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CONFERENCIA 1

Persistence and Degradation of Cyanotoxins

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While cyanotoxins are produced and cause problems in many environments, their occurrence in water for human and animal use is of particular concern (drinking, irrigation, aquaculture). As naturally occurring compounds, it would be expected that a range of microbes have the ability to degrade these toxins, however, although microcystins appear to degrade rapidly in most circumstance (e.g. open water) they have been seen to persist in some systems. Understanding the factors that influence degradation is important to enable us to safely manage toxic bloom occurrences. Cylindrospermopsin, on the other hand, is of growing concern in many water supplies with the potential to cause long-term health effects however; fewer studies have addressed persistence, stability and biodegradation. Experience indicates that cylindrospermopsin can be both released from viable cyanobacteria and remain stable in freshwater for some time. This is of particular concern in warm climates where persistent low levels of cylindrospermopsin may expose people to serious health risks. This highlights that, as we develop processes and plans to protect humans and animals from intoxication, we need to base our decision making on robust evident and not overly extrapolate both between environmental scenarios and between different cyanotoxins.

Key words: degradation, microcystins, cylindrospermopsin

CONFERENCIA 2

Methods for the analysis of cyanobacterial toxins: fit for purpose?

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As our research effort increases, the number of known cyanobacterial toxins grows, as well as the number of analytical methods for the detection of these toxins. Which method is best to use in any given situation depends on how well it is fit for purpose. Using BMAA and microcystin analysis as examples, it will be discussed how the use and development of different methods has influenced our knowledge on these toxins.

Key words: methodology, analysis, BMAA, microcystins

CONFERENCIA 3

Cyanobacteria and cyanotoxins risks via food. Do we know all the hazards?

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Cyanobacteria toxins and other secondary metabolites not yet well known may accumulate in a diverse array of organisms, from plants to aquatic and terrestrial animals and by that way, enter the human food chain. On the other side, the growing consumption of cyanobacteria and microalgae may pose safety questions that are not yet well assessed. Episodes of human intoxication due to these consumptions are scarce but indicate the risk. The global changes impact all over the world indicates that toxins and toxin producing cyanobacteria are spreading to a wider range of geographic areas. An overview of the main toxins and their risks in terms of human health will be presented, highlighting those toxins that would need a special attention. The main and most important toxin vector will be reviewed and the need for legislation concerning toxins in water, plants, fish and shelfish discussed. A significant array of new molecules produced by cyanobacteria as secondary metabolites have been unraveled in the last decade, but their environmental and human health impact is far from been known. The problems associated with the production, commercialization and consumption of algae and cyanobacteria-based supplements will be pointed out, so as to stress the need for an international regulamentation.

Key words: cyanobacteria, cyanotoxins, food contamination, risks, food supplements

SESIÓN ORAL: Detección de cianotoxinas y problemas ambientales.

Moderador: Fernando Cobo

COMUNICACIONES ORALES: 1-5

O1
EXPLORAR - Exploring the aquatic resistome

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Antibiotic resistance is one of the major problems in public health today since the failure of antibiotherapy has dramatic clinical implications such as the increase of mobility/mortality and of health costs. Antibiotic resistance is a dynamic process since antibiotic-resistant microorganisms, and the corresponding genetic material, flows and disseminates among several settings simultaneously: humans, animals and natural habitats. Water environments are recognized as important pools of antibiotic pollution and antibiotic resistance genes, but the water *resistome* (collection of all genes responsible for antibiotic resistance in water environments) is far from being characterized. It is well known that antibiotic pollution affects the structure/functioning/diversity of aquatic ecosystems, namely the cyanobacteria community. However, the role of cyanobacteria in the context of antibiotic resistance was never characterized. In this presentation, the aims, the team, the methodologies and the preliminary results of the national project EXPLORAR – Exploring the Aquatic Resistome (PTDC/BIA-BMA/31451/2017), founded by the Portuguese Foundation for Science and Technology (FCT, PT) will be discussed. In this project, we are investigating the contribution of indigenous freshwater organisms (cyanobacteria and bacteria) to water *resistome*. We expect to identify and characterize ecological niches associated with antibiotic resistance in freshwater environments. This may contribute to define a monitoring strategy to map the antibiotic resistance profiles of national freshwater resources. We expect to contribute to the definition of preventive measures against the dissemination of antibiotic resistance in the environment.

Key words: Freshwater cyanobacteria, Antibiotic pollution, Antibiotic resistance, Aquatic resistome, High-Throughput Sequencing Technologies

*Presenting Author

O2

Monitorización en tiempo real y a remoto de algas y cianobacterias en el embalse de As Conchas. Primeros resultados del proyecto Cianoalert

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El objetivo del proyecto Cianoalert es la generación de un sistema de alerta temprana frente a los afloramientos de algas y cianobacterias en masas de aguas continentales. Este sistema de alerta se basa en la recogida masiva de datos en tiempo real y a remoto mediante el uso de sondas multiparamétricas colocadas *in situ*, y datos de imágenes de satélite (Landsat 8 y Sentinel 2) y de cámaras multiespectrales tomadas con un vuelo de dron. Durante 2018 se han obtenido los primeros resultados de la zona piloto (Embalse de As Conchas, Galicia) y se han comparado con los muestreos manuales realizados por la Confederación Hidrográfica del Miño-Sil. Los datos recogidos por las sondas multiparamétricas permitieron conocer el estado del afloramiento en todo momento, pudiendo identificar los momentos clave previos a la floración, mientras que los muestreos manuales mostraron datos similares pero mucho más escasos. No obstante, existieron momentos puntuales, correspondientes a la mayor concentración de células, donde las sondas multiparamétricas no recogieron unos valores de clorofila total y ficocianina reales. En cambio, sí se observaron cambios en los datos recogidos por los sensores de pH y temperatura. Esto sugiere que un mayor número y variedad de sensores podrían ser necesarios para disminuir las incertidumbres generadas por el sistema autónomo. Los modelos aplicados para los sensores remotos (satélites y dron) confirman la utilidad de estas técnicas de detección remota para el seguimiento de los afloramientos de algas y cianobacterias en masas de aguas continentales, aunque requieren de ajustes y calibraciones adicionales para cada zona de estudio. Por lo tanto, los resultados hasta ahora obtenidos en este piloto muestran que es un sistema válido y con potencial, que requiere de una estrategia de seguimiento a múltiples escalas (manual, sensores *in situ* y satélites) para garantizar su fiabilidad.

Palabras clave: monitorización, imágenes vía satélite, sondas multiparamétricas

*Autor que presenta el trabajo

O3

Cianobacterias potencialmente tóxicas en la Cuenca del Ebro, un caso de estudio

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Una de las mayores amenazas en los sistemas acuáticos es la eutrofización y uno de los signos típicos de la eutrofización son los blooms de las cianobacterias. Estos blooms pueden causar grandes estragos al resto de las comunidades que interactúan con el medio acuático en donde se presentan, ya que generan un desequilibrio en la concentración de oxígeno, cambian la estructura de la comunidad y las cianobacterias potencialmente tóxicas (CPT) pueden producir diferentes cianotoxinas (hepatoxinas, neurotoxinas, dermatoxinas y endotoxinas). La cuenca del Ebro es la segunda cuenca de mayor tamaño de la península Ibérica, y en el presente estudio analizamos las CPT y los blooms que se han registrado en los diferentes embalses de la cuenca. Los muestreos se realizaron de 2010 a 2018 en la época de verano, se midieron las variables ambientales y se tomaron muestras de agua integradas para identificar y cuantificar las diferentes especies de cianobacterias. Los géneros de CPT más frecuentes fueron *Anabaena, Aphanizomenon, Dolichospermum, Microcystis* y *Planktothrix.* Los mayores blooms se registraron en los embalses de Urrunaga en 2017 con >220.000 cel/ml de *Microcystis aeruginosa, s*eguido de Oliana en 2016 y 2017 con 190.000 cel/ml y 90.000 cel/ml respectivamente de *Aphanizomenon gracile.* Los blooms se presentaron tanto en embalses mesotróficos como eutróficos. El análisis de correspondencia canónica (CCA) indica que las variables ambientales más relacionadas con las especies de cianobacterias fueron la temperatura, el pH, los nutrimentos (fósforo y nitrógeno en sus diferentes formas), la ficocianina y la clorofila *a* que son sus integrantes somáticos. El porcentaje de embalses que presentaron al menos una especie de CPT fue del 46%, mientras que embalses con blooms fue de 5%, sin embargo, con el paso del tiempo el número de embalses que presentan blooms y especies potencialmente tóxicas han ido en incremento.

Palabras clave: Cianobacterias, embalses del Ebro, *Microcystis aeruginosa, Aphanizomenon gracile,* cianotoxinas

*Autor que presenta el trabajo

O4

Detection and prevention of cyanobacterial blooms using spectrofluorometry techniques and remote sensing images from Sentinel-2 satellites.

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Cyanobacteria blooms seriously affect water quality and thus it has an impact on its recreational use, fishing, drinking water, and adverse effects from an environmental point of view. Because these blooms are an increasing problem due to water eutrophication and rising temperatures caused by climate change, extended monitoring of cyanobacteria presence and growth is crucial in avoiding the toxicity problems associated with these blooms. A continuous control of cyanobacteria concentration in water bodies could be carried out by *in situ* measuring instruments or using remote sensors, which would serve as warning systems to carry out remedial actions, after confirming the presence of cyanobacteria, quantity and specific composition by means of sampling and microscopic observation by the specialist. It is therefore necessary to develop works linking instrumental methods using phycocyanin as indicator (spectro-fluorometry and remote sensing), with direct sample counting of cyanobacteria. For *in situ* phycocyanin measurements, a C3 submersible fluorometer (Turner Designs) calibrated using phycocyanin standard extract from *Spirulina* (Sigma-Aldrich Chemicals) was used. For remote sensing measurements, images from Sentinel 2, corrected atmospherically with the C2RCC method, were used, adjusting the algorithm previously set-up to assess phycocyanin concentrations in eutrophic water bodies with the phycocyanin data measured *in vivo* in reservoirs of this study. Spectral band ratio 740/665 nm has given the best correlation between remote sensing signal and logarithmically normalized values of phycocyanin ($R2 = 0.9594$, $n = 21$, $p < 0.001$, RMSE 17.21 mg/m^3). A greater number of samples and an augmentation of their range of values would improve the adjustment. This methodology is thought to be very suitable. The use of satellite remote sensing images would function as an early warning system for the presence of cyanobacteria, which, if positive, might enable us to evaluate its potential toxicity, enlarging significantly both in time and space the information available.

