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The 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins

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Editorial

Statement of Peer Review—7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins

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In submitting conference proceedings to *Biology and Life Sciences Forum*, the volume editors of the proceedings certify to the publisher that all papers published in this volume have been subjected to peer review administered by the volume editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal.

- Type of peer review: single-blind.
- Conference submission management system: Authors submitted their abstracts through an online form available on the conference website; abstracts and evaluation forms were sent to reviewers by e-mail; when revisions were requested by reviewers, abstracts were sent back to the authors by email; and reviewed abstracts were re-evaluated by the same reviewer and/or by the editors. The final decision was communicated to the authors by email.
- Number of submissions sent for review: 59.
- Number of submissions accepted: 56.
- Acceptance rate (number of submissions accepted/number of submissions received): 0.949.
- Average number of reviews per paper: 1.
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- Any additional information on the review process: none.

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Abstract

Degradation of *Microcystis* sp. in Surface Water by Ozone †

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Abstract: Due to the gradual increase in the concentration of algae, in general, in continental surface water reservoirs, it is necessary to incorporate advanced technologies in drinking water treatment plants with a view to reduce/eliminate the algae present. This degradation of the algal mass will additionally lead to the destruction of the toxins that could be released into the aqueous medium during the course of potabilisation. In this work, degradation assays of *Microcystis* sp. in surface water by oxidation processes with ozone have been studied. Ozonation experiments have been carried out in a homogeneous regime (mixture of an aqueous solution with dissolved ozone and surface water) and heterogeneous regime (continuous bubbling of a gaseous current with ozone in surface water). Different aqueous matrices were used for the assays (distilled water, Guadiana river as it passes through Badajoz (Spain), Villar del Rey reservoir (Spain) and a stream that circulates through the campus of the University of Extremadura in Badajoz (Spain)), which were doped with different amounts of a culture of *Microcystis* sp., provided by the University of Coimbra (Portugal), to achieve the desired initial load of algal mass. The original aqueous matrices were filtered through 0.45 micrometer filters (Millipore). An ozonizer (Sander, model 300.5) was used, capable of generating from pure oxygen a mass flow rate of 6 g/h with an ozone concentration in the gas phase of 1.6% by volume. In the case of the experiments in a heterogeneous regime, the reactor was a cylindrical column 20 cm high and 8 cm in diameter, fitted with a microporous diffuser. To determine the content of algal mass present in the initial sample and in those treated by ozonation, a portable fluorimeter (Aquafluor, Turner) was used, which allows to measure the content of chlorophyll in vivo between 0.3 and 300 µg/L. Ozone analysis in the aqueous phase was performed by the Karman Indigo method and in the gas phase by iodometry. Experiments carried out in homogeneous regime show that in only 5 minutes the chlorophyll content is reduced by 50% for all types of aqueous matrices and for different initial contents of algal mass between 15 and 50 µg/L. For a time of 30 minutes, the conversion is between 80 and 90%, except for the Guadiana river matrix, which is between 70 and 80%. In the case of the heterogeneous regime, ozonation experiments have been carried out by varying the ozone concentration between 4.5×10^{-5} and 5.4×10^{-4} mol/L. It is observed that this variable has a strong positive influence on the degradation of the algae. Thus, for a time of 30 minutes, the degradation is total with the four matrices for the ozone concentration of 5.4×10^{-4} mol/L, while for the concentration of 4.5×10^{-5} mol/L the degradation is between 80 and 90%. A kinetic study has been carried out in both types of ozonation regime. In the case of the homogeneous regime, the model includes both the self-decomposition of ozone in water and the direct reaction of ozone with the algae, and in the case of the heterogeneous regime, the mass transfer of ozone from the gas to the liquid phase is also incorporated. The resolution of the model with the MATLAB software leads to a value of the kinetic degradation constant of the alga between 9×10^{-4} (Villar del Rey reservoir) and 1.4×10^{-3} (distilled water) L/(µg chlorophyll min).

Keywords: surface water; potabilisation; ozonation; kinetic study; *Microcystis*

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Abstract

Historical Geographical Distribution of Potentially HCBs (Harmful Cyanobacterial Blooms) across the Portuguese Territory under a European Context †

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Abstract: Climate change enhances the occurrence of HCBs (Harmful Cyanobacterial Blooms) worldwide, particularly in freshwater ecosystems, posing increased risks for local populations. The present work intends to conduct a spatiotemporal historical overview on the occurrence of potentially toxic cyanobacterial blooms in Europe and Portugal, particularly over the past four decades. *Microcystis* blooms are widely reported across the globe, and microcystins (MCs) are the most detected cyanotoxins. Nevertheless, this can be greatly attributed to the fact that MC-LR is the main cyanotoxin targeted in most studies over the past 40 years due to the health risk guidelines from World Health Organization (WHO). In Europe, this trend is also observed, with microcystins as the most reported cyanotoxins, followed by anatoxins, saxitoxins, cylindrospermopsin, and nodularin. In Portugal, MCs have been reported since the 1980s in freshwater bodies such as shallow lakes (e.g., Mira and Vela lakes) and reservoirs (e.g., Torrão, Carrapetelo, Aguieira, Alvito, Odivelas, Monte Novo, Enxoé, and Roxo reservoirs), and their occurrence has not only been mainly related with the blooms of *Microcystis* spp. (particularly *M. aeruginosa*) but also with *Aphanizomenon* and *Planktothrix* spp. High levels of anatoxin-a have been detected in strains of *Anabaena* and *Aphanizomenon* spp. isolated from Maranhão reservoir and near Waste Water Treatment Plants. Saxitoxins have been reported from reservoirs such as Montargil and Crestuma, and related to *Aphanizomenon flos-aquae*. Toxic *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*) strains have been reported for the first time in Portugal in 2003. Although cylindrospermopsin was not detected then, it has been later reported to occur at Vela Lake and São Brás Lake (Azores). This also suggests the importance of referring and monitoring species recognized as having toxic strains with a wide variety of toxic compounds rather than only the most known target cyanotoxins. The present compilation of data aims to contribute to a preliminary Portuguese/Iberian database platform on HCBs and future modelling by integrating the available historical data. The persistent incidence of potentially toxic cyanobacteria through the years in target water bodies may help better understand their dynamics and design deeper ecological research approaches as well as more effective management and control strategies.

Keywords: cyanobacterial blooms; cyanotoxins; freshwater bodies; Portugal; spatiotemporal database

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Abstract

Taxonomic Assessment of the Cyanobacteria from the BACA Culture Collection: Contribution to the Knowledge of Their Diversity and Monitoring in the Azores †

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‡ Presenting author (oral communication).

Abstract: Cyanobacteria taxonomy is changing significantly, with many new genera and species having been uncovered from a variety of cultures over the last decade. The use of cultures allowed for genetic studies using molecular markers, e.g., 16S rRNA and 16S-23S internal transcribed spacer (ITS) rRNA, complementing the morphological and ecological information traditionally used in the description of new taxa. Presently, the culture collection BACA (Azorean Bank of Algae and Cyanobacteria) comprises more than 350 strains of cyanobacteria isolated from terrestrial, aquatic, and thermal habitats in the Azores. The phylogenetic study of more than 200 strains shows new phylogenetic clades and possibly many new genera and species. Strains belonging to possible new taxa were selected for further genetic studies by 16S rRNA and 16S-23S ITS rRNA gene amplification using the 27F and 23S30R primers and sequenced. The secondary structure of the ITS gene was predicted using the mFold web server, and the D1-D1′ helix, Box-B helix and V3 helix were compared. Transmission electron microscopy was performed on simple coccoid and filamentous strains to visualize the thylakoid position in the cell. The use of a polyphasic approach enabled the identification of several new cyanobacteria taxa, supported by the phylogenetic analysis and the identification of diacritical morphological characters. The description of these new taxa contributes to the knowledge of cyanobacteria biodiversity and a better understanding of their taxonomy, which is still very confusing and problematic, allowing a better clarification of species and genera distribution.

Keywords: taxonomy; 16S rRNA; 16S-23S rRNA; internal transcribed spacer; biodiversity; phylogeny; transmission electron microscopy

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Abstract

Cyanobacteria and Cyanotoxins in Azorean Lakes: Spatial and Temporal Analysis of Long-Term Monitoring Data (2003–2018) †

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‡ Presenting author (oral communication).

Abstract: Eutrophication became the main environmental problem of Azorean lakes at the end of the 20th century, resulting mainly from the intensification of anthropogenic activities in the catchments. This problem raised great public concern, leading to the implementation of monitoring programs to assess the status of the Azores inland waters. During the monitoring programs, many cyanobacterial species were present in high abundance, and several blooms and cyanotoxins have been recorded over the years. In this work, monitoring data from twenty-three lakes, from 2003 to 2018, were analyzed to understand the distribution and dynamics of the presence of cyanobacteria and cyanotoxins, as well as the importance of local and global environmental factors. Although we found some interannual variability, the results confirm a high abundance of cyanobacteria in many lakes, frequently of toxic species. Besides a high correlation between the lake trophic state and the abundance of cyanobacteria, some changes in the communities, namely regarding the dominant species, suggest the influence of global factors as drivers of these changes. This study contributes to improving cyanotoxin monitoring programs and mitigation actions to control harmful cyanobacterial blooms (HCBs).

Keywords: cyanotoxins; eutrophication; anthropogenic effects; long-term data; HCBs

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Abstract

Testing in Tropical Reservoirs a Remote Sensing Phycocyanin Empirical Model Performed for Temperate Reservoirs: Ahead of Climate Change †

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Abstract: Remote sensing is a tool that is being used increasingly often for both terrestrial and aquatic ecology. For inland waters, most works focus on developing an empirical or analytical model to estimate optical active variables related to water quality. More and more studies use remote sensing as a support tool for ecosystem processes, but developing local specific models is time and resources consuming. The most used method for developing models is the empirical one, which directly relates the remote-sensed signal to the variables of interest using statistical techniques so as to produce robust results for the areas and data sets from which they are derived. Empirical algorithms can be expected to perform well only inside their range of derivation and for the area in which they are derived. Thus, to facilitate their use, it is necessary to have models that are applicable in different climatic zones and types of water. That is why we are going to apply empirical models developed with data from different types of water at temperate zone to different types of water at tropical areas. This will allow us to have algorithms calibrated for the future scenarios that will cause climate change in temperate zones: a decrease in precipitation and an increase in temperature, evaporation and water retention time. To achieve this, between October and December 2021, thirteen reservoirs of the Tiete River basin (Sao Paulo, Brazil) were sampled, and 41 samples were obtained. The sampling points were georeferenced and phycocyanin was measured in situ using a Turner Design C3 Submersible Fluorometer calibrated with Spirulina Standard 40% purity (Sigma-Aldrich CAS 11016-15-2, San Luis, MO, USA). Seven Sentinel-2 images were processed with Sentinel Application Platform (European Space Agency) for resampling, and for atmospheric correction using the neural net C2X-C. The estimated values to be tested from algorithms application were validated with data from these reservoirs, covering a phycocyanin range from 5.4 to 326 µg/L. The results obtained were R^2 0.84, RMSE 63 µg/L, RRMSE 68%, MAPE 42% and bias 46 µg/L. To improve the obtained results, the algorithm was recalculated for these tropical data sets using the same operations for the same spectral bands (*R*704/*R*665 ratio), obtaining better results, R^2 0.85, RMSE 36 µg/L, RRMSE of 39%, MAPE 12% and bias 11 µg/L. With these results, we can see that it is possible to use algorithms from temperate zones in tropical zones, although they require recalculation, so a minimum number of samples should always be taken to validate and identify errors. This process will ultimately serve us to readjust models already tested in previous works built on much more data. This technique will help with remote sensing implementation, as an analysis tool in studies of dynamics and processes taking place in inland waters, and will help us try to stay ahead of climate change.

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Abstract

Toxic Cyanobacteria Impacts on a Eutrophic and Protected Natural Ecosystem (Vela Lagoon) †

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‡ Presenting author (oral communication).

