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**AVALIAÇÃO DO IMPACTO DA DRENAGEM ÁCIDA DE MINA (DAM) EM  
ORGANISMOS FOTOSSINTETIZANTES DE ESTUÁRIOS DE SANTA CATARINA**

Florianópolis – SC

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Dissertação submetida ao Programa de Pós-Graduação em Oceanografia da Universidade Federal de Santa Catarina como parte dos requisitos para a obtenção do Grau de Mestre em Oceanografia.

Orientador: Prof. Dr. Paulo Antunes Horta Junior.

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Victória Silvestre Corrêa

**Avaliação do impacto da drenagem ácida de mina (DAM) em organismos  
fotossintetizantes de estuários de Santa Catarina**

O presente trabalho em nível de Mestrado foi avaliado e aprovado, em 08 de março de 2024,  
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Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado  
adequado para obtenção do título de Mestra em Oceanografia.

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Coordenação do Programa de Pós-Graduação em Oceanografia

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Prof. Paulo Antunes Horta Jr., Dr. Orientador

Florianópolis, 2024

À minha mãe, Sonita, a primeira e mais importante botânica que eu conheci.

## RESUMO

Compreender a extensão dos danos causados pela poluição advinda da mineração de carvão em organismos fotossintetizantes e seus impactos em processos biológicos, químicos e biogeoquímicos de sistemas estuarinos é fundamental. A Bacia Hidrográfica do Rio Araranguá e Bacia Hidrográfica do Rio Urussanga, Sul do Brasil, são regiões que recebem poluentes da mineração de carvão que ocorre em áreas próximas, e carregam águas ácidas para o mar. O objetivo deste estudo foi examinar o impacto do efluente ácido da mineração de carvão, conhecido como drenagem ácida de mina (DAM), na qualidade da água do estuário do Rio Araranguá e Rio Urussanga; que recebem estes rejeitos, e na comunidade de carbono azul, marismas e manguezais de ecossistemas estuarinos de Santa Catarina, além de avaliar o potencial dessa comunidade em mitigar o impacto gerado pela acidificação. Foram realizadas análises de parâmetros biológicos, físico-químicos e biogeoquímicos destes estuários com foco na avaliação ecofisiológica e caracterização *in situ* e também por meio de experimentos controlados em laboratório. As comunidades de plantas estudadas em marismas e manguezais apresentaram comprometimento de sua saúde pela ação da DAM, através de redução da atividade fotossintética, bem como, redução nas taxas de crescimento relativo. Na Bacia Hidrográfica do Rio Araranguá e Bacia Hidrográfica do Rio Urussanga a espécie fotossintetizante dominante foi *Schoenoplectus americanus*. Em experimentos controlados em laboratório também houve redução na atividade fotossintética de *Spartina alterniflora* em contato com a drenagem ácida de mina. *Elodea* sp. também se mostra como uma espécie sensível ao contato com a DAM, uma vez que apresenta redução em suas taxas de crescimento relativo ao ser exposta ao efluente ácido. A flora das áreas de estudo selecionadas para análise apresentou-se como estabilizadora física e química de solo, tamponando a água intersticial e aumentando o pH, em comparação com regiões sem a presença destas comunidades de plantas. O fortalecimento de políticas públicas para preservação e restauração destes ecossistemas estuarinos, bem como uma transição justa para formas limpas de se produzir energia, sem a utilização de combustíveis fósseis e sem a geração destes rejeitos, são necessárias para evitar o comprometimento da saúde de espécies fotossintetizantes, assim como a perda de biodiversidade dos serviços ecossistêmicos associados.

