



Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais



Exploring Pesticide Impacts on Aquatic Microalgae: a Scientometric Analysis and Experimental Assessment of Clomazone Tolerance in Two Species of Microalgae

Alex Lindor

Orientador: Prof. Dr. Junior Borella
Coorientador: Dr. Muryllo Santos Castro

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**ATA DE DEFESA DE DISSERTAÇÃO DE MESTRADO EM BIOLOGIA DE
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A toda minha família,
especialmente à minha mãe, uma guerreira que luta todos os dias pelo bem de sua
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RESUMO

Nas últimas décadas, o uso de agrotóxicos, especialmente herbicidas, aumentou significativamente em todo o mundo, visando controlar doenças, organismos nocivos e atender à crescente demanda alimentar impulsionada pelo aumento populacional. Contudo, o uso excessivo desses produtos tem efeitos adversos sobre organismos não-alvo, como microalgas e cianobactérias, que são fundamentais para a produção de matéria orgânica e oxigênio nos ecossistemas aquáticos. Alterações em suas estruturas ou funções devido à exposição a esses compostos podem gerar sérias repercussões na cadeia trófica. Dada a importância das microalgas e o uso crescente desses produtos, para entender melhor esses impactos, uma análise cienciométrica foi realizada com base nas publicações científicas de 1991 a 2023, (o ano em que Web of Science começou a publicar artigos com resumos), extraídas da base de dados Web of Science, sobre os efeitos de pesticidas/herbicidas em microalgas e cianobactérias. Os resultados indicam um aumento constante nos estudos sobre esse tema, com China, Estados Unidos e Índia liderando as contribuições científicas. A maioria dessas pesquisas (71%) foca nos herbicidas, utilizando principalmente as clorofíceas (*Chlorophytes*) em bioensaios para avaliar os efeitos letais e subletais desses compostos. No entanto, a análise revela lacunas importantes, incluindo uma baixa colaboração internacional e uma falta de diversidade na escolha de pesticidas e grupos de organismos testados. Esses achados destacam a necessidade de diversificar as pesquisas para preencher essas lacunas e compreender melhor os impactos dos herbicidas nos ecossistemas aquáticos. Além disso, um estudo experimental avaliou a tolerância de duas espécies de microalgas verdes, *Desmodesmus communis* e *Pseudopediastrum boryanum*, ao herbicida clomazona, com foco na formulação comercial microencapsulada Gamit 360CS®. Os testes, conduzidos em cinco concentrações (0,25, 0,50, 0,75, 1 e 1,5 mg L⁻¹) sob condições controladas, revelaram respostas diferenciais entre as espécies. *D. communis* apresentou uma redução significativa no teor de clorofila-a e carotenoides, além de um aumento no estresse oxidativo em concentrações mais altas, indicando maior sensibilidade. Por outro lado, *P. boryanum* demonstrou maior tolerância, com manutenção estável dos pigmentos fotossintéticos e ativação de mecanismos antioxidantes, incluindo superóxido dismutase (SOD), catalase (CAT) e glutationa S-transferase (GST). Esses resultados evidenciam as respostas específicas das microalgas ao estresse induzido pelos herbicidas e destacam o potencial de *P. boryanum* para aplicações em biorremediação. Ao combinar uma análise cienciométrica com testes experimentais, este trabalho oferece informações críticas sobre os riscos ecológicos relacionados à exposição aos herbicidas e abre caminho para abordagens estratégicas que visem proteger os ecossistemas aquáticos e mitigar os impactos ambientais.

Palavras-chave: Chlorophyceae; Ecotoxicologia; Agrotóxicos; Produção científica; Toxicidade; Contaminação; Estresse oxidativo

ABSTRACT

In recent decades, the use of pesticides, particularly herbicides, has increased significantly worldwide to control diseases, harmful organisms, and meet the rising food demand driven by population growth. However, the excessive use of these products adversely affects non-target organisms, such as microalgae and cyanobacteria, which play crucial roles in the production of organic matter and oxygen in aquatic ecosystems. Changes in their structures or functions caused by these compounds can have severe consequences for the trophic chain. Given the importance of microalgae and the extensive use of pesticides and herbicides, and to better understand these impacts, a scientometric analysis of scientific publications from 1991 to 2023 (the year Web of Science began publishing articles with abstracts), was conducted using the Web of Science database. The analysis focused on the effects of pesticides and herbicides on microalgae and cyanobacteria during this period. The results indicate a steady increase in studies in this field, with China, the United States, and India leading scientific contributions. Most of these studies (71%) concentrate on herbicides, with chlorophytes (*Chlorophytes*) being the most frequently used organisms in bioassays to assess lethal and sublethal effects of these compounds. However, the analysis also revealed significant gaps, including limited international collaboration and a lack of diversity in the selection of pesticides and groups of test organisms. These findings emphasize the need to diversify research to address these gaps and better understand the impacts of herbicides on aquatic ecosystems. Additionally, in parallel, an experimental study evaluated the tolerance of two green microalgae species, *Desmodesmus communis* and *Pseudopediastrum boryanum*, to the herbicide clomazone, particularly its commercial microencapsulated formulation, Gamit 360CS®. Tests conducted at five concentrations (0.25, 0.50, 0.75, 1, and 1.5 mg/L⁻¹) under controlled conditions revealed species-specific responses. *D. communis* showed a significant reduction in chlorophyll-a and carotenoid content, as well as increased oxidative stress at higher concentrations, indicating heightened sensitivity. In contrast, *P. boryanum* exhibited greater tolerance, maintaining stable photosynthetic pigments and activating antioxidant mechanisms, including superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). These results highlight the specific responses of microalgae to herbicide-induced stress and underscore the potential of *P. boryanum* for bioremediation applications. By combining scientometric analysis and experimental testing, this study provides critical insights into the ecological risks associated with herbicide exposure, while paving the way for strategic approaches to protect aquatic ecosystems and mitigate environmental impacts.

Keywords: Chlorophyceae; Ecotoxicology; Pesticide; Scientific production; Toxicity; Contamination; Oxidative stress

APRESENTAÇÃO

Esse trabalho foi dividido em quatro partes: introdução geral, capítulo 1, capítulo 2, e considerações finais. A formatação seguida foi de acordo com as normas da ABNT, exceto os capítulos, em que a formatação segue as recomendações da revista “*Ecotoxicology*” em que foi submetido o capítulo 1 e da revista “*Environmental Toxicology and Chemistry*” em que será submetido o capítulo 2.

Na introdução geral deste trabalho, apresentamos uma visão global sobre os agrotóxicos, com ênfase especial nos herbicidas, destacando os riscos ambientais associados ao uso excessivo desses produtos, como a contaminação e a poluição dos solos e das águas, que afetam principalmente os organismos não-alvo, incluindo as microalgas e cianobactérias. Também abordamos a importância do estado do Rio Grande do Sul na produção nacional de arroz (*Oryza sativa*). Além disso, apresentamos o herbicida clomazona e sua formulação microencapsulada Gamit 360CS[®], bem como seu modo de ação e efeitos em organismos não-alvo.

O primeiro capítulo intitulado “Understanding the global research landscape of pesticide impacts on aquatic microalgae and cyanobacteria” se trata de um mapa sistemático e análise cienciométrica da produção científica sobre o impacto de agrotóxicos/herbicidas nas microalgas e cianobactérias ao longo dos últimos 32 anos.

O segundo capítulo intitulado “Sensitivity of two green microalgae (*Desmodesmus communis* and *Pseudopediastrum boryanum*) to clomazone herbicide exposure” um estudo experimental abordando sobre o potencial de tolerância das microalgas *D. communis* e *P. boryanum* expostas ao Gamit 360CS[®] (Clomazona).

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Introdução Geral

1. Agrotóxicos

O termo "Agrotóxicos" refere-se a qualquer substância ou mistura de substâncias utilizadas para controlar doenças de plantas e organismos nocivos e/ou indesejáveis nos campos agrícolas, em particular, e no meio ambiente, em geral (Arias-Esteves et al., 2008; Chow et al., 2020). Dependendo do organismo a ser controlado, do modo de ação ou da estrutura química, os pesticidas são classificados em: inseticidas (onde os organismos-alvo são insetos), fungicidas (fungos), parasitoides (parasitas) e herbicidas (plantas daninhas), entre outros (Arias-Esteves et al., 2008; WHO, 2019; Jain et al., 2024). Eles são amplamente utilizados em diversas formulações, especialmente na agricultura, onde melhoram a produtividade (Nsibande et al., 2016; Bujanovic et al., 2020).

2. Herbicidas

Os herbicidas são, entre os pesticidas, os mais amplamente utilizados globalmente em termos de quantidade (Kniss, 2017; Sharma et al., 2019; FAO, 2022; Shattuck et al., 2023). Eles são formulados para eliminar plantas invasoras e indesejáveis, com o objetivo de prevenir, limitar ou destruir os danos que podem causar às culturas agrícolas (Bujanovic et al., 2020). Os herbicidas são aplicados continuamente não apenas na agricultura, mas também em outros locais, como silvicultura, áreas verdes urbanas e na manutenção de estradas e ferrovias (Almeida et al., 2019). Os agricultores os utilizam amplamente para aumentar os lucros, otimizar o controle de ervas daninhas, flexibilizar a produção e reduzir o tempo e a necessidade de mão de obra (Monaco et al., 2002). Contudo, o uso excessivo de herbicidas tem consequências, especialmente para o meio ambiente, animais e seres humanos (Narayanan et al., 2024). Estudos indicam que os herbicidas são importantes contaminantes de solos e águas, causando efeitos adversos à saúde humana (Steffen et al., 2011) e a outros organismos não-alvo, como aves, peixes, invertebrados e microorganismos, incluindo macrófitas, cianobactérias e microalgas (Monaco et al., 2002; Choi et al., 2012; Sanchez et al., 2017; Benegas et al., 2023). Consequentemente, a contaminação e a poluição ambiental por herbicidas tornaram-se um problema global que atrai atenção especial da comunidade científica.

3. Clomazona

Há mais de três décadas, o Brasil é um dos maiores produtores e exportadores de arroz (*Oryza sativa*) da América do Sul (FAO, 2019), graças principalmente ao estado do Rio Grande do Sul, que responde por 71% da produção nacional (CONAB, 2022). Muitas vezes, os vastos campos de arroz são invadidos por plantas daninhas que limitam a produção. Para garantir a produtividade, os agricultores utilizam herbicidas de forma excessiva (Mottes et al., 2017), entre os quais a clomazona (SOSBAI, 2016). As plantações de arroz irrigado são consideradas fontes potenciais de contaminação ambiental, dependendo das quantidades de herbicidas aplicadas (Grutzmacher et al., 2008).

O clomazona [2-(2-clorobenzil)-4,4-dimetil-1,2-isoxazolidin-3-ona] é um herbicida seletivo utilizado em pré ou pós-emergência para controlar plantas daninhas (Dayan et al., 2015) (Fig. 1). Pertencente à família das isoxazolidinonas, é amplamente recomendada para arrozais irrigados e outras culturas, como soja, cana-de-açúcar, milho e tabaco (SOSBAI, 2018; Bujanovic et al., 2020). É o 13º herbicida mais vendido no estado do Rio Grande do Sul e o 16º no Brasil (IBAMA, 2018). Considerado o mais importante entre os inibidores da síntese de carotenoides (De Albuquerque et al., 2020), é comercializado em formulações microencapsuladas, como o Gamit 360CS®, com 360 g/L de ingrediente ativo, ou em formulações mais concentradas anteriores, como Gamit 500EC e Gamit Star, com concentrações respectivas de 500 e 800 g/L (Schreiber et al., 2015) (Figura 2). Trata-se de um herbicida não ionizável, moderadamente persistente, com solubilidade em água de 1,2 g/L-1 a 20 °C (PPDB, 2018) e meia-vida variando de 90 a 276 dias em solos sob condições aeróbicas, diminuindo para 60 dias sob condições anaeróbicas (Lewis et al., 2016). Na água, sua meia-vida é de 1,5 a 2,5 meses. Em condições de baixa concentração de solutos, apresenta volatilidade significativa, aumentando os riscos de contaminação de águas subterrâneas (Van Scoy & Tjeerde, 2013; Escoto et al., 2019). Com peso molecular de 239,70, coeficiente de partição octanol/água (Kow) de 350 e pressão de vapor de $1,92 \times 10^{-2}$ Pa a 25 °C (Worthing & Hance, 1991), a clomazona representa uma ameaça aos ecossistemas aquáticos.

O mecanismo de ação da clomazona baseia-se na inibição de uma enzima chave na biossíntese de pigmentos clorofilas e carotenoides (Fig. 3). Uma vez absorvida, a clomazona é metabolizada em 5-ceto-clomazona pelas hemoproteínas do complexo monoxigenase do citocromo P-450, que a converte em um herbicida ativo (Yun et al., 2005). A 5-ceto-clomazona é responsável por inibir a 1-desoxi-xilulose-5-fosfato sintase (DOXP), uma enzima essencial

na biossíntese de pigmentos, como clorofila e carotenoides, moléculas responsáveis pela fotoproteção da clorofila contra o excesso de luz (Kruse, 2001; Ferhatoglu & Barret, 2006; Dayan et al., 2015). O clomazona também pode interferir na cadeia de transporte de elétrons, impedindo a reoxidação de NADPH e a regeneração de ribulose-1,5-bisfosfato (RuBP), o que leva a uma diminuição na taxa de fotossíntese (Darwish et al., 2015; Piveta et al., 2018). A inibição da biossíntese de carotenoides por este herbicida pode ainda causar formação de espécies reativas de oxigênio e peroxidação lipídica em condições de alta intensidade luminosa (Cobb, 2021).

