Chemosphere 82 (2011) 1010–1016

Contents lists available at ScienceDirect

Chemosphere

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

The effects of the nitrofuran furaltadone on *Ulva lactuca*

Sara Leston [a,b,*], Margarida Nunes [a,b], Ivan Viegas [a,c], Marco F.L. Lemos [d,e], Andreia Freitas [f], Jorge Barbosa [f], Fernando Ramos [b], Miguel A. Pardal [a]

[a] CFE – Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal
[b] CEF – Center for Pharmaceutical Studies, Health Sciences Campus, Pharmacy Faculty, Coimbra University, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal
[c] CNC – Centre for Neurosciences and Cell Biology, Department of Life Sciences, University of Coimbra, 3001-401 Coimbra, Portugal
[d] CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[e] ESTM & GIRM, Polytechnic Institute of Leiria, P-2520-641 Peniche, Portugal
[f] INRB/LNIV – Instituto Nacional de Recursos Biológicos, I.P./Laboratório Nacional de Investigação Veterinária (LNIV), Estrada de Benfica 701, 1549-011 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 14 June 2010
Received in revised form 19 October 2010
Accepted 21 October 2010
Available online 24 November 2010

Keywords:
Nitrofuran
Macroalgae
Bioindicators
Contamination

ABSTRACT

The use of pharmaceuticals in the food production industry as prophylatic and therapeutic agents is necessary to promote animal health, but may entail significant consequences to natural ecosystems, especially in the cases of overdosing and use of banned pharmaceuticals. The vast effects that antibiotics released into the environment have on non-target organisms are already under the scope of researchers but little attention has been given to primary producers such as macroalgae. The present study assessed furaltadone's, an antibacterial agent illegally used for veterinary purposes, uptake capacity by *Ulva lactuca* and its effect in the growth of this cosmopolitan macroalgae. Differences in macroalgal growth were shown when submitted to prophylactic and therapeutic concentrations of furaltadone in the water (16 and 32 $\mu g\ mL^{-1}$, respectively). The therapeutic concentration caused higher growth impairment than the prophylactic treatment did, with 87.5% and 58% reductions respectively. Furthermore, together with data collected from the accumulation assays, with values of internal concentrations as high as 18.84 $\mu g\ g^{-1}$ WW, suggest that the macroalgae *U. lactuca* should be included in field surveys as a biomonitor for the detection of nitrofurans.

1. Introduction

Environmental chemical contamination can occur as a result of human activities, carrying with it potential adverse ecological impacts (Olette et al., 2008). To assess such effects it has become necessary to understand which chemical stressors are present and their likelihood to impact exposed receptors. A vast array of veterinary medicinal products (VMP) is used in animal production serving several purposes from growth-promotion to the treatment of diseases (Stolker et al., 2007). In the last decades the avian industry grew considerably and even though there have been significant improvements and a higher sanitary control, there is still a concern with the leakage of VMPs into the environment (Halling-Sørensen et al., 1998; Wollenberger et al., 2000; Boyd, 2003; Lalumera et al., 2004). Antibiotics are among these potentially harmful substances since they are especially tailored to be biologically active (Halling-Sørensen et al., 1998; Wollenberger et al., 2000; Edhlund et al., 2006; Kümmerer, 2009). Among these is furaltadone (FTD) (Fig. 1), a nitrofuran derivative and a highly effective chemotherapeutic drug, used as an antibacterial agent to fight common bacterial and protozoan infections, which acts by inhibiting microbial enzymes involved in the carbohydrate metabolism (Balizs and Hewitt, 2003; Vass et al., 2008). It is rapidly metabolized resulting in very stable metabolites which have been linked with carcinogenic, mutagenic and teratogenic effects in humans (Jager et al., 1997; Chadfield and Hinton, 2003; Barbosa et al., 2007; Bartel et al., 2009). For this reason the use of FTD together with three other nitrofurans was banned from use in livestock in the EU in 2004 (Barbosa et al., 2007; Verdon et al., 2007; Vass et al., 2008; European Commission Regulation 37/2010). In spite of this, since it is very inexpensive and has a high success rate, in many countries it is illegally used as it can be found on the blackmarket (Report of a Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance, 2006; Vass et al., 2008). According to data predicted by a model (unpublished), it will likely be found in an eventual discharge up to 50 $\mu g\ mL^{-1}$, corresponding to the commonly used therapeutic dosage (prophylactic concentration: 25 $\mu g\ mL^{-1}$).

* Corresponding author at: CFE – Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal. Tel.: +351 239855760.
E-mail address: saraleston@ci.uc.pt (S. Leston).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.10.067

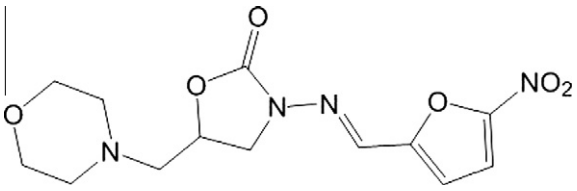

Fig. 1. Structure of furaltadone [5-morpholinomethyl-3-(5-nitrofurfurylidereamino)-2-oxazolidinone].