**Palavras-chave:** Marisma, Manguezal, Vegetação, pH, Biorremediação

## ABSTRACT

Understand the extent of damage caused by pollution from coal mining activity on photosynthetic organisms and its impacts on biological, chemical and biogeochemical processes in estuarine systems is fundamental. The Araranguá Drainage Basin and the Urussanga Drainage Basin, Southern Brazil, are regions that receive pollutants from coal mining activity that takes place in nearby areas, and carry acidic waters towards the sea. The aim of this study was to examine the impact of the coal mining effluent, known as acid mine drainage (AMD), on the water quality of the Araranguá Drainage Basin and Urussanga Drainage Basin estuaries; that receive these acidic wastes, and in the blue carbon community, salt marshes and mangroves of estuarine ecosystems in Santa Catarina, in addition to evaluating the potential of this community in mitigating the impact generated by acidification. Analyses of biological, physicochemical and biogeochemical parameters of these estuaries were carried out with a focus on ecophysiological assessment and characterization *in situ* and also through controlled experiments in the laboratory. The plant communities studied in salt marshes and mangroves presented compromised health to the action of the AMD, through a reduction in photosynthetic activity, as well as a reduction in relative growth rates. In the Araranguá Drainage Basin and Urussanga Drainage Basin, the dominant photosynthetic species was *Schoenoplectus americanus*. In controlled laboratory experiments there was also a reduction in the photosynthetic activity of *Spartina alterniflora* in contact with acid mine drainage. *Elodea* sp. also appears to be a sensitive species in contact with AMD, as it presents a reduction in its relative growth rates when exposed to the acidic effluent. The flora of the study areas selected for analysis presented itself as a physical and chemical soil stabilizer, buffering interstitial water and increasing pH, compared to regions without the presence of these plant communities. The strengthening of public policies for the preservation and restoration of these estuarine ecosystems, as well as a fair transition to green ways of producing energy, without the use of fossil fuels and without the generation of these wastes, are necessary to avoid compromising the health of photosynthetic species, as well as the loss of biodiversity and associated ecosystem services.

**Keywords:** Saltmarsh, Mangrove, Vegetation, pH, Bioremediation.

## **LISTA DE ABREVIATURAS E SIGLAS DA INTRODUÇÃO GERAL**

AMREC	Associação dos Municípios da Região Carbonífera
BC	Blue Carbon
CETEM	Centro de Tecnologia Mineral
DAM	Drenagem Ácida de Mina
IBRAM	Instituto Brasileiro de Mineração
PH	Potencial Hidrogeniônico



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## 1 INTRODUÇÃO GERAL

O carvão mineral é conhecido e utilizado desde os primórdios da história da humanidade por diferentes culturas. Na América do Norte, os primeiros registros datam do século XVII, descobertos no Apalache, região leste dos Estados Unidos (LASSON, 1972). Na Ásia, existem registros da utilização do carvão em 300 d. C. na China, e mais tarde, no século XIII, Marco Polo observou que o uso do minério estava difundido por todo o país, utilizado para aquecimento e metalurgia (DAEMEN, 2004). Na Europa, o primeiro registro escrito está na obra “Meteorologica”, de Aristóteles por volta de 300 a.C., (DAEMEN, 2004), onde o autor menciona a existência de corpos combustíveis que podem ser carbonizados (LEE, 1952).

Entretanto, a exploração e utilização do combustível fóssil torna-se um problema socioambiental a partir da Revolução Industrial (DAEMEN, 2004), onde toneladas de carvão começam a ser queimadas mundialmente (CHESTNEY, 2022), levando a emissões massivas de poluentes atmosféricos, como o gás carbônico (CO<sub>2</sub>) e óxidos de enxofre (BREEZE, 2015). Além disso, a hidrólise do carvão rico em enxofre produz rejeitos ácidos e metais dissolvidos, que impactam ecossistemas aquáticos e terrestres (CASTILHOS; FERNANDES, 2011), como a drenagem ácida de mina (DAEMEN, 2004).

### 1.1 A DRENAGEM ÁCIDA DE MINA (DAM)

A drenagem ácida de mina (DAM) é um passivo ambiental que surge a partir do descarte irresponsável de rejeitos da atividade mineradora de carvão (CASTILHOS; FERNANDES, 2011). A DAM possui alto potencial poluente e é um dos impactos ambientais mais severos que ocorrem em regiões mineradoras, assim sendo:

O carvão mineral é extraído em minas a céu aberto ou subterrâneas e enviado às usinas de beneficiamento, onde são gerados resíduos sólidos e líquidos. Estes, contendo grande quantidade de rejeitos finos, são direcionados para bacias de decantação. Em alguns casos, após a decantação, a água é lançada diretamente no corpo hídrico mais próximo. Os rejeitos grossos são dispostos, mais frequentemente, em áreas planas [...] e também utilizados na pavimentação de pátios (CASTILHOS; FERNANDES, 2011, p. 363).