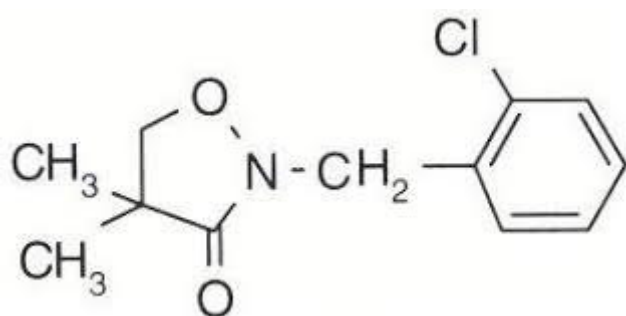


Figura 1 - Estrutura química do herbicida clomazona® (composto puro) [2-(2-clorobenzil)-4,4-dimetil-1,2-isoxazolidin-3-ona]. fonte: IBAMA 2019



Figura 2 - Embalagem comercial do Gamit 360CS®

Os organismos fotossintéticos ou fotoautotróficos, incluindo plantas, microalgas e cianobactérias, desempenham um papel extremamente importante nos ecossistemas por meio

da fotossíntese. Este processo envolve a absorção de luz, transferência de elétrons, fosforilação fotossintética e assimilação de carbono (Stanier & Cohen-Bazire, 1977; Johnson, 2016). Assim, a fotossíntese constitui um fenômeno metabólico essencial, não apenas para a produção de biomassa, mas também para fornecer alimento e energia aos seres vivos (Johnson, 2016). No entanto, ela pode ser afetada por fatores ambientais (Kaiser et al., 2015). Por exemplo, os herbicidas podem induzir mutações no processo fotossintético ao bloquear a formação de isoprenóides nos cloroplastos, como o 2-C-metil-D-eritritol 4-fosfato (MEP), que inclui tocoferol e transportadores de elétrons (Eisenreich et al., 2004). A clomazona, como inibidor de carotenóides, pode perturbar a fluorescência da clorofila (Hess, 2018).

Em altas concentrações, a clomazona afeta não apenas a síntese de carotenóides, mas também o conteúdo de clorofilas *a* e *b* (Darwish et al., 2013; Dayan et al., 2015). Os carotenóides são responsáveis por absorver luz em comprimentos de onda curtos (azul, 400-500 nm) e transferir a energia absorvida para a clorofila no centro de reação (Fontana et al., 2000). Durante a fotossíntese, o herbicida impede a reoxidação do NADPH e a regeneração do RuBP (ribulose-1,5-bifosfato) ao interferir na cadeia de transporte de elétrons no cloroplasto (Darwish et al., 2015; Weber et al., 2017). O NADPH, junto com o ATP produzido durante o transporte de elétrons fotossintético, é utilizado no ciclo de Calvin-Benson para fixar CO₂ por meio do RuBP e produzir açúcares (Roháček & Barták, 2008; Govindjee et al., 2017). Em determinadas condições, a enzima responsável pela fixação de CO₂ (ribulose-1,5-bifosfato carboxilase/oxigenase) pode atuar como oxigenase e iniciar o processo de fotorespiração (Gaudillere, 1982). Para que isso ocorra, grande parte dos produtos gerados durante o processo fotossintético (ATP e NADPH) deve ser consumida (Voss et al., 2013; Hirel et al., 2007), o que pode reduzir a produtividade de plantas ou microalgas (Peterhansel et al., 2013).

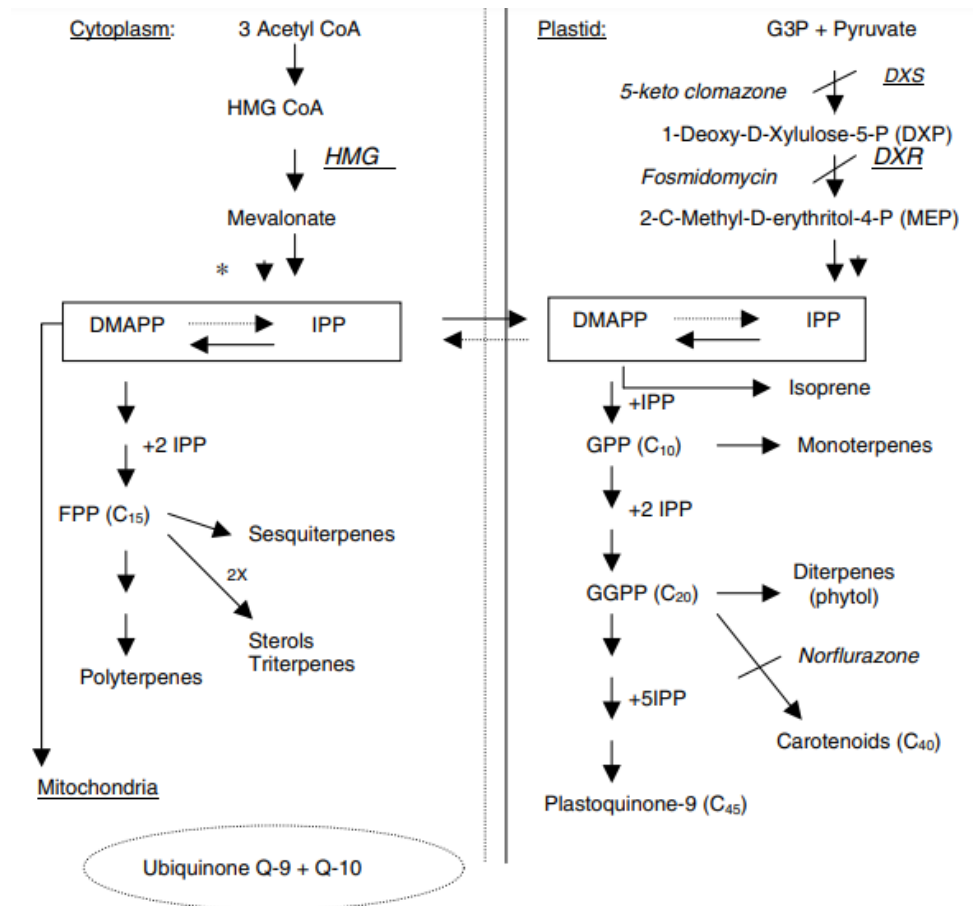


Figura 3: Via de isoprenoides citoplasmática e plastídica demonstrando a inibição da enzima 1-deoxi-xilulose 5-fosfatase sintase (DOXP) pela forma 5-ceto clomazone (Ferhatoglu e Barret, 2006)

Em condições normais de crescimento, a produção de espécies reativas de oxigênio (EROs) nas células é muito baixa. No entanto, sob estresse ou exposição a compostos tóxicos como herbicidas, sua produção aumenta, provocando peroxidação de lipídios na membrana celular e, em alguns casos, morte celular (Dayan & Watson, 2011). Entre as EROs estão o ânion superóxido (O_2^-) e o peróxido de hidrogênio (H_2O_2), gerados pelo escape de elétrons na cadeia de transporte fotossintético (Jackson & Colmer, 2005). O estresse oxidativo também pode causar danos biológicos ou alterar os sistemas de defesa antioxidantes. As células reagem ao estresse por meio de um sistema complexo que inclui enzimas como superóxido dismutase (SOD), ascorbato peroxidase (APX) e catalase (CAT), além da glutathione S-transferase (GST), uma enzima de desintoxicação de xenobióticos. As SODs, metaloenzimas, catalisam a dismutação de dois radicais superóxido (O_2^-) para formar H_2O_2 e O_2 , reduzindo o risco de formação de radicais hidroxila (OH), os oxidantes mais reativos (Dubey, 2011; Dinakar et al.,

2012). As enzimas APX e CAT são cruciais na desintoxicação de H_2O_2 , uma ERO moderadamente reativa (Bhatt & Tripathi, 2011).

O herbicida clomazona é considerado uma ameaça ambiental (IBAMA, 2018) devido ao uso excessivo, sua persistência no solo e na água e sua capacidade de contaminar cursos de água superficiais e subterrâneos (Marchesan et al., 2010). O clomazona pode chegar a esses ambientes por percolação lateral, lixiviação, escoamento superficial e subsuperficial, drenagem ou erosão, e uma vez nesses ambientes, pode afetar vários organismos não alvo provocando efeitos subletais e letais (Devault et al, 2007; Van Der Oost et al., 2003). A grande diversidade de organismos presentes nesses ambientes, como microalgas, pode sofrer desequilíbrios ecológicos. Estudos mostram que herbicidas aplicados em cultivos de arroz afetam a qualidade da água, reduzem o crescimento de microalgas e alteram a comunidade zooplancônica, aumentando a densidade de cladóceros e copépodes (Marques et al., 2009; Reimche et al., 2014). O herbicida clomazona já foi detectado em mais de 50% das amostras de água destinadas ao consumo humano durante um período de monitoramento de quatro anos na região sul do Brasil, em concentrações que variam de valores muito baixos(ou seja , abaixo do limite de quantificação para ser medidas com precisão) até um máximo de 164 ng L^{-1} , com medidas calculadas distribuídas de seguinte forma: $73,8 \text{ ng L}^{-1}$ na água potável e $75,5 \text{ ng L}^{-1}$ na água superficial (Caldas et al., 2019). Armas et al. (2005) e Primel (2005), afirmam que a presença de clomazona também foi comprovada em águas localizadas em áreas de cultivo de cana-de-açúcar e arroz no Estado Rio Grande do Sul.

As microalgas, devido à semelhança das vias metabólicas com as das plantas, são particularmente vulneráveis à contaminação por herbicidas como a clomazona (Rutherford & Krieger Liskay, 2001). Elas são organismos microscópicos fototróficos, com tamanho variando de micrômetros a algumas centenas (Richemond, 2004). Podem ser unicelulares ou multicelulares, procariotas ou eucariotas (Siavle & Steyer, 2013). Possuem organização celular simples, geralmente unicelulares, filamentosas, coloniais ou parenquimatosas sem diferenciação de tecidos, e contêm clorofila (*a* e *b*) e outros pigmentos fotossintéticos semelhantes (Sadi, 2012; Becerra C., 2009). As microalgas estão presentes em diversos habitats, como oceanos, mares, lagos, lagoas e rochas (Basset et al., 2018). Elas desempenham funções ecológicas significativas, participando da fixação de nitrogênio, do ciclo do carbono e da transferência de matéria (Staveley & Smrcek, 2005; Ramakrishnan et al., 2010; Garrido-Cardenas et al., 2018). Além disso, constituem a base das cadeias alimentares e produzem mais

de 60% do oxigênio atmosférico (DeLorenzo et al., 2001; Chisti, 2004; Cid et al., 2012). Muitas espécies de microalgas também são úteis na remediação ambiental devido à sua capacidade de degradar ou imobilizar poluentes como pesticidas (Zhou et al., 2014; Kurade et al., 2016; Chang et al., 2019). Sua sensibilidade aos esses produtos (herbicidas) as torna excelentes bioindicadores para estudos ecotoxicológicos (Lewis, 1995).

4. Espécies de microalgas utilizadas neste estudo

Os gêneros *Desmodesmus* e *Pseudopediastrum* diferenciam-se por características específicas: *Desmodesmus*, da família Scenedesmaceae, forma colônias lineares com 4 ou 8 células. Ela tem uma grande distribuição em águas doces. Sendo esse gênero e essa espécie amplamente utilizados em estudos ecotoxicológicos e biotecnológicos (Bica et al., 2012; Puerta et al., 2020; Hernández-García et al., 2019), enquanto *Pseudopediastrum*, da família Hydrodictaceae ou Sphaeropleales, apresenta colônias em formato de disco com projeções periféricas semelhantes a chifres. O número de células por colônia pode variar (Britanica, 2017). Ela é empregada como organismo teste para avaliar a toxicidade de xenobióticos, como biorremediar no tratamento de águas e na produção de bioprodutos e também compostos farmacêuticos (Park et al., 2014; Puerta et al., 2020; Corrêa da Silva et al., 2020). Esses dois gêneros são cosmopolitas e característicos de ambientes eutróficos (Bicudo & Menezes, 2006; Danieli et al., 2023). *Desmodesmus communis* (Hegew) e *Pseudopediastrum boryanum* (Turpin) E. Hegewald (Fig. 4) pertencem à classe Chlorophyceae, que inclui cerca 8.000 espécies conhecidas, das quais a maioria, em torno de 90%, é encontrada em ambientes lacustres (Esteves, 2011).

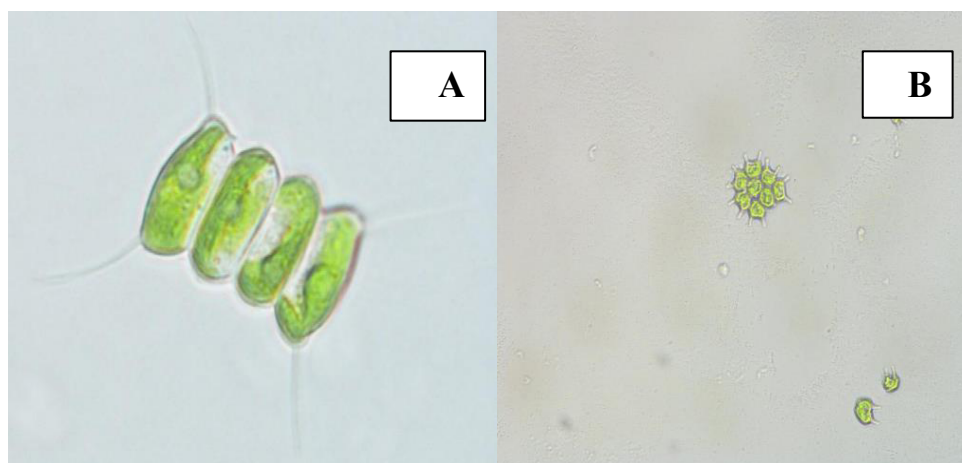


Figura 4. Imagens de microscópio de luz das duas espécies de microalgas da classe Chlorophyceae: A) *Desmodesmus communis* e B) *Pseudopediastrum boryanum*.