The prediction of distribution patterns of a given chemical through the different compartments of an ecosystem is often difficult, thus the pharmacokinetics and effects on individual groups of organisms constituting the system is often preferred (McCarty et al., 2004; Walker et al., 2006), despite the importance of assessing indirect and mixture effects (Geiszinger et al., 2009). Macroalgae represent an ecologically important group serving many relevant functions that include an extensive contribution to the primary production of estuarine ecosystems, a role in nutrient cycling and a source of food and habitat for aquatic biota (Conti and Cecchetti, 2003; Melville and Pulkownik, 2006; Torres et al., 2008).

In aquatic systems macroalgae are continuously exposed to the contaminants present and as they represent a very large biomass they can act as a sink for xenobiotics (Sandermann, 1992; Pflugmacher et al., 1999; Walker et al., 2006) becoming a gateway for higher trophic levels (Walker et al., 2006). They also possess characteristics that allow them to be considered as bioindicators as they are relatively sedentary, easy to identify and with a wide geographic distribution (Melville and Pulkownik, 2006; Torres et al., 2008). Nevertheless, little information is available on bioconcentration and effects of xenobiotics (including antibiotics) on macroalgae (Macrì and Sbardella, 1984; Bierkens et al., 1998; Pflugmacher et al., 1999; La Barre et al., 2004; Carafa et al., 2007; Lai et al., 2009; Torres et al., 2008). In order to better understand the consequences of antibiotics present in estuarine macroalgae, *Ulva lactuca* was selected. The aims of this research were (i) to investigate the behavior of FTD in seawater, (ii) to ascertain the capacity of the macroalgae to uptake nitrofuran from the water, (iii) to assess the influence on growth of *U. lactuca* at two concentrations simulating the prophylactic and therapeutic use, (iv) to investigate the probability of the bioaccumulation in the trophic web, and (v) to evaluate the suitability of using macroalgae as a bioindicator for the presence of nitrofurans.

2. Experimental

2.1. Reagents

The FTD analytical standard and the internal standard nifuroxazide were obtained from Sigma–Aldrich (St. Louis, USA). Methanol, ethyl acetate, acetonitrile and N-hexan were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical-reagent grade except solvents used in mobile phase that were HPLC grade.

2.2. Macroalgae

The green macroalgae *U. lactuca* was collected during low tide in the Mondego estuary (Portugal, 40°80′N, 8°50′W). After being washed and rinsed to remove possible epibionts present on the surface, macroalgae were placed in a refrigerated container along with seawater and transported to the laboratory. After a thorough inspection of the fronds to assure the absence of organisms, 2 kg WW of *U. lactuca* were placed in 40 L glass fiber tanks, which had been previously filled with filtered natural seawater (pore Ø 0.45 μm) and Provasoli's Enriched Medium (PES; L. Provasoli, 1963) modified by Bold and Wynne (1978), and acclimated for 48 h under 80 μmol photons m^{-2} s^{-1} of white fluorescent light, 14:10-h LD photoperiod, 25 °C and 35 psu. Macroalgae were maintained in the same conditions for 4 weeks prior to the beginning of the experiment. Natural seawater was collected 1 week before, filtered through sterile Ø 0.45 μm filters and stored at 4 °C before use.

2.3. Experimental design

The same conditions set during acclimation were kept during all experiments but aeration was replaced by constant and gentle horizontal stir. The day prior to the start of the trial, glass containers were filled with 250 mL of filtered seawater and 5 mL of PES, placed on orbital shakers and left to acclimate for 24 h. Just before beginning, healthy algal disks with Ø 5 cm (approx. 20 cm^2) were cut and a stock solution of FTD was prepared in methanol. The concentrations were then adjusted from the stock solution and added to the flasks to obtain two test concentrations: 16 μg mL^{-1} (hypothetical prophylactic concentration) and 32 μg mL^{-1} (hypothetical therapeutic concentration) designated as groups P and T respectively. These concentrations correspond to 60% of the concentrations used for prophylaxis (25 μg mL^{-1}) and therapy (50 μg mL^{-1}). Each group had three replicates for each sampling time and each replicate contained three algal disks. Additionally four control groups designated as A, B, C and D were prepared. Control A followed the same preparation as for P and T without addition of FTD, to establish the natural growth of *U. lactuca*. Control B was used to verify the natural degradation of the nitrofuran and consisted of seawater with antibiotic for both concentrations P and T. Control C was set to exclude the possible effects of methanol on the macroalgae and the same volumes used in P and T were added to the respective flasks [1.6% and 3.2% (v/v) respectively]. Finally, since the solution has a very strong yellow color which could theoretically interfere with growth, two solutions with yellow dye were prepared with the same wavelengths, corresponding to control D. To avoid evaporation but still allow gas exchange, all containers were loosely covered with glass lids.

Sampling times were as follows: 0, 1, 2, 5, 8, 12, 16, 24, 48, 72, 96 and 120 h.

2.3.1. Water

At each sampling time, after the algae had been removed, temperature, salinity and pH were measured, water was filtered through glass fiber filters (Ø 0.22 mm) and immediately frozen at −20 °C until extraction and kept from light to prevent photodegradation.