A pirita (FeS<sub>2</sub>), mineral que é desassociado do carvão por métodos de separação física (CASTILHOS; FERNANDES, 2011), é um rejeito da mineração, uma vez que não possui valor

econômico verdadeiramente tentador para as mineradoras, sendo despejada em áreas abertas, sem o manejo ambiental correto. À céu aberto, a pirita reage com o oxigênio presente no ar atmosférico e com a água, presente na umidade do ar e na chuva, e vai sendo oxidada lentamente por diferentes reações químicas (EVANGELOU, 1998). Ao final do processo de oxidação, têm-se a formação de ácido sulfúrico ( $H_2SO_4$ ) e sulfato ferroso ( $FeSO_4$ ), e, com o passar do tempo, formam-se grandes piscinas de um rejeito aquoso ácido nas regiões onde houveram os depósitos piritosos (WEILER; SCHNEIDER, 2019). Este rejeito formado da oxidação da pirita, é conhecido como drenagem ácida de mina (DAM).

As características específicas da drenagem ácida podem variar de acordo com o mineral que é explorado (ROBB; ROBINSON, 1995), mas suas características imutáveis são os valores extremamente baixos de pH (FREITAS *et al.*, 2009; HAO *et al.*, 2017; LESSMANN *et al.*, 2000; LUÍS *et al.*, 2009; PRASANNA *et al.*, 2011) e a alta concentração de metais pesados, já que a acidez do efluente possui a capacidade de solubilizar metais na água intersticial do solo e adjacências (CASTILHOS; FERNANDES, 2011). Metais potencialmente tóxicos como: manganês, cobre, chumbo, cromo, mercúrio (UFRGS, 2000), arsênio, ferro (BRUNEEL *et al.*, 2006) e antimônio (LUÍS *et al.*, 2009) podem ser encontrados em regiões de DAM. Em regiões de mineração de carvão, especificamente, são observadas elevadas concentrações de ferro, manganês, sulfato e alumínio (ROBB; ROBINSON, 1995).

Os valores de pH da DAM variam geralmente de 2 a 3 (FREITAS *et al.*, 2009; HAO *et al.*, 2017; LESSMANN *et al.*, 2000; LUÍS *et al.*, 2009; PRASANNA *et al.*, 2011), de elevada acidez. Com o passar do tempo e o escoamento, o efluente pode penetrar no solo, dissolvendo nutrientes e minerais essenciais para os processos vitais de plantas, alterando os ciclos biogeoquímicos dos locais poluídos (RADOVIC; SCHOBERT, 1992). Esse efeito pode ser amplificado quando a DAM ou a água e solo que estiverem em contato com a mesma, escoam para os ecossistemas aquáticos adjacentes, com rios, lagos, e mesmo o lençol freático, impactando a integridade ambiental destes pela acidificação (RADOVIC; SCHOBERT, 1992). A entrada de grandes concentrações de metais pesados também compromete a fauna, aumentando os índices de mortalidade de peixes e moluscos (RADOVIC; SCHOBERT, 1992). Em contato com o solo ou com a água, haverá a mudança no potencial hidrogeniônico (pH) destes ambientes, que serão acidificados.

A combinação de baixos valores de pH e altas concentrações de metais pesados, compromete a fauna e flora das regiões impactadas, fazendo com que haja grande perda de biodiversidade local, comprometimento da saúde dos ecossistemas (RADOVIC; SCHOBERT, 1992), alteração e redução na riqueza de espécies (FREITAS *et al.*, 2009). O passivo ambiental

gerado pela mineração de carvão por si só é um poluente complexo, e a entrada do passivo em diferentes ecossistemas faz com que seja difícil prever e tratar a extensão dos danos que serão gerados (ROBB; ROBINSON, 1995).

