Ecotoxicology and Environmental Safety 99 (2014) 35–44

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Preliminary assessment of terrestrial microalgae isolated from lichens as testing species for environmental monitoring: Lichen phycobionts present high sensitivity to environmental micropollutants

N. Domínguez-Morueco [a,*], H. Moreno [a], E. Barreno [b], M. Catalá [a]

[a] Departamento de Biología y Geología, ESCET, Universidad Rey Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Madrid, Spain
[b] Instituto Cavanilles de Biodiversidad y Biología Evolutiva (ICBIBE), Botánica, Fac. C. Biológicas, Universitat de Valencia, C/ Dr. Moliner 50, 46100 Burjassot, Valencia, Spain

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form
2 October 2013
Accepted 6 October 2013
Available online 29 October 2013

Keywords:
Carbamazepine
Diclofenac
Lichen phycobionts
Microalgae
Microbioassays
Micropollutants

ABSTRACT

Bioassays constitute a tool for pollution analysis providing a holistic approach and high-quality indication of the toxicity. Microbioassays allow evaluating the toxicity of many samples, implying lower costs and enabling routine monitoring and pollution control. But tests conducted so far are limited to the use of a small number of taxa. Lichens are excellent bioindicators of pollution with great ecological significance. Studies show that the phycobiont is more sensitive to pollutants than the mycobiont. Phycobiont have features such as adaptation to anhydrobiosis and relatively rapid growth *in vitro*, making them suitable for microbioassays. Our aim is to determine the sensitivity of phycobionts to the pharmaceutical micropollutants carbamazepine and diclofenac as a preliminary step for the development of a toxicity microbioassay based on phycobionts. Optical dispersion and chlorophyll autofluorescence were used as endpoints of toxicity on two algal species showing that suspensions present cyclic and taxon specific patterns of aggregation. *Trebouxia* TR9 suspensions present a very high grade of aggregation while *Asterochloris erici* cells do not. Both micropollutants alter optical properties of the suspensions of both species. No significant alteration of chlorophyll autofluorescence by carbamazepine is observed. *A. erici* chlorophyll autofluorescence is extremely sensitive to diclofenac but the effect is not dependent on the drug concentration or on the time of exposure. Differently, TR9 only shows punctual chlorophyll alterations. Fluctuations in optical dispersion may indicate changes in the population structure of the species, including reproductive strategy. *A. erici* seems more sensitive to micropollutants, is better characterized and is available from commercial collections.

1. Introduction

Chemical analysis has historically been the most used method to monitor and evaluate the environmental pollution (Wadhia and Thompson, 2007). These measurements can identify compounds in the environment, but do not give an indication of the bioavailability of toxic substances and of the joint effects these compounds may have on the biota (Hendriks et al., 1994, cited in Van der Griten et al., 2010). In this situation, the “bioassay” constitutes a complementary tool of analysis, capable of providing a holistic approach and an indication of the acute toxicity, genotoxicity or chronic effects that the organisms under study undergo (Wadhia and Thompson, 2007; Van der Griten et al., 2010).

When conducting bioassays routinely, there may be problems due to the high number of samples, and space and facilities (greenhouses, pools) required, usually reflected in increased economic costs. This has led to the development of bioassays with miniaturization or micro procedures, known as “microbioassays”. They allow evaluating the toxicity of a high number of samples, implying lower costs and enabling structures for monitoring and pollution control (Wadhia & Thompson, 2007). But tests conducted so far are limited to the use of a small number of taxa, due to either practical or economic criteria, and not by ecological criteria (Catalá et al., 2009). It is therefore necessary to extend the range of action of these studies to more taxa, as usually the most unknown are the most threatened.