Abstract: Toxic cyanobacteria naturally inhabit freshwater resources. The Vela Lagoon is a Natura 2000 protected site located near the Atlantic Ocean and in the central region of Portugal and has well-established recreational impacts. in recent years, the Vela Lagoon has been studied for the occurrence of cyanotoxins, and control studies have demonstrated that cylindrospermopsins were found on the water at maximum concentrations of 12 µg/L. Given these assessed impacts and the nature of this protected site, it becomes essential to review the impact of Vela Lagoon cyanotoxins within a longer period of time that includes 12 months between November 2016 and October 2017. The methods used include bloom occurrence and analysis on cyanotoxins, namely the microcystins variants LR, RR, and YR; cylindrospermopsins; anatoxin-a; and saxitoxins, by applying genomics and chemistry methods. The findings revealed that cylindrospermopsins are still a member of the cyanobacteria toxic community of the Vela Lagoon, that anatoxin-a was found to occur in trace amounts on the water fraction and bloom samples, and that microcystins-LR is the only variant found to occur in the Vela Lagoon. Given the two bloom episodes observed during the sampling season, control measures need to be readily implemented in the Vela Lagoon, since the impacts on the multiple cyanotoxins found can constitute a risk to humans who live near this protected site and frequently use this water for domestic irrigation. Finally, this study illustrates that toxic cyanobacteria can also impair water quality in sustainable environments.

Keywords: cylindrospermopsins; anatoxin-a; microcystins-LR; risk assessment

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Abstract

Bioprospection of Toxin-Producing Cyanobacteria in the BACA Culture Collection †

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† Presented at the 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins, Ponta Delgada, Portugal, 18–20 July 2022.
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Abstract: With the increased eutrophication of water bodies due to anthropogenic activities and climate change, aquatic environments have increased the growth of cyanobacteria blooms worldwide. In addition, these microorganisms may produce toxic secondary metabolites (cyanotoxins), such as microcystins, saxitoxins, cylindrospermopsins, and anatoxin-a. These are harmful to human health and to other organisms that come into contact with contaminated waters, resulting in hepatotoxic, dermatotoxic, neurotoxic pathologies, and death. As we still do not know all the cyanobacteria species that can produce them, this study aimed to search for the presence of cyanotoxin biosynthesis genes in cyanobacteria strains isolated from the lakes of the Azores and to assess the risk of toxicity to public health. Therefore, molecular techniques were used to identify the cyanotoxins biosynthesis genes in thirty cultured strains deposited in the Azorean Bank of Algae and Cyanobacteria (BACA). The results revealed the presence of eleven strains with the the *ana*F gene, of which, two had the the *ana*C gene. Given that the presence of these strains in lakes may represent a public health risk, the continuous monitoring of water quality and cyanotoxin presence in water bodies of the region is essential so that risk can be determined. Measures must be implemented to minimize this problem while preserving the population's life quality.

Keywords: microcystin; saxitoxin; anatoxin-a; cylindrospermopsin; PCR; public health; Azores

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Abstract

Temporal Evolution of the Cyanobacterial Infestation of the Lalla Takerkoust Dam Revealed by Landsat Satellite Imagery †

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Abstract: The Lalla Takerkoust dam in Morocco experiences episodes of cyanobacterial blooms every year. The variation trend of cyanobacterial blooms in this lake was studied. The Normalized Difference Vegetation Index (NDVI), derived from Landsat observations, combined with cyanotoxins, reported in the dam for more than 30 years, from 1990 to 2020, was used to provide a quantified history of the infestation dynamics of the dam water used for crop irrigation and drinking water production. The results showed that the vegetation indices fluctuated significantly over time (Kendall test $p < 0.001$). Summer and autumn were the main seasons for the appearance of cyanobacterial blooms, with higher cyanotoxin concentrations in autumn than in summer, with 159.67 µg/L in November 2020 and 4.79 µg/L in October 2019, respectively. On the other hand, other parameters related to climate change were analyzed to explain the differences in NDVI values over the years. The variation of temperature, quality of the chromatic spectrum, and precipitation seem to contribute to the variation of the NDVI and, consequently, the infestation of algal bloom in the lake. Indeed, in 2010, a year where rainfall was 333.57 mm and the average temperature in October was 18.81 C, we recorded a PAR of 102.84 $W \cdot m^{-2}$ with UVA of 11.51 $W \cdot m^{-2}$ and UVB of 0.25 $W \cdot m^{-2}$. During this year, NDVI was highest by 0.28, and biomass toxicology was highest at 11.5 mg $MCLR \cdot g^{-1}$ DW. Ultimately, this study is the first in Morocco that traces a temporal evolution by satellite imaging of the history of the Takerkoust dam infestation. It will allow an awareness of the effects of climate change on the quality of this freshwater resource.

Keywords: vegetation index; biomass toxicology; cyanobacteria; cyanotoxin; Takerkoust

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V.V., F.E.K., H.-P.G. and B.O.; visualization, R.M.; supervision, B.O., F.E.K. and H.-P.G.; project administration, A.C.; funding acquisition, A.C., V.V. and B.O. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data used here can be publicly accessed on https://power.larc.nasa.gov/data-access-viewer/ (accessed on 26 January 2022) Lalla Takerkoust dam Morocco.

Conflicts of Interest: The authors declare no conflict of interest.

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Abstract

Novel Toxicity Aspects of Cyanotoxins †

Angeles Jos *,‡, Antonio Casas-Rodríguez, Cristina Plata-Calzado, Leticia Diez-Quijada, Concepción Medrano-Padial, María Puerto, Ana I. Prieto and Ana M. Cameán

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‡ Presenting author (key-note talk).

Abstract: The occurrence of toxic cyanobacteria and their cyanotoxins production are increasing phenomena reported worldwide. Cyanotoxins, secondary metabolites of different cyanobacterial species, are considered a potential hazard that can become a risk for human and environmental health after exposure. Global changes (rising temperatures, eutrophication due to human activities, etc.) favour their appearance leading to increased exposures and, consequently, risks. In order to avoid their negative impacts, the World Health Organization has established provisional drinking-water and recreational-water guideline values, and different countries have implemented limits in their regulations. However, these limits are not enough taking into account the increasing number of known cyanotoxins. Also, the establishment of these legal limits is hampered by the gaps in the knowledge of their toxicological profiles. Thus, this work aimed to establish the state of the art on the human toxicity of cyanotoxins (mainly microcystins, cylindrospermopsin and anaxotin-A) and to shed light on aspects not completely elucidated, such as target organ toxicity, immunotoxicity or genotoxicity.

Keywords: cyanotoxins; cylindrospermopsin; microcystins; anatoxin-A; toxicity; risks

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Abstract

Cyanotoxins beyond Plankton and Lacustrine Environments †

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† Presented at the 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins, Ponta Delgada, Portugal, 18–20 July 2022.

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Abstract: The first evidence of cyanotoxins production came from planktonic species, and for a long time planktonic species and blooms were the focus of most toxicological studies. The toxicity of benthic species, and its consequences, has also been known from the very beginning. Still, in the last years, a huge amount of data has been gathered worldwide reinforcing the potential importance of cyanotoxins in benthic community dynamics, their role in modelling the physiognomy of aquatic systems, and the associated potential risks for human populations, especially in a climate change scenario. Cyanobacteria can develop in almost any possible habitat, natural or man-made, representing a potential hazard, but concentrations in benthos are usually very low, minimizing risks of sporadic human exposures. The importance of Cyanobacteria in the food webs of several aquatic systems has been highlighted lately as they may represent a very important food resource during different adverse environmental conditions, opening questions on the timing of toxin production or the effectiveness of detoxification methods of aquatic consumers. Furthermore, microalgae food and supplements have become very popular lately, and their regular consumption may represent a real risk when they contain Cyanobacteria and the presence of toxins is not analyzed. The globalization of markets eases the acquisition of products from everywhere, but there is no clear international legislation to protect consumers.

Keywords: benthos; cyanotoxins; animal and human toxicity; detoxification; toxicity adaptation

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Abstract

Green Nanotechnology for the Remediation of Cyanotoxins from Contaminated Waters †

Jesús M. González-Jartín [1,*,‡], Amparo Alfonso [1], Rebeca Alvariño [1], Inés Rodríguez-Cañás [1], Mercedes R. Vieytes [2], Yolanda Piñeiro [3], Lisandra de Castro [3], Manuel González [3], Jose Rivas [3] and Luis M. Botana [1]

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Abstract: The presence of contaminants in water may involve a risk to human and animal health. Conventional water treatment methods such as coagulation, flocculation, and sedimentation are ineffective for cyanotoxin removal. In addition, high amounts of cyanotoxins can be released during those processes if cells lyse. Thus, new mitigation strategies must be developed to ameliorate the consequences of harmful algal blooms. In this sense, nanotechnology has become a promising tool for the treatment of contaminated water. Several nanomaterials with specific chemical affinities can be combined into hybrid structures, leading to nanostructured agents with a large surface area and with the ability to absorb different contaminants. In addition, these structures can include magnetite, which enables separation from the detoxified substance by magnetic extraction, which is considered a green technique. This approach has been successfully applied to the removal of dyes, endocrine disruptors, and heavy metal ions. Recently, we have described the use of carbon nanoparticles to remove around 60% of microcystins from contaminated solutions, but with a low efficiency in the adsorption of anatoxin-a and cylindrospermopsin. In this work, a new set of biocompatible magnetic nanocomposites were tested using artificially contaminated water. The toxin content in solutions was determined before and after treatment by ultra-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS). With these new nanocomposites, cyanotoxin elimination was highly improved, reaching toxin removal rates of up 80%. Therefore, the implementation of the nanotechnology in water treatment could be a promising approach to reduce the presence of natural toxins in the water.

Keywords: cyanotoxins; nanotechnology; UHPLC-MS/MS; nanoparticles; Microcystis

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Abstract

The AIHABs Project: Towards an Artificial Intelligence-Powered Forecast for Harmful Algal Blooms †

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Abstract: Eutrophication of water bodies in Europe is contributing to the increase of Harmful Algal Blooms (HABs) which pose a serious risk to human health. To address this problem, the AIHABs project will develop an early warning forecasting system to predict the occurrence, spread and fate of cyanotoxins caused by HABs in inland and coastal waters, using Artificial Intelligence (AI) and the latest innovations in mathematical modelling, nanosensors, and remote sensing. The system predictions will allow timely action to minimise the risks of consuming surface waters or using them as recreational resources when the water bodies are prone to producing toxic cyanobacterial blooms. Following a multi-criteria analysis, two sites with a history of HABs (one in Spain and one in the Czech Republic) were identified as the most suitable inland and coastal water sites for the study. The main criteria for site selection were the availability of the catchment required data for modelling, the strong evidence of historical HABs, the ease of satellite monitoring of water bodies and accessibility for water sampling. Samples will be taken, synchronously with satellite image acquisition, during, before and after algal blooms. In addition, current and historical data from the selected catchments will be included in a prediction model using the MIKE HYDRO River software, and innovative nanosensors will be designed to determine the concentration of cyanotoxins. Finally, an early warning forecasting system will be developed to predict the occurrence, spread and fate of cyanotoxins caused by HABs in water bodies.

Keywords: cyanotoxin; modelling; nanosensors; remote sensing

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Abstract

Expansion of Cylindrospermopsin in the Azores: Evidence for New Producing Taxa †

Rita Cordeiro [1,*,‡], Rúben Luz [1,2], Joana Azevedo [3], Vitor Vasconcelos [3,4], Vítor Gonçalves [1,2] and Amélia Fonseca [1,2]

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Abstract: The worldwide expansion of cylindrospermopsin (CYN) has been a public concern due to its potential impacts on the environment, the economy, and mainly on human health. Due to global warming, this toxin has been reported in a wider range of countries, from tropical to temperate regions. The first report of cylindrospermopsin in the Azores came from an environmental sample collected in 2016 and was later found in two nostocalean cultured strains isolated from a eutrophic lake. This work gathers all the CYN data in the Azores and reports new identifications of toxic strains. Selected cultured strains (five) from BACA (Azorean Bank of Algae and Cyanobacteria) were analyzed for the presence of genes cyrA, cyrB, cyrC, and cyrJ by PCR and for toxin identification by ESI-LC-MS/MS. 16S rRNA phylogenetic analysis was assessed for all strains. The main results showed amplification of cyr genes in *Nostoc* sp. BACA0429, *Kamptonema* sp. BACA0455, and nostocalean strain BACA0109. However, ESI-LC-MS/MS did not identify CYN in any of the tested strains. The phylogeny also revealed that BACA0109 is close to previously identified CYN producers BACA0025 and BACA0031, described as potential new cyanobacteria taxa. The presence of CYN and CYN-producing cyanobacteria in the remote Azorean Islands is further evidence of the CYN global dispersion and an alert to the need for cyanotoxins monitoring and mitigation in the Azores' inland waters.