Em algumas minas, a drenagem ácida demora muito tempo para ser detectada e pode continuar sendo gerada por décadas, mesmo após a desativação dos sítios mineradores (MONCUR *et al.*, 2005). O termo “*yellow boy*” pode ser visto na literatura associado à drenagem ácida de mina (RAVENGAI *et al.*, 2005), pois em alguns casos o efluente pode adquirir uma coloração amarelo-alaranjada por conta do hidróxido férrico presente nos rejeitos (ROBB; ROBINSON, 1995), descolorindo as águas naturais afetadas, direta e indiretamente, pela DAM.

## 1.2 O CARVÃO

O carvão mineral é descrito como uma rocha sedimentar constituída principalmente de carbono (VONCKEN, 2020), possuindo também constituintes inorgânicos, como minerais, associados à sua composição e estrutura (RADOVIC; SCHOBERT, 1992). O carvão é formado a partir de restos de plantas e algas de ambientes pré-históricos, por um processo chamado de coalificação (VONCKEN, 2020). Estes fragmentos vegetais devem ser soterrados e compactados em ambientes pobres ou ausentes de oxigênio, geralmente pântanos, para evitar a decomposição completa (VONCKEN, 2020). Após o soterramento e compactação, estes restos orgânicos precisam estar em condições específicas de pressão e temperatura para se transformarem em carvão, e diferenças nas condições físicas as quais o material é submetido podem levar à formação de carvões com propriedades físico-químicas diferentes (LASSON, 1972; RADOVIC; SCHOBERT, 1992). A formação do carvão deu-se há algumas dezenas ou milhões de anos atrás (RADOVIC; SCHOBERT, 1992), e, na natureza, o minério encontra-se presente em formações geológicas que variam dos períodos Carbonífero até o Mioceno (358,9 até 5,33 milhões de anos antes do presente) (VONCKEN, 2020).

A maioria dos carvões possuem algum tipo de mistura mineral associada a eles, e a presença de enxofre é variável de acordo com o tipo de carvão (DAEMEN, 2004; RADOVIC; SCHOBERT, 1992). É comum a ocorrência de enxofre pirítico na rocha sedimentar encontrada na Bacia Carbonífera Catarinense, no Extremo Sul do Brasil. O enxofre pirítico aparece na forma de grãos do mineral pirita ( $\text{FeS}_2$ ) associado à rocha (RADOVIC; SCHOBERT, 1992), assim sendo:

A maioria da pirita no carvão foi formada por reações de ferro e de enxofre na água dos ambientes alagados ou na água que lavou o carvão à medida que ele se acumulava. Uma vez que a pirita está “associada” ao carvão simplesmente por incorporação mecânica ou mistura física com a parte orgânica do carvão, grande parte pode ser removida moendo o carvão para liberar os grãos de pirita, seguido por métodos físicos relativamente simples para separar os grãos de pirita das partículas do carvão (RADOVIC; SCHOEBERT, 1992, p. 199-120, tradução nossa).

No Brasil, as reservas de carvão mineral encontram-se localizadas majoritariamente nos estados da região Sul, e o estado de Santa Catarina possui cerca de 3,3 milhões de toneladas do minério em seu território (BELOLLI *et al.*, 2002; UFRGS, 2000). A Bacia Carbonífera está localizada na região sudeste do estado, e é considerada a maior reserva mineral de carvão brasileiro, possuindo um comprimento maior que 85 km e largura entre 5 e 20 km (CASTILHOS; FERNANDES, 2011). A Bacia Carbonífera Catarinense, localizada no Extremo Sul do estado, é composta por 12 municípios, de acordo com a Associação dos Municípios da Região Carbonífera (AMREC, 2024). Fazem parte da Bacia Carbonífera: Criciúma, Balneário Rincão, Içara, Orleans, Cocal do Sul, Lauro Müller, Siderópolis, Morro da Fumaça, Treviso, Forquilha, Nova Veneza e Urussanga, e embora nem todos os municípios ainda possuam minas em seu território, todos participam direta ou indiretamente de diferentes etapas da atividade mineradora.