Microalgae rank among the most frequently used organisms because they provide features such as high sensitivity and a high reproducibility (Shitanda et al., 2009). The organisms referred to as algae are generally a collection of unrelated organisms that possess plastids (Del Campo et al., 2010) Green algae, or Chlorophyta, constitute a huge and extremely diverse phylum of eukaryotic organisms. These eukaryotes must not be confounded with prokaryotic cyanobacteria, also known as blue-green algae. At present, the

* Corresponding author.
E-mail address: dm.noelia@gmail.com (N. Domínguez-Morueco).

0147-6513/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ecoenv.2013.10.007

toxicity bioassays use free-living aquatic algae as *Pseudokirchneriella subcapitata* or *Chlorella* sp. (Levy et al., 2009) but the possibility of using algae associated with lichens has never been studied.

Lichens are organisms very sensitive to environmental pollution, able to accumulate pollutants from the atmosphere or from soils. Therefore, these have been used as bioindicators of air or soil quality (Bačkor and Váczi, 2002; Bačkor et al., 2007). Besides being excellent bioindicators of pollution, lichens have great ecological significance, because they play a key role in soil formation, since in all cases, the first organisms to appear on the nude rocks are encrusting and foliose lichens (Cooper and Rudolph, 1953).

Lichens are complex organisms with a specific organization. Their thalli are composed of heterotrophic fungi (mycobiont) and photosynthetic algae or cyanobacteria, photobionts, or in the specific case of algae, phycobionts. Environmental studies with lichens show that, generally, the most sensitive element to pollutants is the phycobiont (Bačkor, Váczi (2002)). Over 50% of all lichen species are associated with algae of the genus *Trebouxia* (*sensu lato*, including *Asterochloris*) (Gasulla et al., 2010; Piovár et al., 2011). These algae have features such as the possibility to obtain large quantities at low economic costs and tolerance to a wide range of operating temperatures (Gasulla et al., 2010). In addition unlike free-living algae have high tolerance to desiccation (high tolerance to hydric stress) (Gasulla et al., 2009, 2013), qualities that free-living algae do not possess.

Symbiotic algae (phycobionts) belonging to *Astherochloris* and *Trebouxia* genera (class *Trebouxiophyceae*) can be isolated from lichens. Phycobionts are different to most algae used in toxicity bioassays belonging mostly to classes *Chlorophyceae* or *Chrysophyceae*. Apart from phylogenetic divergences, lichen phycobionts present dramatic ecophysiological differences:

1) Lichens are terrestrial organisms, and therefore, phycobiont (green microalgae) toxicity would be representative of terrestrial ecosystems vs. aquatic green algae currently used.
2) Linked to terrestrial dwelling, lichen phycobionts possess a high tolerance to desiccation (they are adapted to anhydrobiosis), a useful property that free-living algae do not possess, since they belong to aquatic ecosystems.
3) Phycobionts are symbiotic organisms. Recent works demonstrate remarkable similarities in certain biological traits among symbiotic organisms such as lichens, rhizobium or mycorrhizae (Meilhoc et al., 2011; Puppo et al., 2013). These symbioses are of utmost importance for both global nitrogen ecology and crop production. Phycobionts could serve as valuable testing organisms of how environmental pollution may disrupt symbiotic interaction.

These unique properties of phycobionts along with the need to increase the available taxa for use in toxicity bioassays, especially for terrestrial habitats, make them a good choice for the development of microbioassays of toxicity with low associated economic costs, increasing the number of taxa available. However, no study on the utility of lichen phycobionts as testing species in environmental monitoring or toxicology has been addressed yet. The first question to answer is whether they are sensitive to environmental pollutants. If this is the case, the next questions to solve are how to adapt their peculiar characteristics to a bioassay and what are the most appropriate endpoints to measure toxicity.