To determine the concentration of FTD present in the water, the samples were filtered through 0.22 μm pore filters and transferred into amber vials equipped with inserts for high-performance liquid chromatography with UV detection (HPLC–UV) analysis. The equipment consisted of a Dionex HPLC-system with a degasser, quaternary pump (P580), autosampler (ASI-100), column thermostat and a diode array detector (UVD340U). The columns used were a New Guard Perkin Elmer C18 pre-column and a Merck RT 250-4 Lichrospher 100 RP-18 column, both with 5 μm particle size. The mobile phase consisted of ammonium acetate and acetonitrile delivered isocratically. Analyses were performed in the dark to prevent possible degradation due to light.

2.3.2. Furaltadone uptake

To determine the concentration of FTD taken up from the water column, macroalgae were analyzed by liquid chromatography with tandem mass spectrometry (LC–MS/MS) using a method adapted

from McCracken and Kennedy (2007). After removal from the water, algae were paper-dried, weighed and stored at −20 °C, until further analysis. To extract FTD's residues, the samples were minced individually and placed in centrifuge tubes to which 40 μL of nifuroxazide and 10 mL of ethyl acetate were added. After centrifugation, the supernatant was collected and evaporated, followed by addition of 10 mL of acetonitrile and 3 mL of N-hexan. Samples were then left to stand and N-hexan was discarded. The sample was again evaporated to dryness under a nitrogen stream and the resulting residue reconstituted in 500 μL of methanol: water solution. Samples were then transferred to amber vials and placed in the LC autosampler. The equipment was composed of an Agilent 1100 Series HPLC system coupled to a Triple Quadrupole System Sciex API 2000 tandem mass detector with a Turbolon-Spray ion source, operating under the Sciex Analyst 1.4.1 software. The LC columns consisted of a guard column Zorbax Eclipse XDB-C8 and a Zorbax Eclipse XDB-C18 column, with 5 μm particle size.

2.3.3. Growth

To determine the effects of FTD on macroalgal growth, disks were photographed at the beginning and end of each time point. Disk areas were determined using computer-assisted software (Photoshop CS3-extended) and variations calculated. Macroalgal death was considered whenever there were clear signs of decay.

2.4. Statistics

All data were checked for normality and homoscedascity. The effect of the concentration of FTD on growth and the differences in uptake were assessed using the *t* test to determine significant differences between the control and the treatments. When applicable, results are presented as mean ± SE. The significance level was inferred at $P \leqslant 0.05$ for all statistical tests. All calculations were performed using GraphPad Prism® 5 software (Graph Pad Software, Inc.).

3. Results

3.1. Water

The nominal concentrations used in the trial were 16 μg mL^{-1} and 32 μg mL^{-1}, which were confirmed in control B (Fig. 2).

The stability of FTD in saline aqueous solution for both concentrations was determined with control group B, so that it could be compared with the degradation in the presence of *U. lactuca* (Fig. 2). The patterns were similar with and without the algae according to the findings of Edhlund and colleagues (2006) for the natural photolysis of the compound. Nonetheless, the concentrations in the water were much lower in the presence of the plant. For group P the minimum concentration at 120 h was 0.22 μg mL^{-1}, whereas the correspondent control B was much higher with a concentration of 3.48 μg mL^{-1}. Group T followed the same pattern decreasing to 2.11 μg mL^{-1} while the lowest control concentration was 9.64 μg mL^{-1}.

3.2. Furaltadone uptake

In group P, removal of FTD from the water column was relatively slow during the first 2 h after which it peaked attaining a maximum value of 18.84 μg g^{-1} WW within the first 5 h. Subsequently, the uptake decreased gradually until it reached the minimum value of 0.03 μg g^{-1} WW after 72 h, remaining constant thereafter (Fig. 3A).

In group T the maximum internal concentration of 12.18 μg g^{-1} WW was reached within the first hour (Fig. 4A). In the proceeding hours there was a slight decrease to concentrations around 9 μg g^{-1} WW which were constant for 12 h. Following this time, concentrations decreased again to 3.68 μg g^{-1} WW after 48 h which was still much higher than the values found for the same interval of time in group P. Although algal death was determined at 48 h, macroalgae were still left in the water and analyzed to assess their ability to maintain internal amounts of FTD, which was confirmed. Concentrations were higher than those presented by group P until the end of the trial.