Justificativa

O Brasil está classificado entre os quatro (4) maiores consumidores de pesticidas do mundo, o que lhe confere uma posição de liderança (Rigotto et al., 2014; Donley, 2019). As regiões Sul e Centro-Oeste são as que mais consomem pesticidas no âmbito nacional, com 31% e 33% do uso total do país, respectivamente. Somente o estado do Rio Grande do Sul utiliza 11% da quantidade total de pesticidas aplicada no país (IBAMA, 2018). Apesar do Brasil possuir normas que estabelecem os níveis de concentração permitidos em ambientes aquáticos para alguns pesticidas, conforme as resoluções 357/05 e 396/08 do CONAMA, a clomazona não está incluída nessas resoluções nem possui valores de referência que possam ser considerados com segurança (Marchesan et al., 2010). Entretanto, a clomazona é considerada um herbicida de risco ambiental e toxicológico de nível II, sendo o 13º mais vendido no estado do Rio Grande do Sul e o 16º mais comercializado no país (IBAMA, 2018). Devido à sua alta solubilidade, persistência e meia-vida, o herbicida clomazona pode facilmente contaminar recursos hídricos e prejudicar organismos aquáticos não-alvo.

Diversos estudos destacam os impactos dos agrotóxicos nas microalgas, como a inibição do crescimento, alterações enzimáticas e efeitos na viabilidade celular (Lage et al., 2001; Macedo et al., 2008). Contudo, outros aspectos ainda permanecem pouco explorados. Nesse contexto, considerando a importância das microalgas nos ecossistemas aquáticos, sua grande diversidade e sensibilidade aos fatores ambientais, este estudo visa realizar uma análise cienciométrica para avaliar a evolução das pesquisas sobre os efeitos de pesticidas/herbicidas em microalgas e cianobactérias. Além disso, dado o grande uso da clomazona no país como um todo e, particularmente, no Rio Grande do Sul, o objetivo é avaliar a tolerância potencial do Gamit 360CS®, uma formulação microencapsulada do herbicida clomazona, em duas espécies de microalgas verdes. Acreditamos que este trabalho permitirá verificar os possíveis efeitos do Gamit 360CS e o potencial de tolerância dessas microalgas a este produto.

OBJETIVOS

2.1. Objetivo geral

Compreender os efeitos de diferentes agrotóxicos, especialmente do herbicida clomazona (Gamit 360CS®), sobre microalgas através de cienciométrica e experimento laboratorial.

2.2. Objetivos específicos

- O primeiro capítulo teve como objetivo: (i) Quantificar os trabalhos na literatura científica que abordam os efeitos de pesticidas sobre microalgas; (ii) Identificar os países que mais publicam; (iii) analisar os tipos de colaborações realizadas; (iv) identificar as principais revistas que publicaram artigos nesse tema; (v) levantar as palavras-chaves mais utilizadas; (vi) conhecer quais microalgas e cianobactérias (habitat, classe e espécie) são mais utilizadas em testes para avaliar efeitos de herbicidas; (vii) identificar qual classe de pesticidas mais estudada e (viii) averiguar quais os herbicidas mais estudados; (ix) quais os testes mais frequentemente utilizados e (x) os efeitos mais avaliados.
- O segundo capítulo teve como objetivo: a avaliação a tolerância potencial de duas espécies de microalgas verdes *D. communis* e *P. boryanum* expostas ao Gamit 360CS[®], com base na análise dos conteúdos dos pigmentos e das atividades das enzimas antioxidantes.

3. Hipóteses

Para o capítulo 2, duas hipóteses foram formuladas:

H1: O herbicida clomazona (Gamit 360CS[®]) diminui conteúdo dos pigmentos das microalgas.

H2: O herbicida clomazona (Gamit 360CS[®]) aumenta a produção de EROs e induz a atividade de enzimas antioxidantes (Superóxido dismutase, ascorbato peroxidase e catalase), além da Glutathione S-transferase para detoxificação do herbicida.

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CAPÍTULO 1

O manuscrito segue a formatação solicitada pela revista *Ecotoxicology*, a qual esse trabalho foi submetido. No entanto, para facilitar a visualização, as imagens foram adicionadas ao longo do texto.

Understanding the global research landscape of pesticide impacts on aquatic microalgae and cyanobacteria

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Abstract

In recent decades, the global use of pesticides – particularly herbicides – to control economically harmful organisms and meet food demands has increased significantly. However, this increased use of pesticides can affect non-target organisms, such as microalgae and cyanobacteria, which play critical roles in aquatic ecosystems. To assess the scientific progress on the effects of pesticides, especially herbicides, on microalgae and cyanobacteria, a scientometric and systematic map was conducted, analyzing publications from 1991 to 2023 in the Web of Science database. The findings show a steady increase in publications over the years, with China, the United States, and India ranking as the top three countries in research output on this topic. These studies were published in high-impact journals, with 71% of the publications focusing on herbicides as experimental substances, Atrazine, Glyphosate and Diuron are the most widely used herbicides. Chlorophytes were the most commonly used organisms in bioassays to assess the lethal and sublethal effects of these compounds. The study also identified several gaps, including limited international collaboration, and the lack of diversity in the selection of keywords indicates the need to explore different pesticides or mixtures of them, as well as the use of different groups of microalgae as test organisms. These insights can raise researchers' awareness of existing research gaps, encouraging future studies to address them. Furthermore, the findings provide valuable guidance for environmental organizations in developing strategic decisions and policies to protect aquatic ecosystems.

Keywords: algae; ecotoxicology; pesticides; pollution; scientific production.

1. Introduction

Pesticides are natural or synthetic substances designed to eliminate organisms deemed harmful or undesirable, such as those that damage crops (Sharma et al., 2019). They can be classified according to their specific target into herbicides, insecticides, fungicides, and others (Abhilash and Singh, 2009; Shrivastava and Bajpai, 2015). In agriculture, the global use of pesticides has surged in recent decades due to their effectiveness and their role in enhancing crop productivity (Settimi et al., 2016; Bombardi, 2017; Kniss, 2017; European Parliament (Sarkar), 2021). According to the Food and Agriculture Organization (FAO), over four megatonnes of pesticides are used annually across 164 countries (FAO, 2017).

Among pesticides, the herbicides group are the most widely used (FAO, 2022; Shattuck et al., 2023). Over the past years, herbicides have significantly supported global food supply by controlling economically undesirable plants (Powles, 2014). However, their use causes environmental problems, such soil and water contamination, posing risks to human health and biodiversity (Monaco et al., 2002). Notably, these chemicals can spread beyond their intended targets, dispersing into the atmosphere, infiltrating soils, and reaching aquatic environments (Patel et al., 2020).

In aquatic ecosystems, pesticides can cause both lethal and sublethal effects on non-target organisms, such as macrophytes, fish, microalgae, and cyanobacteria (Choi et al., 2012; Sanchez et al., 2017; Benegas et al., 2023), with the latter being particularly impacted by herbicides (Rutherford and Krieger Liskay, 2001; Castro et al., 2021). Certain herbicides, such as atrazine, glyphosate, and diuron, significantly inhibit microalgal growth and photosynthesis by blocking electron transport in photosystem II (PSII) (Grasso et al., 2022 ; Castro et al., 2022; Zhang et al., 2022). Microalgae and cyanobacteria are photosynthetic autotrophs with unique structures (chloroplasts) that enable the process of photosynthesis (Sisman-Aydin, 2019). These organisms are crucial for ecosystems as primary producers in aquatic environments, contributing to carbon and silicon cycles, with microalgae responsible for about 20–25% of global carbon fixation and 40% of primary production (Cid et al., 2012; Sisman-Aydin, 2022). They also produce nearly 60% of atmospheric oxygen, marking a significant contribution to life on Earth (Chisti, 2004). In ecotoxicological studies, microalgae and cyanobacteria are frequently used as model organisms due to their sensitivity to various chemicals, including pesticides and metals, as well as their ease of laboratory culture and short life cycle (Lewis, 1995; Castro et al., 2021).

Despite the rapid advances in studies investigating the impact of pesticide exposure on microalgae and cyanobacteria, particularly herbicides, there are significant questions and gaps in the current research landscape that require further investigation. These include identifying which herbicides have already been studied and which remain largely overlooked, as well as understanding the specific groups of microalgae and cyanobacteria that have been most researched. In that context, the scientometric is a branch of information science focused on evaluating the scientific and technological output of the scientific community. A recent methodological advance proposed combining bibliometric analysis with systematic mapping, known as research weaving (Nakagawa et al., 2019). Research weaving enhances the utility of a systematic map by assessing research connectivity, revealing relationships among authors, countries, and institutions (Donthu et al., 2021). By providing insights into collaborative networks, intellectual trends, and research impact within a specific topic or research context, research weaving can guide future studies.

Several scientometric and systematic mapping studies have been conducted in the field of ecotoxicology, providing an overview of publications on the effects of herbicides on organisms, including glyphosate and paraquat (Zyoud et al., 2017; Zyoud, 2018), 2,4-D (Zuanazzi et al., 2020), and atrazine (Castro et al., 2021). However, few studies focused specifically on the effects of herbicide on microalgae and cyanobacteria. Those studies that do examine these effects frequently emphasize research gaps, including the need for increased international collaboration and more studies utilizing these organisms in ecotoxicological research (Castro et al., 2021).

Given the importance of assessing scientific progress and considering the essential ecological roles of microalgae and cyanobacteria, as well as the environmental concerns surrounding increased pesticide use, this study undertakes a scientometric and systematic map of research on the effects of pesticides and herbicides, in particular on microalgae and cyanobacteria from 1991 to 2023. This study aims to provide a comprehensive overview of research trends in this field, addressing key questions: (i) Has the number of relevant articles increased over recent years? (ii) Which countries are leading in publication output? (iii) What are the patterns of international collaboration? (iv) Which journals publish most frequently on this topic? (v) What keywords are most commonly used? (vi) Which pesticide classes are most studied? (vii) What herbicides are most frequently examined? (viii) Which classes of microalgae and cyanobacteria are most studied? (ix) What test types are commonly employed?

(x) What effects are most frequently assessed? We expect that these insights will guide future research and support informed decisions in this critical area.

2. Research method

2.1. Data collection and analysis

For this scientometric and systematic map analysis, we used the Web of Science (WoS) database from Clarivate Analytics, updated as of January 2024. WoS is widely recognized as one of the leading, reliable databases in scientific research, offering three major citation indices for prominent journals and providing multidisciplinary information from over 18,000 high-impact journals (Li et al., 2018; Zhu and Liu, 2020). The scientometric and systematic map was conducted in line with the Reporting Standards for Systematic Evidence Synthesis (Haddaway et al., 2018; checklist provided in Supplementary File 1) (Fig. 1) to enhance the clarity and rigor of the analysis. This structured approach aids in the systematic identification, selection, and synthesis of literature (Page et al., 2021). The following keywords were used for the search: (((TS=(Effect* OR Toxicity*) AND ((TS=(Herbicide* OR Fungicide* OR Insecticide* OR Pesticide* OR Bactericide*))) AND ((TS=(microalgae* OR Algae* OR Cyanobacteria*))))). This search strategy was designed to identify studies that examined the effects of pesticides and herbicides on microalgae and cyanobacteria. The keywords were applied to titles, abstracts, and keywords fields. We applied several filters: language (English), document type (research articles), and time range (1991 to 2023). We selected 1991 as the starting year, as this was when Web of Science began indexing article abstracts.

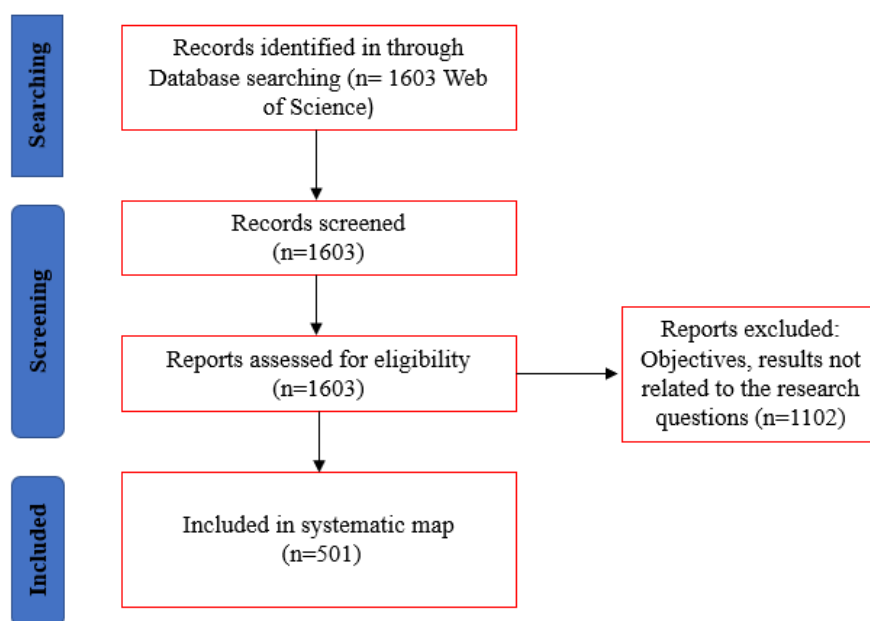


Figure 1. Screening process, in alignment with Reporting standards for Systematic Evidence Syntheses (ROSE) methodology.

After entering the keywords into the WoS search engine, we initially retrieved a list of 1,603 articles. We then conducted a thorough review of each article to exclude those unrelated to our objective, resulting in a refined list of 501 relevant articles. In line with our specific objectives, we collected the following information for each article: (i) publication year; (ii) country of publication and international collaborations (according to the country of the corresponding author); (iii) journal of publication; and (iv) keywords most used (vi) habitat type of microalgae or

cyanobacteria (marine or freshwater); (vii) pesticide classes used (herbicides, insecticides, fungicides, etc.); (viii) herbicide most used; (ix) classes of microalgae and cyanobacteria used; (x) analysis methods (e.g., biological toxicity tests and biomarkers) and (xi) evaluated effects.

