DAD (n = 5)			LC-MS/MS (n = 3)		
	Conc. / (μg L^{-1})	R / %	RSD / %	Conc. / (μg L^{-1})	R / %	RSD / %
Diuron	0.1	96.7	7.2	0.004	118.0	13.0
	1.0	100.0	1.4	0.02	104.0	15.0
	5.0	100.2	2.4			
Irgarol	0.1	99.7	5.6	0.004	107.0	15.0
	1.0	100.7	4.6	0.02	100.0	4.0
	5.0	99.8	4.2			

procedures were also satisfactory. Thus, although both methods were similar considering the accuracy and repeatability, the LC-ESI-MS/MS was the selected method for the analyses of seawater samples due to the lower sensitivity. However, if no other sensitive technique was available liquid chromatography with diode array detection may be used, provided that, for such, larger volumes of sample pre-concentrated injected volumes were used.

Environmental seawater analysis

The results of the water samples collected from Port of Itaqui are shown in Table 4. The seawater samples were taken twice *per* year during 2 years. The samples were initially analyzed by both methods, however most of the results were lower than limits of detection of HPLC-DAD, so the LC-ESI-MS/MS was chosen due to better sensitivity for the analysis of the biocides in seawater samples. The presence of Diuron and Irgarol was detected in most samples collected over the period of two years, showing for most sampled a slight decrease on the concentrations from 2010 to 2011 (Table 4). Thirteen out of 24 samples showed concentrations of Diuron ranging between 0.05 and 7.80 μg L^{-1}, while nineteen samples presented concentrations of Irgarol between 0.02 to 4.80 μg L^{-1}. The average concentration of Diuron was lower than those of Irgarol. Corresponding concentration pattern seems to be observed for studies cited at the literature,[24,28] but comparison between analytical results was not supported by statistical evaluation due to lack of data for such corrected evaluation. At this moment, the results only show preliminary records of antifouling in the studied area.

Although Diuron is also used as herbicide in agricultural fields, contributions of this source were considered unlikely and therefore negligible for the studied region. The highest values were systematically found on the sampling sites "Am1", "Am2" while for "Am5", in April 2011, there was the highest Diuron concentration in the sample collected in that period. Diuron was not detected in any sample collected in November 2011. Large ships were moored during all four campaigns at these sites. Furthermore, the samples were collected next to metallic structures of the moored vessels, which might explain the highest concentrations found in those sites for both antifouling compounds. Diuron and Irgarol were also found at site Am.4, located in a mangrove area on the opposite side of the pier where there were no ships. The concentrations ranged from < LOD and 0.37 μg L^{-1}. Taking into account that all the samples were collected during high tide, it was assumed that samples were relatively homogeneous and influence of freshwater flow was negligible. This was evidenced by slight variations on the physical-chemical parameters of seawater sampled during rainy and dry periods (salinity varied between 36 and 36.5, pH from 6.95 to 8.35 and temperature from 25 to 29 °C). The results indicated that both biocides were reasonably dispersed into such environment of maritime trafic.

When comparing the results obtained with several studies performed on harbor and marina areas around the world, concentrations of Diuron and Irgarol might appear at first glance very high. However, there are some aspects that must be considered. For example, in a study performed along the coast of Singapure, Basheer *et al.* found Irgarol concentration as high as 4.0 μg L^{-1} in harbor areas with intense maritime vessels flux.[1] In another monitoring study, Irgarol was found in concentration higher than 2.0 μg L^{-1} in marina and harbor areas with a high density of boats and during yachting season.[24] Lambert *et al.* also found Diuron and Irgarol in water samples collected from rivers and shallow freshwater lakes of East Anglia, UK. They also reported that highest concentration were observed in areas used for berthing and mooring of boats and vessels.[19] For Diuron, concentrations as high as 6.7 μg L^{-1} were observed on studies performed in the same area, at different campaigns.[6] More recently, several studies have showed that

Table 4. Concentrations of Diuron and Irgarol in seawater samples collected from five harbor areas and one from mangrove area (Am. 4) in Port of Itaqui (São Luis, Maranhão, Brazil). Sampling was made twice *per* year (from 2010 to 2011), at rainy (April) and dry (November) season