Keywords: PCR; 16S rRNA; cyr; ESI-LC-MS/MS; monitoring

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Abstract

Cyanobacteria as a Source of Eco-Friendly Bioactive Ingredients for Antifouling Marine Coatings †

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Abstract: Marine biofouling is defined as the undesirable colonization of submerged man-made surfaces by fouling organisms (microfoulers and macrofoulers) and represents a major economic nuisance for maritime industries worldwide on account of the drag friction increase on ships' hulls resulting in the over-consumption of fuel and high maintenance costs. The most commonly used strategy to prevent marine biofouling is based on antifouling (AF) paints containing bioactive compounds. However, some of the AF compounds used have been found to be toxic towards target and non-target organisms, which raises many environmental issues. Thus, the development of new eco-friendly AF agents has been a priority. Portoamides (PAs), natural cyclic dodecapeptides isolated in our group from the cyanobacterium *Phormidium* sp. LEGE 05292 from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC), have shown strong potential as a more sustainable active ingredient in AF compositions. These PAs showed high effectiveness in the prevention of mussel larvae settlement (EC50 = 3.16 µM), and also bioactivity towards growth and biofilm disruption of marine biofouling bacterial strains, while not showing toxicity towards both target and non-target species. Considering the great potential of these natural products in the field of antifouling solutions, in this work, the incorporation of the PAs in commercial polyurethane and silicone (PDMS)-based marine coatings, followed by a proof-of-concept test in real sea conditions (Leixões Port), was carried out to demonstrate their industrial applicability. The in situ test showed effectiveness in the ability to prevent the colonization of fouling organisms on substrates coated with PAs-based marine coating when compared with control, and even compared with the commercial biocide Econea. These results highlight the potential of natural products as active ingredients in new more environmentally friendly marine coatings to prevent biofouling.

Keywords: antifouling; cyanobacteria; portoamides; marine coatings; eco-friendly

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Abstract

Cyanotoxin Removal from Water Using Activated Carbon Magnetic Beads †

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Abstract: There are many contaminants in water that may damage the health of people and animals, such as naturally occurring cyanotoxins, which have increased their presence in recent years due to climate change and eutrophication. Although water must pass through a treatment station before consumption, none of the treatment methods used are totally effective for the elimination of cyanotoxins. In this study, a complementary method for toxin removal is investigated which consists of using nanostructured beads with a magnetic core coated by an adsorption material. In contact with water, the beads are capable of adsorbing different toxic compounds on their surface and can be easily separated from water, afterwards, by a magnet. Adsorption spheres are prepared with nanostructured magnetite cores coated with activated carbon using sodium alginate as an agglutinating compound. The adsorption capacity of these magnetic beads is assessed with water solutions of microcystin-LR, cylindrospermopsin, and anatoxin-A. Toxin removal from water is evaluated by quantification using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. The results show that these activated carbon-coated magnetic beads can remove approximately 20% of microcystin-LR from mili-Q water at concentration levels 60 times higher than the WHO recommended level of 1 µg/L. With the same conditions, 20 % of cylindrospermopsin is also captured. For anatoxin-A, with a much smaller molecular weight, 70% is removed at a six-fold lower concentration. Toxin removal increases throughout the 2-h duration of the experiments. Microcystin-LR adsorption is affected by pH, with a higher removal at highly acidic or alkaline pHs. In addition, these beads can be regenerated and reused for several adsorption cycles. In summary, activated carbon magnetic beads can be used to remove cyanotoxins from water with varying effectiveness, depending on toxin size and solution pH, and they can be reused for several removal cycles after optimized regeneration protocols.

Keywords: magnetic beads; activated carbon; cyanotoxins; detoxification

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Abstract

Microcoleus autumnalis Cyclopeptides Present Protective Properties against Oxidative Stress †

Rebeca Alvariño [1,*,‡], Amparo Alfonso [1], Shauna O'Brien [2], Jesús González-Jartín [1], Nadia Pérez-Fuentes [1], Sandra Gegunde [1], Brian Kennedy [3], Olivier Thomas [2] and Luis M. Botana [1]

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Abstract: Oxidative stress is a common pathological mechanism in neurodegenerative diseases. Aging leads to mitochondrial dysfunction and produces a reduction in endogenous antioxidant systems efficiency, with a consequent increase in reactive oxygen species (ROS) release. This unbalance among protecting and damaging molecules impairs neuronal function, even causing cell death. Therefore, the search for new drugs with antioxidant and neuroprotective effects is a great challenge. In this work, the bioactivity of three new cyclopeptides (1–3), isolated from the freshwater cyanobacterium *Microcoleus autumnalis*, was tested in SH-SY5Y human neuroblastoma cells. This species is a prolific source of bioactive metabolites, anatoxin being the best-characterized molecule due to its neurotoxicity. In this context, cytotoxicity of compounds was firstly analyzed. None of the *M. autumnalis* metabolites induced toxic effects up to 50 μM after 24 h of incubation. In view of the lack of neurotoxicity produced by compounds 1–3, their antioxidant and neuroprotective ability was analyzed. With this objective, SH-SY5Y cells were co-treated with compounds (0.001, 0.01, 0.1, and 1 μM) and 150 μM H_2O_2 for 6 h. The effect of compounds 1–3 on cell viability under oxidative stress conditions was determined, finding that the three compounds were able to protect neuronal cells from oxidative damage. Next, mitochondrial function was evaluated by monitoring mitochondrial membrane potential. In this assay, only compound 3 recovered the organelles from the depolarization generated by H_2O_2. Finally, intracellular ROS content was assessed, observing that 1–3 decreased the levels of these harmful molecules. In conclusion, *M. autumnalis* cyclopeptides presented neuroprotective effects mainly mediated by their ability to reduce ROS levels. These results suggest that compounds 1–3 act as direct antioxidants and could be interesting compounds for further studies in neurodegenerative diseases.

Keywords: *Microcoleus autumnalis*; cyanobacteria; antioxidant; neuroprotection

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Abstract

Effects of Refrigeration and Freezing in Cylindrospermopsin and Microcystin Concentrations on Leaves of Lettuce (*Lactuca sativa*) and Spinach (*Spinacia oleracea*) †

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Abstract: As a consequence of climate change, an increase in the occurrence of cyanotoxins is happening. These toxins are a large group of secondary metabolites with different chemical structures, mechanisms of action and worldwide distribution, and can cause harmful effects in humans and animals. Humans can come in contact with these toxins through the oral route through the consumption of contaminated water and food. Frequently, in order to improve their shelf life, many foods, such as vegetables, are refrigerated or frozen before being consumed. Therefore, the aim of this work was to assess the potential effect of refrigeration (4 °C for 24 h, 48 h and 7 days) and freezing (−20 °C for 7 days, 1 month and 3 months) on the concentration of cylindrospermopsin (CYN) and microcystins (MCs) (MC-LR, MC-RR and MC-YR) in lettuce and spinach leaves. Vegetable samples were spiked with a stock solution of each toxin containing 0.75 µg toxin/mL (equivalent to 0.75 µg/g fresh weight) and subjected to the corresponding conservation process. Samples were extracted and quantified with ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). In the case of CYN, only in spinach samples the refrigeration process caused a significant time-dependent decreased in its concentration (48 h and 7 days). However, this preservation process was not shown to be effective in reducing the concentration of this toxin in lettuce. Moreover, the freezing process in spinach didn´t show differences in the CYN content between the control and the experimental groups. In general, results obtained in MC concentrations showed that the refrigeration process in both lettuce and spinach caused a significant decrease in toxin concentrations, and this decrease was higher in lettuce. Moreover, freezing caused a significant reduction in MC concentrations in spinach after 3 months. These results showed the differences in the toxin content depending on the type of process selected (refrigeration versus freezing) and vegetable species. Furthermore, more studies are needed to study the influence of different storage processes on cyanotoxin concentrations in vegetables humans consume to assess the risk of human exposure to these cyanotoxins in a more realistic way.

Keywords: cylindrospermopsin; microcystins; spinach; lettuce; refrigeration; freezing

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Abstract

Effects of Physicochemical Variables on the Cyanobacteria Biovolume in Iberian Peninsula †

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Abstract: The growing need for water supply, because of the massive population growth, causes continuous problems. Water is subjected to great pressures, diminishing its quality and affecting the flora and fauna that depend directly or indirectly on it, as well as humans. These aspects increase the cost of its treatment or the loss of water bodies for human consumption. One of the main problems that appear in inland water bodies is nutrient enrichment, which is associated with problems of eutrophication or even hypertrophy, which can cause massive blooms of cyanobacteria, altering or destroying aquatic ecosystems. This work focuses on the study of different physicochemical variables of inland water bodies in the NE of the Iberian Peninsula in the Ebro river basin, such as temperature, residence time, pH, total nitrogen, inorganic nitrogen, total phosphorus, orthophosphates, silicates and phycocyanin, in order to establish a relationship with cyanobacterial biovolumes and how they affect their proliferation. These variables were analyzed in the laboratory in order to, subsequently, carry out a correlation between variables and a multistatistical analysis of components. On the other hand, remote sensing was used by applying a previously developed algorithm to evaluate phycocyanin concentrations and compare them with in situ measurements. This provided significant correlations between the temperature, total nitrogen, total phosphorus and residence time according to the biovolume of cyanobacteria; we also obtained a negative correlation, for example, in the case of silicates. The results showed alarming levels of cyanobacteria in a large part of the reservoirs, which should be studied due to the danger associated with the possible appearance of cyanotoxins.

Keywords: cyanobacterial harmful algae blooms; remote sensing; phycocyanin; cyanobacterial biovolume; reservoirs

Citation: Pérez-González, R.; Sòria-Perpinyà, X.; Soria, J.; Sendra, M.D.; Vicente, E. Effects of Physicochemical Variables on the Cyanobacteria Biovolume in Iberian Peninsula. *Biol. Life Sci. Forum* **2022**, *14*, 19. https://doi.org/10.3390/blsf2022014019

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Abstract

Analytical and Functional Profiles of Paralytic Shellfish Samples Extracted from *Semele proficua* and *Senilia senilis* †

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Abstract: Paralityc shellfish poisoning (PSP) is a human illness associated with the consumption of contaminated seafood products with the toxins known as saxitoxins and congeners. The PSP syndrome is associated to three groups of toxins: N-sulfocarbamoyl, carbamate and decarbamoyl, produced by dinoflagellates, generally by the genus *Gymnodinium*, *Alexandrium* and *Pyrodinium*. Between 2007 and 2008, episodes of PSPs contaminations in bivalves in Angola were reported. In this work, ten samples were extracted from *Semele proficua* captured in Luanda Bay and *Senilia senilis* catched in Mussulo Bay. This samples were analyzed by HPLC and functional electrophysiology in order to detect possible benzoate paralytic shellfish poisoning toxins and to determine their activity on sodium channels. These compounds were detected at higher amounts after peroxide oxidation than after periodate oxidation. All the compounds presented STX-like activity at concentrations of 6.996 µg STX eq/kg. Three of the ten samples showed an unknown peak after 8 min of peroxide oxidation which means that this unknown compound did not affect the functionality of sodium channels in cerebellar granule cells obtained in our laboratory from 7-day old mice.

Keywords: saxitoxin; decarbamoylsaxitoxin; sodium channels; cerebellar neurons; high-performance liquid chromatography

Citation: Raposo-Garcia, S.; Botana, A.M.; Rey, V.; Louzao, M.C.; Vale, C.; Botana, L.M. Analytical and Functional Profiles of Paralytic Shellfish Samples Extracted from *Semele proficua* and *Senilia senilis*. *Biol. Life Sci. Forum* **2022**, *14*, 20. https://doi.org/10.3390/blsf2022014020

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Abstract

Cyanotoxins (Microcystins) in Water Irrigation: Evaluation of Contamination, Detoxification Process in Plant-Animal Food Chain Model and Human Health Risk †

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Abstract: Under the effect of climate change and eutrophication, cyanobacterial blooms occur in surface waters; these cyanobacteria are capable of producing secondary metabolites called cyanotoxins. Among these cyanotoxins, microcystins (MCs) are the most common and most harmful to human and animal health. Agricultural plants come into direct contact with microcystins (MCs) during irrigation with waters containing cyanotoxins (MCs), whereas animals and humans may come across MCs following the consumption of initially contaminated agricultural products. The objective of this work was to evaluate the bioaccumulation, transfer and detoxification of MCs in plants (*Fragaria vulgaris* L.) and a nuisance animal (*Meriones shawii*). *F. vulgaris* culture was conducted under hydroponic conditions. All plants were irrigated using 0, 1, 5, 10 and 20 µg/L of MCs for 60 days. Besides, aliquots (10 g) of fruit from the treated plants were prepared to feed *M. shawii* previously divided into five groups with six animals each for 4 consecutive weeks. All experimental procedures were performed in accordance with Cadi Ayyad University's animal care guidelines and European Decree 1, February 2013, for the ethical use of animals for experimental purposes (NOR: AGRG1238767). We made efforts to reduce pain and the number of *Meriones* used in this study. The results reported a decreasing accumulation of toxins in different organs of the plant (perlite > roots > leaves > stems > fruits). With the obtained results, the bioconcentration factor (BCF) and the acceptable daily intake (ADI) were determined in order to gauge the health risk. Furthermore, the glutathione S-transferase (GST) and glutathione reductase (GR) activities were also evaluated. The highest activities were recorded in F. vulgaris roots and M. shawii liver. The results of this study indicated the possible bioaccumulation and transfer of MCs throughout the food chain, in addition to the involvement of enzymes such as GSH and GR in the detoxification process of MCs.