For scientometric and systematic map analyses, we downloaded the data in BibTex format and analyzed it using the *bibliometrix* package (Aria and Cuccurullo, 2017) in R software (R Core Team, 2019). We employed VOSviewer software to create an international collaboration map and to identify the most frequently used keywords (Van Eck and Waltman, 2022). Collaboration types were classified based on the corresponding author's country: articles with authors from a single country were designated as "Single Country Publications (SCP)," while those with authors from multiple countries were labeled as "Multiple Country Publications (MCP)" (Li et al., 2009). To interpret the collaboration map, we used the following visual features: (i) circles represent countries, with size proportional to the number of publications; (ii) lines between circles denote international collaborations, with thickness indicating collaboration frequency. The keyword map included keywords with at least five occurrences. The keywords used as search terms in the search for articles used in this study, which would undoubtedly be the most frequent ones, were excluded from the analysis (Barbosa and Lanari, 2022). For the collaboration map, we included articles with a maximum of 25 countries listed among the authors and only considered countries that contributed at least 5 articles. We also used Pearson correlation to evaluate whether the number of publications per country is related to the Gross Domestic Product (GDP) invested in research in 2021. Data on country GDP were obtained from Word Bank Groups ([link](#)), with exception of Australia, Switzerland, Pakistan, and New Zealand for which we did not find reliable information. The correlation was performed in the software R (R Core Team, 2020).

In the analysis of the countries and journals with the highest number of publications on the effects of pesticides on microalgae and cyanobacteria, we focused on the top 10. This approach aims to provide a comprehensive and representative overview of the most significant scientific contributions in the field, ensuring a more effective and focused analysis of the highest-impact publications. Journal impact factors (IF) were obtained from the Journal Citation Reports (JCR) 2023. To account for the information gathered (organisms, pesticide classes, main herbicides, types of tests, and evaluated effects), we used the following approach: if a study included more than one group of organisms, each group was counted individually. This same approach was applied to the other data analyzed. For the "type of tests" category, we counted articles that exclusively used toxicity tests, those that focused only on biomarkers, and those that employed both. Toxicity tests included those that assessed organism mortality or growth inhibition, while biomarkers were those that evaluated sublethal effects on organisms. Toxicity tests are laboratory experiments conducted under controlled conditions to estimate the toxicity of substances, and environmental samples (water or sediments). Biomarkers are defined as biological responses to stressors, reflected in biochemical, cellular, histological/morphological, physiological, and behavioral changes (Depledge, 1990).

3. Results and Discussion

3.1. Temporal trends and evolution

Between 1991 and 2023, a total of 501 articles examining the effects of pesticides on microalgae and cyanobacteria were published with abstracts in journals indexed in the Web of Science database. In 1991, the year Web of Science

began indexing abstracts, 11 articles were published; this number dropped to 7 the following year but returned to 11 in 1993. Since then, the number of annual publications has fluctuated, with a notable low in 2000, when only 2 articles were published. Starting in 2001, the number of publications gradually increased, although annual fluctuations continued. From 2007 to 2012, despite these variations, a notable upward trend emerged, peaking at 27 articles in both 2011 and 2012 (Fig. 2). This trend underscores the sustained relevance and timeliness of research on the impact of pesticides on aquatic ecosystems within the scientific community. Studies by Köhler and Triebkorn (2013) and Van Der Kraak et al. (2014) have mentioned that publications on the potential effects of pesticides, have not only increased steadily, but have also become a subject of interest since the 1990s, particularly on triazines.

Several socio-economic, environmental, and research-related factors could explain the increase in publications on pesticides during this period. Herbicide use in agriculture is often regarded as a highly efficacious solution for combating economically harmful and undesirable organisms, increasing crop yields, or improving soil environmental conditions (e.g., reducing erosion, fuel consumption, and greenhouse gas emissions), as highlighted by Gianessi (2013) and Begum et al. (2017). However, the growing awareness of environmental risks and the need for sustainable management of aquatic ecosystems has undoubtedly led to an increase in studies evaluating the effects of pesticides on non-target organisms. For example, concerns about water eutrophication and pollution caused by agricultural pesticides have driven studies on microalgae and cyanobacteria, which are sensitive bioindicators, to better understand their effects and fill knowledge gaps (De Souza et al., 2020; Benegas et al., 2023; Narayanan et al., 2024). Additionally, new environmental regulations adopted in Europe and the United States since 1993 and 2000 likely contributed to the increase in research to meet the requirements of environmental protection agencies (European Commission, 2000; Köhler and Triebkorn, 2013; Singh et al., 2023). The rise in research output may also be attributed to the emergence of microalgae in biotechnology, with their potential use as biofuels and in bioremediation processes, which has heightened the need to examine the influence of pesticides on these organisms (De Morais et al., 2022). Moreover, advancements in analytical techniques such as spectroscopy have enabled more detailed studies of the sublethal effects of pesticides (Almeida et al., 2019). Furthermore, the complexity of these products continues to capture the attention of scientists regarding their effects on humans and non-target organisms. Scientometric studies have also been conducted on widely used herbicides, such as glyphosate (De Castilhos Ghisi et al., 2020), paraquat (Zyoud, 2018), 2,4-dichlorophenoxyacetic acid (2,4-D) (Zuanazzi et al., 2020), and atrazine (Castro et al., 2021), to evaluate their potential effects on various types of organisms and habitats. This is particularly relevant as herbicides are among the most widely used and sold pesticides worldwide (Kniss, 2017; FAO, 2022; WHO, 2023).

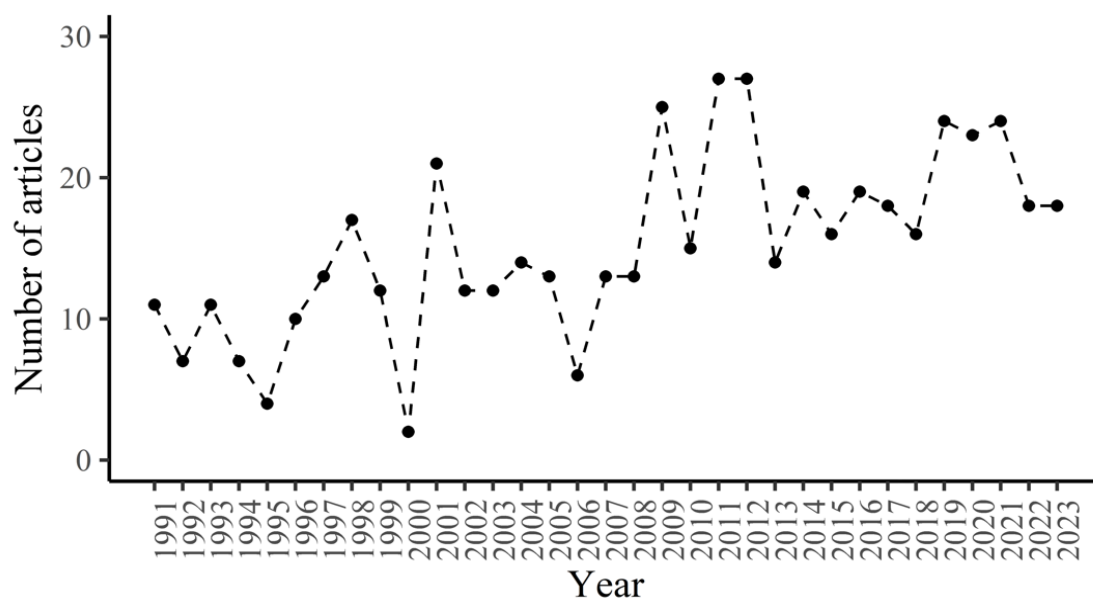


Figure 2. Number of articles published on Web of Science database between 1991 to 2023 analyzing the effects of pesticide on microalgae and cyanobacteria.

3.2. Countries

The findings of this study indicate that from 1991 to 2023, researchers from 38 countries published work on the effects of pesticides on microalgae and cyanobacteria in journals indexed in the Web of Science database (Table 1). The top ten contributing countries (both in sole and collaborative publications) accounted for 71.45% of the total publications. Among these, four are European, two American, two Asian, one Oceanian, and one Eurasian. The geographical diversity in scientific publications indicates a broad global awareness of the importance of pesticides and herbicides and their environmental implications, especially for aquatic organisms such as microalgae and cyanobacteria. This suggests that the impact of pesticides is a global concern, transcending specific regions, likely due to the universality of agricultural practices that use these products and the growing recognition of their adverse effects on aquatic ecosystems.

Table 1. The 10 countries with the highest number of published articles on the effects of pesticides on microalgae and cyanobacteria from 1991 to 2023 in the Web of Science database.

Rank	Country	Numbers of articles	SCP	(%) ¹	MCP	(%) ²
1	<i>China</i>	110	95	86	15	14
2	<i>USA</i>	50	43	86	7	14
3	<i>India</i>	44	42	95	2	5
4	<i>Spain</i>	34	33	97	1	3
5	<i>France</i>	33	29	88	4	12
6	<i>Portugal</i>	22	15	68	7	32
7	<i>Germany</i>	21	16	76	5	24
8	<i>Australia</i>	15	11	73	4	27
9	<i>Canada</i>	15	11	73	4	27
10	<i>Turkey</i>	14	14	100	0	0
Total³		358	309		49	
Average (%)⁴				86		14

USA: United States of America. SCP: number of single country publications. MCP: number of multiple country publications. ¹Percentages of SCP in respect to total publication in the country, ²percentages of MCP in respect to total publication in the country, ³ total number of published articles, ⁴ average percentage total.

China (22% of publications), the United States (10%), and India (9%) form the leading trio on the list of countries with the highest number of publications on pesticide impacts on microalgae and cyanobacteria, collectively accounting for 55.86% of the publications from the top 10 countries, with a combined total of 204 articles. The elevated publication rates observed in these countries may be attributed to a number of factors, including investments in research and development (R&D), the extensive use of pesticides, and institutional frameworks that promote research on the environmental impacts of pesticides. This prominence may also relate to their respective R&D expenditures as a percentage of GDP, often considered a key indicator of economic development, innovation, and scientific productivity (Torres et al., 2021). Specifically, China and the United States rank among countries investing more than 1% of their GDP in R&D, with China at 2.41% and the United States at 3.49% in 2020 (World Bank, 2020). However, the correlation between the percentage of GDP invested in R&D and the number of articles was weak and non-significant ($r = 0.083$, $p = 0.640$), indicating that GDP percentage may not be the sole determining factor in these countries' high publication rates. Among the other factors that may play a role is population size. China, India, and the United States are among the most populous countries globally, with populations of approximately 1.428 billion (India), 1.411 billion (China), and 334.914 million (United States) in 2023 (World Bank, 2024). The need to sustain large populations has driven these countries toward intensive agricultural practices, heavily reliant on pesticides and herbicides. Notably, these nations are also among the world's top pesticide consumers: in 2019, China consumed 1.76 million metric tons, the United States 408,000 tons (Pozo et al., 2011; Fernández, 2021; Pathak et al., 2022). The extensive use of these substances requires substantial research into their environmental impact, which is likely to result in a higher publication rate. Finally, these publication trends may also reflect policy measures governing pesticide use and research. China has implemented policies to regulate pesticides and support their scientific management, coupled with a growing budget from the National Science Foundation of China since 2000 (Pan Xing-Lu et al., 2019; Yang, 2016). In the United States, institutions like the Environmental Protection Agency (EPA) and federal laws promote both pesticide regulation and research (De Albuquerque et al., 2020). India's Council of Agricultural Research (ICAR) conducts extensive research on the effects of pesticides on soil, water, and biodiversity, strengthening the country's agricultural research output and its commitment to understanding these impacts.

3.2.1. International collaboration networks between countries

Scientific collaboration has grown substantially in recent years, becoming a hallmark of innovation in research practices (Huang, 2015). This shift is largely due to the increasing complexity of scientific inquiries, which often require specialized skills from diverse fields (Abt, 2007). However, our findings reveal a different trend: among the 10 countries that publish the most, 86% published without collaboration while only 14% published in collaboration with other countries (Table 1). This is a clear gap that needs to be addressed. The contrast between the global trend of collaboration and the reality observed in our analysis suggests that, although the increase in scientific collaboration is a general trend, there are research areas, such as evaluating the impacts of pesticides on aquatic ecosystems, where international collaboration remains limited. Our data indicate that, for this specific field,

the collaborative process needs to be strengthened. More collaborative research could enhance data and methodology sharing, enrich approaches with diverse perspectives, and facilitate access to resources and infrastructure. This would contribute to a more comprehensive and effective understanding of the global impacts of pesticides, as well as strengthen environmental policies. Therefore, encouraging international partnerships is crucial to overcoming these limitations and addressing the challenges posed by pesticides.

The three top-publishing countries show very low percentages of international collaboration: China at 13.64%, the United States at 14%, and India at 5%. In contrast, Australia and Canada, despite their small number of publications, showed higher collaboration rate of 27% compared to these three countries. These findings indicate limited international cooperation on research into the effects of pesticides on microalgae and cyanobacteria, even though this topic is globally significant. This trend of low international collaboration has also been observed in studies focused on herbicides like atrazine (Castro et al., 2021) and 2,4-D (Zuanazzi et al., 2020). The lack of strong international collaborations on pesticides can be attributed to several interconnected factors. Regulatory discrepancies across countries, particularly regarding pesticide authorization and usage, pose methodological and conceptual challenges (De Albuquerque et al., 2020). Developing countries, often hindered by limited research infrastructure, face difficulties in participating in international projects, thereby amplifying global scientific disparities (Chilvers and Kearnes, 2020). Furthermore, national research agendas, closely aligned with local agricultural and environmental issues, along with the geographical closeness of research partners, significantly influence collaboration dynamics (Gulbrandsen and Smeby, 2005; Katz, 1994).