No início do século 19 é descoberta a existência no minério na região Sul, e geólogos foram enviados para analisar a qualidade do material das jazidas. Mesmo não sendo um carvão de alta qualidade, iniciou-se a extração (MONTEIRO, 2020). O carvão encontrado na Bacia Carbonífera Catarinense é classificado como hulha (BELOLLI *et al.*, 2002), e possui de 69 a 86% de teor de carbono em sua composição (GRAMMELIS *et al.*, 2016). A hulha é um tipo de carvão betuminoso, formado cerca de 300 milhões de anos atrás (RADOVIC; SCHOEBERT, 1992). Inicialmente, o carvão catarinense era extraído nas encostas de morros e riachos. Com o passar do tempo e o aumento da procura, surgiram então as primeiras carboníferas da região (MONTEIRO, 2020).

A atividade mineradora estimulou fortemente o desenvolvimento econômico do Sul do Brasil nos séculos 19 e 20, e, em 2020, cerca de 462 milhões de dólares foram investidos na importação do minério (IBRAM, 2021), de acordo com o Instituto Brasileiro de Mineração. Importantes questões histórico-políticas como a Primeira Guerra Mundial (BELOLLI *et al.*, 2002; DAEMEN, 2004), leis incentivando o uso do carvão mineral brasileiro, a criação de carboníferas na região Sul do Brasil durante o governo Vargas (MONTEIRO, 2020) e a crise

do petróleo nos anos 1970 (DAEMEN, 2004), ajudaram na exploração do combustível fóssil. À princípio, o carvão passa a ter grande importância econômica com a criação da máquina a vapor, durante o processo histórico-social chamado de Revolução Industrial, onde a combustão do carvão gerava energia térmica e mecânica (PAUL, 2004), podendo posteriormente ser convertida em eletricidade. Hoje, o carvão continua sendo utilizado para geração de energia em usinas termoelétricas (IBRAM, 2021), mas também como matéria-prima na indústria siderúrgica na fabricação do aço (Belolli et al., 2002; UFRGS, 2000).

As maiores reservas de carvão no mundo encontram-se localizadas nos Estados Unidos, Rússia, China, Austrália e Índia (ARAÚJO, 2014), e todos estes países aparecem associados a diversos tipos de problemas ambientais provenientes da atividade mineradora (DAEMEN, 2004). No Brasil, o estímulo à extração e exploração do minério também estimulou a degradação e poluição ambiental. Identificar a extensão dos danos ambientais que a mineração causa não é uma tarefa fácil, uma vez que diferentes etapas da atividade mineradora, como a pesquisa, a extração do carvão, seu beneficiamento, o transporte e a utilização podem ser realizados em municípios distintos (CASTILHOS; FERNANDES, 2011).

O carvão mineral é o combustível fóssil com maior potencial poluente da água, solo e atmosfera (REVESZ; LIENKE, 2016), e embora atualmente estejam disponíveis formas de se obter energia de maneira renovável, causando menos impacto ao meio ambiente, sua utilização e exploração continua sendo incentivada no Brasil (BRASIL, 2022). Ao longo de mais de 100 anos de exploração, diferentes tipos de impactos ambientais e sociais foram causados, e a partir dos anos 2000, muitas mineradoras catarinenses priorizaram políticas de recuperação e proteção ambiental (CARBONÍFERA BELLUNO, 2024; CARBONÍFERA CATARINENSE, 2024; CARBONÍFERA METROPOLITANA, 2024; RIO DESERTO, 2024). Entretanto, o que ainda se observa no Sul Catarinense é um grande rastro de degradação ambiental causado pela mineração de carvão, uma vez que a exploração do combustível fóssil continua a ser estimulada e nem todas as regiões impactadas foram ou estão sendo recuperadas.