Sample	Diuron / (μg L^{-1})				Irgarol / (μg L^{-1})			
	2010		2011		2010		2011	
	Apr.	Nov.	Apr.	Nov.	Apr.	Nov.	Apr.	Nov.
Am1	0.27	7.80	0.21	< LOD	3.13	4.80	0.09	0.02
Am2	0.15	3.70	0.19	< LOD	3.05	2.60	< LOQ	0.08
Am3	0.12	0.06	0.05	< LOD	0.95	0.25	< LOD	0.02
Am4	< LOD	0.05	< LOD	< LOD	0.37	0.25	< LOD	< LOQ
Am5	< LOD	0.10	1.34	< LOD	0.06	0.16	< LOD	< LOD
Am6	< LOD	0.70	< LOD	< LOD	0.41	0.11	< LOD	< LOQ

Concentrated volume: 250 mL. Relative standard deviation (n = 3) ranged from 5 and 12%. LOD: 0.0012 μg L^{-1} for Diuron and 0.006 μg L^{-1} for Irgarol.

maximum measured values of these antifouling compounds were decreased, mainly for Irgarol, and it was supposed to be due to prohibited use in some countries.[10,28,29] This was noted in a previous study by one of the authors of this work, in which biocides concentrations were between not detected and 0.02 μg L^{-1} and not detected and 0.006 μg L^{-1} for Diuron and Irgarol, respectively, in Rio Grande harbor, located on south of Brazil.

It is important to mention that monitoring studies for Diuron and Irgarol have been performed in different climatic regions, mainly in temperate and Mediterranean regions. To our knowledge, this work presents the first evidence about the presence of the antifouling booster biocides in harbor areas located on northeast of Brazil. This finding is relevant, since several studies have demonstrated that Diuron and Irgarol can cause acute toxicity on the aquatic biota at concentrations similar to those observed in several marine environments.[9,10,12,23,29] Recently, a study performed with eight native marine species of subtropical regions showed that concentrations higher than 1 μg L^{-1} are prone to cause severe impacts on the growth of autotrophic aquatic species such as microalgae and corals.[13] On the other hand, ecologically important grazer of algae and sea grasses, such as the sea urchin *Lytechinus variegatus*, were slightly affected by Irgarol and Diuron.[30] In a study conducted by Perina *et al*. the estimated inhibitory concentration values (IC50) for both biocides were between 1.49 and 3.75 mg L^{-1}, respectively, which were considerably above the obtained for another antifouling biocides such as organotins.[30] Thereby, the toxicity of Diuron and Irgarol on marine organisms is still poorly understood and must be more evaluated. The results obtained in our work should be considered as a first assessment about the presence of Diuron and Irgarol on peculiar tropical marine ecosystem and this can be used as concentration reference for further ecotoxicological studies.

Conclusions

Although the analytical methods presented similar accuracy and precision, the determination of Diuron and Irgarol in seawater samples by using solid phase C-18 cartridges followed by LC-ESI-MS/MS proved to be the best choice, considering parameters such as sensitivity and selectivity. For such method, limits of detection for Diuron and Irgarol were, respectively, twenty-five and twice times lower than that obtained for the HPLC-DAD method. However, due to matrix effect, the analytical curves were made on spiked seawater samples.

The selected method was applied in monitoring study performed in a harbor area located in northeast of Brazil, in a peculiar mangrove area. In general, the observed concentrations were higher than those usually found in harbor environments, although values on the same magnitude have been recorded in the literature. These data are preliminary but relevant, considering the toxicity and persistence of both biocide compounds that can be potentially dangerous in mangrove areas under heavy flow navigation.

Acknowledgments

Lia G. R. Diniz and Marcelle S. Jesus thank financial support provided by CAPES and FAPEMA. The authors thank the local port company (Empresa Maranhense de Administração Portuária - EMAP) for permission to collect seawater samples and the LACOM/FURG for LC-ESI-MS/MS analysis. G. Fillmann was sponsored by CNPq (Brazilian National Research Council) (314335/2009-9).