Key words: Cyanobacteria-blooms, phycocyanin, spectro-fluorometry, remote-sensing, Sentinel-2, cyanotoxins

*Presenting Author

O5

Modelling of chlorophyll-a and *Microcystis aeruginosa* decay under the effect of different oxidants in culture media

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Blooms of the cyanobacterium *Microcystis aeruginosa* are common in many eutrophic lakes and pose a serious threat to water quality, potentially giving rise to high turbidity, food web alterations, increased production of toxic Microcystin (MC) and odorous compounds. The comparative effectiveness of oxidant treatment of *M. aeruginosa* cells in culture media was evaluated by applying a mathematical model of chlorophyll-a (Chl-a), cell and MC removal. The oxidants were chlorine (1-5 mgL^{-1}), hydrogen peroxide (HP: 50- 150 mgL^{-1}), percitric acid (PCA: 10- 50 mgL^{-1}), and peracetic acid (PAA: 1.5-7.5 mgL^{-1}). The Weibull distribution model was applied to assess the degree of inactivation of *M. aeruginosa* viability under different oxidant treatments. First-order kinetics was successfully applied to the experimental data for Chl-a decay. Using the Weibull model, it was possible to predict the required exposure time for oxidants to achieve a 99.9% reduction in viable *M. aeruginosa* cells with respect to the initial value (Tr). Five milligram per litre (5 mgL^{-1}) chlorine produced a 77% degradation of MC after 72 h, with an exposure time (Tr) of 141 h. Among the peroxide treatments (HP, PCA and PAA), PCA (10-50 mgL^{-1}) produced the highest level of MC degradation (66-69%), with low exposure times (Tr=119-125 h). This is the first modelling report of *M. aeruginosa* decay by oxidant treatment.

Palabras clave: *Microcystis aeruginosa*, Microcystin, mathematical model, oxidants, chlorophyll-a decay, Weilbull model

*Presenting Author

SESIÓN ORAL: Cianotoxicidad y Gestión de los Recursos Acuáticos 1

Moderadora: Leda Giannuzzi

COMUNICACIONES ORALES: 6-7

O6

High levels of Anabaenopeptins detected in a *Microcystis* bloom by LC-HRMS

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The appearance of a massive growth of cyanobacteria in the Sau-Susqueda-Pasteral system in the autumn of 2015 has been the most recent episode of bloom in Catalonia. This system of reservoirs is located in the middle reaches of the River Ter (Catalonia, NE Spain) and is devoted mainly to urban supply, regulation of the River Ter, irrigation and production of hydroelectric energy. In fact, it is the main supply system for the metropolitan area of cities such as Barcelona and Girona. The presence of *Microcystis* cyanobacteria in the bloom was confirmed by taxonomic study. Additionally, low and high toxicity was detected in the extracellular and intracellular fractions of some samples, respectively. The reservoir was confined and periodic sampling in the reservoir was carried out. Analysis studies were performed by enzyme-linked immunosorbent assay (ELISA) and liquid chromatography-high resolution mass spectrometry (LC-HRMS). The results for the analysis of microcystins were negative (<0.3 µg/L) in all the surface samples. Only traces of microcystin-LR and -dmLR were detected in concentrations estimated to be close 10 ng/L. In contrast, the presence of different anabaenopeptins and oscillamides at unusually high concentrations, in the order of mg/L were observed. To our knowledge, no previous studies have detected these bioactive peptides at such high levels. The reliable identification of these metabolites was achieved by HRMS. Although these peptides are not included in any regulations or recommendations and they are considered less toxic than microcystins, current data show that they can give a positive response in some toxicity tests. Specifically, anabaenopeptins have been shown to be inhibitors of protein phosphatases and carboxypeptidase A. These results agree with recent studies in which analysis of cyanobacterial metabolites in surface waters reveals more than microcystin. However, these cyanopeptides have received little attention.

Key words: Anabaenopeptins, HRMS, Bloom control

*Presenting Author

O7

Cianobacterias y cianotoxinas en ambientes eutrofizados que pueden repercutir en la actividad acuícola camaronera

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La camaronicultura en Ecuador es una actividad primordial en la exportación de productos y generación de divisas para el país. Dicha acuicultura crece sometida a dificultades en el rendimiento de la producción por diferentes motivos. Uno de los inconvenientes son los *blooms* de cianobacterias que ingresan a las piscinas camaroneras, lo que dificulta superar los controles de calidad del producto para su exportación. Las cianobacterias causan preocupación por la calidad del agua y salud humana, especialmente en ecosistemas acuáticos eutrofizados, creciendo como floraciones nocivas y produciendo peligrosas toxinas para el ser humano y animales. Algunos géneros producen metabolitos volátiles que confieren olores y sabores al agua, e incluso a camarones y peces en sistemas de cultivo, alterando sus caracteres organolépticos. Optimizamos la extracción de DNA de muestras de agua y camarón *(Penaeus vannamei),* y la amplificación por PCR de fragmentos de genes de referencia en cianobacterias *(ntcA* y *glnB)* y camarón *(abdA* y *betacatenin)*, e identificación por tamaño molecular de fragmentos amplificados. Además, se diseñaron oligonucleótidos para la amplificación de fragmentos de genes implicados en la producción de cianotoxinas: *mcyA, mcyE, cyrA y cyrC.* Relacionamos la incidencia de cianobacterias con la eutrofización, tomando parámetros físico-químicos in situ (temperatura, pH, salinidad, oxígeno disuelto, conductividad, sólidos disueltos totales) y análisis de nutrientes utilizados en los índices de evaluación (fósforo reactivo soluble, fosfato, amonio, nitrito, nitrato).

Palabras clave: blooms, camarón, PCR, cianotoxinas, eutrofización, parámetros

*Autor que presenta el trabajo.

SESIÓN ORAL: Cianotoxicidad y Gestión de los Recursos Acuáticos 2

Moderadora: Elsa Dias

COMUNICACIONES ORALES: 8-11

O8

Toxins determination in seawater and seaweed-based supplemental foods using ionic liquids and liquid chromatography coupled to time of flight-mass spectrometry

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An analytical procedure for the determination of three cyanotoxins, microcystin LR, microcystin RR and nodularin, and two phycotoxins, domoic acid (neurotoxin) and okadaic acid (diarrhetic toxin) in seawater and seaweed-based supplemental foods is proposed. The toxins were prior isolated from the seaweed samples by using a salting out liquid extraction with acetonitrile, formic acid, sodium chloride and magnesium sulphate. Due to the low concentrations of these compounds, a preconcentration step was included using dispersive liquid-liquid microextraction with an ionic liquid based on 1-hexyl-3-methylimidazolium hexafluorophosphate as extractant solvent (80 mg) and acetonitrile as disperser solvent (0.5 mL) at pH 1.5, following the principles of green analytical chemistry. Liquid chromatography with electrospray ionization and quadrupole time of flight-mass spectrometry (Q-TOF-MS) was used for separation and identification of toxins, the selectivity of the detection system provided unequivocal identification based on accurate mass measurements. The TOF-MS scan mass spectra and TOF-MS/MS scan mass spectra were recorded in the positive ion mode and the quantification was carried out using the protonated molecule of each compound. Retention times ranged between 6.2 and 18.0 min using a mobile phase composed by a mixture of methanol and formic acid (0.1%) and gradient elution. None of the target toxins was detected in any of the seawater samples analysed, above their corresponding detection limits. However, different seaweed-based supplemental foods composed on marine phytoplankton (*Nannochoropsis gaditana*), blue-green algae, *Spirulina* (from ecological agriculture) and *Spirulina maxima* (rich in chlorophyll) were also analysed and microcystin LR was detected in the sample of blue-green algae.

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Key words: Toxins, seawater, seaweed-based foods, ionic liquids, liquid chromatography, time of flight-mass spectrometry

*Presenting Author

O9

Cyanotoxins in the Azores: new identifications?

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Cyanobacteria are photosynthetic prokaryotes, capable of toxin production, that can be found in a variety of habitats, including freshwater, marine, terrestrial and extreme environments (e.g. hot springs, hypersaline). Cyanobacteria blooms are very common during the last decades, these are a serious environmental and public health problem. Toxic strains are not visually different from non-toxin producing ones, hence the necessity to use molecular methods that have been used to identify cyanotoxin producing genes and identify cyanotoxin production risk. In order to assess cyanobacteria biodiversity and to determine neurotoxins production potential in the Azores, several habitats from these islands were sampled: 25 volcanic freshwater lakes, seven streams, 21 thermal water and six terrestrial sampling sites. Cyanobacteria isolation was made with an inverted microscope, by serial dilutions, after adaptation in BG-11 medium. Saxitoxin (STX) and Anatoxin-a (ATX-a) genes (sxtA, sxtG, sxtH, anaC and anaF) were targeted using specific primer pairs. A total of 153 cyanobacteria strains from 30 genera were isolated, e.g. *Aphanizomenon* and *Mastigocladus*, and are maintained in unicyanobacterial cultures in the Azorean Bank of Algae and Cyanobacteria created in the framework of the REBECA project (MAC/1.1a/060).
All targeted genes were detected in the tested strains, identifying 9 strains with ATX-a production potential. Regarding STX, this works reveals incoherent results regarding sxtA gene, finding that not all the strains with this gene (19) had the presence of either sxtG (11) and/or sxtH (3), enhancing once again the necessity to study deeper the molecular behind STX production and sxtA dispersion among different cyanobacteria and other organisms as dinoflagellates. This work brings continuously new information regarding cyanotoxins, since some of the results were in well-known cyanotoxins producing strains such as *Aphanizomenon gracile*, however others were in non-reported cyanotoxin producers such as *Planktolyngbya limnetica*. These are the first indications of neurotoxins production in isolated cyanobacteria from the Azores.