### 1.3 A FLORA E A DAM

Dentre os produtores primários encontram-se espécies sensíveis ou resistentes às condições adversas impostas durante a exposição ou influência da DAM, com destaque aos organismos chamados de extremófilos (VARSHNEY *et al.*, 2015). Organismos extremófilos podem ser classificados de acordo com o parâmetro físico-químico extremo, como a acidez no



caso da poluição por DAM, a qual são submetidos e sobrevivem (VARSHNEY *et al.*, 2015). Podem ser vistas espécies de plantas vasculares e algas extremófilas em regiões de DAM (FREITAS *et al.*, 2009; HAO *et al.*, 2017; LESSMANN *et al.*, 2000; LUÍS *et al.*, 2009; PRASANNA *et al.*, 2011), e, os organismos que vivem nestas regiões, além de possuírem aparatos fisiológicos que os ajudam a sobreviver em ambientes mais inóspitos, também podem ser conhecidos como acidófilos ou ácido-tolerantes, uma vez que sobrevivem em baixos valores de pH.

Encontra-se baixa diversidade e riqueza de algas (FREITAS *et al.*, 2009; LUÍS *et al.*, 2009; VARSHNEY *et al.*, 2015) e plantas em ambientes poluídos pela drenagem ácida. A DAM possui a capacidade de causar impactos no funcionamento de organismos fotossintetizantes, observados através de perda de desempenho fisiológico, redução de estruturas morfológicas e comprometimento do crescimento em algumas espécies, como *Phragmites australis* e *Scirpus validus* (WU *et al.*, 2022b). Em *Eleocharis laevigulumis* observa-se acúmulo de metais pesados em suas raízes e diminuição de tecido fotossintético, um indicativo de stress por altas concentrações de metais (SILVA, 2015).

#### 1.4 OS ECOSISTEMAS COSTEIROS DE TRANSIÇÃO E A DAM

A distribuição de marismas e manguezais abrange praticamente todo o litoral brasileiro. Enquanto as marismas predominam na zona subtropical, os manguezais prevalecem na zona tropical e equatorial equivalente (SCHAEFFER-NOVELLI, 1999). Ecossistemas costeiros como manguezais e marismas são reconhecidos como Blue Carbon (BC), pois possuem uma capacidade maior de sequestrar e estocar carbono (DESTRI, 2023), e a vegetação, solo e água associados a estes ambientes desempenham papel importante no armazenamento de carbono (NOAA, 2022) e no tamponamento do sistema (CABRAL *et al.*, 2021). São ecossistemas complexos, altamente resilientes e considerados como um dos ecossistemas mais produtivos do planeta (VALE; SCHAEFFER-NOVELLI, 2018).

O sequestro do carbono ocorre durante a fotossíntese, quando o dióxido de carbono é absorvido pelas macrófitas e convertido através de reações metabólicas e de sintetização em glicose e oxigênio (BOWYER; LEEGOOD, 1997). Assim, ocorre diminuição nas concentrações de dióxido de carbono na atmosfera e transformação do carbono em moléculas orgânicas que compõem a estrutura morfológica das plantas (WHITTINGHAM, 1970). O oxigênio, um dos produtos das reações, é liberado na atmosfera e torna-se disponível para ser utilizado por outros organismos, incluindo os próprios fotossintetizantes durante a respiração

celular (YAMORI, 2020). E a remoção de dióxido de carbono que ocorre através da fotossíntese em ecossistemas costeiros de transição pode criar um efeito detectável no pH da água (SANTOS *et al.*, 2021).

Ecossistemas costeiros de transição como marismas e manguezais, são descritos como ambientes que sofrem amplas flutuações ambientais relacionadas aos fatores físico-químicos, como a influência dos ciclos de subida e descida das marés, que interfere na amplitude térmica e na variação de salinidade, também relacionada com a biodisponibilidade de metais (PINTO-COELHO; HAVENS, 2014), e estão fortemente associados à corpos de águas estuarinos ou águas costeiras (VALE *et al.*, 2018). Marismas e manguezais também podem liberar carbono e nutrientes através de trocas que ocorrem a cada ciclo de mudança das marés (SANTOS *et al.*, 2021).

Os marismas e manguezais do Brasil abrigam cerca de 170 espécies vegetais (BINFARÉ, 2016; SCHAEFFER-NOVELLI, 1999), e a maioria das marismas é dominada por poucas ou apenas uma espécie de macrófitas (SCHAEFFER-NOVELLI, 1999). A morfologia das raízes destes ecossistemas faz com que o sedimento fique preso em seus espaços vazios, e desta forma os poluentes que escoam até estes ambientes também ficam presos no solo do manguezal (VALE *et al.*, 2018). Assim, o manguezal previne e minimiza a poluição sobre a região estuarina, melhorando a qualidade da água (SCHAEFFER-NOVELLI; JUNIOR, 2018).