The revolutionary development of resources and new technologies has led to increased release of chemicals that may pose a threat to the environment and biodiversity (Bolong et al., 2009). Some of these compounds have been frequently detected in different environmental compartments (rivers, lakes or sediments) at low concentration (ng to $\mu g\ L^{-1}$ and μg to $mg\ kg\ L^{-1}$ dry matter) and for this reason they are known as micropollutants (Delgadillo-Mirquez et al., 2011). These include pharmaceutical compounds and their metabolites (Fent et al., 2006). Pharmaceuticals may reach agricultural fields both through irrigation with micropolluted surface water and amendments with compost or biosolids derived from sewage sludges (Vazquez-Roig et al., 2011; Martin Ruel et al., 2012). Irrigation itself can generate water aerosols that directly reach lichens and soil tillage will produce contaminated soil aerosols. Terrestrial organisms can thus be exposed to these drugs by dry/wet deposition of aerosols generated in nearby agricultural fields (Fig. 1).

The antiepileptic carbamazepine is a Central Nervous System (CNS) drug that helps to quiet the abnormal firing of nerves. It blocks the sodium voltage-dependent channel of excitatory neurons. It is often used as an anticonvulsant and mood stabilizer (Van den Brandhof and Montforts, 2010). Diclofenac is a drug belonging to the family of Nonsteroidal Anti-inflammatory Drugs (NSAIDs). This is a phenylacetic acid derivative that inhibits cyclooxygenases, key enzymes catalyzing the biosynthesis of prostaglandin. Both drugs have been detected in water bodies at concentrations in the order of $ng\ L^{-1}$ or even $\mu g\ L^{-1}$ (Zhang et al., 2008; González Alonso et al., 2010; Valcárcel et al., 2010, 2011). Studies such as that conducted by Feito et al., (2012) highlight the potential threat that, these drugs can be for wild organisms, especially for terrestrial plants. Specifically, environmental concentrations of diclofenac can cause acute lethal and chronic sublethal toxicity in higher plant development (fern spore bioassay). Therefore, it is essential to monitor and evaluate the impact of drugs on different components of the ecosystems, especially if their presence is continued (Van der Griten et al., 2010).

Thus, the objective of this study is to determine the sensitivity of phycobionts to carbamazepine and diclofenac as a preliminary step for the development of a toxicity microbioassay based on isolated lichen phycobionts. Optical dispersion (O.D.) and chlorophyll autofluorescence were used as endpoints of toxicity.

2. Materials and method

2.1. Biological material

Two strains were used in this study, *Asterochloris erici* and *Trebouxia* sp. TR9. *A. erici* described by Skaloud and Peksa, 2010, (formerly known as *Trebouxia erici* Ahmadjian, 1960) is a phycobiont isolated from the lichen *Cladonia cristatella*. It has a growth temperature between 17 °C and 23 °C, with an optimum at 20 °C

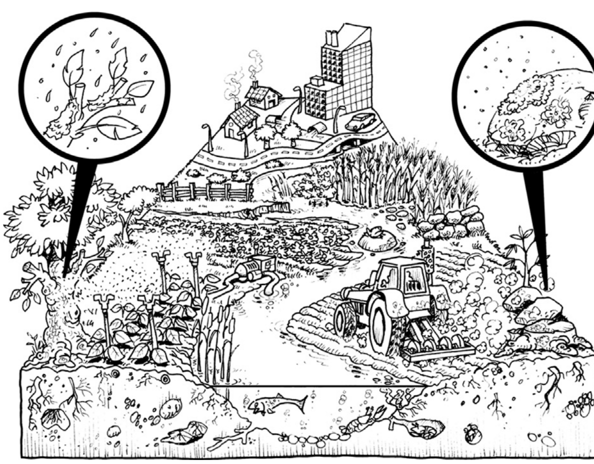

Fig. 1. Urban discharges and inefficacy of sewage treatment plants lead to pollution of surface waters with pharmaceuticals. Irrigation with these micropolluted waters can generate aerosols that may reach terrestrial organisms of the agro-ecosystem. Besides irrigation, pharmaceuticals can reach soils by the amendments done with compost or biosolids containing sewage sludge. Soil tillage also generates aerosols of contaminated dust that can deposit on adjacent ecosystems.