Palavras chave: BACA, Culture collection, Toxic cyanobacteria, Molecular tools, Cyanotoxins monitoring

*Presenting Author

O10

Cyanotoxins from Portuguese freshwater systems: a recent report on its proliferation

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Environmental health is a key factor in promoting water quality. Cyanobacteria on the contrary releases harmful cyanotoxins that impair freshwater ecosystems. Taking into account that these have several impacts such as water provision, irrigation and recreation it is well demanded the surveillance of freshwater systems for safety and health purposes as well as to improve water quality. To achieve this, faster and accurate methodologies such as the molecular and immunological methods are required. Following this between April and September of 2017 a surveillance campaign was carried out in seven freshwater systems located in the North and Centre Regions of Portugal. The main aim was to assess environmental safety and health regarding cyanotoxicity. In our data both methods showed that microcystins are still problematic followed by anatoxin-a in both regions. Cylindrospermopsins appear to be spreading to North freshwater systems while saxitoxins also showed a high prevalence in North and Centre Regions both through the molecular technique. Our data additionally shows that cyanotoxicity in Portugal has proliferated since neurotoxins were circumscribed to the South part (saxitoxins), cylindrospermopsins to the Centre and South Regions and microcystins are well distributed. Besides the ecological damage that cyanotoxins can bring to ecosystems our study reflects the lack of environmental safety and health of Portuguese freshwater ecosystems and also a high risk of exposure to humans and welfare with direct effects in the development of new socio-economic activities in the surveyed ecosystems (tourism and aquaculture).

Key words: Cyanobacteria, Cyanotoxins, Environment, Risk assessment

*Presenting Author

O11

Proyecto IMPREX. Identificación de blooms algales mediante indicadores meteorológicos y de proceso de tratamiento en la ETAP La Contraparada (Murcia).

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De 2016-19 se desarrolla el Proyecto IMPREX (Improving PRedictions of hydrological Extremes), correspondiente al programa H2020. Dentro del consorcio creado para el proyecto se encuentra Cetaqua como socio, Aquatec es un "Third-party", y Aguas de Murcia actúa como "Stakeholder".
Aquatec realiza una asistencia técnica en la ETAP La Contraparada (Aguas de Murcia), y en el Proyecto IMPREX en concreto en la ejecución de los muestreos y análisis planificados.
Las tareas realizadas en la ETAP La Contraparada, se han centrado en: evaluar cómo afectan los condicionantes meteorológicos extremos a la calidad del agua superficial, analizar toxinas algales que se desconoce si están presentes en el embalse de regulación de agua bruta de la ETAP (anatoxinas, saxitoxinas), así como desarrollar indicadores que ayuden a predecir la formación de blooms algales.
Se ha trabajado en el tratamiento estadístico con el programa Orange del histórico de datos físico-químicos, biológicos y meteorológicos, encontrando diversas relaciones que serán de utilidad para la monitorización y prevención de episodios de bloom algales (con concentración de clorofila "a" superior a 10 µg/l) en el embalse de la ETAP, permitiendo la ejecución de prácticas operativas en el agua de captación y en el tratamiento, que minimicen el impacto algal en el proceso de potabilización.
En octubre de 2018, se detecta en el Embalse de la ETAP y en el Azud de Ojós, la cianofícea *Planktothrix* sp., típica de los blooms algales del norte de España y resto de Europa y muy rara en el sur. Se realiza monitorización hasta su desaparición de manera natural.

Palabras clave: agua superficial, potabilización, predicción, indicadores meteorológicos, *Planktothrix sp.*, prácticas operativas

*Autor que presenta el trabajo

SESIÓN ORAL: Compuestos aleloquímicos y efectos ambientales.

Moderadora: Remedios Guzmán-Guillén

COMUNICACIONES ORALES: 12-14

O12

Efectos tóxicos de [D-Leu[1]]MC-LR sobre tejidos animales y vegetales: menor capacidad inhibitoria sobre PP1 y mayor potencia tóxica que MC-LR.

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MC-LR y [D-Leu[1]]MC-LR son congéneres de hepatotoxinas llamadas microcystinas presentes en los florecimientos cianobacterianos de la Cuenca del Plata; diferenciadas en la sustitución de Alanina por D-Leucina en la posición 1, involucrada en la unión al sitio activo de las proteínas fosfatasas; su principal mecanismo de acción. El objetivo fue evaluar los efectos de exposiciones agudas a MC-LR y [D-Leu[1]]MC-LR, sobre un modelo animal (ratones N:NIH Swiss) y uno vegetal (*Phaseolus vulgaris*). Hemos observado variaciones en las dosis letales de [D-Leu[1]]MC-LR (50 µg/kg) respecto de las publicadas para MC-LR (100 µg/kg), presentando aumento del % en peso del hígado y hemorragias intrahepáticas en ratones expuestos a dosis de 50 a 200 µg[D-Leu[1]]MC-LR/kg; y un principio de esteatosis luego de una única dosis i.p. de 25 µg [D-Leu[1]]MC-LR/kg, ausente en el mismo tratamiento con MC-LR. Los estudios en modelo vegetal, de contacto único durante la imbibición (3,5 y 15 ppm) también mostraron diferencias en cuanto a la germinación, desarrollo y morfología de las plantas y niveles de TBARs; siendo más afectadas las tratadas con [D-Leu[1]]MC-LR para una misma dosis. Los valores de IC50 para PP1 fueron 35,4 ppb para [D-Leu[1]]MC-LR y 10 ppb para MC-LR. Al evaluar la actividad fosfatasa en un homogenato de raíces se vio que [D-Leu1]MC-LR inhibió hasta un 60% la actividad de todas las fosfatasas presentes; mientras que MC-LR inhibió un 12%. En un homogenato de hígado la inhibición efectuada por [D-Leu[1]]MC-LR fue del 50% y por MC-LR del 40%. Nuestros resultados indican que es necesario profundizar en la toxicidad de [D-Leu[1]]MC-LR, dado que evidentemente puede estar inhibiendo otras fosfatasas presentes, fundamentales para el funcionamiento de las células; que sumado al estrés oxidativo podría explicar las diferencias de toxicidad de ambas toxinas sobre los tejidos estudiados.

Palabras clave: [D-Leu[1]]MC-LR, MC-LR, Potencia tóxica, Inhibición de fosfatasas

*Autor que presenta el trabajo

O13

Caracterización de floraciones de cianobacetrias nocivas en la Laguna de Cajititlán, Jal. Análisis de abundancia , distribución de especies y análisis bioquímicos

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Para este trabajo se ha monitoreado la laguna Cajititlan, en Tlajomulco de Zúñiga, Jalisco, la cual experimenta constantes floraciones fitoplanctónicas y fenómenos paralelos de altas mortalidades de fauna acuática. Los monitoreos inicial nos indican la alta presencia de cianobacterias en especial del género *Cylindrospermopsis*, con especies descritas como productoras de cianotoxinas del tipo cilindrospermopsina, saxitoxinas, anatoxina-a. El objetivo de este estudio en una primera etapa consistió en monitorear durante un año el fenómeno, identificar las características ambientales del cuerpo de agua, la distribución y abundancia de las floraciones de cianobacterias en la laguna. El trabajo esta orientado en aislar y determinar la especie del género *Cylindrospermopsis* presente en las floraciones por medio de técnicas moleculares para luego realizar estudios de caracterización bioquímica por los métodos de Bligh & Dyer para lípidos como GC para perfil de ácidos grasos, espectrometría para proteínas y carbohidratos.

Palabras clave: *Cylindrospermopsis*

*Autor que presenta el trabajo

O14

Sensibilidad de diferentes cepas tóxicas de *Microcystis aeruginosa* frente a compuestos aleloquímicos generados por *Phormidium* sp.

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El género de cianobacterias *Microcystis* constituye una grave amenaza para la salud humana a escala planetaria debido a sus proliferaciones en aguas continentales. Sus principales efectos nocivos se deben a la producción de hepatotoxinas (microcistina). En las últimas décadas se han realizado ensayos de diversas metodologías para el control de estas proliferaciones tóxicas; entre ellas, la inducción de mezcla en la columna de agua, el uso de compuestos químicos (detergentes, peróxido de hidrógeno) y el empleo de quelantes para el secuestro de nutrientes. El objetivo principal de esta investigación consiste en demostrar el efecto de aleloquímicos producidos por la cianobacteria *Phormidium* sp. sobre diferentes cepas tóxicas de *M. aeruginosa,* explorando su posible aplicación para biorremediación de proliferaciones de dicha cianobacteria. Para testar la eficacia de los compuestos alelopáticos, se han empleado dos tipos de metodología: bioensayos y experimentos de microcosmos en cultivos continuos (quimiostato) de larga duración. En ambas prácticas experimentales, empleamos 9 cepas tóxicas de *M. aeruginosa.* En relación a los bioensayos, cada cepa de *M. aeruginosa* fue expuesta a diferentes concentraciones de filtrado de cultivos con *Phormidium* sp. En estos experimentos, todas las cepas mostraron un descenso significativo de su tasa de crecimiento a medida que se incrementa la concentración del filtrado. La cepa de *Phormidium* sp. empleada produce unos compuestos con actividad alelopática demostrada, denominados portoamidas. En un bioensayo realizado con estos compuestos, hemos demostrado que pueden ser los responsables de la actividad alelopática mostrada en este trabajo. Por otro lado, en los experimentos de microcosmos de larga duración, se estudió el efecto simultáneo de la competencia interespecífica por un recurso limitante (nitrato) y por alelopatía entre ambas especies. Con este procedimiento conseguimos demostrar que, en condiciones similares a una proliferación de *M. aeruginosa*, esta cianobacteria es eliminada por los compuestos alopáticos producidos por *Phormidium* sp.

Palabras clave: Biorremediación de proliferaciones, *Microcystis aeruginosa*, cianobacteria, *Phormidium* sp., alelopatía, competencia interespecífica

*Autor que presenta el trabajo

SESIÓN ORAL: Cianobacterias en alimentación humana

Moderadora: Rosario Martins

SESIONES ORALES: 15-17

O15

Cyanobacteria as fatty acid producers in human nutrition. Potentiality of semiarid environments

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Semiarid regions are usually neglected in projects searching for new biological resources but evidences are being accumulated about the high biodiversity they may harbour mainly composed of organisms very well adapted to fairly extreme conditions. Most semiarid habitats (terrestrial or aquatic) are dominated by algae (included cyanobacteria) that may represent sources of environmentally and economically valuable compounds. There is an increasing interest in fatty acids in nutrition beacuse some of them are essential in different steps of development of most animals, including humans, an their potential health benefits have been extensively studied. Two streams (Alhárabe and Chícamo rivers) in a climatic gradient between 200 and 600 mm rainfall were compared from the point of view of fatty acid composition their algal communities. Lipids were extracted, trans-metylated and FAME were separated and identified by gas-liquid chromatography. All collected algae taxa contained fatty acids considered as essential for human consumption including DHA. Permanova analysis showed significant differences between the two streams studied and between winter and summer in both streams. Simper analysis showed differences between both streams in summer and also showed significant differences in fatty acid proportions between taxonomic algae groups and macroinvertebrate feeding groups. Cyanophyta contributed significantly to the differences found between streams in summer. Our data show that macroinvertebrate feed on dominant algal communities in these rivers and that cyanophyta represent an important resource part of year. Much more research is needed before generalisations could be made but fatty acids can be used as markers of fluvial communities changes in time and space and algae from semiarid environments should be taken into account for biotechnological purposes.