Keywords: microcystins; *Fragaria vulgaris*; *Meriones shawii*; bioaconcentration; irrigation; hydroponic culture; bioconcentration factor; acceptable daily intake

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Abstract

Assessment of Cyanobacterial Biomass as Sustainable Agricultural Fertilizer: Soil Experiment with Plants in Pot †

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Abstract: Providing food to the growing human population in a sustainable way is one of the greatest challenges of modern society. In this context, cyanobacterial biomass (CB) can function as a source of macronutrients to increase soil productivity. These organisms can be collected from the environment in considerable amounts, since they tend to grow in large blooms. However, some of these cyanobacterial strains produce toxins that need to be carefully monitored to avoid food accumulation. The objective of this work was to evaluate the possible use of toxic and non-toxic strains of CB as fertilizer supplement in the growth of economically relevant vegetables. One-month-old *Raphanus sativus* (radish) and *Spinacia oleracea* (spinach) plants were grown in pots in indoor controlled conditions. Six experimental conditions were set: (1) a control with no nutrient addition, (2) a recommended dose of a NK commercial fertilizer (CF), 0.6g of lyophilized CB of (3) a non-toxic strain of *Cylindrospermopsis raciborskii*, (4) a toxin-producing strain of *C. raciborskii*, (5) *Microcystis aeruginosa*, and (6) *Anabaena* sp. Several variables were estimated: in CB, this included the NPK dose addition, and in plants, the height, dry weight (dw) of the shoot and root, and the mineral content of plant edible parts. The mineral content in CB was estimated and compared with the recommended dose of CF, according to the information given by the fabricant label. We found no significative differences in N composition; nevertheless, there was a significative higher content in P and significative lower content in K in the CB. In the plants, we found no significative statistical differences between the treatments for the dw of radish root and spinach height. In spinach, the dw of the shoot in the *M. aeruginosa* treatment was significantly lower than the control, CF, and both the toxic and non-toxic *C. raciborskii* biomass. Additionally, in radish, the plant height and dw of the shoot *M. aeruginosa* treatment were significantly lower than in the toxic strain of *C. raciborskii* treatment. When analyzing mineral content in edible parts, we found that spinach treated with control and CF showed a higher content of Ca, Mo, N, P, and K, while in radish, the same two treatments plus the *C. raciborskii* toxic had higher Co and Fe content. *M. aeruginosa* amendment seems to impair shoot growth in both plant species. On the contrary, the toxic *C. raciborskii* CB seems to have a beneficial effect on growth and in mineral uptake on radish plants.

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Keywords: radish; spinach; fertilizer; *Anabaena*; *Cylindrospermopsis*; *Microcystis*

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Abstract

Presence of *Microcystis* sp. and Microcystins in Alqueva Reservoirs Assessed by Chemical and Molecular Methods †

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Abstract: The Alqueva reservoir, located in the Alentejo region, in the south of Portugal, is considered the largest artificial lake in Europe. It has been in operation since 2002, and it is used to produce energy and supply water for agriculture and to the populations in this region of Portugal. The water distribution system, starting from the main reservoir, includes 19 reservoirs of smaller capacity and a network of waterways totaling 382 km in length. Furthermore, the occurrence of cyanobacteria in water reservoirs has been recognized as an environmental concern due to the potential presence of their related toxins that can cause severe health effects. This work aimed to monitor the presence of cyanobacteria and the commonly associated cyanotoxin microcystin (MC) in three reservoirs belonging to the Alqueva water system, namely São Pedro, Magra, and Pisão, located in the district of Beja. These reservoirs were selected considering the historical data of phytoplankton provided by EDIA, the entity in charge of the management of this infrastructure. The field work was carried out in July, August, and September, the months with the highest risk of outgrowth of cyanobacteria, in the year 2020. Two or three samples of water (5 L) were collected in different locations of the reservoirs, once per month, by boat. Samples were collected at different depths in the photic zone using a Van Dorn bottle and pooled. The water samples were then processed in the laboratory. Molecular biology techniques were used to detect the presence of cyanobacteria (16S rRNA) and MC-related genes. Moreover, chemical analysis techniques based on liquid chromatography and mass spectrometry (MS) were used to identify and quantify MCs. The results revealed the presence of MCs in the three reservoirs in the three months monitored. Concentrations of this toxin varied between 0.01 µg/L and 0.1 µg/L, with São Pedro being the reservoir displaying the highest concentrations of MCs in all of the months monitored. These results are consistent with the molecular study based on the analysis of *Microcystis* sp. 16 rRNA and MC biosynthetic genes (mcya–mcyG), suggesting the presence of putative toxic *Microcystis* sp. strains in the three reservoirs. Despite the low concentrations of MCs detected in these reservoirs, their recurrent presence in Alqueva waters serve as a reminder of the need to monitor cyanobacteria and cyanotoxins on a regular basis.

Keywords: toxic cyanobacteria; cyanotoxins; water quality; Alqueva; crop irrigation

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Abstract

Alternative Methods of Treatment of Cyanobacterial Biomasses to Reduce Toxin Content †

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† Presented at the 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins, Ponta Delgada, Portugal, 18–20 July 2022.
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Abstract: Microalgae blooms are natural processes that occur in eutrophic aquatic ecosystems. Microalgae blooms, namely those constituted by cyanobacteria, are undergoing a significant expansion as a result of anthropogenic pollution and climate change. Many of these blooms cause environmental and public health concerns due to the production and accumulation of toxic substances by some cyanobacterial species. Despite the burdens that cyanobacteria may cause in the environment and human health, cyanobacterial biomasses are interesting sources of compounds in biotechnology. Cyanobacteria also have interesting plant growth properties, and their biomass is an excellent soil amendment. In order to promote safe use of this type of material in biotechnology and agriculture, a research work was outlined, which consisted in seeking inexpensive and environmentally sustainable methods of treatment of *Microcystis aeruginosa* biomass and to reduce the content of the toxin microcystin (MC) in the biomass. Lyophilized or hydrated biomass from laboratory cultures of *M. aeruginosa* were subjected to treatments by heat (50 °C), ultraviolet radiation, ozone, and solar radiation for periods ranging from 2 to 12 h. The results demonstrate a significant reduction in the amount of MC in the biomass exposed to natural radiation for 12 h, from 0.0042 to 0.0028 mg of MC-LR/mg of dry biomass, equivalent to a reduction of about 33% of the total toxin. Efforts are currently being made to characterize the chemical transformation of the toxin catalyzed by natural radiation. No other treatment allowed us to reduce the amount of toxin present in the biomass, which suggests a strong chemical resistance of MC. This method of treatment of cyanobacterial biomass is quite interesting, and its use on a large scale depends on a confirmation of the preservation of the biotechnological properties of biomass after the applied treatment.

Keywords: cyanobacteria; cyanotoxins; biotechnology; bio-fertilizer; sustainable agriculture

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Abstract

Are Cyanobacteria an Overlooked Risk for Ecosystems and Visitors in Spanish National Parks? †

Albano Diez-Chiappe [1,*,‡], Samuel Cirés [1], Felipe Bolgenhagen [1], María de los Ángeles Muñoz-Martín [1], Ana Justel [2], Antonio Quesada [1] and Elvira Perona [1]

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Abstract: Potentially toxic cyanobacterial communities are prolific in freshwater ecosystems and in national parks where vulnerable fauna tend to be found. This study focuses on the proliferation of toxic cyanobacteria and the risks they represent in the rivers and reservoirs of two Spanish national parks. As far as we know, this is the first time that an ecological, taxonomical and toxicological characterization of planktonic and benthic cyanotoxin-producing cyanobacteria is carried out in this type of protected areas in Spain. Our results, obtained during 2021 sampling campaigns, have confirmed the occurrence of cyanobacteria in these ecosystems and assessed the risks of these cyanobacteria to these ecosystems. An alarming occurrence of benthic mats, dominated mainly by *Phormidium autumnale* (also called *Microcoleus autumnalis*), a potentially anatoxin producer, has been observed in rivers from Sierra de Guadarrama National Park. In reservoirs from Monfragüe National Park, the planktonic communities have been dominated by *Microcystis*, *Aphanizomenom*, *Arthrospira* and *Planktothrix*. Genetic screening by PCR and sequencing have confirmed the presence of cyanotoxin biosynthesis genes (*mcy*E, *ana*F and *sxt*A) in all communities studied. The relationship among community diversity, the presence/concentration of cyanotoxins (microcystins, saxitoxins and anatoxins) and the environmental parameters measured is discussed. These results will contribute to preparing protocols for evaluating and managing the potential risk to visitors to, workers in and fauna of these protected ecosystems.

Keywords: planktonic; benthic; *Phormidium*; anatoxin; microcystins; saxitoxins

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Abstract

Occurrence of Cyanotoxins in Mineral Water Sources and Hot Springs from NW Iberian Peninsula †

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Abstract: Balneotherapy can cause adverse reactions to the usual doses of application of treatments, and consists of a nonspecific clinical picture, the so-called "thermal crisis" or "balneointoxication". Despite its clinical similarity (gastric discomfort, hepatic congestive outbreaks, cutaneous reactions, etc.) with that observed in acute cyanotoxin poisonings, a thermal crisis has never been associated with the abundant growth of potentially toxic cyanobacteria in mineral water sources. The aim of this work was to verify the hypothetical involvement of cyanotoxins in this clinical picture. Several samples (21) of 18 sources, representative of the different mineral–medicinal waters present in Galicia (northwest Spain) and mostly used for balneotherapy, were collected during September 2018. Samples were filtered and the algae retained were extracted with sonication using acidified methanol and analysed with LC-ESI-HRMS. The target analysis of the cellular matrix samples (limit of detection = 0.01–0.05 μg L^{-1}) did not show nodularin or any of the microcystins (MCs) for which standards are available (MC-dmRR, RR, dmLR, YR, LR, WR, LA, LY, LW and LF). The presence of other MCs, nodularins and related cyanobacterial peptides (CPs) was observed with a suspect screening analysis of cyanotoxins, based on an HRMS home-made database of 157 MCs, 10 nodularins, cylindrospermopsin and 29 CPs previously described in the literature. Signals not referenced in the literature were identified as CPs. Based on HRMS and restrictive criteria (accuracy, isotopic pattern, diagnostic fragments, elements considered, charge, ring plus double bond equivalents and nitrogen rule), the signals were confirmed. In summary, 12 MCs, 2 nodularins and 2 CPs were qualitatively detected. A list of all tentatively identified cyanopeptides in each sample was reported, including the retention times, ion signal type, proposed molecular formula, theoretical *m/z*, samples where each signal was detected, mass accuracy of measures and their isotopic pattern scores. CP-2 was detected in 14 samples, and it was confirmed as a signal from a cyanobacterial peptide, but with more unsaturations than analogous MCs. The presence of MC-OiaA and MC-OiaAba in three samples was also noteworthy. In addition, [seco-2/3]NOD-R was detected in five samples.