The collaboration map visually illustrates the relationships between countries (Fig. 3). China, the country with the most publications, collaborates with a broad range of nations like the USA and Egypt. The United States, which ranks second, collaborates with fewer countries than China, while India has collaborated with England, Australia, and Thailand. Both China and the United States, as leading countries in publication numbers, show collaborative activity, albeit limited. Geographical proximity or shared scientific and technological interests often drive these international partnerships (Katz, 1994; Wagner, 2015). This was notably the case for the collaboration between China and the United States, which began in the late 1970s and was rooted in mutual scientific interests (Xiaoming Jin, 2003).

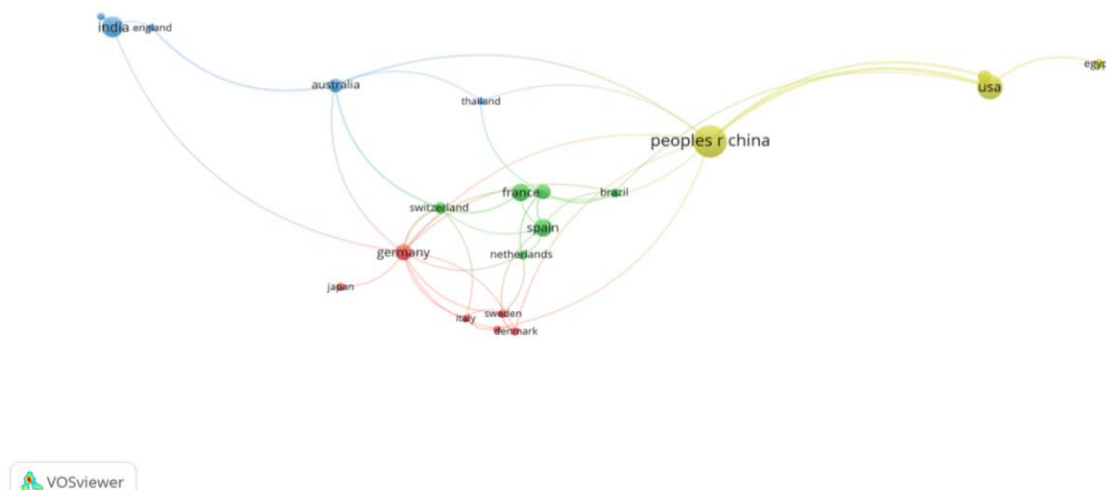


Figure 3. Map of the international collaboration network between countries that analyzed the effects of pesticides on microalgae and cyanobacteria between 1991 and 2023. On the map, the circles and their sizes represent the countries and the number of articles, respectively, and the lines and their thickness represent their cooperation and the degree of collaboration, respectively

3.3. Journals

The 501 articles published on the effects of pesticides on microalgae and cyanobacteria have been published in 29 different journals. Notably, more than half of these publications – 253 articles, representing 50.49% of the total – originated from just 10 of these journals. The journal with the highest number of articles is *Aquatic Toxicology* (46 articles and an impact factor (IF) of 4.1), followed by *Ecotoxicology and Environmental Safety* (43 articles; IF = 6.2). and in third place, *Chemosphere* (35 articles; IF = 8.1) (Table 2). These results reveal a significant concentration of publications in a limited number of journals, which may indicate that research on the effects of pesticides on microalgae and cyanobacteria is being centralized in journals specializing in ecotoxicology and aquatic toxicology. The predominance of *Aquatic Toxicology* and *Ecotoxicology and Environmental Safety* among the most relevant journals, with a high number of published articles, suggests that these journals are key references in the field, attracting a substantial portion of the publications on the topic. The high impact factor of these journals further underscores the credibility and impact of the research addressing the effects of pesticides on aquatic environments. This scenario provides insight into how research in ecotoxicology, particularly on pesticides and their consequences for aquatic organisms, is structured within the realm of scientific publications, highlighting both the dominance of high-impact journals and the contribution of more specialized ones.

Table 2. Characteristics of the ten journals that most published articles on the effects of pesticides on microalgae and cyanobacteria from 1991 to 2023 from Clarivate Analytics Web of Science database. IF = Impact Factor of 2023 (JCR)

Rank	Journal	Number of articles	IF
1	<i>Aquatic Toxicology</i>	46	4.1
2	<i>Ecotoxicology and Environmental Safety</i>	43	6.2
3	<i>Chemosphere</i>	35	8.1
4	<i>Environmental Toxicology and Chemistry</i>	35	3.6
5	<i>Science of the Total Environment</i>	20	8.2
6	<i>Bulletin of Environmental Contamination and Toxicology</i>	18	2.7
7	<i>Journal of Environment Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes</i>	17	1.4
8	<i>Ecotoxicology</i>	14	2.4
9	<i>Pesticide Biochemistry and Physiology</i>	13	4.2
10	<i>Environmental Science and Pollution Research</i>	12	-

3.4. Network of keywords

In scientific publications and various types of research, keywords play a crucial role. They enable readers to quickly engage with the text and assess its relevance to their interests. Analyzing associated keywords and their

frequency can help identify research topics, evaluate emerging trends, and track developments within a specific field of knowledge (Yu et al., 2017; Lin et al., 2019). Therefore, selecting and using appropriate keywords is essential. In the keyword network, based on the 501 retrieved articles, the terms “photosynthesis”, “atrazine”, and “glyphosate” emerge as the three most frequent keywords (Fig. 4). Our results confirm that atrazine and glyphosate are indeed the herbicides most frequently used in studies involving microalgae and cyanobacteria (see Pesticides section), justifying the choice of these keywords. The prominent presence of the term “photosynthesis” in our dataset also reflects its use as a widely employed physiological biomarker in environmental impact analyses on these organisms.

Our keyword network is divided into seven distinct clusters, each characterized by a different color. Each cluster groups a set of keywords that clarifies important subjects within each research area. Cluster 1 connects the keywords “photosynthesis” with “oxidative stress”, “growth”, and others suggesting that some studies are focused on understanding the physiological effects of pesticide impact on the photosynthetic capacity and growth of target organisms. The presence of terms such as “oxidative stress” also indicates an analysis of the underlying mechanisms of the physiological changes caused by pesticides. Cluster 2 links “atrazine” with “phytoplankton” and “ecotoxicology”, among others showing that some studies highlight the relevance of examining the interaction between specific pesticides and aquatic organisms, especially those that play a vital role at the base of the aquatic food chain. Phytoplankton are crucial for the balance of aquatic ecosystems, and the effects of pesticides on these organisms can have cascading consequences for the health of the aquatic environment (Naselli-Flores and Padisák, 2022). Clusters such as 3 and 4, which involve “glyphosate”, “diuron”, “paraquat”, and “*Chlorella vulgaris*”, reflect the focus of many studies on common herbicides and specific organisms, such as *Chlorella vulgaris*, which is widely used as a bioindicator in toxicity testing (Schwab et al., 2013; Shi et al., 2014; Zhang et al., 2022; Osters et al., 2022). These clusters highlight the need to investigate the impact of commonly used herbicides on key organisms in aquatic ecosystems. Cluster 5 associates “degradation” with “metabolite” and “biodegradation” highlighting the importance of understanding not only the immediate effects of pesticides but also how these compounds degrade in the environment and the products resulting from this degradation, which may have additional effects on environmental health. Finally, Clusters 6 and 7, with terms such as “chlorophyll fluorescence”, “bioassay”, and “EC50”, indicate the importance of experimental methods used to evaluate pesticide effects and their toxicity. The use of the terms “*Pseudokirchneriella subcapitata*” and “EC50” (a measure of the effective concentration of a substance that causes 50% of the maximum effect) indicates that both the model organism and the measure are widely used in toxicological studies, reinforcing the relevance of standard techniques for measuring pesticide effects on aquatic organisms (Yeh and Chen, 2006; Pérez et al., 2011; Machado and Soares, 2020; Machado and Soares, 2021).

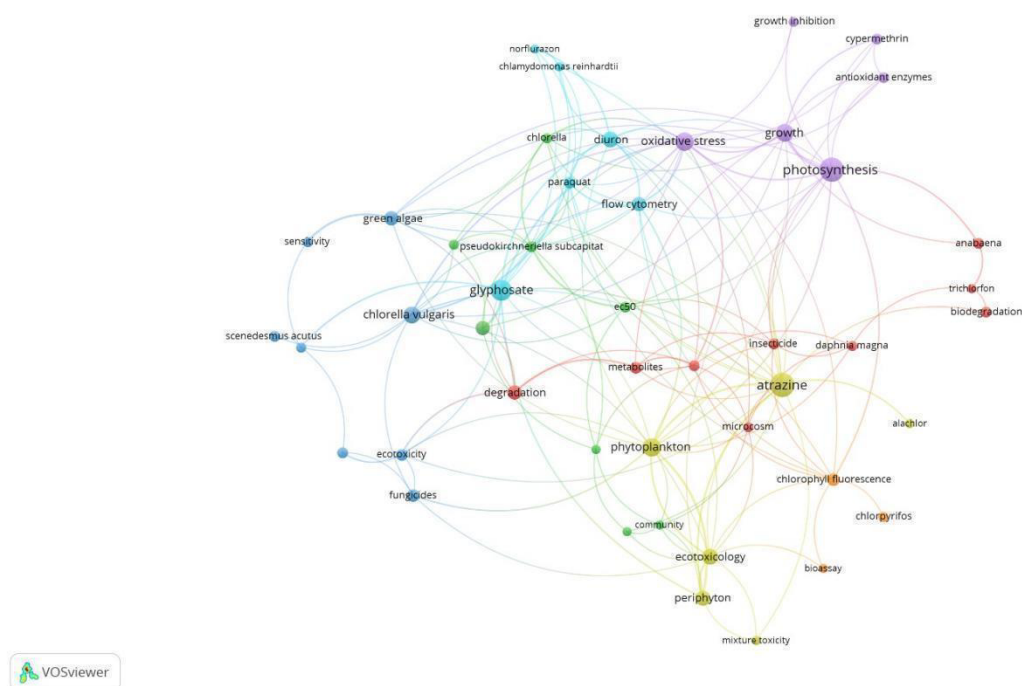


Figure 4. Main keywords from studies that analyzed the effects of pesticides on microalgae and cyanobacteria between 1991 and 2023, retrieved from the Web of Science database. The size of the circles indicates the frequency of occurrence of these words in the analyzed studies. The colors represent different clusters or groups of related words. The lines between words indicate co-occurrences.

3.5. Pesticides

In examining the most commonly studied classes of pesticides, our findings reveal that herbicides dominate published research on pesticide effects on microalgae and cyanobacteria over the past 32 years. Of the 501 publications analyzed, 356 articles representing 71% of the total evaluated the effects of one or more herbicides on microalgae or cyanobacteria. This was followed by insecticides, studied in 140 publications (28%), fungicides in 62 (12%), acaricides in 5, and algaecides in 3. Notably, some studies evaluated a combination of herbicides, insecticides, and fungicides. The predominance of studies using the herbicide class is likely due to this class being the most widely used among pesticides, as shown by the findings of Kniss (2017) and the FAO reports (2021, 2022).

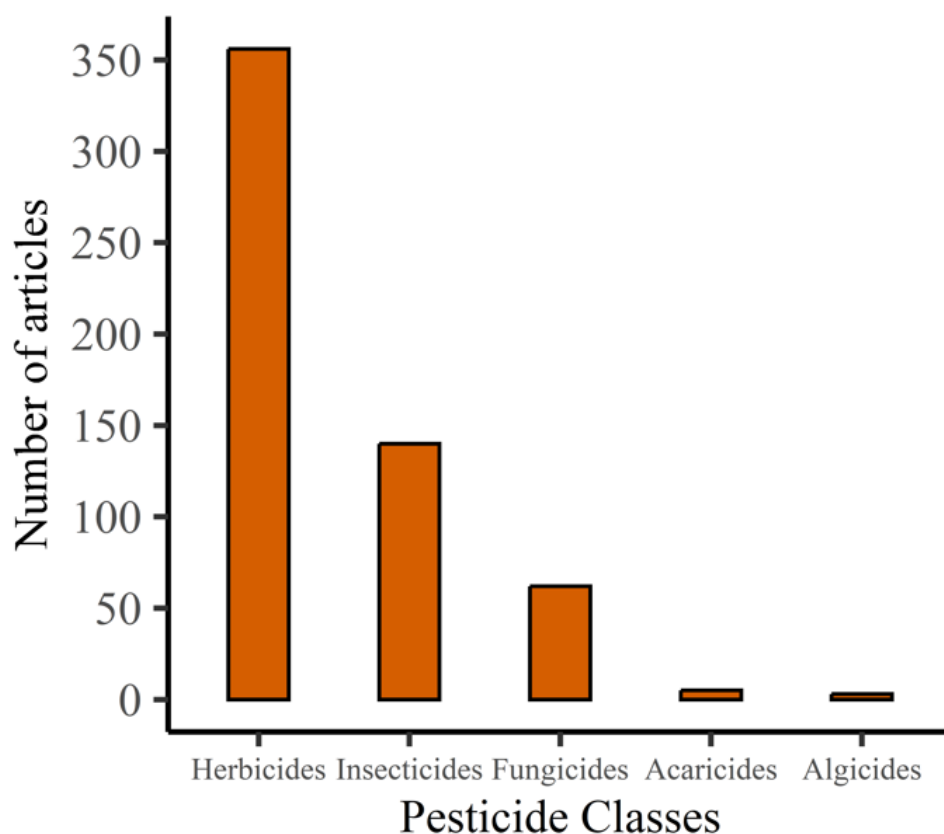


Figure 5. The most used pesticide classes in articles examining the effects of pesticide on microalgae and cyanobacteria published between 1991 to 2023 and retrieved from the Web of Science database.