Key words: benthic algae, cyanophyta, fatty acids, semiarid streams

O16
Studies of the possible effects of a producer and a non-producer extracts of cylindrospermopsin in the SH-SY5Y cell line

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Cylindrospermopsin (CYN) is a cyanotoxin whose presence is increasing in the last decades as a consequence of human activity and climate change, supposing a risk for human and animal health. Due to its toxic effects, this toxin is classified as a cytotoxin. In fact, CYN has proven to exert damage at several levels in the organism, including the nervous system as previous studies have shown an alteration in the cholinergic neurotransmission and oxidative stress parameters. For this, the aim of the present work was to assess the cytotoxic capacity of a producer (PE) and a non-producer (NPE) extracts of CYN (*Chrysosporum ovalisporum* and *Cylindrospermopsis raciborskii*, respectively), to study their capacity to produce oxidative stress and acetylcholinesterase activity (AChE) alterations in the human neuroblastoma SH-SY5Y cells. Our results show a greater sensitivity of the cells to the MTS biomarker, providing a half effective concentration (EC_{50}) of 1.111 ± 0.325 µg/mL in the case of the PE, and 5.658 ± 1.180 µg/mL in the case of the NPE after 24 hours of exposure. On the other hand, PE led to a rise in the oxidative stress biomarkers, by increasing the reactive oxygen species and decreasing the glutathione levels, which is in agreement with the response obtained for NPE at higher time and concentrations of exposure. In the case of the AChE, only the PE caused a rise in the activity. Thus, both extracts have the capacity to induce oxidative damage, whereas the PE can, moreover, cause effects in the neurotransmission.

Acknowledgments: Spanish Ministerio de Economía y Competitividad for the project AGL2015-64558-R,MINECO/FEDER, UE.

Keywords: cylindrospermopsin, SH-SY5Y, acetylcholinesterase, oxidative stress, cytotoxicity

*Presenting Author

O17
Bank of Algae and Cyanobacteria of Azores (BACA) a culture collection for biotechnological and pharmaceutical research

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The Azores have a singular biota, mainly due to the unique character of the oceanic islands ecosystems and the diversity of habitats found in the archipelago, including freshwater, terrestrial, thermal and marine environments. The high diversity and quantity of suitable habitats for the development of microalgae and cyanobacteria makes the Azores an important site for discover new species and biomolecules of increased value. With the aim to build a culture collection of these organisms, under the REBECA project (INTERREG MAC/1.1a/060), we gather the existing collections in the University of the Azores (144 cyanobacteria strains and 112 microalgae strains) and create the BACA - Azorean Algae and Cyanobacteria Bank. With the bioprospection in several islands and habitats across the archipelago, BACA accounts presently with 402 strains, including 188 cyanobacteria and 214 microalgae. All strains in BACA are being identified by traditional methods based on morphology and by molecular analysis. About 50 cyanobacteria strains are already identified by the combination of both approaches. BACA also produces biomass of selected strains in bioreactors at laboratory scale for biochemical characterization and search of bioactive compounds with biotechnological and/or pharmaceutical applications.

Keywords: Microalgae, Cyanobacteria, Bioactivity, Azores, Extremophiles

*Presenting Author

PANELES

P1

A novel cyanobacterial geosmin producer, revising *Geo*A distribution and dispersion patterns in Bacteria

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Cyanobacteria are well known for their part in worldwide occurrence of aquatic blooms, while producing a myriad of natural compounds, some with toxic potential, but others of high biotechnological potential, as geosmin. We performed an environmental survey of cyanobacterial soil colonies to identify interesting metabolic pathways and adaptation strategies used by these microorganisms and isolated, sequenced and assembled the genome of a cyanobacterium that displayed a distinctive earthy/musty smell, typical of geosmin, confirmed by GC-MS analysis of the culture's volatile extract. Morphological studies pointed to a new Oscillatoriales soil ecotype confirmed by phylogenetic analysis. Our studies of geosmin gene presence in bacteria revealed a scattered distribution among cyanobacteria, actinobacteria, delta and gammaproteobacteria, covering different niches. Careful analysis of the geosmin gene tree points to an ancient bacterial origin of the gene that was probably successively lost in different time frames. The high sequence similarities in cyanobacterial geosmin gene amidst freshwater and soil strains reinforces the idea of an evolutionary history of geosmin that is intimately connected to niche adaptation.

Key words: Geosmin, *geo*A, genome sequencing, Biological Soil Crusts, Cyanobacteria, Volatile Organic Compounds

*Presenting Author

P2

Comparison of saxitoxin-genes expression and production profiles between *Aphanizomenon gracile* and *Cuspidotrix issastchenkoi* strains, isolated from freshwater reservoirs

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Saxitoxins (STX) are a group of carbamate alkaloids known to inhibit the axons sodium ion channels, thus affecting the nervous system in vertebrates. These neurotoxins cause paralysis and respiratory failure, ultimately ending in death. They are produced by organisms belonging to two different kingdoms: marine eukaryotic dinoflagellates and freshwater prokaryotic cyanobacteria. The unique biosynthetic pathway, which is responsible for encoding proteins that allow synthesizing and exporting STX, is the cluster *sxt*, already described in several cyanobacterial species. It has been observed that diverse environmental factors affect differently STX production in cyanobacteria. Among those, temperature is the one that aroused greater interest, since it directly affects cyanobacterial growth rates, however controversial results have been reported. Furthermore, studies on the environmental regulation of STX synthesis and transport are quite rare. In this work, we tested the influence of temperature in two STX producers, *Aphanizomenon gracile* LMECYA40 and *Cuspidothrix issatschenkoi* LMECYA31, isolated from Portuguese freshwaters and maintained in the ESSACC. Total STX concentration, the expression of genes linked to STX biosynthesis (*sxtA*) and transport (*sxtM* and *sxtPer*) and the percentage of heterocytes per trichome were determined, in four different phases of cyanobacterial growth (lag, early exponential, late exponential and stationary phases). At 20ºC, a higher expression of the *sxt* genes and higher production of STX in the late exponential and stationary phase was observed in both species. Also, *C. issatschenkoi* produced more STX throughout the growth cycle than *A. gracile.* In *A. gracile* the percentage of heterocytes per trichome was higher in the lag phase, decreasing along the cell cycle. *C. issatschenkoi* did not form heterocytes throughout the growth cycle. Further experiments are being performed at 10ºC and 30°C in order to understand the effect of temperatures environmentally relevant in STX producers.

Keywords: Saxitoxin, temperature, *Aphanizomenon gracile*, *Cuspidothrix issatschenkoi*, heterocytes, gene expression

*Presenting Author

P3

Cyanotoxins and water residence in a coastal wetland

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The National Park of the Albufera is an important Iberian and Mediterranean coastal wetland (Valencia, Spain) with severe environmental impacts (agriculture, urban and industrial pressure) in its catchment area. Pollution is the cause of this wetland progressive degradation, because of the diversity of uses and economic policies. This wetland has also the largest Iberian coastal lagoon (23 km^2 and 1.2 m mean depth). From late 1950s several impacts like eutrophication, pesticides, introduction of exotic fish species and hydrological control for agriculture in the surrounded ricefield area, have severely affected and modified the lake ecology. In 1991, a restoration plan started by nutrient diversion, but nutrient loadings and pollution still high preventing an improvement in the lake ecological status. Climate and agriculture enhance negative effects for the lagoon restoration. In the last decade, hydrological changes have introduced new stressors and conditions for toxic algae to develop in the lagoon. Cyanotoxins are in the lake water and accumulated in the plankton and the top levels of the food web (fish). They can constitute a threat for fisheries and wildlife inhabiting the wetland. The results of some of the studied carried out in the lagoon are showed in the present work. This wetland is located in a transitional zone that links continental and marine waters and consequently pollution and toxins can affect to several ecosystems. This makes its restoration and the design of management programmes complex. The presentation of this study was financially supported by a Prometeo project from the Generalitat Valenciana.

Key words: wetland, *Microcystis*, miscrocystin, toxin quota, agriculture, restoration

*Presenting Author

P4

Effects of cylindrospermopsin and glyphosate at environmental concentrations on growth, photosynthesis, phenolic compounds and mineral content in lettuce plants

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The intensification of agriculture has increased water eutrophication and the presence of cyanobacterial toxins, such as cylindrospermopsin (CYN). Glyphosate (GLY) is the most widely used herbicide, mainly due to the extensive cultivation of GLY-resistant plants. Previous studies support the hypothesis that, individually, CYN and GLY can affect the yield of crop plants, depending on the exposure concentration. Lettuce (*Lactuca sativa L.*) is a commercial leafy vegetable, extensively consumed worldwide with major importance for human nourishment and economy. Given the relevance of the interaction of these two compounds in risk assessment, this study aimed to assess the effects of environmentally relevant concentrations of CYN (50µg/L), GLY (750 µg/L) and the CYN/GLY mixture on growth, photosynthesis, phenolic compounds and mineral content in lettuce plants grown in soil and hydroponic system. In general, for all the treatments, the plants exposed in soil system resulted in a decrease in fresh weights of the shoots and roots; however an increase in the fresh weight of roots was observed in plants exposed to CYN. The plants in hydroponic system showed an increasing trend in shoot weight and negligible differences in root weight. No negative effect on photosynthesis was observed, even leading to an increase in this parameter in lettuce grown in soil system. The amount of phenolic compounds was not significant different in lettuce plants exposed in soil system, however it tended to be higher in plants grown in hydroponics, mainly when exposed to CYN. In both the soil and hydroponic grown plant leaves, a general decline in mineral content was observed, with a few elements showing an enhanced concentration. Our results suggest that CYN and GLY can change yield and nutritional quality of lettuce when present in environmentally relevant concentrations. Further research is needed to understand the under-lying impacts.