Keywords: thermal crisis; microcystins; nodularins; cyanobacterial peptides; mineral–medicinal waters; LC-ESI-HRMS

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Abstract

Contributions of TOXICROP Project for the Assessment of the Impacts of Toxic Cyanobacteria in Agriculture †

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Abstract: Water contaminated with microcystins (MCs) or other cyanotoxins is recurrently used in agriculture and for crop irrigation. Several deleterious effects of MCs in plants that may impair crop productivity, including a decrease in growth and tissue necrosis, as well as an inhibition of photosynthesis and metabolic changes, have been reported. Studies also revealed a significant accumulation of MCs in edible tissues and plant organs, which raise concerns related to food safety. The European project TOXICROP precisely tackles this environmental problem. The main aims of the project are to map agricultural risk areas of cyanotoxin occurrence, to assess the fate of cyanotoxins in crops, and evaluate the impacts of using low-quality water for crop irrigation. The project also develops research on water remediation, exploring nature-based technologies. Here, we review part of the research carried out in the project, concerning the toxicity of cyanotoxins in crops. The research from TOXICROP Consortium has revealed for instance that adult strawberry or faba bean plants are susceptible to moderate concentrations of MCs (10 to 20 µg MCs/L). Furthermore, experiments with faba bean and common wheat grown in sterile (microorganism-free) and non-sterile (microorganism-rich) soil, watered with 100 µg MCs/L, revealed that native rhizospheric microbiota play an important role in the mitigation of the phytotoxic impact of MCs on plant growth, reducing toxin accumulation in both soils and plant tissues. Our studies also revealed that leaf vegetables, such as lettuce and spinach, growing in hydroponics are more susceptible to MCs than to the toxin, cylindrospermopsin (CYN). The lowest toxin concentrations affecting spinach and lettuce growth were 5 + 5 and 25 + 25 µg/L CYN/MC mixtures, respectively. The results also reveal that the accumulation of MCs and CYN in plants depends on the conditions in which plants grow and concentrations of toxins in the irrigation water. In some cases, MCs are accumulated in plant tissues and exceed the tolerable daily intake proposed by the World Health Organization. We highlight the importance and contributions of this research to the definition and implementation of regulatory limits for cyanotoxins in irrigation waters.

Keywords: toxic cyanobacteria; cyanotoxins; water quality; alqueva; crop irrigation

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Abstract

Antibacterial Activity of Cyanobacterial Extracts against *Legionella* spp. †

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Abstract: Cyanobacteria are recognized sources of natural compounds with a pharmaceutical interest, namely for their antimicrobial activity. Several studies have shown the inhibitory effect of cyanobacteria against the most common bacterial pathogens. However, the bioactivity against *Legionella pneumophila* was never reported. *L. pneumophila* is ubiquitous in water environments and causes respiratory infections through water–air transmission. A rise in *Legionella* outbreaks might be expected, considering that climate changes will exacerbate water-borne diseases. In this work, we evaluated the antibacterial potential of 25 freshwater cyanobacteria from ESSACC collection, against seven *Legionella* strains (two reference, two clinical and three environmental). Cyanobacterial biomass was extracted with n-hexane, dicloromethane:methanol (2:1), methanol 70%, and water and extracts were dried and dissolved in DMSO (25%). The disk diffusion method was adapted to *Legionella* growth using 1 McFarland suspension in BCYE plates. Levofloxacin (10 µg) and DMSO (25%) were used as positive/negative controls, respectively. Methanolic extracts from *Dolichospermum flos-aquae* (LMECYA 165), *Limnothrix redekei* (LMECYA 145), *Microcystis aeruginosa* (LMECYA 127) and *Planktothrix agardhii* (LMECYA 257) induced inhibition zones ≥10 mm, demonstrating their antibacterial activity against *L. pneumophila*. These results encourage us to further investigate the potentiality of cyanobacteria as natural sources of antibiotics and/or water disinfectants, to overcome the occurrence of pathogenic *Legionella* in water environments.

Keywords: antimicrobials; *Legionella*; cyanobacterial extracts; natural compounds; freshwater cyanobacteria

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Abstract

Capillary Electrophoresis–Tandem Mass Spectrometry as an Analytical Technique for the Simultaneous Determination of Multiclass Cyanotoxins †

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Abstract: Cyanotoxins are toxic metabolites produced by most cyanobacteria. In recent years, the occurrence of cyanobacterial blooms in aquatic ecosystems has temporally and spatially increased because of nutrient oversupply caused by human and also by climatic changes. This increase has a negative impact on water quality, ecosystem integrity, and human health. Cyanotoxins constitute a group of compounds with diverse physicochemical properties and their presence in drinkable, fishable, and recreational water is the main health-damaging cause. They are also able to bioaccumulate in plants and vegetables irrigated with contaminated water. Research on the development of suitable analytical methods is needed to establish early-warning strategies for the improved protectionof humans and ecosystems health. Liquid chromatography coupled with mass spectrometry (LC-MS) has been the preferred option for the control of these compounds, mainly using reverse-phase mode or hydrophilic interaction liquid chromatography (HILIC) in order to separate multiclass cyanotoxins of varying polarity, which cannot be handled by the commonly used reverse phase columns. In this work, we propose the use of capillary electrophoresis (CE) coupled with tandem mass spectrometry using triple quadrupole and positive electrospray ionization (CE-(ESI)-MS/MS) to determine a mixture of cyanotoxins with different polarity. CE is an advantageous alternative to LC given its short analysis times, high resolution, low sample and reagent volumes, and the use of silica capillaries and buffers as separation media, resulting in lower cost and low environmental impact. Moreover, CE allows the analysis of molecules hardly affordable by LC, such as polar and very similar compounds (e.g., isomers). The method is designed for the simultaneous determination of eight cyanotoxins belonging to three different classes: cyclic peptides (microcystin-LR, microcystin-RR, and nodularin), alkaloids (cylindrospermopsin, anatoxin-a), and three non-protein amino acids isomers (β-methylamino-L-alanine, 2,4-diaminobutyric acid, and N-(2-aminoethyl) glycine). Separation was achieved using an acidic background electrolyte (BGE) consisting in 2 M of formic acid (FA) and 20% acetonitrile in water. The proper separation and resolution of the three non-protein amino acid isomers was one of the main challenges of the method. This was overcome by applying a voltage of 30 kV in a 90 cm length capillary at 20 °C. Parameters affecting MS detection and the sheath–liquid interface were also studied. Finally, the fixed values were: a sheath gas flow rate of 5 L/min at 195 °C; sheath–liquid consists of MeOH/H2O/FA (50:49.95:0.05 $v/v/v$), a flow rate of 15 µL/min; and a nozzle voltage of 2000 V; N_2 dry gas rate of 11 L/min at 150 °C; a nebulizer pressure of 10 psi; and a capillary voltage of 2000 V. Online pre-concentration approaches were tested in order to achieve higher sensitivity, obtaining a enrichment factor of 4 with a mixed technique of pH-junction and Field Amplied Sample Stacking (FASS).

Keywords: capillary electrophoresis; multiclass cyanotoxins; online pre-concentration; tandem mass spectrometry

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Abstract

Cyanotoxins Dynamics on Portuguese Freshwater Ecosystems on Current Global Changes †

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Abstract: Cyanotoxins are chemical pollutants produced and released by Cyanobacteria, the oldest living prokaryotes, and have well-established implications for human and aquatic biota health. Due to current global conditions, it has become essential to offer an overview of the dynamics of cyanotoxins occurrence in order to improve the prediction and control of our water resources. In Portugal, in recent years, the impacts on global warming have contributed to hot abnormal events and a rise in temperature. Between May and October in two consecutive years (2017–2018), seven freshwater ecosystems located in the north and central regions of Portugal were assessed for the occurrence of microcystins (mcyA), cylindrospermopsins (cyrC), anatoxin-a (anaC), and saxitoxins (sxtI) by applying genetics methods. Our findings illustrate that, in a year with two heat waves (2017), the occurrence of blooms increased, as did the number on cyanotoxins detected compared with the less warm year (2018). Despite this trend, two ecosystems escaped and maintained an elevated risk with regard to cyanotoxin detection. DNA sequencing revealed the presence of potentially toxic cyanobacteria in all sampled ecosystems. The data retrieved highlighted genotypes for all main cyanotoxins. Continuous monitoring efforts are demanded in Portugal for improving knowledge of the occurrence of cyanotoxins and for future national regulations. Preliminary data also revealed that the impact of global change on Portugal has improved cyanotoxin detection.

Keywords: microcystins; cylindrospermopsins; anatoxin-a; saxitoxins; PCR; risk assessment

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Abstract

Monitoring Cyanobacteria and Phycocyanin: A Case Study in the Albufera of Valencia †

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Abstract: Some cyanobacteria are considered beneficial to humans, while others are considered harmful due to their ability to synthesize and release cyanotoxins. Cyanobacterial blooms are proliferations of cyanobacteria due to the high concentration of nutrients such as nitrogen and phosphorus, as well as the intervention of other physicochemical parameters such as solar radiation and temperature, worsening the trophic state of the water body. Cyanobacterial bloom can produce cyanotoxins, which directly decrease water quality for human use. Since the 1980s, urban and agricultural intensification, together with the effects of climate change, produced the current poor trophic state of the Albufera of Valencia. It is a coastal lagoon located in the east of the Iberian Peninsula, which requires continuous monitoring in order to monitor its ecological quality. The objective of this study is to monitor the concentration of phycocyanin and its relationship with the density of cyanobacteria during the winter–spring of 2022. For this, physicochemical variables were measured, and phytoplankton communities were determined; finally, the presence of phycocyanin was measured in situ and by remote sensing using Sentinel-2 imagery. The lagoon presents a state of permanent turbidity with an average transparency of 30 cm according to the Secchi Disk. The results obtained show an increasing concentration of phycocyanin from winter to summer related to higher phosphorus concentrations, reaching values of 561.3 µg/L for phycocyanin and 8.7 mg P/L of total phosphorus. According to the trophic state reference values of the Water Framework Directive, the concentration of total phosphorus is excessive, indicating a hypertrophic state. According to the World Health Organization, the concentration of phycocyanin found would indicate a state of high alert. Remote sensing serves as a suitable tool for monitoring and tracking the status of the lagoon.

Keywords: coastal lagoon; cyanobacteria; phycocyanin; toxicity; remote sensing; eutrophication

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Abstract

The Role of Cyanobacteria in the Aquatic Resistome †

Tânia Rosado [1], Duarte Balata [2], Rani de la Rivière [1,3], Vera Manageiro [4,5,6], Dina Carpinteiro [7], Luís Vieira [7], Francisco Pina-Martins [2], Carina Menezes [1], Octávio S. Paulo [2], Manuela Caniça [4,5,6] and Elsa Dias [1,5,6,*,‡]

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Abstract: The aquatic environment is a reservoir of antibiotics, pathogenic and native microorganisms, and antibiotic resistance genes (ARGs), constituting a key aspect of the One Health approach. Thus, the problem of antibiotic resistance is no longer restricted to bacterial pathogens but is a consequence of the interplay between distinct clinical and environmental drivers. In the project "ExplorAR—Exploring the Aquatic Resistome", we investigated the putative role of cyanobacteria (CB) in the freshwater resistome, using a multidisciplinary approach based on phenotypic/genotypic/genomic tools. In this presentation, we disclose the main results of the project: (i) the development of a microdilution-based antibiotic susceptibility assay for CB; (ii) the establishment of an antibiotic susceptibility profile of CB genus/species; (iii) the identification of CB strains with reduced susceptibility to multiple antibiotic classes; (iv) the identification of ARGs in CB genomes; (v) the characterization of the antibiotic resistance profile of CB-associated bacteria; (vi) mapping the occurrence of CB and ARGs in surface freshwater reservoirs by high-throughput sequencing approaches. Overall, ExplorAR strongly supports the hypothesis that CB are environmental players in the emergence and dissemination of ARGs in water environments, contributing to the problem, and challenges, of antibiotic resistance.