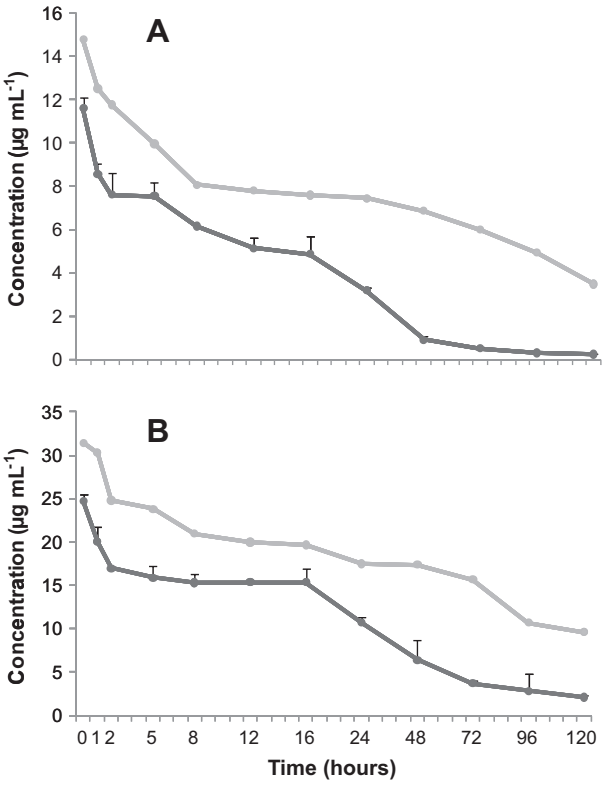

Fig. 2. (A) Stability test of furaltadone in aqueous solution with (dark line) and without (grey line) the presence of *U. lactuca* at 16 μg mL^{-1}, during the course of the experiment. Data represent mean values of three independent replicates. (B) Stability test of furaltadone in aqueous solution with (dark line) and without (grey line) the presence of *U. lactuca* at 32 μg mL^{-1} during the course of the experiment. Data represent mean values of three independent replicates.

3.3. Growth

The results provide evidence that the growth of *U. lactuca* was significantly affected by the presence of FTD in the water. Figs. 3B and 4B present the variations in growth during the 5-d exposure to the antibiotic at both concentrations and in comparison with the control. In the prophylactic treatment the variation followed the same increasing pattern as the control for the first 24 h ($P > 0.05$) and significantly different thereafter ($P < 0.05$). Growth still continued however, although less pronounced than the control group, suggesting that the presence of FTD inhibited growth (Fig. 2A). On the contrary, group T exhibited a very distinct growth pattern. During the first 12 h it showed a variation in disk area slightly higher than the control however not statistically different ($P > 0.05$). After 16 h a decrease in growth rates was observed, coinciding with the first signs of algal decay, becoming statistically different from the control group ($P < 0.05$). Regarding growth, the test was terminated after 48 h, but macroalgae were still maintained for the length of the experiment to determine water and internal concentrations.

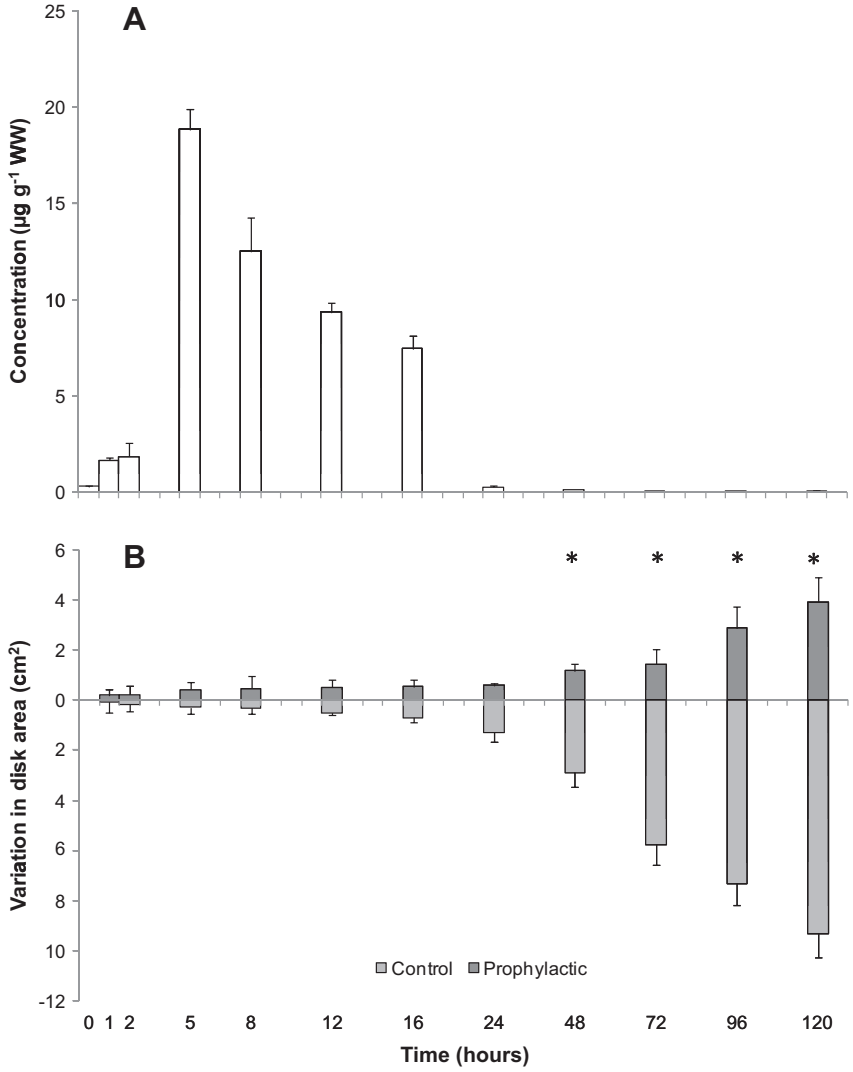

Fig. 3. (A) Internal concentrations of furaltadone (µg g^{-1} WW) for *U. lactuca* at each sampling time for the prophylactic group P. Data represent mean values of three independent replicates. (B) Growth measured as variation in disk area (cm^2) at each sampling time for group P, plotted against the control. Data represent mean values of three independent replicates. Asterisk (*) indicates results significantly different from the control.