In the context of herbicide research, atrazine is the most extensively studied substance, featuring in 94 articles and representing 26% of all publications on the effects of herbicides on microalgae and cyanobacteria. Glyphosate and diuron follow, both occupying the second position with 51 articles each, representing 14% of the herbicide-focused publications (Fig. 5). These findings reinforce conclusions from prior studies that have identified glyphosate and atrazine as among the most widely used herbicides worldwide (Kumar et al., 2013; Singh et al., 2018), due to their ease of application, effectiveness, and affordability in both local and international markets (Woodburn, 2000; WHO, 2023; FAO, 2022).

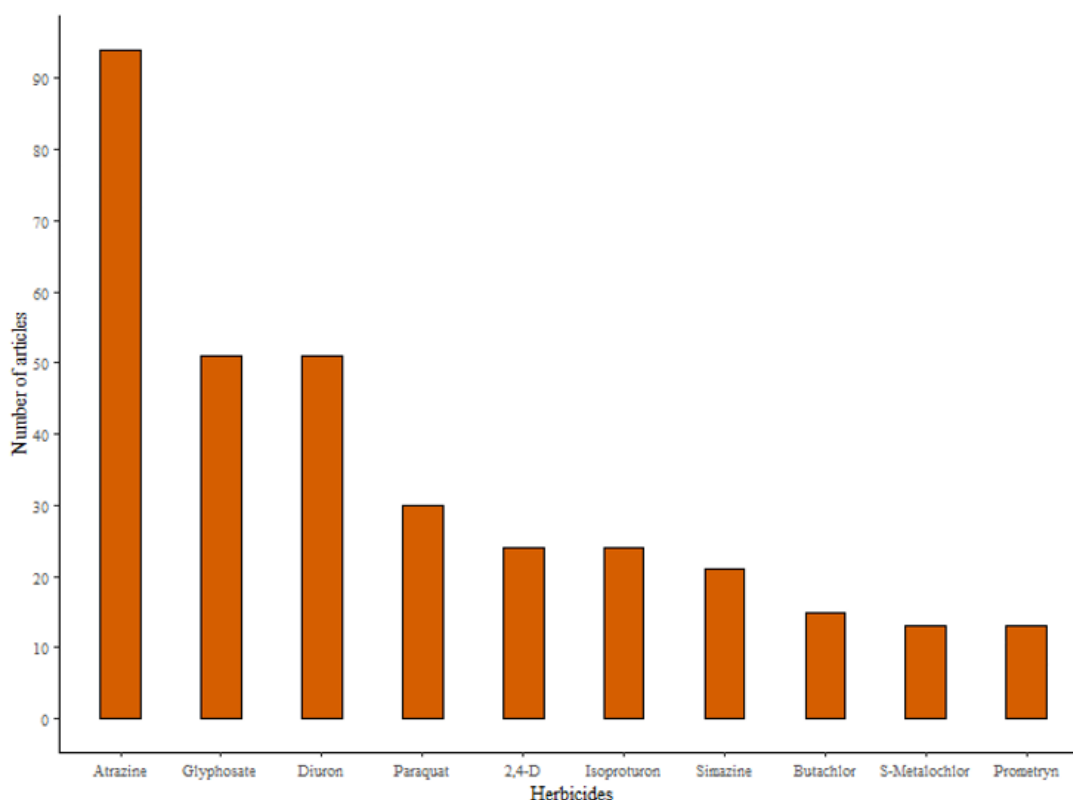


Figure 6. Main herbicides studied in articles that analyzed the effects of pesticides on microalgae and cyanobacteria between 1991 and 2023 retrieved from the Web of Science database.

3.6. Microorganism Classes

Regarding the classes of microorganisms that have been most studied, the results of this study indicate that Chlorophyceae, Cyanophyceae, and Bacillariophyceae represent the three most commonly examined in scientific studies that assess the impact of pesticides, particularly herbicides, over the past 32 years in the Web of Science database (Fig. 6). Among these, Chlorophyceae significantly surpasses the other two classes in the number of studies published. This predominance can be attributed to several factors, such as the sensitivity of many species to pollutants, including pesticides and herbicides. For example, Chlorophyceae (green algae) exhibit greater sensitivity to herbicides such as the atrazine, than Bacillariophyceae (Tang et al., 1998), and further studies corroborate that green algae are generally more sensitive than cyanobacteria (Miquel and Roessink, 2006; Ma et al., 2008; AbdEl-Aty and El-Dib, 2009). Additionally, Chlorophyceae play an essential ecological and trophic role as primary producers in aquatic food webs. Their ease of manipulation in laboratory, along with their morphological and physiological diversity (Cid et al., 2012), further supports their selection for research. Historically, due to their heightened sensitivity to various pollutants, green algae were among the first organisms chosen for toxicological studies as early as the 1960s (Nalewajko and Olaveson, 1998). Therefore, it is unsurprising that there are significantly more published articles utilizing one or more green algae species as experimental organisms in studies of the impact of pesticide on photosynthetic microorganisms.

However, the focus on these three classes limits a broader understanding of the effects of herbicides on microalgal biodiversity and the ecological dynamics of aquatic ecosystems. Other microalgal groups, such as Zygnematophyceae and Cryptophyceae, remain understudied, despite playing important roles in various aquatic habitats. Expanding research to include species from a variety of algal groups could provide a more holistic view

of pesticide impacts and contribute to a more comprehensive understanding of the ecological risks associated with the use of agrochemicals in aquatic environments.

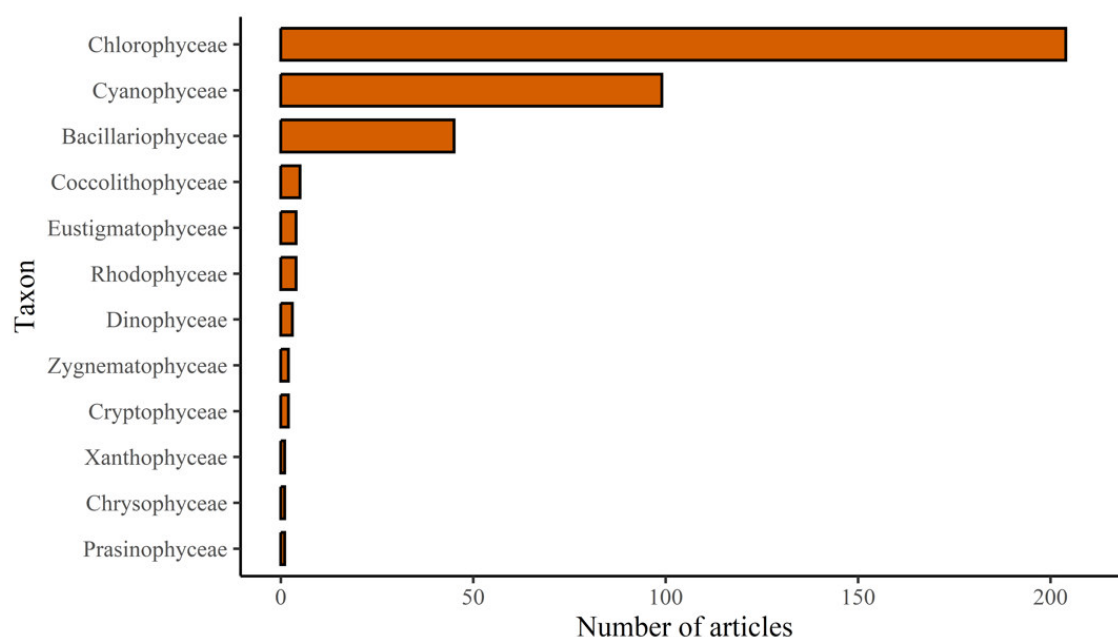


Figure 7. Main taxa studied in articles that analyzed the effects of pesticides on microalgae and cyanobacteria between 1991 and 2023, retrieved from the Web of Science database.

3.7. Tests and evaluated effects of herbicides

Among the 356 articles published on the effects of herbicides on microalgae and cyanobacteria, 133 (37%) employed toxicity tests to assess various parameters, including mortality, growth inhibition, reproduction, biomass, and photosynthetic efficiency. Meanwhile, 142 articles (40%) utilized biomarker tests to measure oxidative stress indicators (including reactive oxygen species and enzymes, such as catalase, superoxide dismutase, ascorbate peroxidase, and so on), and 81 articles (28%) combined both types of tests (Fig. 8a). Interestingly, biomarker-based tests slightly outnumber toxicity tests, which is notable considering that biomarker tests began to gain traction in toxicological studies only after 1997 (Blaise and Gagné, 2009).

The rise in the use of biomarker tests can be attributed to their ability to complement toxicity tests by providing insights into the mechanisms through which contaminants affect organisms - insights that conventional toxicity tests may overlook. Biomarkers facilitate the early detection of sublethal effects and enhance understanding of the mechanisms of action of contaminants (Almeida et al., 2019). Furthermore, the incorporation of biomarkers assessing DNA damage and oxidative stress offers a more comprehensive view of sublethal effects (Míguez et al., 2021). When used in tandem (toxicity tests and biomarkers), these approaches provide a clearer understanding of sublethal effects and pollutant mechanisms before visible or lethal damage occurs.

The physiological and biochemical impacts were the most frequently evaluated effects throughout the study period, accounting for 85.39% and 31.17% of the evaluations in the articles, respectively. This preference can be attributed to their capacity for rapid and precise detection of sublethal responses, providing critical toxicity indicators and insights into herbicide action mechanisms on microalgae and cyanobacteria (Didur et al., 2012; Vallotton et al., 2008; Almeida et al., 2019). Therefore, these analyses are essential for thoroughly and proactively evaluating the

impacts of contaminants on microalgae and cyanobacteria within ecosystems. We observed a low number of studies investigating effects on biological communities, a main gap of the global research landscape on the effects of pesticides on aquatic ecosystems. The study of communities enables the assessment of both the direct impacts on individual species and the broader consequences for community structure and dynamics (Dos Santos et al., 2024). Communities of microalgae, cyanobacteria, and other aquatic organisms play essential roles in biogeochemical cycles, primary productivity, and ecological balance. Disruptions in these communities can impact the entire food web, causing ecological imbalances that jeopardize biodiversity and ecosystem resilience. By focusing on community-level effects, these studies help identify shifts in population composition and structure, such as the loss of sensitive species and the proliferation of resistant ones, which can lead to cascading effects that impact other organisms and ecological processes. Furthermore, community-based studies offer a more comprehensive understanding of the ecological risks associated with pesticide use, enabling more effective, evidence-based management and conservation strategies. Thus, expanding research on the effects of pesticides on ecological communities is essential to safeguard the health of aquatic ecosystems and their environmental services (Floriane et al., 2014; Naicheng et al., 2017).

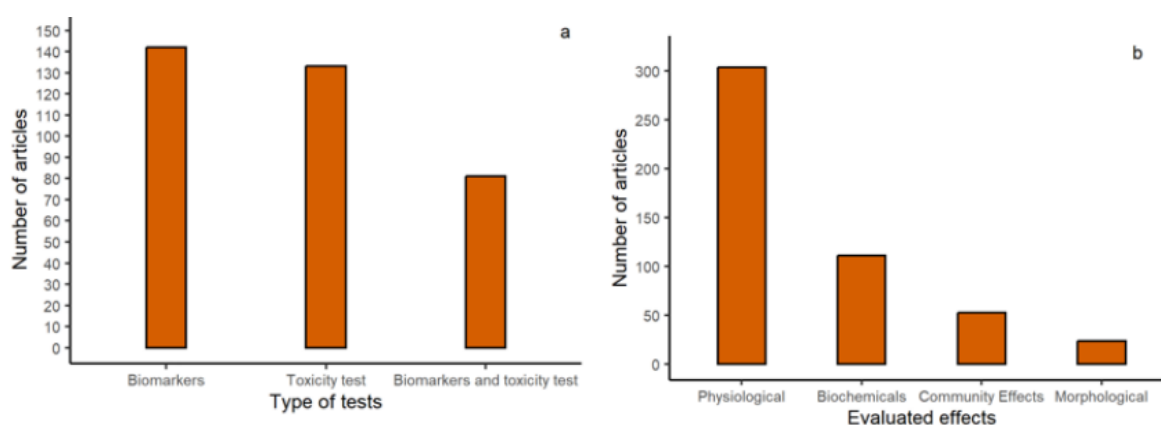


Figure 8. a) Most commonly used test types and b) most evaluated effects in articles that analyzed the effects of pesticides on microalgae and cyanobacteria between 1991 and 2023, retrieved from Web of Science database.

4. Conclusion and future perspectives

This scientometric and systematic map study has demonstrated a steady increase in research evaluating the effects of pesticides, particularly herbicides, on microalgae and cyanobacteria from 1991 to 2023 showing the global relevance of the topic, with numerous researchers from various countries examining and assessing the potential impacts of these compounds on diverse organisms within aquatic ecosystems. Our analysis identified the leading countries in publication output—China, the United States, and India—both for independent and collaborative efforts. These studies have been published in prestigious journals. Herbicides were the most studied class of pesticides, with atrazine, glyphosate and diuron being the most frequently used herbicides in the retrieved studies. Chlorophytes emerged as the most frequently studied class of microorganisms in laboratory experiments.

Additionally, we should emphasize that we have highlighted the most studied topics within the general subject, based on the keyword network, the most commonly used bioassays, and the effects most frequently evaluated.