References

1. Basheer, C.; Tan, K. S.; Lee, H. K.; *Mar. Pollut. Bull.* **2002**, *44*, 697.
2. Paradas, W. C.; Amado Filho, G. M.; *Braz. J. Oceanogr.* **2007**, *55*, 51.
3. Godoi, A. F. L.; Favoreto, R.; Silva, M. S.; *Quim. Nova* **2003**, *26*, 708.
4. Castro, I. B.; Westphal, E.; Fillmann, G.; *Quim. Nova* **2011**, *34,* 1021.
5. Mohr, S.; Berghahn, R.; Mailahn, W.; Schmiediche, R.; Feibicke, M.; Schmidt, R.; *Environ. Sci. Technol.* **2009**, *43,* 6838.
6. Thomas, K. V.; Fileman, T. W.; Readman, J. W.; Waldock, M. J.; *Mar. Pollut. Bull.* **2001***, 42*, 677.
7. Manzo, S.; Buono, S.; Cremisini, C.; *Arch. Environ. Cont. Tox*. **2006**, *51*, 61.
8. Sapozhnikova, Y.; Wirth, E.; Schiff, K.; Brown, J.; Fulton, H.; *Mar. Pollut. Bull.* **2007***, 54,* 1972.
9. Bao, V. W. W.; Leung, K. M. Y.; Lui, G. C. S.; Lam, M. H. W.; *Chemosphere* **2013**, *90,* 1140.
10. Di Landa, G.; Parrella, L.; Avagliano, S.; Ansanelli, G.; Maiello, E.; Cremisini, C.; *Water Air Soil Poll.* **2009***, 200*, 305.
11. Lee, S.; Chung, F.; Won, H.; Lee, D.; Lee, Y-W.; *J. Hazard. Mat.* **2011**, 185, 1818.
12. Konstantinou, I. K.; Albanis, T. A.; *Environ. Int*. **2004**, *30,* 2035.
13. Bao, V. W. W.; Leung, K. M. Y.; Qiu, J-W.; Lam, M. H. W.; *Mar. Pollut. Bull.* **2011**, *62,* 1147.
14. Tóth, S.; Slooten, K. B.; Spack, L.; Alencastro, L. F.; Tarradellas, J.; *Bull. Environ. Contam. Toxicol.* **1996**, *57,* 426.
15. Gatidou, G.; Thomaidis, N. S.; Zhou, J. L.; *Environ. Int.* **2007**, *33*, 70.

16. Dyer, R. A.; Tolhurst, L. E.; Hilton, M. J.; Thomas, K. V.; *Bull. Environ. Contam. Toxicol.* **2006**, *77,* 524.
17. DPR Pesticide Chemistry Database. Environmental Monitoring Branch, Department of Pesticide Regulation, http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/Diuron.pdf accessed in October 2013.
18. Gatidou, G.; Kotrikla, A. K.; Thomaidis, N. T.; Lekkas, T. D.; *Anal. Chim. Acta* **2005**, *528*, 89.
19. Lambert, S. J.; Thomas, K. V.; Davy, A. J.; *Chemosphere* **2006***, 63*, 734.
20. Menezes, M. P. M.; Berger, U.; Mehlig, U.; *Acta Amaz.* **2008**, 403.
21. Teixeira, S. G.; Souza Filho, P. W. M.; *Rev. Bras. Geof.* **2009**, *27,* 69.
22. Rodríguez, A. S.; Ferrera, Z. S.; Rodríguez, J. J. S.; *Cent. Eur. J. Chem.* **2012**, *10*, 521.
23. Rodríguez, A. S.; Ferrera, Z. S.; Rodríguez, J. J. S.; *Environ. Chem. Lett.* **2011**, *9*, 203.
24. Chesworth, J. C.; Donkin, M. E.; Brown, M. T.; *Aquat. Toxicol.* **2006**, *66,* 293.
25. Koutsaftis, A.; Aoyama, I.; *Environ. Toxicol*. **2006**, *21,* 432.
26. Sapozhnikova, Y.; Wirth, E.; Singhasemanon, N.; Baceyd, J.; Fulton, B. C. M.; *J. Environ. Monitor.* **2009**, *10*, 1069.
27. Demoliner, A.; Caldas, S. S.; Costa, F. P.; Gonçalves, F. F.; Clementin, R. M.; Milani, M. R.; Primel, E. G.; *J. Brazil Chem. Soc.* **2010**, *21,* 1424.
28. Ferrer, I.; Ballesteros, B.; Marco, M. P.; Barcelo, D.; *Environ. Sci. Technol*. **1997**, *31,* 3530.
29. Cima, F.; Ballarin, L.; *Chemosphere* **2012**, *89*, 19.
30. Perina, F. C.; Abessa, D. M. S.; Pinho, G. L. L.; Fillmann, G.; *Ecotoxicol.* **2011***,* 20, 1870.

Submitted: August 28, 2013
Published online: December 10, 2013

FAPESP has sponsored the publication of this article.