representative cells of the immune system, the Jurkat cell line (lymphocytes). The results showed that both extracts reduced cell viability after 24 hours of exposure. The mean effective concentrations 50 (EC50) were 55.20 ± 7.06 µg/mL for HE1 and 56.86 ± 4.05 µg/mL for HE2. In addition, cell death mechanisms were investigated by flow cytometry, being the apoptosis and late apoptosis the predominant processes after 24 and 48 hours of exposure, respectively. Moreover, the mRNA expression of various cytokines was studied by RT-qPCR. Among them, interleukin 2 (IL-2) and interferon-gamma (INF-?) levels were up-regulated after 24 hours of exposure to both HE1 and HE2, while a down-regulation was observed in the tumor necrosis factor-alpha (TNF-?) under the same experimental conditions. After 48 hours of exposure, only up-regulation of IL-2 was observed. Overall, these findings suggest that both extracts could have immunomodulatory effects in vitro. Further research is needed to determine the mechanisms involved and the impact of HE1 and HE2 on the human immune system.

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4.16.P-We409 A MALDI-MSI-Based Approach to Characterize the Spatial Distribution of Cylindrospermopsin and Lipid Alterations in Rat Intestinal Tissue

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Anthropogenic activities and climate change are exacerbating the proliferation and distribution of cyanobacteria, which produce harmful cyanotoxins like cylindrospermopsin (CYN). This toxin poses significant risks to both ecosystems and human health. Ingestion of contaminated food and water is the primary route of CYN exposure, underscoring the importance of monitoring its absorption, distribution, and potential health effects. In that sense, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging (MALDI-MSI) offers a powerful tool for visualizing the spatial distribution of a wide range of molecules. This study aimed to develop and optimize a novel MALDI-MSI method for CYN detection in rat intestinal tissue. To investigate the quantitative spatio-temporal distribution of the toxin, mid-intestinal samples were collected from rats exposed to 500 µg CYN/kg body weight and sacrificed at 0, 2, 4, 6, and 24 hours post-exposure. Cryosections of fresh intestine tissue (jejunum) were obtained at 10 µm, mounted in MALDI IntelliSlides and frozen until use. The slides were allowed to reach room temperature during a 30 min drying step using a vacuum pump, and the DHB matrix was applied over the entire surface of the slide by using a HTX TM-Sprayer. MALDI-MSI experiments were carried out in positive ionization mode, within a m/z range of 300-1350 and an ion mobility range from 0.8 1.68, using a timsTOF-FleX mass spectrometer. Additionally, the impact of CYN on the intestinal lipid profile was assessed. The results showed that the method developed was useful to detect and quantify CYN and its sodium and potassium adducts in rat intestine. Moreover, the method was validated for linearity, sensitivity, and precision using mimetic tissue sections spiked with various CYN concentrations (0.1-100 ppm), ensuring its suitability for visualizing CYN, and its sodium and potassium adducts distribution in the rat tissue. Regarding lipid profile, significant alterations in several lipid families were observed, suggesting an inflammatory response, increased oxidative stress, and progressive damage to cell membrane integrity. Further research is necessary to elucidate the distribution of CYN in other vital organs, such as the liver, kidney, and stomach.

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4.16.P-We410 Assessing the Effects of Phycotoxin Mixtures on Marine Zooplankton: Insights from Copepod Responses at Different Life Stages

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The expansion of human activities into oceans and lakes has profoundly disrupted aquatic ecosystems. Over recent decades, HAB occurrences have increased due to mounting anthropogenic pressures. Harmful algal blooms (HABs) are a phenomenon occurring hen certain primary producers, capable of generating toxic metabolites (phycotoxins) proliferate excessively. These phycotoxins can accumulate in fish and shellfish, subsequently moving up the food web and adversely affecting organisms at higher trophic levels, ultimately posing significant risks to human health. Phycotoxins are classified into five main groups based on their effects: paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), and azaspiracid poisoning (AZP). While the individual effects of these toxins are well-documented, the combined effects on the marine food chain remain less understood. Copepods are key primary consumers in marine ecosystems, acting as vital links to higher trophic levels, such as planktivorous fish. They also play an essential role in oceanic biogeochemical cycling, for example through carbon C and nitrogen export to deeper waters. This study, therefore, examines the effects of mixed phycotoxin exposure on two copepod species: the epibenthic copepod Nitokra spinipes and the planktonic copepod Acartia clausi. We investigated the impacts of two harmful algal species, Protoceratium reticulatum and Alexandrium minumtum, using a full factorial design that included environmentally relevant concentrations of both living algae, as well as their extracts. After 48 hours of exposure to these mixtures, we evaluated the response of adult copepods for swimming speed, inactivity, and mortality using the ZebraBox device, while naupliar immobility was assessed under a light microscope. Our research aims to enhance understanding of the impacts and mechanisms of mixed HAB exposure on copepods, contributing to broader insights into potential risks to ecosystems and human health.

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4.16.P-We411 A Step Closer to Link LC-PUFA Fatty Acids and Toxin Biosynthesis in Toxic Dinoflagellates

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Long-chain polyunsaturated fatty acids (LC-PUFAs) are essential for many physiological processes in marine organisms. While these compounds are typically associated with health benefits in higher trophic levels, emerging evidence suggests that their presence in toxic harmful algae species (HABs) may be related to toxicity. While direct evidence is limited, PUFAs might serve as biochemical precursors or modulators in the biosynthetic pathways of certain algal toxins. In dinoflagellates it is believed that synthesis of some FAs and toxins share the same anaerobic Polyketide Synthase (PKS) pathway. This study explores the potential role of LC-PUFAs as proxies for toxin production in the benthic harmful dinoflagellates Prorocentrum lima and Amphidinium carterae. Both species are known for their production of bioactive secondary metabolites such as okadaic acid and amphidinols, respectively, potentially harmful to marine biota. Here, the toxicity of dinoflagellate species was firstly evaluated and validated through lethal effects (LC50) in marine microinvertebrates (artemia and amphipods). The levels of specific fatty acids were then correlated with the most common toxins in both dinoflagellate species, after culturing them at different temperatures (15ºC, 19ºC and 24ºC), until both reached the end of their exponential growth phase. Results revealed strong positive correlations, mostly between EPA and DHA, and th e quantity of toxins identified in both species.

Understanding these mechanisms is critical for predicting the ecological impacts of HABs, as disentangling the specific relation of particular fatty acids and other algal metabolites with overall toxins and their toxicity may become crucial in understanding and mitigating HABs effects on marine food webs.

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4.16.P-We412 A Hyalella azteca Transgenerational Biotest to Screen Reproductive and Embryonic Development Effects of MC-LR and Exudates from Cyanobaterial Toxic Strains