4. Discussion

Antibiotics can easily enter aquatic environments mainly through wastewater runoffs, representing a potential threat to aquatic biota. The present study focuses on the behavior of the nitrofuran FTD in seawater and its effects on the green macroalgae *U. lactuca*.

Based on the results from this experiment, FTD has a very low-persistency in seawater when compared to other drugs that can remain for weeks and even years (Lai et al., 2009). This was expected since other studies have referred the fast metabolism of the molecule itself (Zuidema et al., 2005; Edhlund et al., 2006; Verdon et al., 2007; Pimpitak et al., 2009). Moreover, Edhlund and colleagues (2006) found that FTD, as well as other nitrofuran antibiotics, is photochemically degraded to nitrofuraldehyde (NFA) in aqueous solution, predominantly via photolysis which is due to the overlap of the absorption spectrum with the solar spectral output (Edhlund et al., 2006). Also, according to these authors there is a pH dependence with higher degradation occurring at acidic values (Edhlund et al., 2006). However, the pH values for the experiment were in the alkaline range to ensure the optimum conditions for growth and to be in accordance with the environmental range, which means that in natural ecosystems FTD photodegradation rate is probably slower than in other mediums. In this scenario, this nitrofuran could be available for longer periods and be absorbed in higher concentrations.

The ability of the macroalgae to remove FTD from the water was tested at two different concentrations, 16 and 32 µg mL^{-1}. FTD is a lipophilic compound with an additional morpholinomethyl ring that enhances its water solubility (Stammati et al., 1997), presenting a K_{ow} of 0.2 (Moffat et al., 2004) indicating that it may be efficiently taken up by plants, as is the case with many other xenobiotics (Coleman et al., 1997; Walker et al., 2006). The tested green macroalgae showed an efficient removal of the nitrofuran, involving absorption and/or adsorption processes. *U. lactuca* was able to take up FTD in both treatments and this uptake would account for the differences in concentration of the antibiotic in solution found between the controls and groups P and T. One important aspect to be discussed is the fact that both groups reflected the concentration present in solution. The continuous decline of FTD in both solutions was accompanied by a gradual decrease in the internal concentration. It is unclear however, whether this reduction in the amount taken up is due to passive diffusion to the water or if there is a mechanism by which the

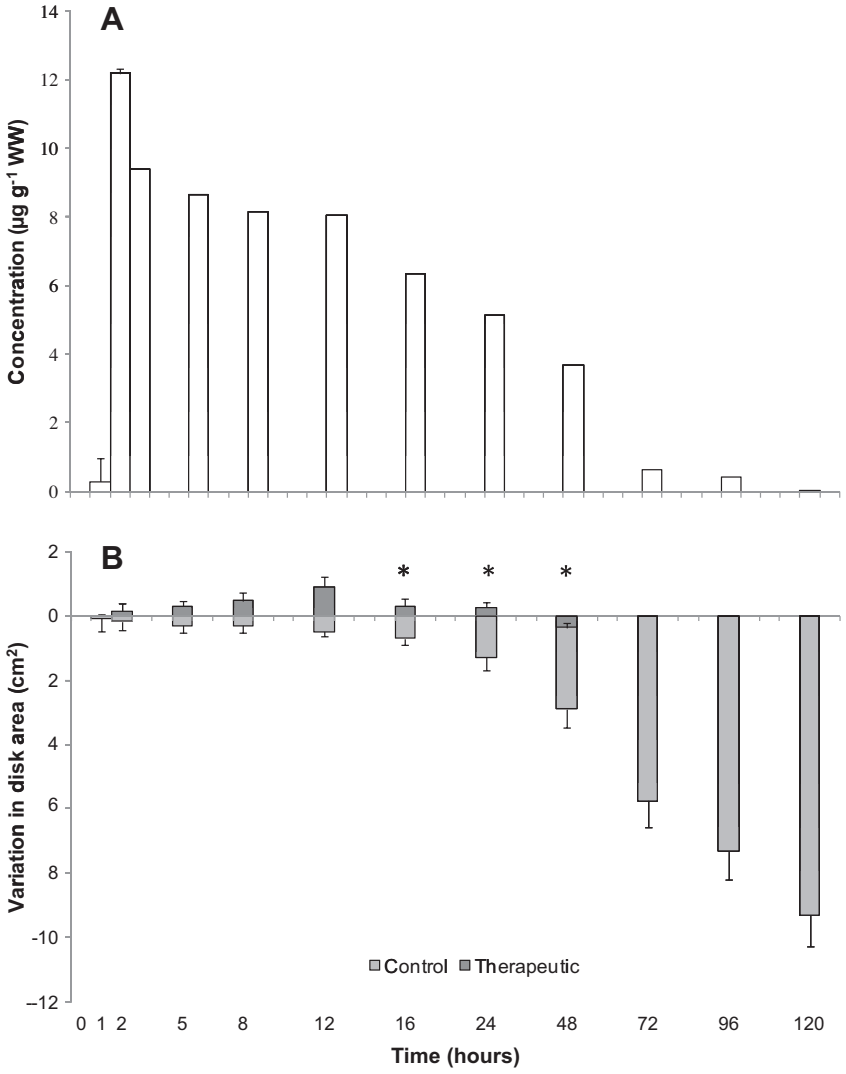

Fig. 4. (A) Internal concentrations of furaltadone (µg g^{-1} WW) for *U. lactuca* at each sampling time for the therapeutic group T. Data represent mean values of three independent replicates. (B) Growth measured as variation in disk area (cm^2) at each sampling time for group T, plotted against the control. Data represent mean values of three independent replicates. Asterisk (*) indicates results significantly different from the control.