São ecossistemas extremamente importantes pois abrigam grande diversidade e riqueza biológica, uma vez que servem como berçário para espécies de crustáceos, moluscos e peixes, muitas vezes, endêmicos destes locais (SCHAEFFER-NOVELLI, 1999). São ambientes que também recebem animais migradores que passam ao menos uma fase do seu ciclo de vida, como a desova, por exemplo, nestas áreas (SCHAEFFER-NOVELLI, 1999), e a fauna local possui as adaptações necessárias para sobreviver às oscilações diárias de fatores abióticos comuns em marismas e manguezais (PINTO-COELHO; HAVENS, 2014).

Em ecossistemas costeiros acidificados ou impactados por altos teores de metais pesados associados à mineração de carvão e de outros minérios, pode ser observada a dominância de espécies como: *Spartina alterniflora*, *Spartina densiflora*, *Sarcocornia perennis*, *Limonium brasiliensis*, *Atriplex* sp. na Argentina (IDASZKIN *et al.*, 2017), *Spartina alterniflora* na China (PANG *et al.*, 2017), *Ruppia megacarpa*, *Zostera muelleri* na Austrália (FARIAS *et al.*, 2018), *Salicornia* spp. no Reino Unido (SMILLIE, 2015), *Cyperus articulatus* na Nigéria (ELIJAH; OHIMAIN, 2003), *Avicennia officinalis* e *Sonneratia alba* na Índia (NORONHA-D'MELLO; NAYAK, 2016), *Bruguiera cylindrica* e *Rhizophora mucronata* nas Filipinas (BARNUEVO, 2011) e *Phragmites australis* na África do Sul (VAN DEVENTER

and CHO, 2014). Em alguns locais impactados por diferentes tipos de drenagem ácida, a diversidade da vegetação pode ser reduzida em até 80% em relação aos locais próximos não impactados pela atividade mineradora (XU *et al.*, 2022).

Estuários são ambientes que podem, com o passar do tempo, servir como destino final dos produtos naturais ou antrópicos fruto das atividades humanas que ocorrem ao longo de toda a bacia hidrográfica (SILVESTRINI; D'AQUINO, 2020). As comunidades de vegetação, que se encontram nas proximidades de locais que servem como áreas de depósito de rejeitos de mineração, ajudam naturalmente a mitigar a contaminação de metais pesados causada pela drenagem ácida (IDASZKIN *et al.*, 2017).

Em marismas e manguezais, parte dos metais pesados são absorvidos pelas plantas existentes na região (IDASZKIN *et al.*, 2017; SILVA, 2015), sem o comprometimento total dos seus sistemas vitais. A capacidade fisiológica inerente a estas espécies resistentes à DAM é utilizada como base para a biorremediação do local, visando a melhoria da qualidade da água do sistema. *Phragmites australis* e *Typha latifolia*, macrófitas típicas de ecossistemas costeiros de transição, são utilizadas em tratamentos aeróbicos em regiões de minas abandonadas profundas (ROBB; ROBINSON, 1995). A manutenção da fisiologia de produtores primários que habitam regiões de DAM faz com que estes também sejam opções para aumentar o sequestro de carbono e reverter ou minimizar outros tipos de impactos ambientais associados à atividade mineradora (WU *et al.*, 2022).

Portanto, organismos fotossintetizantes de marismas e manguezais podem ajudar a minimizar os problemas advindos da exploração de carvão mineral e da entrada da drenagem ácida de mina em diferentes ecossistemas, por meio da absorção de metais pesados e aumento de pH na coluna d'água de ambientes acidificados. Assim, a acidificação e a toxicidade produzidas pela atividade mineradora poderiam ser remediadas de forma sustentável. Sabendo que o rejeito ácido da mineração do carvão possui a capacidade de causar variações na qualidade da água, a qual induz mudanças nas comunidades de regiões impactadas, este estudo visa investigar os impactos da drenagem ácida de mina nos organismos fotossintetizantes em estuários subtropicais adjacentes ao Oceano Atlântico Sul Ocidental.