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Key words: cylindrospermopsin, glyphosate, lettuce, mineral content, phenolic compounds, photosynthesis

*Presenting Autho

P5

Problems of the cyanobacteria associated with the presence of cyanotoxins in subtropical reservoirs (São Paulo, Brazil)

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The aim of this study was to evaluate the water quality in a set of subtropical reservoirs (Igaratá - IGA, Atibainha - ATI, Paiva Castro - PC, Rio Grande - RG, Itupararanga ITU, Broa - BR, Barra Bonita - BB, Guarapiranga – GUA and Salto Grande – SG (State of São Paulo, Brazil), focusing on cyanobacteria and cyanotoxins. Physical, chemical and biological variables of water were measured. Phytoplankton samples were analyzed by inverted microscopy for calculation of density and biovolume. The samples with microcystin (MC-LR, MC-RR, MC-LA e MC-YR) were analyzed and quantified in a chromatographic system coupled to a mass spectrometer. We identified 15 taxa of Cyanobacteria (Chroococcales, Nostocales, Oscillatoriales). In the ITU, BR, BB and SG reservoirs, *M. aeruginosa* and *M. panniformis* was recorded in all sampling stations. *Cylindrospermopsis raciborskii* was found in the reservoirs ATI, PC and ITU. The biomass of cyanobacteria varied from 0.035 mgL^{-1} to 145.0 mgL^{-1}. The presence of MC-LA, MC-LR, MC-YR and MC-RR variants was detected in different concentrations in 5 of the 9 studied reservoirs. The concentration of MC varied from 0.0023 μgL^{-1} to 1.08 μgL^{-1}. At the dam area of BB reservoir, the highest concentrations of MC-YR (1.08 μgL^{-1}) and MC-RR (0.70 μgL^{-1}) were recorded. The MC-LR was higher at the dam area of BR reservoir (0.39 μgL^{-1}). MC-LR type was considered the most common at 10 sampling points and MC-YR and MC-RR type occurred only at 5 from the 27 points shown. According to CCA the most important variables related to the presence of cyanobacteria were suspended solids, water transparency, electrical conductivity and total phosphorus. The occurrence of a gradient between the better quality reservoirs (IGA, ATI, PC, RG) and the worst quality water reservoirs (GUA, BB, ITU, SG and BR) was detected according to the metric measures and presence of microcystins.

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Key words: Cyanobacteria, reservoir, microcystin, Brazil

*Presenting Author

P6

Variação espacial dos Grupos Funcionais fitoplanctônicos de um reservatório tropical de usos múltiplos (São Paulo, Brasil): impacto das cianobactérias

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A determinação dos grupos funcionais (GFs) fitoplanctônicos foi investigada no reservatório tropical Broa, localizado no estado de São Paulo (Brasil). Análises *in situ* e laboratoriais possibilitaram a caracterização limnológica e fitoplanctônica, sendo a mesma realizada a partir de amostragem única e da água superficial em nove pontos ao longo do perfil longitudinal no período seco. Determinou-se como espécies descritoras àquelas que contribuíram com biomassa total >1%, considerando biovolume celular. Como resposta à tendência de eutrofia, houve destaque das cianobactérias na estrutura da comunidade em relação à densidade, biomassa e espécies descritoras a partir dos grupos funcionais H1, K, Lm e Sn, representados pelas espécies: *Aphanizomenon gracile, Aphanocapsa elachista, A. delicatissima, Dolichospermum sp., D. solitarium, Microcystis sp., M. aeruginosa*, e *Cylindropermopsis raciborskii*. A CCA indicou compartimentalização dos pontos amostrais com formação de duas zonas principais. A mesma foi inconclusiva quanto às variáveis que mais influenciaram a biomassa algal, porém indicou que a comunidade não esteve limitada pelos nutrientes. As vantagens adaptativas das cianobactérias sobre as demais classes culminaram na dominância das mesmas, visto que as oito espécies descritoras representam tal grupo. O GF H1 se destacou com 94,43% da biomassa fitoplanctônica total, ocorrendo ao longo de todo o reservatório, tendo como principal representante a espécie *A. gracile*. Todas as espécies descritoras da comunidade amostrada já foram relatadas como potenciais produtoras de toxinas na literatura (microcistinas, anatoxina-a, cilindrospermopsina e saxitoxinas). Reforçando que o estudo das cianobactérias e seu monitoramento não devem ser negligenciados, particularmente por terem sido detectadas variantes de microcistinas na zona da barragem. Conclui-se que o reservatório apresenta problemas sanitários que podem ser estudados a partir de abordagem ecológica pela aplicação da metodologia de Grupos Funcionais, que se mostrou uma ferramenta simplificada e satisfatória para contribuir para o gerenciamento de reservatórios.

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P7

Efectos del cambio climático en la síntesis de polisacáridos de *Nostoc commune*

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Los polisacáridos se sitúan en la parte externa de la pared celular de cianoprocariotas. Se cree que regulan la pérdida y absorción de agua y sirven como una matriz que protege a todo el organismo. Debido a las propiedades únicas que presentan, los polisacáridos son utilizados en numerosas aplicaciones biotecnológicas y farmacéuticas como emulsificantes, estabilizantes o agentes espesantes. Se sometió *Nostoc commune* a diferentes condiciones ambientales ligadas al cambio climático como incremento de nitrógeno, de radiación ultravioleta, de salinidad y de temperatura con el fin de conocer el efecto que tienen sobre su síntesis de polisacáridos. Las colonias de *Nostoc commune* se incubaron durante 10 días en medio BG11 con diferentes concentraciones de amonio (0, 50, 500 mM NH_4Cl), temperaturas (20, 30, 40 C), conductividades (2.09, 4.11, 8.17 mS/cm) y radiaciones (PAR=50.3, UVA=6.44, UVB=1.51 W/m^2) con un fotoperiodo de 18:6 h excepto las que recibían UVB con 04:20 h. Se extrajeron y determinaron los polisacáridos según Pacepavicius y col. (1997). Los resultados indicaron que la síntesis de polisacáridos aumentó significativamente con una concentración de amonio de 50 µM NH_4Cl ($F=45.706$; $p<0.01$), una radiación UVB de 1.51 W/m^2 ($F=62.691$; $p<0.01$) y conductividades de 4.11 mS/cm y 8.17 mS/cm ($F=4.816$; $p<0.1$). Además, el contenido de polisacáridos en *Nostoc commune* también se incrementó con una temperatura de 30 ºC. Estos resultados sugieren que los polisacáridos podrían jugar un importante papel ante condiciones extremas permitiendo el desarrollo de estos organismos en hábitats áridos y semiáridos e inducen a investigar su perfil glucídico enfocado a la industria alimentaria y farmacológica.

Referencia:

Pacepavicius, G., Lau, Y. L., Liu, D., Okamura, H. & Aoyama, 1. (1997). A rapid biochemical method for estimating microbial biomass. Environ Toxicol Water Qua1 12, 97-100.

Palabras clave: cambio climático, cianobacteria, cianofícea, factores ambientales, polisacáridos

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P8

The possible role of microcystin (D-Leu[1] MC-LR) as antioxidant on *Microcystis aeruginosa. In vitro* and *in vivo* evidences

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Microcystins constitute a serious threat to the quality of drinking water worldwide. However, the eco-physiological role of them is not yet completely known and it is suggested that MC can play a significant role in the antioxidant protection. The objective of this study was to evaluate the MC antioxidant capacity *in vitro* by Electronic Paramagnetic Resonance (EPR), highly specific for the different reactive oxygen species (ROS) and *in vivo* by long term (7 days) exposure of a *Microcystis aeruginosa* to high (29°C) temperature in addition to a 26°C control. It was observed an effective antioxidant activity of [D-Leu[1]]MC-LR against both radicals. In addition, significant increase in cellular biomass was observed under 29ºC being higher in [D-Leu[1]]MC-LR added cultures. The oxidation rate of 2,7-dichlorofluorescein di-acetate (DCFH-DA) was decreased during days 2, 5 and 7, in presence of MC, being significantly lower in the maximum exponential growth. In conditions of maximum ROS, at high temperature, the activity of the enzymatic antioxidant Catalase was significantly lower in [D-Leu[1]]MC-LR supplementation.
Overall, [D-Leu[1]]MC-LR prevented cell damage in models of increased temperature by a possible ROS scavenger supporting the hypothesis of an antioxidant function of MC at least at the hydrophilic level.

Keywords: *Microcystis aeruginosa*, microcystins, ROS, catalase, temperatura

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P9

Modificación en la producción de ficobiliproteínas en *Nostoc commune* como adaptación a escenarios de calentamiento global

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Los productores primarios son especialmente sensibles al cambio climático, fundamentalmente por la alteración del proceso fotosintético. Las ficobiliproteínas son pigmentos fotosintéticos que se ensamblan en complejos supramoleculares denominados ficobilisomas que se sitúan en la cara externa de la membrana tilacoidal. Las ficobiliproteínas se clasifican en aloficocianinas (APC) verde-azuladas (Amáx = 650-655nm), ficocianinas (PC) azules (Amáx = 610-620nm) y ficoeritrinas (PE) rojas (Amáx = 540-570nm). Las ficobiliproteínas, debido a sus propiedades terapéuticas, tienen una importante aplicación potencial en la industria farmacéutica y alimentaria. Se sometió *Nostoc commune* a diferentes condiciones ambientales ligadas al cambio climático como incremento de nitrógeno, de radiación ultravioleta, de salinidad y de temperatura con el fin de conocer el efecto que tienen sobre su síntesis de ficobiliproteínas. Las colonias de *Nostoc commune* se incubaron durante 10 días en medio BG11 con diferentes concentraciones de amonio (0, 50, 300 mM NH_4Cl), temperaturas (20, 30, 40 C), conductividades (2.09, 4.11, 8.17 mS/cm) y radiaciones (PAR=50.3, UVA=6.44, UVB=1.51 W/m^2) con un fotoperiodo de 18:6 h excepto las que recibían UVB con 04:20 h. Se extrajeron y determinaron las ficobiliproteinas según Doke (2005) y Bennett y Bogorad (1973), respectivamente. Los resultados indicaron que el contenido de ficobiliproteínas aumentó significativamente con una concentración de amonio de 50 μM NH_4Cl ($F=5.043$; $p<0.01$) y una conductividad de 4.11 mS/cm ($F=23.686$; $p<0.01$). Además, la síntesis de ficobiliproteínas en *Nostoc commune* se incrementó con una exposición a radiación UVA de 6.44 W/m^2 y una temperatura de 30 ºC. Estos resultados sugieren que las ficobiliproteinas podrían jugar un importante papel como sustancias de reserva ante condiciones extremas e inducen a investigar su perfil proteínico enfocado a la industria alimentaria y farmacológica.