Although research on the impacts of pesticides, particularly herbicides, on microalgae and cyanobacteria has advanced significantly in recent decades, several gaps still need to be addressed to deepen our understanding of the effects of these compounds on aquatic ecosystems. International collaboration among countries remains limited, resulting in restricted research networks and repetitive partnerships, which hinders a broader exchange of knowledge and approaches. The keyword analysis revealed several research gaps regarding the effects of herbicides on microalgae. While herbicides such as atrazine and glyphosate have been extensively studied, there is a need for further exploration of pesticide mixtures, additionally, underexplored groups like cyanobacteria should be prioritized. While classes of microalgae such as Chlorophyceae and Cyanophyceae have been extensively studied, other groups remain understudied. Expanding research to include other microalgal groups could provide a more realistic view of pesticide impacts and contribute to a more comprehensive understanding of the ecological risks associated with the use of agrochemicals in aquatic environments. We encourage the development of studies that use toxicity tests in conjunction with biomarkers. The combination of these methods can enhance the accuracy and sensitivity of ecotoxicological assessments, enabling better prediction of the long-term impacts of pesticides and other agrochemicals on aquatic ecosystems, with implications for conservation and environmental management. Additionally, the development of studies evaluating the effects on ecological communities is of utmost importance to understand the impacts of pesticides on aquatic ecosystems and their functioning. Finally, this work has the potential to guide researchers and decision-makers by providing valuable information that can influence future research directions and inform policies aimed at protecting aquatic ecosystems from the harmful effects of pesticides, with a particular focus on organisms essential to primary production. Its phytoremediation aspect could be incorporated into public environmental management policies as a sustainable strategy for the decontamination of aquatic ecosystems polluted by herbicides. This would involve identifying resistant microalgae species capable of absorbing and degrading these pollutants, promoting their use in the treatment of agricultural and industrial wastewater. This study can also contribute to shaping regulations on the use of pesticides by encouraging more environmentally friendly approaches and strengthening water quality monitoring standards. Ultimately, this work could serve as a cornerstone for developing ecological restoration alternatives to mitigate the harmful effects of herbicides on aquatic biodiversity.

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CAPÍTULO 2

O manuscrito segue a formatação solicitada pela revista *Environmental Toxicology and Chemistry*, a qual esse trabalho será submetido no formato de *Short communication*. No entanto, para facilitar a visualização, as imagens foram adicionadas ao longo do texto.

Sensitivity of two green microalgae (*Desmodesmus communis* and *Pseudopediastrum boryanum*) to clomazone herbicide exposure

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Abstract

The excessive use of herbicides in agriculture poses an increasing threat to aquatic ecosystems, leading to the contamination of these environments and subsequently impacting non-target organisms such as microalgae. This study evaluated the tolerance of two green microalgae species, *Desmodesmus communis* and *Pseudopediastrum boryanum*, to the herbicide clomazone. The effects of clomazone on photosynthetic pigments, oxidative stress indicators, and enzymatic activity were assessed at five concentrations (0.25, 0.50, 0.75, 1, and 1.5 mg L⁻¹) under controlled laboratory conditions. The results revealed species-specific responses: *D. communis* exhibited a decrease in chlorophyll-a and carotenoid content, as well as an increase in oxidative stress at higher concentrations, indicating heightened sensitivity in this species. In contrast, *P. boryanum* demonstrated greater tolerance, maintaining stable photosynthetic pigments and activating antioxidant defense mechanisms, notably superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). These findings highlight the differential responses of microalgae to herbicide-induced stress and underscore their potential for bioremediation applications, particularly that of *P. boryanum*. This work provides critical insights into the ecological risks of herbicide exposure, including clomazone, and emphasizes the importance of exploring microalgal resilience to mitigate environmental impacts.

Keywords: Chlorophyceae; Herbicide; Oxidative stress; Toxic effects.

INTRODUCTION

Environmental contamination caused by the use of herbicides in agriculture represents an ongoing threat to various ecosystems, particularly aquatic one, which serve as habitats for numerous organisms (Devault et al., 2007; Beketov et al., 2013). Among these herbicides, clomazone is a selective herbicide extensively used in pre - or post-emergence to control weed plants in irrigated rice, soybean, sugarcane, tabaco and corn crops (Dayan et al., 2015; SOSBAI, 2018; Bujanovic et al., 2020), and is of particular concern for aquatic organisms due to its persistence and solubility (1.2 g L⁻¹ at 20°C) in soil and water (Stevanović et al. 2021). Its half-life varies with environmental conditions, ranging from 90 to 276 days in aerobic soil, decreasing to 60 days under anaerobic conditions, and approximately 1.5 to 2.5 months in water (Lewis et al., 2016). Like other herbicides, clomazone reaches aquatic ecosystems through leaching, surface and subsurface runoff, lateral percolation, erosion, washing, etc. (Gavrilescu, 2005; Nsibande and Forbes, 2016; Triegel and Guo, 2018; Pandey et al., 2020 ; Jing et al., 2021). Thus, they affect various non-target organisms through lethal and sub-lethal effects, particularly microalgae, which play a crucial ecological role as primary producers in aquatic food chains, regulators of water quality, producers of atmospheric oxygen, and carbon fixers (Lürling, 2011; Deng et al., 2015; Blaise et al., 2004).

Clomazone, in its pure formulation [2-(2-chlorobenzyl)-4,4-dimethyl-1,2-isoxazolidin-3-one], belongs to the Isoxazolidinone family of herbicides and primarily acts by inhibiting 1-deoxy-xylulose-5-phosphate synthase

(DOXP), a key enzyme in carotenoid biosynthesis. Carotenoids are essential for protecting photosynthetic cells from oxidative stress and excess light (Dayan et al., 2015). This leads to the progressive reduction of essential photosynthetic pigments (chlorosis) in plants and microalgae. However, it is also sold under various microencapsulated commercial formulations, including Gamit 360CS[®], which is less volatile and caused lower phytotoxicity for bioindicator species (Schreiber et al., 2015). Nonetheless, microalgae exposed to clomazone may undergo biochemical alterations in their enzymes and chlorophyll pigments, impairing their ability to perform photosynthesis and sustain their metabolism. However, studies also show that some species of green microalgae, such as *Chlorella vulgaris* and *Scenedesmus obliquus*, exhibit tolerance to pollutants and pesticides by inducing enzymatic detoxification mechanisms, highlighting the bioremediation capacity of these organisms (Zhou et al., 2014; Kurade et al., 2016; Chandra et al., 2021; de Morais et al., 2022).

Despite the widespread use of clomazone, few scientific studies have explored its impact on green microalgae, and even fewer have investigated their potential capacity for bioremediation. Given the environmental importance of these organisms and concerns regarding the effects of this herbicide on aquatic ecosystems. The aim of this study is to evaluate sensitivity of two species of green microalgae - *Desmodesmus communis* (Turpin) E. Hegewald and *Pseudopediastrum boryanum* (Hegew) - to the microencapsulated formulation Gamit 360CS[®]. This study will help to better understand the effects of clomazone, particularly one of its microencapsulate formulations known as Gamit 360CS[®], on non-target organisms, as well as to highlight their potential role in reducing the environmental impacts of this herbicide.

MATERIAL AND METHODS

The species *D. communis* and *P. boryanum* were obtained from the Continental Microalgae Culture Collection at the Institute of Biological Sciences (CCMD-FURG). The microalgae were maintained at a light intensity of 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, 12:12-h light:dark cycle, and 23 ± 2 °C. The experiments were conducted with axenic cultures obtained by washing in a Dakin solution (Vieira, 1983), and the species were cultured in WC medium (Guillard and Lorenzen, 1972). The tests started when the initial cell concentration of algae reached 10^5 – 10^6 cells ml^{-1} , determined by counting using a Neubauer chamber with 40 $\times$ magnification.

Gamit 360CS[®], one of the commercial formulations of the herbicide Clomazone, was the product used to conduct the experiment. The required formulation volume was calculated based on the clomazone concentration in the product (360 g/L). Six experimental groups were established, an untreated check (control) and five herbicide concentrations (0.250; 0.50; 0.750; 1; 1.50 mg /L), determined based on the literature and preliminary tests. The tests were performed in a transparent glass Erlenmeyer flask (100 ml) filled with 50 ml of experimental medium, which was the WC culture medium. The tests lasted 72 h. All treatments were performed with five replicates and were maintained in the same conditions as described for cultivations and precultures. Culture flasks were gently shaken (manually) twice a day until the end of the experiment.

To determine the chlorophyll-a, chlorophyll-b, and carotenoid content, cells from each species were collected by centrifugation for each treatment, and the pigments were extracted using DMSO + CaCo₃ (Welbur, 1994). The concentrations of Chl-a, Chl-b, and carotenoids were calculated according to the same author.

Superoxide ($\text{O}_2^{\bullet-}$) levels were measured following the methodology of Li et al. (2010). Absorbance was recorded using a standard curve prepared with known concentrations of sodium nitrite. Hydrogen peroxide (H_2O_2) levels were determined using the method proposed by Velikova et al., (2000). H_2O_2 content was quantified using a

standard curve prepared with known concentrations. Lipid peroxidation was estimated by quantifying thiobarbituric acid-reactive substances (TBARS) in the supernatant, as described by Cakmak and Horst (1991). Absorbances were recorded at 535 and 600 nm using a spectrophotometer. The MDA-TBA adduct (red pigment) rate was calculated using the molar extinction coefficient ($\epsilon_{535\text{ nm}} = 155 \times 10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$), and lipid peroxidation was expressed as $\mu\text{mol MDA-TBA mg}^{-1}$ fresh weight.

To measure the activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), enzymatic extracts were obtained from approximately 50 mL of microalgal cultures, previously separated from the herbicide solution. Samples were ground with liquid nitrogen and about 5% (w/w) polyvinylpolypyrrolidone (PVPP) and homogenized in 1.5 mL of extraction buffer containing 100 mM potassium phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetra acetic acid (EDTA), and 20 mM sodium ascorbate. The extract was centrifuged at 12,000 g for 20 minutes at 4°C, and the supernatant was used for enzymatic activity assessment.

SOD, CAT, and APX activities were determined respectively by the methods of Giannopolitis and Ries (1977), Azevedo Neto et al. (2006), and Nakano and Asada (1981). Enzymatic extracts were obtained from 50 mL of microalgal cultures previously separated from the herbicide solution to measure GST activity. The total protein concentration was determined using a biuret-based method (Labtest Kit, MG, Brazil) (Gornall et al., 1949). GST activity is expressed as mU mg^{-1} protein, where one unit (U) of GST is defined as the amount of enzyme capable of forming 1 μmol of 2,4-dinitrophenyl glutathione product per minute. Glycolate oxidase (GO) activity was determined according to Bai et al. (2014). Reactions were monitored by the formation of glyoxylate phenylhydrazone ($\epsilon_{324\text{ nm}} = 17 \text{ mM}^{-1} \text{ cm}^{-1}$) at 30°C, and absorbance was measured at 324 nm at 10-second intervals over 2 minutes.

One-way ANOVA was used to evaluate differences between treatments for each species compared to the control, followed by Tukey's post-hoc test. The significance level was set at 95% ($\alpha = 0.05$). Data analysis was performed using the R statistical software (R Core Team, 2019).

RESULTS

Effects of clomazone on photosynthetic pigments in *D. communis* and *P. boryanum*

The effects of different concentrations of the herbicide clomazone on photosynthetic pigments (Chl-a, Chl-b, and carotenoids) in the species, *D. communis* and *P. boryanum* are shown in Fig. 1. A significant reduction in chlorophyll-a content was detected in *D. communis* at 1.5 mg/L, while *P. boryanum* showed no changes across all tested concentrations (Fig 1 A). The herbicide clomazone showed no effect on the chlorophyll-b content in either of the two studied species at any of the tested concentrations (Fig. 1B). Regarding carotenoids, similar to chlorophyll-a content, a decrease was observed only at the highest concentration of 1.5 mg/L of clomazone, and this effect was exclusively on *D. communis* (Fig. 1C). These results suggest that at low concentrations ($\leq 1 \text{ mg/L}$), the herbicide clomazone does not affect the photosynthetic pigments of these two microalgae species. However, at 1.5 mg/L, a decrease in chlorophyll-a and carotenoid content was observed in *D. communis*. On the other hand, for *P. boryanum*, even at the highest concentration, clomazone showed no effect on photosynthetic pigments.

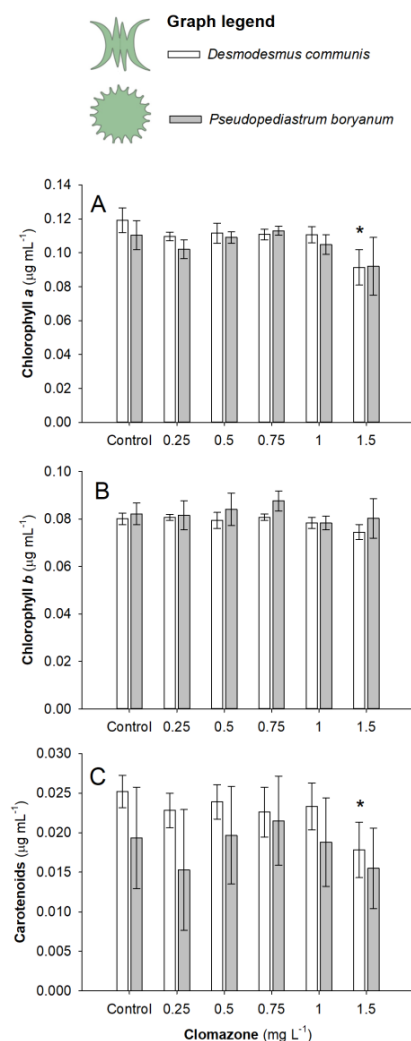


Figure 1. Effects of the herbicide clomazone on photosynthetic pigment content (chlorophyll-*a*, chlorophyll-*b* and carotenoids) in two species of green microalgae, *Desmodesmus communis* (white bars) and *Pseudopediastrum boryanum* (gray bars), as a function of different clomazone concentrations (from 0,25 mg/L to 1.5 mg/L).

Biochemical and enzymatic responses to clomazone-induced oxidative stress in *D. communis* and *P. boryanum*.

Oxidative Stress and Lipid Peroxidation

For *D. communis*, an increase in superoxide production was observed at the concentrations of 1 mg/L and 1.5 mg/L, the highest levels tested, indicating oxidative stress. In contrast, for *P. boryanum*, variations in superoxide levels were minor or nearly nonexistent at all concentrations. This suggests that *D. communis* experiences oxidative stress at higher clomazone concentrations, while *P. boryanum* shows little to no impact, indicating a higher tolerance to clomazone (Fig. 2A). For hydrogen peroxide, no significant increase was observed in either *D. communis* or *P. boryanum* at any of the concentrations tested (Fig. 2B). No significant changes in lipid peroxidation were observed for either species at any clomazone concentration. This indicates that cellular membranes were not damaged by the herbicide (Fig. 2C).