Keywords: antibiotic pollution; antibiotic resistance; aquatic resistome; cyanobacteria; minimum inhibitory concentration; next-generation sequencing

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biology and life sciences forum

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Abstract

Assessing the Reason Why Heterotrophic Bacteria Present in Aquatic Environments Are Not Affected by Microcystins and Unraveling Alternative Genes for Microcystin Degradation †

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Abstract: Cyanobacteria are a ubiquitous and diverse group of phototrophic prokaryotes, which mainly inhabit aquatic ecosystems. In certain optimal environmental conditions, there may be a rapid increase in cyanobacteria populations, leading to the formation of blooms, which are frequently associated with the presence of cyanotoxins. Microcystins (MCs) are the most frequent hepatotoxin produced by cyanobacteria. Scarce previous studies have shown that the growth of aquatic heterotrophic bacteria, which co-occur with cyanobacteria, may not be affected by the presence of MCs, or may present a reduction, never being totally inhibited by their presence. In this study, we examined the effects of three microcystin variants (MCLR, MCRR and MCYR) on a set of heterotrophic aquatic bacteria living in the same ecosystem as cyanobacteria. In particular, the impact of microcystins on the growth of heterotrophic bacteria was tested, and a PCR screening for the presence of microcystin-degrading genes (*mlr*) was performed. The growth assays supported the hypothesis from previous studies, where most heterotrophic bacteria were only slightly or not at all affected by exposure to MCs. Moreover, it seems that the behavior of the isolates when exposed to these cyanotoxins was strain specific. A new bacteria, *mlr*+, was identified, belonging to *Flectobacillus* sp. Furthermore, we decided to perform a genomic study of 14 isolates from a set of potentially interesting bacteria, including *Flavobacterium* spp. and *Aeromonas* spp., to search for xenobiotic-related genes that could be involved in MC degradation. The whole-genome sequencing analysis of these 14 isolates revealed that no COG genes (COG0625; COG0841; COG1566) were present; however, genes similar to CAAX genes were present in the *Aeromonas* spp. isolates analyzed. These results shed new light into alternative molecular mechanisms for microcystin degradation.

Keywords: microcystins; biodegradation; heterotrophic bacteria; *mlr* genes; CAAX genes

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biology and life sciences forum

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Abstract

The Problem of Cyanotoxins in Reservoirs of São Paulo State, Brazil †

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Abstract: Eutrophication process and phytoplankton primary productivity have intensified in continental aquatic ecosystems because of climate change. As a consequence, the proliferation of potentially toxic cyanobacteria is increasing in frequency, magnitude, and duration. For water sources used in public supply, this growth represents an ecological risk to ecosystems and human health. From October 2021 to February 2022, integrated samples of surface water were obtained from 11 reservoirs in São Paulo State, Brazil (Jaguari, Jacarei, Atibainha, Paiva Castro, Rio Grande, Guarapiranga, Barra Bonita, Bariri, Broa, Salto Grande, and Itupararanga). Limnological variables were obtained using the Troll 500 probe, in addition to depth, turbidity (Tur), chlorophyll a (Chla), and phycocyanin (Phy) concentrations (Turner C3 probe). In the laboratory, chlorophyll-a concentrations (ChlaABS) were analyzed. Phytoplankton biovolume (Utermöhl method) was estimated. The concentrations of microcystins (MCs) and saxitoxins (STXs) were analyzed with Beacon kits, in ELISA microplate reader. For the studied reservoirs, the Secchi disc water transparency ranged from 0.6 to 2.3 m. The average values of water temperature, electrical conductivity, pH, and dissolved oxygen were, respectively, 24.8 °C, 162.9 µS/cm, and 8.4 and 9.5 mg/L. For Tur, Chla, Phy, and ChlaABS, ranged from 1.86 to 24.6 NTU, 3.3 to 105.1 µg/L, 12.4 to 445.2 µg/L, and 4.2 to 84.9 µg/L, respectively. Cyanobacteria was the more representative phytoplankton class in biovolume, from 0.07 to 51.7 mm^3/L. STXs and MCs were found in most sampled stations. For STXs it ranged from 0.016 µg/L to 0.308 µg/L, and for MCs in some stations it was higher than 200 µg/L. According to the World Health Organization and Brazilian legislation, in the 11 studied reservoirs, the concentrations of saxitoxins are within the maximum allowed limits (3 µg/L), while for microcystins the concentrations are for most reservoirs above the maximum allowed value (1 µg/L). Considering the analyzed information in relation to water quality and the cyanobacterial community, we verify that most of these environments present a worrying water quality, which can represent a risk for public health.

Keywords: reservoirs; eutrophication; cyanobacteria; cyanotoxins

Citation: Moschini-Carlos, V.; Sòria-Perpinyà, X.; Vicente, E.; Sendra, M.D.; de Araujo, M.K.C.; do Carmo Bitencourt, M.; de Leles Almagro, V.; Pompêo, M. The Problem of Cyanotoxins in Reservoirs of São Paulo State, Brazil. *Biol. Life Sci. Forum* **2022**, *14*, 34. https://doi.org/10.3390/blsf2022014034

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Abstract

Cyanobacterial Biomass Used as Biofertilizer in Lettuce Plants: Effects on Growth and Cyanotoxin Accumulation †

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Abstract: The use of cyanobacterial biomass as a biofertilizer is promising in terms of sustainable agriculture. Nevertheless, cyanobacteria can be considered a threat to human and environmental health due to the potential presence of cyanotoxins, since some studies report that the use of contaminated water for agricultural irrigation can impair plant growth and lead to contamination of food products. Interestingly, at environmentally relevant concentrations, cylindrospermopsin (CYN) seems to cause no deleterious effects in plants, and it might even promote their yield. However, studies assessing CYN accumulation in the edible tissues at environmental concentrations are lacking. The objective of this work was to evaluate the effects of cyanobacterial biomass CYN producing or non-producing on lettuce plant growth, and that of CYN accumulation in edible tissues. This study consisted of growing lettuce plants, under controlled conditions, for 25 days in soil (1) with no extra nutrient addition (control) and supplementation with (2) cyanobacterial biomass that did not produce CYN, (3) cyanobacterial biomass that produced CYN (~10 µg of dissolved CYN), and (4) cyanobacterial biomass that produced CYN, treated by boiling for 5 min (~25 µg of dissolved CYN). At the end of the exposure, lettuce growth was assessed, as well as CYN accumulation in tissues and soil. The results showed that leaf growth was significantly increased ($p < 0.05$) in lettuce plants supplemented with cyanobacterial biomass, especially at condition (3), which was five-fold higher compared with the control group. Regarding CYN accumulation, for conditions (3) and (4), the toxin was detected in the tissues of plants, as well as in soil at the following decreasing order of concentrations: soil > roots > leaves. Interestingly, the concentration determined in lettuce leaves in condition (4) was three-fold lower when compared with the condition (3). Nevertheless, for both conditions, although CYN has been detected in lettuce leaves, the concentration in the edible part did not exceed the proposed provisional tolerable daily intake (TDI) of 0.03 µg/kg/BW. In conclusion, these results suggest that the use of cyanobacterial biomass as lettuce biofertilizer, even containing CYN at environmentally relevant concentrations, can positively influence plant growth and development without compromising the safety of edible tissues.

Keywords: cyanobacteria; cylindrospermopsin; biofertilizer; plant growth; accumulation; *Lactuca sativa*

Author Contributions: Conceptualization, A.C., M.F. and V.V.; methodology, É.S., A.M., J.A., D.M., M.R., M.F. and A.C.; software, É.S., A.M., J.A. and A.C.; validation, É.S., A.M., J.A., M.F. and A.C.; formal analysis, É.S., A.M. and J.A.; investigation, É.S., A.M., J.A., M.F. and A.C.; resources, É.S., A.M., J.A., D.M., M.R., M.F., A.C. and V.V.; data curation, É.S., A.M. and J.A.; writing—original draft preparation, É.S., A.M. and M.F.; writing—review and editing, É.S., A.M., A.C., M.F. and V.V.; supervision, M.F. and A.C.; project administration, A.C. and V.V.; funding acquisition, A.C. and V.V. All authors have read and agreed to the published version of the manuscript.

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Abstract

The Role of Vitamin K Family in Obesity †

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Abstract: Environmental and lifestyle adaptations over the last decades have contributed to enhanced man´s longevity, however it also paved the ground for different diseases to unfold. Today one of the main public health challenges is obesity and its related metabolic dysfunctions. In recent years, the pharmacological options are reported as being insufficient, therefore, the discovery and pharmacological development of new therapeutic approaches are required to overcome this epidemic. Vitamin K are a fat-soluble family of compounds implicated in a number of essential biological functions. Vitamin K1 and K2 are two naturally occurring compounds, while vitamin K3 is the most common synthetic form. A bioactivity-guided approach was used with the Nile red fat metabolism assay in zebrafish larvae to successfully isolate an analog of vitamin K1 for the first time from Tychonema sp. LEGE 07196. The structure confirmation was based on NMR spectroscopy and mass spectrometry. This analog was first isolated in 1965 from the cyanobacteria *Synechococcus elongatus*, but little is known on its bioactivity. The anti-obesity effects of all vitamin K forms, including the K1-analog, were studied. The compounds did not cause any general toxicity or malformations and showed significant neutral lipid-reducing activity after 48 h of exposure. The different vitamin forms displayed different levels of activity which shows the importance of the naphthoquinone ring, as well as the impact of the saturation and polarity of the aliphatic side-chain for the structure–activity relationship. Metabolomics approaches were employed to establish the distinct bioavailability and biotransformation of the different forms of vitamins in the organisms. The metabolite profiling was achieved using different databases and MetaboAnalyst was used for associated analysis. The organism has a clear preference to transform the various vitamins into K1 and K1-analog forms, regardless of the supplementation, and each exposed vitamin significatively altered the expression of different metabolites indicating that different metabolic targets are involved. This work is ongoing, and the final aim is to understand the effects of vitamin K family on obesity and related co-morbidities, which could lay the ground to develop a future nutraceutical with lipid reduction activity.

Keywords: vitamin K family; obesity; metabolic dysfunctions; Nile red fat metabolism assay; metabolomics

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Abstract

Cyanobacteria as a Source of New Antifouling Sustainable Solutions †

Sandra Pereira [1,2,‡], Leonor Ferreira [2], Catarina Gonçalves [2], Vitor Vasconcelos [1,2], Mariana Reis [2] and Joana R. Almeida [2,*]

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Abstract: The usage of paints and coatings with toxic components for the mitigation of marine biofouling in submerged surfaces continues to cause economic, environmental and human health-related problems worldwide. Natural products have the potential to provide solutions for antifouling applications that are effective and ecologically compatible. The diversity of the secondary metabolites that are produced by cyanobacteria make these organisms a promising source of bioactive compounds, especially when antifouling activity has already been documented. The purpose of this study was to explore the metabolic diversity of a range of cyanobacterial strains from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) in search of eco-friendly bioactive compounds for antifouling purposes. A library of fractions, derived from methanolic extracts, belonging to different cyanobacterial strains, was tested towards a prominent macrofouling organism settlement (*Mytilus galloprovincialis* larvae). Promising fractions were submitted to a bioassay guided sub-fractioning that led to the isolation of two compounds. Their structure elucidation was determined by 1D and 2D nuclear magnetic resonance and by mass spectrometry. Anti-settlement effectiveness was assessed through an EC50 bioassay with mussel larvae, as well as antifouling bioactivity towards the growth of five marine biofilm-forming bacteria. The results showed bioactivity against the mussel larvae settlement and low toxicity, but no bacterial growth inhibition was found for the nucleosides (<10% of inhibition). Moreover, general ecotoxicity to the marine environment was evaluated, and the compounds also presented no toxicity against *Artemia salina*, proving them to be ecologically compatible. These promising results confirm the inherent potential of cyanobacteria to provide more sustainable antifouling ingredients to be incorporated in marine coatings.

Keywords: cyanobacteria; antifouling; marine biotechnology; bioactive metabolites

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Abstract

Machine Learning Approach towards the Early Warning of Cyanobacterial Blooms in Drinking Water Reservoirs †

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Abstract: Cyanobacterial harmful algal blooms (CyanoHABs) are expanding globally, representing a major risk for lakes and reservoirs due to their toxicity and economic impacts. Therefore, anticipating their occurrence and understanding the main factors related to CyanoHABs are critical to improve decision-making processes and water resource management. In this context, we present two modelling options for the analysis and prediction of cyanoHABs in two drinking water reservoirs from Spain. This case represents a unique opportunity to combine efforts from different academic disciplines (i.e., aquatic ecology and data science), environmental companies, and public water managers to address this increasingly severe issue. Susqueda (Ter basin, Catalonia) is a eutrophic, large and deep reservoir (Z_{max} = 110 m) where monitoring efforts in recent years have focused on a monthly measurement in more than 30 physico-chemical, hydrological, meteorological and biological parameters, some of them involving expert intervention and costly efforts that could not be held at a higher temporary frequency. Cuerda del Pozo (Duero basin, Castilla y León) is a deep reservoir (Z_{max} = 30 m) where monitoring efforts have focused on daily data collection through probes mounted in automatic profilers. This strategy allowed a higher monitoring frequency for fewer parameters and a narrower time span. In both cases, the parameter chosen as a proxy of cyanobacterial proliferation (output of models) is fluorometric measurements of chlorophyll-a and phycocyanin. The results of our machine-learning-based analyses suggest that the selected modelling path mainly depends on two aspects: (1) the time span where data are collected, and (2) the frequency and type of data measured (i.e., one discrete measurement at the surface vs. many measurements along the water column). Thus, a Susqueda dataset analysis led to more interpretative results, allowing for a better understanding of the system and the main factors related to CyanoHABs with limited predictive capacity. Meanwhile, the Cuerda del Pozo dataset is treated as a time series where autoregressive forecasting techniques, combined with information of exogenous parameters, are applied to foresee cyanobacterial blooms before they occur, losing part of the interpretability in the process. The results from this work are expected to provide an effective tool to boost smart and goal-orientated sampling planning, while improving data-driven decision-making processes essential for the water management of cyanobacterial blooms.