Palabras clave: aloficocianina, cambio climático, cianobacteria, cianofícea, ficocianina, ficoeritrina

*Autor que presenta el trabajo

P10

Screening of the antioxidant activity of LMECYA cyanobacterial strains

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The biotechnological potential of cyanobacteria is becoming increasingly recognized. The antioxidant activity is one of the many bioactivities of cyanobacterial species with promising application in the food and pharmaceutical industries. Indeed, antioxidant compounds from cyanobacteria constitute a natural alternative to current synthetic compounds, which contain preservatives and suspected toxicity. The focus of the present work was the evaluation of the antioxidant profile of seven freshwater cyanobacterial strains, maintained in the Estela Sousa e Silva Algae Culture Collection (ESSACC) and belonging to the genera *Aphanizomenon* (LMECYA9, LMECYA 88), *Dolichospermum* (LMECYA180), *Leptolyngbya* (LMECYA 173), *Microcystis* (LMECYA 127), *Nostoc* (LMECYA 291) and *Planktothrix* (LMECYA 316). Cyanobacteria biomass was obtained from cultures in exponential phase, growing under laboratory-controlled conditions (20ºC, 14/10 h light/dark cycle and light intensity16 ± 4 μEm−2s−1). Biomass was freeze-dried prior to the extraction of cyanobacterial compounds with ethanol and methanol. The antioxidant activity of those extracts was evaluated by four spectrophotometric procedures: DPPH scavenging method, β-carotene bleaching assay, determination of total phenolic and total flavonoid compounds. Preliminary results show that the methanol is more effective than ethanol in the extraction of antioxidant compounds from cyanobacteria and that the antioxidant profile differed among the strains and the antioxidant endpoint. The strains LMECYA 180 and LMECYA 316 exhibited the highest antioxidant activity evaluated by the DPPH scavenging method. The strains LMECYA 173, LMECYA 127 and LMECYA 291 showed the highest activity according to the β-carotene bleaching assay and the phenolic and flavonoid content, respectively. The strains LMECYA 9 and LMECYA 257 presented the poorest antioxidant profile. The access to a collection of cyanobacteria isolates will allow the exploitation of these properties in numerous strains of varied species occurring in national natural resources. These results emphasises the importance of cyanobacteria as potential sources of natural antioxidant compounds.

Key words: Freshwater cyanobacteria, antioxidant potential, natural antioxidants

*Presenting Author

P11

Variación en la composición de sustancias protectoras de la radiación ultravioleta de *Scytonema javanicum* en un contexto de cambio climático

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Las cianoprocariotas suelen desarrollarse en ambientes con intensa radiación solar. Entre los mecanismos para prevenir o contrarrestar los efectos nocivos de la radiación ultravioleta destaca la síntesis de aminoácidos tipo micosporina (MAAs) y escitoneminas. Debido a sus coeficientes altos de absorción UV y su capacidad para proteger la piel de los rayos UV, las micosporinas son candidatas para su uso en aplicaciones farmacéuticas y cosméticas. Asimismo, las propiedades antiinflamatorias y antiproliferativas combinadas con su propiedad fotoprotectora hacen de la escitonemina un candidato excelente como cosmético natural frente a radiaciones ultravioleta en humanos. Se sometió *Scytonema javanicum* a diferentes condiciones ambientales ligadas al cambio climático como incremento de nitrógeno, de radiación, de salinidad y de temperatura con el fin de conocer el efecto que tienen sobre su síntesis de sustancias protectoras de la radiación ultravioleta como escitoneminas y micosporinas. Los tapetes de *Scytonema javanicum* se incubaron durante 8 días en medio BG11 con diferentes concentraciones de amonio (0, 50, 300 mM NH_4Cl), temperaturas (20, 30, 40 C), conductividades (3.7, 5.2, 7.4 mS/cm) y radiaciones (PAR=50.3, UVA=6.44, UVB=1.51 W/m^2) con un fotoperiodo de 18:6 h excepto las que recibían UVB con 04:20 h. Se extrajeron y determinaron las micosporinas y escitoneminas según García-Pichel y Castenholz (1991, 1993). Los resultados indicaron que con la radiación aumentaba significativamente la síntesis de micosporinas ($F= 16.187$; $p<0.01$) y escitoneminas ($F= 6.465$; $p <0.1$). Así, el incremento de salinidad favorecía significativamente la producción tanto de micosporinas ($F= 13.390$; $p<0.01$) como de escitoneminas ($F= 4.920$; $p<0.05$). Otros parámetros estudiados que aumentaron significativamente la producción de micosporinas fueron la temperatura ($F= 4.231$; $p<0.1$) y la concentración de amonio ($F= 5.802$; $p<0.05$). Estos resultados sugieren que tanto micosporinas como escitoneminas podrían jugar un importante papel como sustancias de reserva ante condiciones extremas en *Scytonema javanicum*.

Palabras clave: cambio climático, cianobacteria, cianofícea, escitoneminas, micosporinas, parámetros ambientales

*Autor que presenta el trabajo

P12

Risk Assessment of Cyanotoxins in Irrigation Waters: A Field Research Plan in the Framework of the European Project TOXICROP

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Fresh water resources in many countries are vulnerable due to their biogeographical and climatic characteristics. Moreover higher water consumption and higher human impacts in the downstream water bodies is leading to a higher eutrophication with increased incidence and intensity of cyanobacteria blooms and their toxins. TOXICROP is a European project funded by the Marie Skłodowska-Curie Research and Innovation Staff Exchange (RISE) programme which aims to map agricultural risk areas of cyanotoxin occurrence, to access the fate of cyanotoxins in crops including bioaccumulation and impacts on food contamination related to the use of eutrophic waters in irrigation. Environment-friendly, low-cost technologies of water treatment will also be developed, and methods to detect and assess toxicity of cyanotoxins improved. To develop TOXICROP a multidisciplinary consortium was constituted by leading EU, South-Mediterranean and South-American academic organizations and 3 stakeholders. In the framework of workpackage a field research will take place with the aim of assessing cyanotoxins in main irrigation reservoirs, as well as the dispersion of these natural water pollutants in agricultural soils and cultured horticultural products. The field research will be carried out in selected regions of the consortium countries, El Pañe lake in Perú, Alqueva Lake in Portugal, Takerkoust in Morocco and Surface and groundwaters in Egypt. We will study the occurrence and diversity of toxic cyanobacteria and cyanotoxins, and toxin levels in water, soil and vegetable products will be used to determine the toxicological and contamination levels of these materials in order to assess the risk of human exposure and impacts in the environment. Here we present the research plan to be implemented during 2020 and 2021, in the framework of Toxicrop Project.

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Key words: Cyanobacteria, cyanotoxins, environmental monitoring, food safety, risk assessment

P13

[DLeu[1]]MC-LR y MC-LR, similares pero diferentes. Estudio de toxicidad por contacto único en un modelo vegetal *Phaseolus vulgaris* L. (Fabaceae)

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[DLeu[1]]MC-LR es una microcistina frecuentemente presente en la cuenca Del Plata junto con su congénere MC-LR, de quien se diferencia por la sustitución de Alanina por D-Leucina en la posición 1 de la molécula. Nuestro objetivo fue estudiar los efectos tóxicos de ambas toxinas en un modelo vegetal (*Phaseolus vulgaris*). Las semillas fueron expuestas a 3,5 ppm de [DLeu1]MC-LR o MC-LR, por contacto único durante la etapa de imbibición. Luego se colocaron en arena y fueron regadas con agua libre de toxinas. 10 días post-imbibición el % germinación fue 63% para [DLeu[1]]MC-LR y 91% para MC-LR, el % de desarrollo fue 71% para [DLeu[1]]MC-LR y 80% para MC-LR; y el % de plantas con alteraciones fue 80% para [DLeu[1]]MC-LR y 37,5% para MC-LR. Ambos tratamientos presentaron disminución en el área de raíz y el largo de tallo; y aumento en la clorosis de hojas correlacionándose con un descenso de clorofila más intenso en el tratamiento con [DLeu[1]]MC-LR. Se observaron alteraciones como emisión de raíces y estomas en columna en el hipocotilo y ondulaciones en láminas foliares más pronunciadas en [DLeu[1]]MC-LR. Se evidenció un retraso en la respuesta fototrópica y una disminución del ángulo de curvatura alcanzado en ambos tratamientos siendo más afectadas las expuestas a [DLeu[1]]MC-LR. Esta alteración se mantuvo 30 días post-imbibición. La actividad fosfatasa se presentó disminuida en todos los tejidos de las plantas expuestas a [DLeu[1]]MC-LR, y en tallo y raíz de las plantas expuestas a MC-LR. Los TBARs se encontraron aumentados sólo en tallo de ambos tratamientos. 30 días post-imbibición la actividad fosfatasa continuó disminuida en hojas y tallo de las plantas tratadas con [DLeu[1]]MC-LR y los TBARs aumentaron en todos los tejidos analizados para ambos tratamientos. Por lo tanto debemos profundizar en el estudio de la toxicidad de [DLeu[1]]MC-LR y MC-LR y en los mecanismos subyacentes.

Palabras clave: Microcystina-LR (MC-LR), [DLeu[1]]MC-LR, *Phaseolus vulgaris*, fototropismo, actividad fosfatasa

*Autor que presenta el trabajo

P14

Effects of *Myriophyllum spicatum* ethyl-acetate extract on growth, toxicity and oxidative damage of *Microcystis aeruginosa* in N- limited chemostat

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In all previous studies on macrophyte allelopathy against toxic cyanobacteria *Microcystis aeruginosa*, often batch or semi-continuous cultures have been used. Clearly, this technique does not apply to the study of the long-term effects of macrophytes on microalgae growth. The continuous culture technique in chemostat system seems more appropriate because steady state growth is achieved when new nutrients are added continuously at the same rate as the culture medium is removed. It also allows a constant supply of macrophyte extract to a fixed population of cyanobacteria. The objectives of this work are to evaluate the growth, toxicity and oxidative stress of *M. aeruginosa* exposed to several concentration of *Myriophyllum spicatum* ethyl acetate extracts, by using a continuous culture in N- limited chemostat. Results showed that, with addition of 25 mg/L of macrophyte extract to the BG11 nutrient medium, the cell density, growth rate has stimulated and the physiological parameter were still constant through the 5 days of exposure. More pronounced inhibitory effects are observed on growth of M. aeruginosa after exposures with high concentration of macrophyte extract (50, 75 and 100 mg/L), which inducing damage on antioxidant defense system than the low concentration extracts, based on the activity of, superoxidase dismutase, catalase enzymes and MDA level contrary to the degradation of protein.