nitrofuran is broken down into its metabolite, 5-methylmorfolino-3-amino-2-oxazolidinone (AMOZ) being the primary metabolite and the most toxic. According to the concept of the "green liver" introduced by Sandermann (1992), plants possess a set of enzymes very similar to those found in animals that are able to metabolize xenobiotics (Sandermann, 1992, 1994; Coleman et al., 1997; Suresh and Ravishankar, 2004; Torres et al., 2008). The process consists of three phases divided in transformation (I) mostly in cytochrome P-450 monooxygenases, conjugation (II) under the activity of glutathione S-transferases and glucosyltransferases, and compartmentation (III) either in vacuoles or in the cell wall fraction (Sandermann, 1992, 1994; Coleman et al., 1997; Mitsou et al., 2006; Suresh and Ravishankar, 2004). Although there are currently few studies, this detoxification mechanism was already confirmed for other xenobiotics in marine macroalgae including *U. lactuca* (Mehrtens, 1994; Pflugmacher and Sandermann, 1998a,b; Pflugmacher et al., 1999; Lewis et al., 2001; Mitsou et al., 2006). To establish which mechanism is involved, further studies focusing on enzymatic variation are required since data on the metabolism of nitrofurans in plants are not actually available. Also, the determination of AMOZ should be conducted for both macroalgae and water.

The effects of FTD on growth were also analyzed in this study. Based on the results, *U. lactuca* showed different sensitivities to FTD dependent on the concentrations present. At the lowest concentration, macroalgae were able to grow although less than the control which indicates inhibition. As for the higher concentration, macroalgae died after 48 h. The outcome of the trial points to severe inhibition of growth and also to lethal toxicity. Growth inhibition and toxicity are therefore dependent on the concentrations of FTD present with values in the range of 25 µg mL^{-1} being tolerated by *U. lactuca* whereas higher concentrations proved to be lethal. Similar findings were reported for the microalgae *Selenastrum capricornutum* which presented a value of EC_{90} after 96 h for concentrations of FTD higher than 34 µg mL^{-1} (Macrì and Sbardella, 1984). Lai and colleagues (2009) used three different phenicol antibiotics to test the effects on growth of three algae and the results indicate different sensitivities dependent on the concentrations present. In the same study the growth rate during the first 24 h was similar to the control but after that, it decreased as the concentrations increased, a situation very similar to what was reported in this experiment.

Antibiotics in general are considered as potential micropollutants as the concentrations in which they may occur in natural

environments are very low, in the ppb range (Halling-Sørensen et al., 1998; Le Bris and Pouliquen, 2004). The concentrations used in this study were much higher (ppm range) and may occur only in exceptional conditions in the environment as a result of inadequate treatment of effluents or neglect of the safety rules regarding wastewater disposal (Hektoen et al., 1995; Wollenberger et al., 2000; Boyd, 2003; Radjenović et al., 2007). Still, the possibility of the presence of nitrofurans in the ecosystems is very high. Since *U. lactuca* can sustain growth at nominal concentrations of 25 $\mu g\ mL^{-1}$ it is not anticipated that it will be severely affected in natural ecosystems (Walker et al., 2006). Furthermore, since *U. lactuca* is able to lower the internal concentration of FTD it is not likely that it will be passed along the food chain in high amounts. However, if the compound is being metabolized by the macroalgae then the resulting metabolites (predominantly AMOZ) can be bioaccumulated into higher trophic levels, as their stability is much higher (Leitner et al., 2001; Barbosa et al., 2007; Verdon et al., 2007).

The potential accumulation of FTD by green macroalgae could represent a potential risk of biomagnification of nitrofurans through the trophic web, but in the present case a decrease in internal concentration in *U. lactuca* was observed. Nevertheless, future studies about the mechanism of depuration and metabolism of FTD could give a more clear picture of the environmental risk associated to this compound. As a primary producer, it represents an important link in the food web as it is consumed by organisms in higher trophic levels which in many cases represent economically important species. Moreover, the probability of metabolism of FTD to AMOZ increases with the higher levels which represents an additional and serious risk to the trophic web. Furthermore, the fact that *U. lactuca* is a free-floating species may play an important role in transport of these substances from contaminated to non-contaminated sites.

5. Conclusions

Since green macroalgae can reflect the concentrations present in the water they can be considered as potential indicator tools for the presence of FTD in natural ecosystems. Nevertheless, the use of macroalgae as bioindicator and/or biomonitor of FTD contamination will entail more work. The mechanism of depuration and metabolism are still not clarified and the coupling of laboratory and field experiment will be required to validate green macroalgae as a prospective tool in environmental risk assessment.

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia through a PhD grant attributed to S. Leston (SFRH/BD/42057/2007). The authors wish to thank all the colleagues that contributed to this work.