Keywords: CyanoHABs; monitoring; machine learning; predictive modeling; water management

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Abstract

Cyanobacterial Toxins—An Update of Toxins from Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) †

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Abstract: Cyanobacteria are microorganisms that have remarkable adaptability and can inhabit various types of aquatic and terrestrial ecosystems worldwide, including extreme environments. This group of organisms is considered a rich source of secondary metabolites with potential biotechnological applications and has the capability to produce some potent cyanotoxins that can induce consequences to human health. The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) is a biological resource center located at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), comprising more than 1200 different cyanobacterial and microalgae strains. Until now, 36 strains have been reported as producers of cyanotoxins distributed within different cyanobacterial orders. Recently, LEGE-CC has increased in numbers due to the isolation effort that has been made. In this work, a screening of more than 200 cyanobacterial isolates from subaerial and freshwater environments targeted the genes involved in the biosynthesis of cyanotoxins. As expected, genes involved in cylindrospermopsin, saxitoxin, anatoxin and microcystin production were detected by molecular biology tools. The strains where the genes were detected were grown and sent to liquid chromatography–mass spectrometry (LC-MS) to confirm the production of cyanotoxins. As a culture collection, the screening of cyanotoxins is an essential aspect of cyanobacterial research and provides a comprehensive idea of the production of these toxins for future works.

Keywords: cyanotoxins; cyanobacteria; culture collection; LEGE-CC

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Abstract

Sampling Campaign along Moroccan Atlantic Coast: Cyanobacteria Isolation and Molecular Screening of Cyanotoxins †

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‡ Presenting author (poster).
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Abstract: Cyanobacteria are considered a rich source of secondary metabolites with potential biotechnological applications. Additionally, they have the capability to produce some potent toxins (cyanotoxins) that can have consequences for both environmental and human health. This group of microorganisms with a long evolutionary history and a remarkable adaptability can be found both in aquatic and terrestrial ecosystems, including extreme environments (e.g., freshwater, marine, terrestrial, hot springs, deserts, etc.). Cyanotoxins can be classified into five functional groups according to their primary target organ or effects being designated as hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, and irritant toxins. In this work, the presence of genes involved in the biosynthesis of cyanotoxins (microcystin, saxitoxin, cylindrospermopsin, and anatoxin) were screened from more than 200 strains of cyanobacteria. The isolates were obtained from different samples along the Moroccan Atlantic coast (several sampling sites from El Jadida to Essaouira), and from an ancient Portuguese cistern located at El Jadida. There is a gap in the literature regarding the presence of cyanotoxins in this region and some of the isolated strains are related to genera (e.g., *Phormidium* sp., *Pseudanabaena* sp., *Leptolyngbya* sp., *Lyngbya* sp., and *Geitlerinema* sp.) that have been reported as potential cyanotoxins producers. Future work will include detection by liquid chromatography–mass spectrometry (LC-MS) to confirm the production of cyanotoxins. Furthermore, the isolates will be deposited in our in-house culture collection (LEGE-CC), and will be available for future studies, increasing the size and diversity of the collection.

Keywords: cyanobacteria; cyanotoxins; screening; Morocco; LEGE-CC

Citation: Silva, R.; Morais, J.; Oliveira, F.; Sabour, B.; Vasconcelos, V. Sampling Campaign along Moroccan Atlantic Coast: Cyanobacteria Isolation and Molecular Screening of Cyanotoxins. *Biol. Life Sci. Forum* **2022**, *14*, 40. https://doi.org/10.3390/blsf2022014040

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Abstract

Survey of Freshwater Cyanobacteria and Related Toxin Genes on Coastal and Transitional Waters in Portugal Mainland †

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† Presented at the 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins, Ponta Delgada, Portugal, 18–20 July 2022.

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Abstract: Marine toxic microalgae frequently bloom on the Portuguese coast causing toxin accumulation in shellfish with the consequent harvesting interdiction in the affected production area. Likewise, freshwater cyanobacteria blooms are a constant in Portuguese inland waters, with high levels of toxins reported in the reservoirs. With this constant and persistent eutrophication of freshwater reservoirs, concerns exist whether toxic freshwater cyanobacteria are reaching marine shellfish production areas. For this purpose, a screening was made crossing information from microscopical observations from monitoring samples with cyanobacterial toxin gene presence across several periods in time. Toxin gene presence was based in conventional PCR using primers selected from previous reports. The results showed that freshwater toxin genes markers are present in marine and transitional waters across Portugal, and that the presence of potential toxic freshwater cyanobacteria is recurrent in microscopical observations in monitoring samples. This preliminary information gives us clues to where possible incidences of toxic freshwater cyanobacteria in marine shellfish production areas might occur, in order to assess the areas at greatest risk for shellfish toxification from freshwater blooms transport and remains.

Keywords: cyanobacteria; dinoflagellates; toxin genes; transitional waters

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Abstract

Determination of Multiclass Cyanotoxins in Spirulina-Based Dietary Supplements Using a SLE-Tandem-SPE Procedure Followed by HILIC-MS/MS †

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Abstract: Cyanobacteria are a diverse group of oxygenic photosynthetic prokaryotes, which are believed to be one of the oldest life forms on Earth. They live in a wide range of ecosystems and withstand extreme environmental conditions. An important proportion of cyanobacteria is known to be producers of harmful cyanotoxins, which are toxic secondary metabolites that can impact the ecosystem and human health. The oral route is one of the main ways whereby humans can be exposed to cyanotoxins. Therefore, the consumption of contaminated algae-based food supplements is becoming more relevant due to its upsurge, which underlines the importance of controlling these toxins in this kind of products. This work describes the simultaneous determination of seven cyanotoxins belonging to three different classes: the cyclic peptides microcystin-LR (MC-LR), microcystin-RR (MC-RR) and nodularin (NOD); the alkaloid anatoxin-a (ANA) and three non-protein amino acids isomers β-methylamine-L-alanine (BMAA), 2,4-diaminobutyric acid (DAB) and N-(2-aminoethyl)glycine (AEG). These have been determined in spirulina-derived food supplements using a novel solid–liquid extraction coupled with a solid phase extraction procedure for clean up and preconcentration (SLE-tandem-SPE) and analysis by hydrophilic interaction liquid chromatography with tandem mass spectrometry detection (HILIC-MS/MS). A SeQuant®Zwitterionic Hydrophilic Interaction Liquid Chromatography (ZIC-HILIC) column (EMD Millipore, Billerica, MA, USA) was employed to achieve the chromatographic separation in less than 12 min using water and acetonitrile, both acidified with 0.3% of formic acid, as mobile phase. Previously, an SLE was developed, using 4 mL of aqueous 5% formic acid to extract the most polar compounds, followed by 4 mL of 80% MeOH. Both extracts were combined and submitted to a tandem-SPE using mixed-mode cation exchange (MCX) and Strata-X cartridges. Elution from both cartridges was performed using 10% $NH_3 \cdot H_2O$ in MeOH. Method validation was carried out in terms of linearity, limit of detection (LOD) and quantification (LOQ), recoveries, matrix effect and repeatability and intermediate precision. LOQs in the range of 50–300 $\mu g \cdot kg^{-1}$ and recoveries ranging between 64.2% and 102.9% with an associated relative standard deviation < 19.2% were achieved. Satisfactory precision was obtained with RSD values lower than 19.6% in all cases, with the exception of BMAA, which reported the highest RSD values, reaching 25.1%. The method was satisfactorily applied to determine the occurrence of cyanotoxins in blue green algae (BGA) dietary supplements. DAB was the most frequently detected cyanotoxin, at concentrations up to 2408 $\mu g \cdot kg^{-1}$, and AEG was found in few samples at concentrations up to 194 $\mu g \cdot kg^{-1}$. However, MC-LR and MC-RR were found in one sample at concentration levels higher than 5 $mg \cdot kg^{-1}$, which underlines the need to control these substances in these matrices.

Keywords: cyanotoxins; HILIC-MS/MS; spirulina-based dietary supplements; tandem-SPE

Citation: del Olmo-Iruela, M.; Aparicio-Muriana, M.d.M.; Lara, F.J.; García-Campaña, A.M. Determination of Multiclass Cyanotoxins in Spirulina-Based Dietary Supplements Using a SLE-Tandem-SPE Procedure Followed by HILIC-MS/MS. *Biol. Life Sci. Forum* **2022**, *14*, 42. https://doi.org/10.3390/blsf2022014042

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Abstract

How Intelligent ASVs Can Help Us to Support Cyanobacteria Blooms Detection, Predictions, and Early Warning? †

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Abstract: The automation revolution provides access to robotized water-surface vehicles, which support behaviors of different autonomy and intelligence levels, ranging from those achieved by Remotely Piloted Surface Vehicle (RPSV) to those supported by Autonomous Surface Vehicles (ASVs) and Intelligent ASVs (IASVs). In short, the staff in charge of the RPSVs makes all the decisions and drives them from the shore, while ASVs and IASVs can take control of the situation and move themselves autonomously. Equipped with probes, all of them can be used to collect information about physical parameters and substances, and therefore to monitor water bodies where there is a high probability of Cyanobacteria Blooms (CBs). These vehicles are especially useful for this purpose, as CBs are dynamic biological processes that can occur inside many locations of the water body and become only visible when they emerge into the water surface. In addition, as they produce toxic metabolites that threaten the life of multiple species and limit the recreational use and human consumption of water, the authorities should anticipate their evolution or detect them as soon as possible to minimize the exposure of the population and animals to their harmful effects. Early warning systems in use today cannot capture the temporal-space evolution of CBs, because their fixed probes do not provide information from any Point of Interest (POI) of the water body. In addition, taking personnel to the POIs with boats is an expensive impractical solution to frequently monitor enough water-body locations in order to understand the current state of the CB. An alternative solution consists of: (1) frequently sending the ASVs on their own to any POI of the water body, (2) making them systematically explore Regions of Interest (ROIs), or (3) asking them to intelligently search for relevant information within the water body. In the third option, IASV displacements can adapt themselves to different circumstances, such as the information provided by simulators of the CBs evolution, by the IASVs onboard sensors, or by both. Finally, the diurnal vertical cyanobacteria displacements in the water column can be investigated by attaching the probe to an automatic winch that can also be remotely, automatically or intelligently controlled to be able to explore the water body in its third dimension. During this presentation we will discuss different possibilities that ASVs and IASVs can open in the field of cyanobacteria management.

Keywords: autonomous surface vehicles; artificial intelligence; modelling & simulations; early warning systems

Citation: López-Orozco, J.A.; Carazo-Barbero, G.; García-Pérez, L.; Girón-Sierra, J.M.; Besada-Portas, E. How Intelligent ASVs Can Help Us to Support Cyanobacteria Blooms Detection, Predictions, and Early Warning? *Biol. Life Sci. Forum* **2022**, *14*, 43. https://doi.org/10.3390/blsf2022014043

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Abstract

Exploring the Appetite Inhibition Potential of Bioactive Metabolites from Cyanobacterial Strains †

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Abstract: Higher food intake promotes obesity, a critical public health challenge with increasing prevalence worldwide. Selective modulators of appetite can be applied as therapeutic intervention. Nevertheless, currently the appetite suppressant drugs trigger severe side effects, such as anxiety and depression. For that reason, there is a priority to discover new pharmaceuticals. In this study, a library of 117 cyanobacterial fractions from marine and freshwater environments belonging to The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) of CIIMAR (Interdisciplinary Centre of Marine and Environmental Research) were screened for their ability to interfere in the food intake behavior of zebrafish larvae—a whole small animal model. Two different bioassays were performed for appetite-reducing activity, using fluorescent stained liposomes (passive food intake) or *Paramecia bursaria* (active food intake). Three cyanobacterial fractions from the order Nostocales, Chroococcidiopsidales and Pleurocapsales expressed appetite-suppression bioactivity in the liposomes assay, while three different fractions from the order Synechococcales, Oscilatoriales and Nostocales significantly reduced the appetite in the Paramecium assay. To highlight putatively associated metabolites for the bioactivities, dereplication by metabolomics approaches (LC-MS/MS) was performed, as well as a bioactivity-guided feature-based molecular networking using GNPS, and four compounds were positively correlated to the bioactivity. No matches were found in any database for these molecules, indicating putatively new compounds. Molecular analyses are currently ongoing to discover the involved genes that regulate the passive and active food intake in zebrafish larvae, and consequently uncover the mechanisms of action.