Key words: *Microcystis aeruginosa*, *Myriophyllum spicatum*, N-limited chemostat, Allelopathy, oxidative stress, toxicity

*Presenting Author

P15

Estudios de bioaccesibilidad de cianotoxinas en mejillones crudos y cocinados mediante un modelo de digestión *in vitro*

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La ruta de exposición más importante a cianotoxinas es la oral, principalmente mediante el consumo de agua y organismos acuáticos contaminados, como los moluscos. En este trabajo se evaluó la bioaccesibilidad de 4 cianotoxinas: Microcistina (MC)-LR, MC-RR, MC-YR y Cilindrospermopsina (CYN), mediante un modelo de digestión *in vitro* en mejillones crudos y cocinados (vapor, 2 min) contaminados con 250 ng/g peso fresco. El proceso de digestión humano se imitó incluyendo una fase salivar (con saliva artificial y masticador mecánico), fase gástrica (con pepsina y pH ácido), fase duodenal (con pancreatina, sales biliares, pH alcalino) y fase colónica (con bacterias ácido-lácticas, BAL). Todas las fases se analizaron por UPLC-MS/MS para evaluar la degradación de las cianotoxinas en condiciones digestivas. Los resultados mostraron una reducción continua desde la fase de salivar a la fase colónica. Los porcentajes de bioaccesibilidad descienden en mejillones crudos y cocinados, respectivamente, quedando en un 32 y 26% en el caso de la CYN, en 21 y 22% para MC-LR, en 37 y 42% para MC-RR y en 22 y 26% para MC-YR. Las disminuciones más acusadas se dieron para la MC-LR, a partir de la fase salivar. Los resultados demostraron que el proceso de digestión y jugos digestivos juegan un papel importante en la degradación de estas cianotoxinas.

Agradecimientos: Ministerio de Economía y Competitividad (AGL2015-64558-R, MINECO/FEDER, UE). Leticia Díez-Quijada Jiménez también agradece al Ministerio por la beca (BES-2016-078773) asociada a dicho Proyecto.

Palabras clave: Microcistinas, Cilindrospermopsina, bioaccesibilidad, digestión *in vitro*, mejillones, Cocinado

*Autor que presenta el trabajo

P16

Detección de cilindrospermopsina a través de sus productos de descomposición en músculo de pescado crudo y cocinado: Utilidad de la pirólisis analítica (Py-GC/MS)

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Debido a la capacidad que tiene la citotoxina cilindrospermopsina (CYN) de acumularse en diversos organismos acuáticos, como los peces, es importante disponer de métodos analíticos adecuados que permitan su detección en muestras de pescado contaminado con la toxina. El objetivo de este trabajo fue poner a punto un método de pirólisis analítica (Py-GC/MS) con el que detectar CYN y algunos de sus fragmentos de descomposición en músculo de pescado crudo y cocinado. Músculos de tilapia (*Oreochromis niloticus*) (4g; n=5) contaminados con 50 ng CYN/g peso seco fueron cocinados 2 min por distintas técnicas (microondas, asado, hervido o vapor). Se tomó como control positivo músculo con CYN sin cocinar y como control negativo músculo sin CYN y no cocinado. Todas las muestras fueron congeladas (-80°C) y posteriormente liofilizadas hasta análisis. Las muestras se pirolizaron durante 1 minuto a 350 °C en ausencia de oxígeno en un pirolizador de doble disparo acoplado a un sistema de cromatografía de gases con detector selectivo de masas. La Py-GC/MS directa permitió la detección de CYN (PM 416) en músculo de pescado contaminado a un tiempo de retención de 24,2 min, así como de 3 posibles fragmentos de descomposición característicos de la toxina con PM 290 (15,9 min), 169 (22,4 min) y 336 (25,25 min). Se observaron además variaciones en la abundancia relativa de cada fragmento según el tipo de cocinado, siendo característico el aumento del fragmento con PM 336 tras el cocinado en microondas. El fragmento con PM 290 se observó cuando el pescado fue cocinado por técnicas que no implican agua. De manera general, las técnicas de cocinado que conllevan el empleo de agua (especialmente el hervido), mostraron una abundancia relativa menor tanto de CYN, como de los distintos fragmentos, lo que sugiere la pérdida de la toxina a través del agua del cocinado.

Agradecimientos: Ministerio de Economía y Competitividad (AGL2015-64558-R y CGL2016-78937-R, MINECO/FEDER, UE).

Palabras clave: Cilindrospermopsina, Productos descomposición, Pirólisis analítica, Tilapia, Cocinado

*Autor que presenta el trabajo

P17

Crisis termal, microcistinas y toxicidad potencial en surgencias termales e hipotermales de Galicia (NW España)

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La crenoterapia puede provocar respuestas anormales que se incluyen en la denominada "crisis termal", que consiste en un cuadro clínico inespecífico muy similar al producido por las intoxicaciones por cianotoxinas (molestias gástricas, despeños diarreicos, brotes congestivos hepáticos, reacciones cutáneas, etc.). Las cianobacterias bentónicas de las aguas mineromedicinales constituyen una comunidad cuya diversidad y composición específica están ligadas a la naturaleza geoquímica de la surgencia. En esas condiciones se generan biofilms compuestos por epsilonproteobacterias y gammaproteobacterias del azufre o diferentes siderobacterias y otros organismos quimioautótrofos, en los que se desarrollan también determinadas bacilariofíceas y clorofitas y especialmente cianobacterias. Aunque la identificación morfológica enmascara el reconocimiento de especies con notables diferencias adaptativas, se pueden observar morfoespecies de *Chroococcus, Fischerella, Merismopedia, Phormidium, Oscillatoria, Microcystis*, etc. La frecuencia de las crisis termales depende de la mineralización y la termalidad, pero nunca se ha asociado al crecimiento de cianobacterias en las surgencias. El objetivo de este trabajo consistió en la comprobación de la supuesta intervención de cianotoxinas en el cuadro clínico, estudiando la existencia de microcistina LR y equivalentes, tanto en disolución como en la matriz celular, y el análisis de la toxicidad potencial mediante, respectivamente, enzimoinmunoensayos y bioensayos normalizados que utilizan una bacteria luminiscente (*Vibrio fischeri*) como organismo indicador de la toxicidad. Se analizaron 21 muestras de 18 fuentes distribuidas por Galicia (NW España). En su mayoría sulfurosas poco o moderadamente mineralizadas, con pHs entre 7,9 y 9,3; cuyas características térmicas oscilan entre aguas frías de 15ºC a 18ºC; hipotermales de 25ºC a 31,8 ºC; mesotermales 37ºC a 40ºC e hipertermales con temperaturas entre 41,1ºC y 63,8ºC. Los resultados no permiten relacionar la sintomatología descrita con la existencia de microcistinas en el agua, pero señalan una respuesta hormética en la curva de los bioensayos de toxicidad que es objeto de discusión en este trabajo.

Palabras clave: Crisis termal, microcistinas, toxicidad, hormesis, aguas mineromedicinales

*Autor que presenta el trabajo

P18

Description of an outbreak of cattle intoxication by cyanobacteria (blue-green algae) in the South of Portugal

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Twenty-five cows from a beef herd consisting of 54 healthy animals from Aberdeen Angus breed x Charolais were found dead. Cattle broke the electric fence that bordered on a field of stubble and had access to stagnant water from a small river (Ribeira de Oeiras) that run across the herd. In a lapse of less than 19 hours, 20 cows died and 5 were found sick with clinical signs of ataxia, recumbence, abdominal distension, opisthotonus, paddling and masticatory movements, frothing at the mouth and bloody diarrhea. Clinical and pathological findings revealed the presence of hepatic and renal necrosis. In the water samples, several cyanobacterial species were identified, predominantly belonging to the toxic genera *Microcystis.* Altogether, these observations led us to suspect of acute hepatoxicity caused by cyanotoxins. The diagnosis was confirmed by the detection of microcystin-LR in the kidney from one animal. As far as we know this is the first report of mycrocistin-LR animal poisoning in Portugal.

Key words: Cattle, Cyanobacteria, intoxication, microcystins, mortality

*Presenting Author

P19

Microcystins contents in microalgae food supplements. Results from ecologic and non-ecologic products

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Food supplements with microalgae are increasingly abundant and can easily be found at any supermarket, herbalist, pharmacy or in the Internet. The most pospular products are based on cyanophyceae such as *Aphanizomenon flos-aquae*, *Spirulina platensis* and *Spirulina maxima* or chlorophytes like *Chlorella* and are considered all of them very beneficial for health. However this type of products may represent a health hazard. Even when some of them are labelled as ecologic it is very difficult to find information about the procedence of the material or the methods of production (which can be an important focus of contamination) or processing. In an attempt to know more about the safety of common food supplements 10 dietary supplements with microalgae were studied, selected from common ecologic and non-ecologic products. Detection and quantification of microcystins/nodularins present were made with an ADDA-ELISA kit and HPLC-MS was used for confirmation of positive samples. At the same time a light microscopic study was made to analyse the general contents of the samples. Three of the samples were positive in microcystins/nodularins (exceeding the IDT proposed by WHO) and the presence of microcystins was confirmed in two of them (the two containing *Aphanizomenon flos-aque*). In the other sample (of *Chlorella)* positive to microcystins/nodularins, a large contamination with different microorganisms was observed in the microscopic study, including the presence of *Clostridium* spores. The need for greater control over food products with microalgae and to establish a standard methodology for the analysis of cyanotoxins in order to protect the health of consumers is highlighted.