References

Balizs, G., Hewitt, A., 2003. Determination of veterinary drug residues by liquid chromatography and tandem mass spectrometry. Anal. Chim. Acta 492, 105–131.

Barbosa, J., Ferreira, M.L., Ramos, F., Noronha da Silveira, M.I., 2007. Determination of the furaltadone metabolite 5-methylmorpholino-3-amino-2-oxazolidinone (AMOZ) using liquid chromatography coupled to electrospray tandem mass spectrometry during the nitrofuran crisis in Portugal. Accredit. Qual. Assur. 12, 543–551.

Bartel, L.C., Montalto de Mecca, M., Castro, J.A., 2009. Nitroreductive metabolic activation of some carcinogenic nitro heterocyclic food contaminants in rat mammary tissue cellular fractions. Food Chem. Toxicol. 47, 140–144.

Bierkens, J., Maes, J., Plaetse, F.V., 1998. Dose-dependent induction of heat shock protein 70 synthesis in (*Raphidocelis subcapitata*) following exposure to different classes of environmental pollutants. Environ. Pollut. 101, 91–97.

Boyd, C.E., 2003. Guidelines for aquaculture effluent management at the farm-level. Aquaculture 226, 101–112.

Carafa, R., Wollgast, J., Canuti, E., Ligthart, J., Dueri, S., Hanke, G., Eisenreich, S.J., Viaroli, P., Zaldívar, J.M., 2007. Seasonal variations of selected herbicides and related metabolites in water, sediment, seaweed and clams in the Sacca di Goro coastal lagoon (Northern Adriatic). Chemosphere 69, 1625–1637.

Chadfield, M.S., Hinton, M.H., 2003. Evaluation of treatment and prophylaxis with nitrofurans and comparison with alternative antimicrobial agents in experimental *Salmonella enterica*, *Serovar enteritidis* infection in chicks. Vet. Res. Commun. 27, 257–273.

Coleman, J.O.D., Blake-Kalff, M.M.A., Emyr Davies, T.G., 1997. Detoxification of xenobiotics by plants: chemical modification and vacuolar compartmentation. Trends Plant Sci. 4, 144–151.

Conti, M.E., Cecchetti, G., 2003. A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. Environ. Res. 93, 99–112.

Edhlund, B.L., Arnold, W.A., McNeill, K., 2006. Aquatic photochemistry of nitrofuran antibiotics. Environ. Sci. Technol. 40, 5422–5427.

European Commission, Council Regulation 2377/90/EC, Off. J. Eur. Union, L224 (18 August 1990) (Consolidated Version of the Annexes I to IV Updated up to 22.12.2004 Obtained from www.emea.eu.int).

Geiszinger, A., Bonnineau, C., Faggiano, L., Guasch, H., López-Doval, J.C., Proia, L., Ricart, M., Ricciardi, F., Romaní, A., Rotter, S., Muñoz, I., Schmitt-Jansen, M., Sabater, S., 2009. The relevance of the community approach linking chemical and biological analyses in pollution assessment. Trends Anal. Chem. 28, 619–625.

Halling-Sørensen, B., Nors Nielsen, S., Lansky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E., 1998. Occurrence, fate and effects of pharmaceutical substances in the environment – a review. Chemosphere 36, 357–393.

Hektoen, H., Berge, J.A., Samuelsen, O., Yndstad, M., 1995. Resistance of antibacterial agents in marine sediments. Aquaculture 133, 175–184.

Jager, L.P., de Graaf, G.J., Widjaja-Greefkes, H.C.A., 1997. Differential effects of nitrofurans on the production/release of steroid hormones by porcine adrenocortical cells in vitro. Eur. J. Pharmacol. 331, 325–331.

Kümmerer, K., 2009. Antibiotics in the aquatic environment – a review – part I. Chemosphere 75, 417–434.

La Barre, S.L., Weinberger, F., Kervarec, N., Potin, P., 2004. Monitoring defensive responses in macroalgae – limitations and perspectives. Phytochem. Rev. 3, 371–379.

Lai, H.T., Hou, J.H., Su, C.I., Chen, C.L., 2009. Effects of chloramphenicol, florfenicol, and thiamphenicol on growth of algae *Chlorella pyrenoidosa*, *Isochrysis galbana*, and *Tetraselmis chui*. Ecotoxicol. Environ. Saf. 72, 329–334.

Lalumera, G.M., Calamari, D., Galli, P., Castiglioni, S., Crosa, G., Fanelli, R., 2004. Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. Chemosphere 54, 661–668.

Le Bris, H., Pouliquen, H., 2004. Experimental study on the bioaccumulation of oxytetracycline and oxolinic acid by the blue mussel (*Mytilus edulis*). An evaluation of its ability to bio-monitor antibiotics in the marine environment. Mar. Pollut. Bull. 48, 434–440.

Leitner, A., Zöllner, P., Lindner, W., 2001. Determination of the metabolites of nitrofuran antibiotics in animal tissue by high-performance liquid chromatography–tandem mass spectrometry. J. Chromatogr., A 939, 49–58.

Lewis, S., Donkin, M.E., Depledge, M.H., 2001. Hsp70 expression in *Enteromorpha intestinalis* (Chlorophyta) exposed to environmental stressors. Aquat. Toxicol. 51, 277–291.