Keywords: cyanobacteria; obesity; appetite inhibition bioassay; bioactive metabolites; zebrafish larvae; biotechnological applications

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Institutional Review Board Statement: According to the EC Directive 86/609/EEC for animal experiments, zebrafish larvae in non-independent feeding stages of development are not considered animal experimentation. Hence, ethical review and approval were not necessary.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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Abstract

30 Years of a Collection of Cyanobacterial and Microalgae Cultures: LEGE-CC and Its Contributions to Cyanobacterial Ecotoxicology and Biotechnology †

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† Presented at the 7th Iberian Congress on Cyanotoxins/3rd Iberoamerican Congress on Cyanotoxins, Ponta Delgada, Portugal, 18–20 July 2022.

‡ Presenting author (key-note talk).

Abstract: The LEGE-CC- Blue Biotechnology and Ecotoxicology Culture Collection is a cyanobacteria and microalgae culture collection containing mainly Portuguese strains but with a biodiversity comprising strains from all over the world. It was started in 1991, aiming to collect and maintain toxin-producing cyanobacteria, in its first few years being mostly dedicated to *Microcystis aeruginosa* strains that were not previously represented in national collections. It evolved into having a theme of freshwater toxin-producing cyanobacteria during its first few years, and then other environments (brackish and saltwater) were added. Today, it comprises strains producing most of the known cyanotoxins, e.g., microcystins, anatoxin-a, cylindrospermopsins, saxitoxins and BMAA, as well as other peptides which do have toxic properties but are not well studied from an ecotoxicological point of view, e.g., aeruginosin, microginin, cyanopeptilin, anabaenopeptin and microviridin. More recently, we diversified the collection by adding freshwater microalgae strains that may have interesting biotechnological applications, the collection now comprising nearly 2000 strains. Apart from the initial information regarding the occurrence and prevalence of microcystins in Portuguese freshwaters—which allowed us to develop and implement the first national cyanobacteria monitoring program, together with the ministries of Environmental and Health—it was possible to establish and legislate a guideline value for microcystin-LR in Portugal in 2007 (DL nº 306/2007). The toxin-producing strains were also the bases of many ecotoxicological studies on the impact of the toxins in organisms at different trophic levels. Around 2006, other applications for the LEGE-CC started to be envisaged, including using the non-toxin-producing strains for applications such as antimicrobial, cytotoxic and allelopathic application. In the last ten years, more intense research has been conducted on the biotechnological applications of the cyanobacteria strains in diverse areas such as antifouling, anticancer and anti-obesity, as well as in sensor development, cosmetics and skin diseases.

Keywords: LEGE-CC; cyanobacteria; microalgae; toxins; biotechnology

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Abstract

Discovery of a Novel Potent Cytotoxic Compound from *Leptothoe* sp. †

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‡ Presenting author (oral communication).

Abstract: One of the top causes of worldwide mortality is cancer. In many cases, the effectiveness of traditional chemotherapy is hampered given the emergence of drug resistance alongside a wide range of unwanted side effects. To overcome this, it is essential to search for new drugs that can lead to a more successful cancer treatment. Cyanobacteria are a diverse group of photosynthetic prokaryotes known to produce bioactive metabolites, with various interesting biotechnological applications. Currently, four FDA (food and drug administration)-approved anticancer drugs derived from a cyanobacterial metabolite are used in the clinical setting. CIIMAR (Interdisciplinary Centre of Marine and Environmental Research) hosts LEGE-CC (Blue Biotechnology and Ecotoxicology Culture Collection), a collection of over 700 strains of cyanobacteria with underexplored biotechnological potential. To uncover it, a new method to quicken the discovery of bioactive metabolites was recently developed, leading to the creation of a library of cyanobacterial fractions, readily available for bioactivity assays. In this work, we will present the results of the cytotoxic screening that led to the selection of one strain that decreased cell viability to 10%. This strain, isolated from an environmental sample collected at Cape Verde and identified as *Leptothoe* sp., was then selected for bioactivity-guided fractionation aided by LC–MS (liquid chromatography-mass spectroscopy). Using numerous chromatography techniques, two macrolide-type compounds were isolated: the previously reported phormidolide as well as a new derivative. The structure of the new compound was elucidated by 1D and 2D NMR (nuclear magnetic resonance) and the cytotoxicity was measured against HCT 116 human colon carcinoma cells. A potent cytotoxic activity was observed for the new compound. These interesting results are important for developing new anticancer drugs from cyanobacteria, so more work is being developed to isolate new derivatives.

Keywords: cancer; cyanobacteria; cytotoxicity; library screening; phormidolide

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Abstract

Water Quality Assessment Using Sentinel-2 Imagery Estimating Chlorophyll A, Secchi Disk Depth, and Cyanobacteria Cell Number in Brazilian Reservoirs †

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Abstract: Satellite images were used to assess surface water quality based on the concentration of chlorophyll a (chla), light penetration measured by the Secchi disk method (SD), and the Cyanobacteria cells number per mL (cyano). Nine reservoirs are studied in São Paulo State (Brazil); six reservoirs are interconnected, comprising the Cantareira System (CS), and three others are isolated, the Broa, Salto Grande (SG) and Itupararanga (Itu) Reservoirs. For this study, Sentinel-2 images were employed, alongside SNAP image processing software, and the native products conc_chl and kd_z90max, treated using Case 2 Regional Coast Color (C2RCC) atmospheric correction. The database for chla, SD and cyano was obtained from CETESB, the agency legally responsible for operation of the Inland Water Quality Monitoring Network in São Paulo State. For CS, the results demonstrated robustness in the estimates of chla (RMSE = 3.73; NRMSE% = 19%) and SD (RMSE = 2,26; NRMSE% = 14%). Due to the strong relationship between cyano and chla (R^2 = 0.84, $p < 0.01$, n = 90), both obtained from field measurements, it was also possible to estimate cyano, based on the estimates of chla from the satellite images. For CS, the estimates revealed a clear pattern, with the upstream reservoirs being more eutrophic, compared to those downstream, particularly due to the high cyano. For Broa, a high correlation was also observed between chla and cyano (R^2 = 0.6052, RNMSE% = 27, n = 8). Based on the estimates, Broa showed a eutrophic pattern in practically the entire year of 2020, with a predominance of cyanobacteria in the entire water body (from 10,000 to 20,000 cells/mL). For SG, it was possible to observe robustness only for DS, but not for chla. The restricted database available was considered the main explanatory factor for the low robustness observed for (SG), despite the relationships between the field data. For Itu, the C2RCC-Nets demonstrated robustness in the estimates of Chla (RMSE = 4.0 mg/m^3; NRMSE = 16.7%) and SD (RMSE = 0.78 m; NRMSE = 19.1%). Despite the good fit of the allometric relationship relating the Chla and Cyano field data, it did not allow validation of the cyano estimates using the conc_chl native S2 product, for Itu. Thus, it is concluded that automatic products are excellent tools for estimating chla and SD, and as a result of the solid relationships between chla and cyano, it is possible to estimate the cyano and observe spatial heterogeneity in water quality, based on SD, cyano, and chla.

Keywords: remote sensing; chlorophyll; empirical models; cyanobacteria number; Secchi disc

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Abstract

IA-GES-BLOOM-CM: Towards a Comprehensive Warning and Management System for Cyanobacterial Blooms †

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Abstract: Cyanobacterial Blooms (CBs) are an ecological and public health problem since they may be followed by the production of secondary metabolites, which are toxic for humans and other animals. This threatens the life of multiple species and prevents the use of water resources for recreational and consumption purposes. Therefore, their proper management is essential to minimize the exposure of the population and ecosystems to the harmful effects of CBs. The ability to predict the formation of CBs in a specific water body is limited by the difficulty of acquiring enough data to determine their state with the appropriate temporal and spatial granularity. Moreover, as CBs are complex phenomena that are influenced by many factors, the conclusions derived for a certain water body are hard to extrapolate to others. IA-GES-BLOOM-CM is a synergy project funded by the Community of Madrid, Spain, for boosting the collaboration of researchers from different fields (including biology, automation, and information and communication technologies) to develop disruptive solutions for CB prediction and management. Its aim is to develop a comprehensive and reliable system to automatically and efficiently characterize continental water bodies, predict where and when the CBs are expected to occur, determine their potential risks, and provide the authorities with early warnings of CB breakouts. To this end, we are conceiving a system, supported by Autonomous Surface Vehicles (ASVs, a kind of robotized boats), Modeling and Simulation (M&S) tools, and the Internet of Things (IoT). More specifically, on one hand, the ASVs, which are equipped with probes, will be (1) responsible for capturing information related to the CBs from any point of the water column and surface and will be (2) intelligently guided to the points of interest to make relevant observations in order to optimize the monitoring efforts. On the other hand, M&S tools, including dynamical models and machine learning, will be in charge of predicting the CB temporal and spatial evolution in order to guide the ASVs (whose data, in turn, will be used to fine-tune the models) and warn the authorities about relevant CBs. Finally, an IoT infrastructure will support the communications and deployment of the system, closing the gap between the authorities in charge of the water bodies and the information provided by the different elements of the system. In this paper, we will provide an overview of the main ideas of the project and of its initial developments.

Keywords: autonomous surface vehicles; modeling and simulation; optimization; artificial intelligence; Internet of Things

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Abstract

Uncovering the Cyanobacterial Chemical Diversity: The Search for Novel Anticancer Compounds †

Leonor Ferreira [1], Maria Ligía Sousa [1], Tiago Ribeiro [1], João Morais [1], Abel Forero [2], Raquel Castelo-Branco [1], Pedro N. Leão [1], Marco Preto [1], Ralph Urbatzka [1], Nikoletta Szemerédi [3], Gabriella Spengler [3], Carlos Jiménez [2], Jaime Rodriguez [2], Vitor Vasconcelos [1,4] and Mariana Reis [1,*,‡]

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‡ Presenting author (oral communication).

Abstract: Cancer has a tremendous negative socio-economic impact on our society. Thus, the discovery of new and more effective anticancer drugs is of utmost importance. To address this societal challenge, the main goal of the CYANCAN project was the discovery of anticancer compounds from cyanobacteria. These photosynthetic bacteria are considered among the most promising groups capable of producing metabolites with pharmaceutical applications. A valuable and underexplored natural resource that can underpin the discovery of promising compounds can be found in the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) at CIIMAR (Interdisciplinary Centre of Marine and Environmental Research), comprising more than 700 different cyanobacterial strains. Herein, we present the recent advances implemented for finding robust anticancer lead compounds from LEGE-CC cyanobacteria. For this purpose, we developed a natural product library. Sixty cyanobacterial strains, representative of LEGE-CC biodiversity, were chromatographed to yield 480 fractions that were tested for their cytotoxic activity against 2D and 3D models of human colon carcinoma (HCT 116). The conjugation of monolayer assays and 3D cancer spheroids led to the selection of 11 active fractions, of which the chemical space was studied using an untargeted metabolomics approach. The putative annotation and identification of several compounds led to the selection of two marine strains for compound isolation: *Leptothoe* sp. and *Lusitaniella coriacea*. The isolation of the compounds was guided through bioactivity assays and mass spectrometry. These molecules were purified from the crudes by employing several chromatography methods, and the chemical structures were determined by means of NMR (nuclear magnetic resonance) and MS/MS (tandem mass spectrometry) experiments. Thus, a novel macrolide-type compound was isolated from *Leptothoe* sp., which presented a highly cytotoxic activity against our cancer cell models. Its effects on cancer vascularization and metastasis were studied using the zebrafish model. Moreover, from *L. coriacea*, five unprecedented salicyl-capped thiazol(in)e NRPs-PKs (nonribosomal peptides-polyketides) compounds were isolated. These compounds showed the potential to act as reversers of P-glycoprotein efflux activity.

Keywords: cyanobacteria; anticancer compounds; cytotoxic; multidrug resistance

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