Palabras clave: microcystins, food supplements, ELISA, food security

*Presenting Author

P20

Molecular screening of the potential production of cyanotoxins and cyanobacterial natural products in environmental samples from Cabo Verde Islands

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The Cabo Verde archipelago is located in the central Atlantic Ocean in the Sahel region of Africa. It is comprised by diverse volcanic islands, formed as a result of eruptions from a hotspot under a submarine platform. Cabo Verde islands represent underexplored habitats in what concerns cyanobacteria and their natural products, including toxins. With the aim of determining the presence of cyanotoxin genes and explore the hidden bioactive potential of marine cyanobacteria from these islands, we have conducted sampling campaigns at different sites in São Vicente (Baía das Gatas, Cova de Inglesa, Calhau and Salamansa) and Santo Antão (Ponta do Sol) islands, during April 2018. A PCR-based screening was performed in the collected environmental samples to evaluate the presence of (1) cyanobacterial and cyanotoxin genes; and (2) genes encoding non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKSs). All the samples contained cyanobacterial DNA whereas cyanotoxin genes related with microcystins (MC)/nodularins (NOD), cylindrospermopsins (CYL), saxitoxins (SXT) and anatoxin (ATX) production were spread by several samples collected in São Vicente Island. The one sample collected in Santo Antão Island exclusively revealed the presence of CYL related genes. Both PKS and NRPS genes were detected in several samples from São Vicente island whereas for the sample collected in Santo Antão island we could not confirm the presence of these genes. The results of this study demonstrate the potential risks of the appearance of cyanotoxins in the Cabo Verde coastal marine areas as well as the potential for the discovery of novel bioactive compounds.

Key words: marine cyanobacteria, Cabo Verde, molecular screening, toxin potential

*Presenting Author

P21

A case report on hepatotoxicity induced by paclitaxel interaction with turmeric in association with a microcystin from a contaminated dietary supplement

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A 67-year-old Caucasian male with lung cancer was presented to the Emergency Department with asthenia, anorexia, jaundice and choluria. The patient's lung cancer was being treated medically by a combination of paclitaxel/carboplatin with bi-monthly frequency. The patient was also self-medicating with several natural products, including *Chlorella* (520 mg /day), *Sillybum marianum* (total of 13.5 mg silymarin/ day), zinc sulphate (5.5 mg), selenium (50 µg) and 15 g/day of *Curcuma longa*. In first chemotherapy cycle no toxicity was observed even he was taking other medications as budesonide and sitagliptin. The toxic events started only after the introduction of the dietary products. *Chlorella* had contamination with cyanobacteria (Oscillatoriales) and 1.08 µg of cyanotoxin Microcystin-LR (MC-LR) per gram of biomass was found. Patient was consuming ca 0.01 µg MC-LR/kg/day. This case report describes the first known case of paclitaxel toxicity probably related to pharmacokinetic interaction with Turmeric and a contaminated *Chlorella* supplement resulting in an acute toxic hepatitis and the impact on oncologic patient health.

Key words: Drug-herb interactions, Cyanobacteria, *Chlorella*, Dietary supplement, Microcystin-LR, Toxic hepatitis

*Presenting Author

P22

First report on the anti-cyanobacterial effects of medicinal and aromatic plants essential oil : an ecological approach for cyanobacteria growth regulation (cyano-hab control)

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The occurrence of toxic cyanobacterial blooms in eutrophic lakes, reservoirs, and recreational waters has become a worldwide problem. In order to settle this problem, various strategies, including physical, chemical and biological methods have been proposed. Nevertheless, the use of those strategies is usually not effective. The isolation of natural compounds from many terrestrial plants becomes a promising approach for harmful algae bloom control in aquatic systems. In this study, we highlighted and evaluated the potential algicidal (anti-cyanobacterial) activity of essential oils, extracted from differents Moroccan aromatic and medicinal plants that are widely used for bactericidal, virucidal, fungicidal treatment, against unicellular *Microcystis aeruginosa*. The algicidal activity was tested using the agar diffusion technique in Petri dishes and the counting technique in liquid medium. The results in solid medium indicated that the essential oils of *Chenopodium ambrosioides*, *Thymus broussonetii*, *Thymus maroccanus* and *Thymus satureioides* inhibit totally the growth of *Microcystis aeruginosa* (90 ± 0,00mm). Whereas, the lowest growth inhibition activity was recorded by *Thymus pallidus* essential oil (18,3 ± 0,00mm). In liquid medium, the results revealed that all the essential oils tested have a significant inhibitory effect on *M. aeruginosa* compared to the negative control (copper sulphate). The growth of the tested cyanobacteria was inhibited until 100% by *Thymus broussonetii, Thymus maroccanus* and *Thymus satureioides* essential oils at the end of the experiment. The lowest inhibition rates were observed for *Thymus pallidus* essential oil with 8.8±0.47%. The obtained results will confirm the potent use of plant essential oil, as an alternative ecological process for Cyanobacteria growth regulation (Cyano-Hab bio-control)

Key words: Essential oils, Aromatic and medicinal plants, anti-cyanobacteria, bloom biocontrol

*Presenting Author

P23

Monitoring of cyanotoxins in waters from cultures of hypersaline microalgae by UHPLC with UV and MS/MS detection following salting-out liquid-liquid extraction

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In this study two different analytical approaches have been developed to determine the presence of several cyanotoxins in saline water samples from a continental salt marsh. A salting-out assisted liquid-liquid extraction (SALLE) has been used in combination with ultra-high performance liquid chromatography-tandem mass spectrometry and UV-diode array detection (UHPLC-MS/MS and UHPLC-DAD). The target analytes are eight microcystins named MC-RR, MC-YR, MC-LR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF and nodularin (NOD), covering a wide range of polarities. The separation was achieved using a Zorbax Eclipse Plus RRHD C18 column (50 × 2.1 mm, 1.8 μm) in less than 7.5 and 5.5 min for UV and MS/MS detection, respectively. The mobile phase used consisted of water (solvent A) and acetonitrile (MeCN) (solvent B), both containing 0.01% of formic acid for DAD and 0.4% of formic acid for MS/MS detection, at a flow rate of 0.4 mL min^{-1}. The temperature of the column was set at 25°C and 20 μL of sample were injected. The main parameters affecting the SALLE procedure were studied and the following optimum values were obtained: neutral pH, 2 mL of acetonitrile as extraction solvent and 1,2 g of ammonium sulfate as salting-out agent for 4 mL of water sample. The validation protocols for both methods were accomplished with real water samples obtaining LODs ranging from 1.0 to 3.4 μg L^{-1} and 0.03 to 0.07 μg L^{-1} for DAD and MS/MS respectively. Although the SALLE-UHPLC-DAD methodology is easier and cheaper than UHPLC-MS/MS significantly better detection limits were achieved with tandem mass spectrometry as well as allowing for unambiguous identification. Extraction recoveries were higher than 77.0% (except for MC-RR and NOD which were 53.2% and 54.3, respectively) with satisfactory inter-day and intra-day precisions (RSD below 13.3%).

Key words: Cyanotoxins, Diode array detection, Salting-out assisted liquid-liquid extraction, Saline waters, Tandem mass spectrometry, Ultra-high performance liquid chromatography

*Presenting Author

P24

Fatty acid characterization of cyanobacterial strains isolated from Algerian thermal water

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Geothermal springs in Algeria are well known since the Roman Empire. There are more than 200 thermal springs in the Algerian territory that are mainly located in the Eastern part of the country. Thermal spring mats are composed of well adapted thermophilic organisms usually dominated by cyanobacteria, green algae and diatoms but neither their taxonomic composition nor their chemical composition or their potential use in biotechnology have been studied. The present investigation focus on cyanobacterial mats composition and characterization of their fatty acids. Cyanobacteria were isolated from 3 hot springs in Algeria with water temperatures ranging from 35 to 55 ºC. Four strains preliminarly identified as *Mastigocladus laminosus* were maintained in culture at 35 ºC, 75 μM m^{-2} s^{-1}, 16:8 hours of light/dark and BG11. Lipids were extracted according to Christie (2003) and then trans-metylated. FAME were separated and identified by gas-liquid chromatography. The content of saturated fatty acids ranged from 43.47 to 59.37 %, while the content of polyunsaturated fatty acids ranged from 0.57 to 28.91 %. Oleic acid represented from 9.49 to 36.60 of monosaturated fatty acids. Docosahexaenoic acid (DHA) was not detected in any of the strains but linolenic (LA) and arachidonic (ARA) acids were present in all of them and predominated among w6 (0.69-26.31 % and 0.02-0.10 %) while alfa-linolenic (ALA), and eicosapentanoic (EPA) among w3 (0.05-2.16 and 0.04-0.10 %). The ratios w3/w6 varied from 0.09-0.30 and ARA/EPA from 0.00-2.40 %. The results of this research show the interest of the studied strains in different fields of biotechnology from biofuel production to animal or human nutrition.

Key words: Algeria, cyanobacteria, fatty acids, thermal springs, thermophilic species

*Presenting Author

P25

Cyanobacteria and cyanotoxins (saxitoxin and microcystin) in a subtropical reservoir

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The objective of this study was to identify the cyanobacteria present in the Itupararanga reservoir (São Paulo State, Brazil), to quantify microcystins (MC) and saxitoxins (STX). Water samples were collected in August 2017 at 7 sampling stations along of the reservoir. Phytoplankton samples were analyzed by inverted microscopy for calculation of density and biovolume. The quantitative analysis of STX was performed by the ELISA method, MC-LR was quantified by the SPE and LC-MS method. *Cylindrospermopsis raciborskii* was classified as dominant in the region near the dam and classified as abundant in the medium/upstream reservoir area. Biomass of *C. raciborskii* varied from 84 mm^3L^{-1} to 951.8 mm^3L^{-1}, among the sampling stations. Other cyanobacteria were abundant: *Aphanizomenon gracile* (3 mm^3L^{-1} to 8.7 mm^3L^{-1}); *Pseudanabaena catenata* (0.25 mm^3L^{-1} to 2.10 mm^3L^{-1}); *Phormidium* sp (43.6 mm^3L^{-1} to 174.3 mm^3L^{-1}). The invasive dinoflagellate *Ceratium* (52.3 mm^3L^{-1} to 33.6 mm^3L^{-1}) was also abundant. STX and MC-LR were detected at low concentrations along the reservoir. It was observed a trend towards a gradual increase of STX upstream concentrations for the dam region (0.04 μgL^{-1} to 0.12 μgL^{-1}) and there was a significant correlation with the biomass variation of *C. raciborskii* ($p < 0.05$). The MC-LR concentration remained constant with a slight increase in the dam region (0.004 μgL^{-1} to 0.007 μgL^{-1}). The canonical correspondence analysis indicated that the occurrence of Cyanobacteria was correlated with NH_4^+. The dominance of *C. raciborskii* in the reservoir is responsible for the constant levels of STX in the environment. In this case, the environment is permanently exposed to the risk of toxicity due to the high ecophysiological plasticity of these organisms. The environmental conditions reported in this this study may favor the occurrence of blooms, increasing toxicity in the environment in the future.

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Key words: eutrophication, reservoirs, Cyanobacteria, microcystin, saxitoxin, Brazil

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