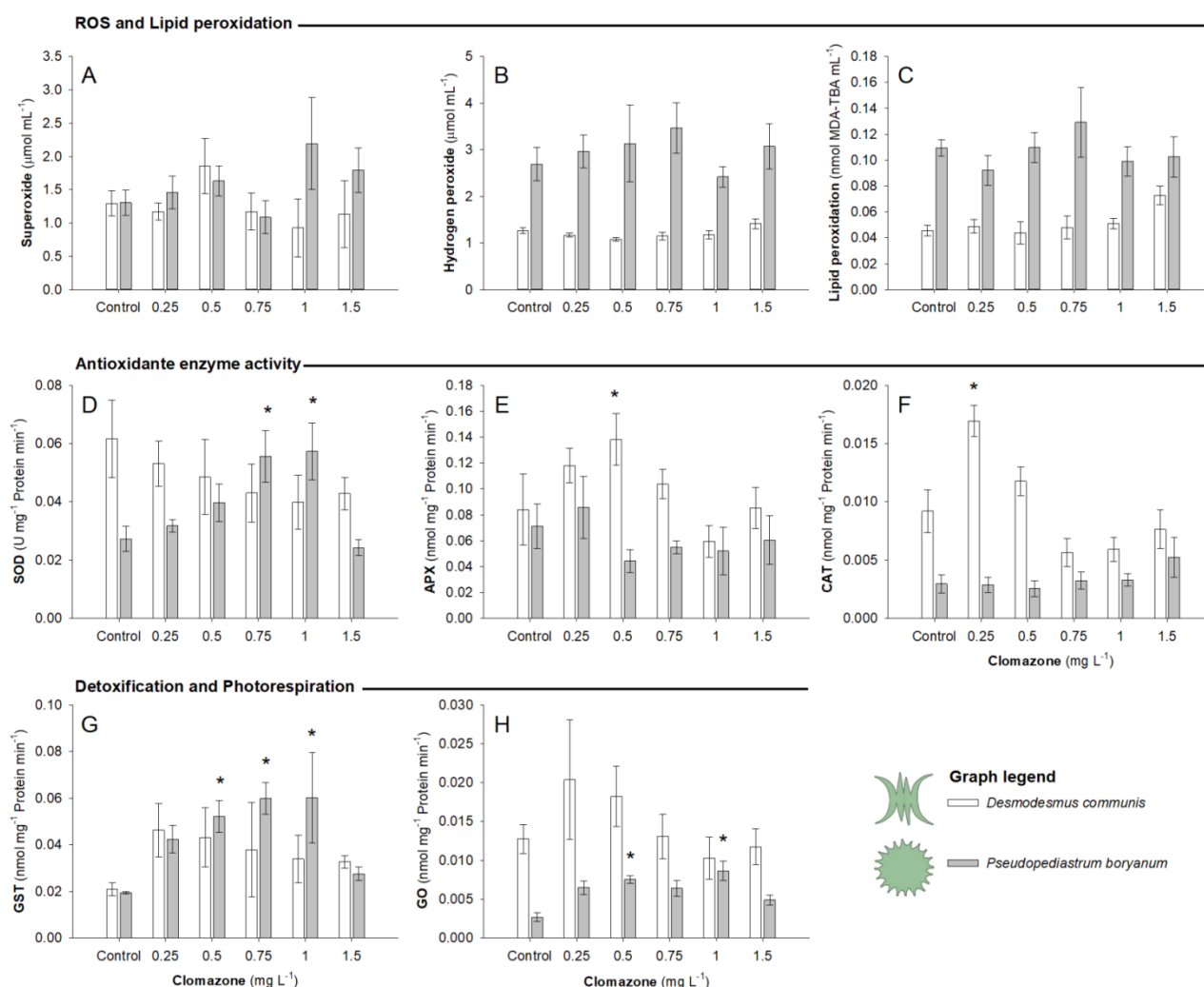


Figure 2. Effects of clomazone on oxidative stress, antioxidant enzyme activity, detoxification and photorespiration in two species of green microalgae *Desmodesmus communis* (white bars) and *Pseudopediastrum boryanum* (gray bars), as a function of different clomazone concentrations (from 0.25 mg/L to 1.5 mg/L).

Antioxidant Enzyme Activities

In *D. communis*, SOD activity showed no significant changes across the different concentrations of clomazone. However, in *P. boryanum*, a significant increase in SOD activity was observed at 0.75 mg/L and 1 mg/L, suggesting that *P. boryanum* responds to oxidative stress caused by clomazone by increasing SOD activity to neutralize free radicals (Fig. 2D). A significant increase in APX activity was detected in *D. communis* at 0.5 mg/L, indicating a response to oxidative stress induced by clomazone at this concentration. No changes were observed at other concentrations or in the control. For *Pseudopediastrum boryanum*, no changes in APX activity were detected at any concentration (Fig. 2E). At the lowest concentration, 0.25 mg/L, a significant increase in catalase activity was observed in *D. communis*, where it activated its antioxidant mechanism to detoxify reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2), generated by oxidative stress. No changes were observed

at other concentrations or in the control. For *P. boryanum*, no significant changes in catalase activity were observed at any concentration (Fig. 2F).

Detoxification and Photorespiration

In *D. communis*, no significant changes in GST activity were observed, indicating that GST activity remained stable across all clomazone concentrations. However, in *P. boryanum*, a significant increase in GST activity was noted at concentrations 0.5 mg/L, 0.75 mg/L, and 1 mg/L, suggesting that *P. boryanum* actively uses GST at moderate to high concentrations to counteract the toxic effects of clomazone (Fig. 2G). The activity of glycolate oxidase remained stable in *D. communis*, indicating that clomazone had no effect on GO activity in this species at any tested concentration compared to the control. However, for *P. boryanum*, a significant increase in GO activity was observed at concentrations 0.5 mg/L and 1 mg/L, suggesting that *P. boryanum* utilizes glycolate oxidase as a defense mechanism against clomazone at these concentrations (Fig. 2H).

DISCUSSION

The results presented in this study highlight the different behaviors of the microalgae *D. communis* and *P. boryanum* under the influence of various concentrations of the herbicide clomazone, particularly showcasing the differential responses between the two species in terms of sensitivity and defense mechanisms against oxidative stress. The observations are line with previous studies such as Almeida et al. (2019) and Míguez et al. (2021) have also investigated the impact of herbicides on microalgae and their tolerance potential.

At high concentrations, clomazone caused a decrease in Chl-a and carotenoid content in *Desmodesmus communis*; consequently, this decrease impairs photosynthetic capacity, likely by affecting thylakoid membranes or the photosynthetic complex and inhibiting carotenoid synthesis. In fact, these photosynthetic pigments are extremely important for plants and microalgae, as they are essential for photosynthesis and protecting cells from oxidative damage. Studies by Kumar et al. (2014); Deng et al. (2015), and Dayan et al. (2015) have indicated that prolonged exposure to carotenoid-inhibiting herbicides such as clomazone can damage the photosynthetic system. However, for *P. boryanum*, clomazone did not show any significant effect on pigments, even at the highest concentrations. This suggests that *D. communis* is probably more sensitive to clomazone, due to a lower capacity to protect its essential photosynthetic pigments for survival and growth.

The increase in superoxide ($O_2^{\bullet-}$) production at the highest concentrations (1 mgL⁻¹ and 1.5 mgL⁻¹) in *D. communis* indicates significant oxidative stress induced by clomazone, as reported by Deng et al. (2015), who found that microalgae exposed to herbicides can suffer from perturbations at multiple levels due to increased ROS production. In contrast, the moderation and stability shown by *P. boryanum* regarding superoxide production reflects an inherent resistance to this herbicide, probably thanks to an intrinsic ability to limit ROS production. No significant effect was observed on hydrogen peroxide (H₂O₂) and lipid peroxidation (MDA) (Fig. 2B and 2C) for either species, which could mean that the cell membranes were not damaged by clomazone, even under the influence of high concentrations. This suggests that the antioxidant defense system has controlled and stabilized oxidative stress. These findings are similar to those reported by Deng et al. (2015) and Velikova (2000), who demonstrated that membrane stability is a key indicator of stress tolerance in microalgae.

From a biochemical standpoint, the oxidative stress induced by clomazone is mainly marked by an increase in reactive oxygen species (ROS) and the activation of enzymatic defense systems, however, each of the two species reacts differently; *Desmodesmus communis*, through the activation of APX and CAT at low concentrations, shows

an early response to moderate stress, reflecting the crucial roles of these enzymes (APX) in reducing hydrogen peroxide (H₂O₂) to water and ascorbate to limit oxidative damage according to (Nakano & Asada, 1981) and CAT in the rapid decomposition of hydrogen peroxide to prevent its accumulation (Azevedo Neto et al., 2006), even at a low level. On the other hand, *Pseudopediastrum boryanum* mobilizes antioxidant mechanisms such as superoxide dismutase (SOD), glutathione S-transferase (GST) and glycolate oxidase (GO), enzymes that play a crucial role in the detoxification pathways of reactive oxygen species (ROS) and the protection of biomolecules, conferring it a better tolerance to the herbicide clomazone.

The results reveal that *Pseudopediastrum boryanum* deploys more robust and varied defense mechanisms such as (SOD, GST, and GO) which confer it a reputation of possible tolerance to the herbicide clomazone. On the other hand, *Desmodesmus communis* shows increased sensitivity, through targeted but limited responses (APX, CAT). These differences observed between the two species could be explained by variations in their enzymatic arsenal, the content of their photosynthetic pigments, or their ability to modulate the expression of genes involved in stress responses. Moreover, they highlight the differential potential of microalgae in phycoremediation, with *P. boryanum* positioning itself as a promising candidate for the detoxification of environments contaminated with clomazone, as the study by Daniéli Saul da Luz et al. (2024) has indicated.

CONCLUSION

In this study, we evaluated the tolerance potential of two green microalgae species (*D. communis* and *P. boryanum*) to the herbicide clomazone, indicating that *P. boryanum* exhibits greater tolerance to clomazone, characterized by the stability of its photosynthetic pigments, lower ROS production, and efficient activation of its enzymatic defense systems through GST enzyme. This suggests that this species possesses more effective mechanisms for detoxification and repair of oxidative damage. In contrast, *D. communis* proved to be more sensitive to clomazone, particularly at higher concentrations, as evidenced by the degradation of its photosynthetic pigments and a marked increase in oxidative stress. These interspecific differences underscore the importance of ecotoxicological approaches to understanding the impacts of herbicides on microalgae.

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CONSIDERAÇÕES FINAIS

Este estudo abordou os efeitos de pesticidas e herbicidas em microalgas e cianobactérias por meio de duas abordagens distintas. a primeira, teórica, foi realizada através de um mapa sistemático e cienciométrico, cobrindo os últimos 32 anos de pesquisas sobre o tema. a segunda abordagem foi experimental, na qual microalgas verdes foram expostas a um herbicida, permitindo a avaliação direta dos impactos desse composto em organismos aquáticos essenciais para os ecossistemas.

Os resultados do mapa sistemático e cienciométrico revelaram que as pesquisas sobre os impactos dos pesticidas e herbicidas nas microalgas aumentaram de forma constante ao longo desse período. Além disso, destacaram o papel relevante de países como China, Estados Unidos e Índia nesse campo de pesquisa. No entanto, a análise também apontou lacunas significativas, como a falta de colaboração entre países e a concentração de estudos em um número limitado de pesticidas e organismos, o que limita a generalização dos resultados obtidos.

Os resultados do estudo experimental evidenciaram respostas diferenciais entre as duas espécies, *Desmodesmus communis* e *Pseudopediastrum boryanum*, expostas ao herbicida clomazona (Gamit 360CS®) em cinco concentrações. Isso demonstra que as microalgas não reagem de maneira uniforme aos herbicidas. Em concentrações mais elevadas, *D. communis* apresentou maior sensibilidade, com redução de seus pigmentos fotossintéticos e aumento do estresse oxidativo. Em contrapartida, *P. boryanum* demonstrou maior tolerância, ativando mecanismos antioxidantes, como as enzimas SOD, CAT e GST. Esses resultados destacam o potencial de *P. boryanum* para aplicações em biorremediação. Os achados atestam que o uso excessivo de herbicidas, incluindo a clomazona, pode alterar gravemente as funções ecológicas das microalgas, com potenciais impactos na cadeia trófica e na qualidade da água. De fato, essas conclusões reforçam a importância de regulamentações mais rigorosas em relação ao uso desses produtos em ambientes agrícolas, principalmente em regiões onde os ecossistemas aquáticos são particularmente vulneráveis, sugerindo o desenvolvimento de estratégias de manejo mais sustentáveis. Além disso, a seleção de espécies de microalgas tolerantes a herbicidas pode ser considerada como uma solução para a restauração de ambientes contaminados ou para a produção de biomassa em condições adversas. Certamente, os resultados deste estudo oferecem contribuições valiosas. No entanto, ele possui limitações, uma vez que se concentrou exclusivamente em duas espécies de microalgas e em uma formulação comercial específica de herbicida. Isso indica que futuras pesquisas devem ampliar o escopo dos estudos, explorando

uma maior diversidade de herbicidas, microalgas e condições ambientais, permitindo uma melhor compreensão dos impactos globais. Como perspectivas, é necessário investigar mais profundamente os efeitos crônicos dos herbicidas em diferentes espécies de microalgas e cianobactérias; aprofundar o conhecimento sobre os mecanismos moleculares envolvidos na tolerância de certas microalgas para reforçar sua utilização em aplicações de biorremediação; fomentar o aumento da colaboração internacional para uma melhor disseminação do conhecimento; e desenvolver abordagens metodológicas mais diversificadas e eficazes.