Macrì, A., Sbardella, E., 1984. Toxicological evaluation of nitrofurazone and furaltadone on *Selenastrum capricornutum*, *Daphnia magna* and *Musca domestica*. Ecotoxicol. Environ. Saf. 8, 101–105.

McCarty, L.S., Power, M., Munkittrick, K.R., 2004. Bioindicators versus biomarkers in ecological risk assessment. Hum. Ecol. Risk Assess. 8, 1159–1164.

McCracken, R.J., Kennedy, D.G., 2007. Detection, accumulation and distribution of nitrofuran residues in egg yolk, albumen and shell. Food Addit. Contam. 24, 26–33.

Mehrtens, G., 1994. Haloperoxidase activity in arctic macroalgae. Polar Biol. 14, 351–354.

Melville, F., Pulkownik, A., 2006. Investigation of mangrove macroalgae as bioindicators of estuarine contamination. Mar. Pollut. Bull. 52, 1260–1269.

Mitsou, K., Koulianou, A., Lambropoulou, D., Pappas, P., Albanis, T., Lekka, M., 2006. Growth rate effects, responses of antioxidant enzymes and metabolic fate of the herbicide propanil in the aquatic plant *Lemna minor*. Chemosphere 62, 275–284.

Moffat, A.C., Osselton, D.M., Widdop, B., 2004. Clarke's Analysis of Drugs and Poisons: in Pharmaceuticals, Body Fluids, and Postmortem Material, third ed. Pharmaceutical Press, London. pp. 1248.

Olette, R., Couderchet, M., Biagianti, S., Eullaffroy, P., 2008. Toxicity and removal of pesticides by selected aquatic plants. Chemosphere 70, 1414–1421.

Pflugmacher, S., Sandermann, H., 1998a. Cytochrome P450 monooxygenases for fatty acids and xenobiotics in marine macroalgae. Plant Physiol. 117, 123–128.

Pflugmacher, S., Sandermann, H., 1998b. Taxonomic distribution of plant glucosyltransferases acting on xenobiotics. Phytochemistry 49, 507–511.

Pflugmacher, S., Wiencke, C., Sandermann, H., 1999. Activity of phase I and phase II detoxication enzymes in Antarctic and Arctic macroalgae. Mar. Environ. Res. 48, 23–36.

Pimpitak, U., Putong, S., Komolpis, L., Petsom, A., Palaga, T., 2009. Development of a monoclonal antibody-based enzyme-linked immunosorbent assay for detection of the furaltadone metabolite, AMOZ, in fortified shrimp samples. Food Chem. 116, 785–791.

Radjenović, J., Petrović, M., Barceló, D., 2007. Advanced mass spectrometry methods applied to the study of fate and removal of pharmaceuticals in wastewater treatment. Trends Anal. Chem. 26, 1132–1143.

Report of a Joint FAO/OIE/WHO Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance, 2006.
Sandermann, H., 1992. Plant metabolism of xenobiotics. Trends Biochem. Sci. 17, 82–84.
Sandermann, H., 1994. Higher plant metabolism of xenobiotics: the 'green liver' concept. Pharmocogenetics 4, 225–241.
Stammati, A., Zampaglioni, F., Zucco, F., 1997. Furaltadone cytotoxicity on three cell lines in the presence or absence of DMSO: comparison to furazolidone. Cell Biol. Toxicol. 13, 125–130.
Stolker, A.A.M., Zuidema, T., Nielen, M.W.F., 2007. Residue analysis of veterinary drugs and growth-promoting agents. Trends Anal. Chem. 26, 967–979.
Suresh, B., Ravishankar, G.A., 2004. Phytoremediation – a novel and promising approach for environmental clean-up. Crit. Rev. Biotechnol. 24, 97–124.
Torres, M.A., Barros, M.P., Campos, S.C.G., Pinto, E., Rajamani, S., Sayre, R.T., Colepicolo, P., 2008. Biochemical biomarkers in algae and marine pollution: a review. Ecotoxicol. Environ. Saf. 71, 1–15.
Vass, M., Hruska, K., Franek, M., 2008. Nitrofuran antibiotics: a review on the application, prohibition and residual analysis. Vet. Med. Czech. 53, 469–500.
Verdon, E., Couedor, P., Sanders, P., 2007. Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoine, nifursol) in poultry muscle tissue through the detection of their five major metabolites (AOZ, AMOZ, SEM, AHD, DNSAH) by liquid chromatography coupled to electrospray tandem mass spectrometry—in-house validation in line with Commission Decision 657/2002/EC. Anal. Chim. Acta 586, 336–347.
Walker, C.H., Hopkin, S.P., Sibly, R.M., Peakall, D.B., 2006. Principles of Ecotoxicology, third ed. CRC Press Taylor and Francis Group, Boca Raton.
Wollenberger, L., Halling-Sorensen, B., Kusk, K.O., 2000. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere 40, 723–730.
Zuidema, T., Mulder, P.P.J., van Rhijn, J.A., Keestra, N.G.M., Hoogenboom, L.A.P., Schat, B., Kennedy, D.G., 2005. Metabolism and depletion of nifursol in broilers. Anal. Chim. Acta 529, 339–346.