No Extremo Sul de Santa Catarina, A Bacia Hidrográfica do Rio Araranguá e a Bacia Hidrográfica do Rio Urussanga são consideradas intensamente impactadas pela atividade carbonífera, de acordo com o CETEM (2001 apud CASTILHOS; FERNANDES, 2011).

## 1.5 HIPÓTESE

O presente estudo parte da hipótese de que espécies de macrófitas variam de acordo com a tolerância que possuem para sobreviver em ambientes aquáticos poluídos e com características físico-químicas extremas, como baixos valores de pH e concentração elevada de metais. Considerando o cenário teórico, podemos pressupor que regiões impactadas pela drenagem ácida de mina possuem perda da riqueza específica e mudança na fisiologia, estrutura e composição das comunidades fotossintetizantes, com favorecimento de espécies acidófilas ou ácido-tolerantes, em comparação com regiões que não recebem efluentes ácidos da atividade mineradora. Ademais, o funcionamento das macrófitas de marisma possui a capacidade de aumentar o pH de ambientes acidificados pela atividade carbonífera.

## 2 OBJETIVOS

### 2.1 OBJETIVO GERAL

A presente dissertação tem por objetivo geral contribuir para o entendimento dos impactos da DAM sobre a flora de ambientes aquáticos costeiros, como foco nas marismas, a partir de experimentos de campo e de laboratório. Assim, pretende-se analisar parâmetros biológicos, físico-químicos e biogeoquímicos de estuários impactados pela drenagem ácida de mina na região Sul do Brasil, com a finalidade de examinar o impacto do efluente ácido nos organismos fotossintetizantes das regiões afetadas.

### 2.2 OBJETIVOS ESPECÍFICOS

- a) Realizar a identificação taxonômica (gênero e espécie) de macrófitas e comparar a diversidade de organismos entre locais impactados e locais controle (CAPÍTULO 1);
- b) Caracterizar a eficiência fotossintética (rendimento quântico fotossintético) das macrófitas e comparar os valores em situações e/ou locais impactados e em situações e/ou locais controle (CAPÍTULO 1 e CAPÍTULO 3);
- c) Quantificar os teores de pigmentos fotossintetizantes e comparar os valores entre situações impactadas e situações controle (CAPÍTULO 3);
- d) Quantificar a biomassa aérea e radicular e comparar os valores entre situações e/ou locais impactados e situações e/ou locais controle (CAPÍTULO 1 e CAPÍTULO 3);
- e) Caracterizar as taxas de crescimento relativo e comparar os valores entre situações impactadas e situações controle (CAPÍTULO 2);
- f) Analisar parâmetros físico-químicos da água, como: pH, temperatura, concentração de oxigênio e salinidade e comparar os valores em situações/locais impactados e em situações/locais controle (CAPÍTULO 1 e CAPÍTULO 3);
- g) Examinar parâmetros biogeoquímicos como: teor de carbonato no sedimento e concentração de nutrientes na coluna d'água e comparar os valores entre situações impactadas e controle (CAPÍTULO 3).

## CAPÍTULO 1

(Submetido à *Aquatic Botany* em novembro/2023, formatado de acordo com os padrões do periódico).

### **Acid mine drainage: the contemporary status of affected estuaries in southern Brazil**

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**Abstract:** Acid mine drainage (AMD) produces one of the most severe environmental impacts related to coal mining. In Southern Brazil, the Araranguá Drainage Basin and the Urussanga Drainage Basin, are highly polluted by the mining activities. In acidified places, there is a low diversity of photosynthetic organisms, and water quality becomes compromised. Furthermore, acid wastes negatively impact the morphology and physiology of the plants. In locations impacted by different types of acid drainage, vegetation diversity may be reduced in relation to nearby locations not impacted by mining activity. Therefore, it is necessary to investigate the impacts of AMD contamination on the composition and ecophysiology of the estuarine flora in Southern Brazil. For this purpose, estuaries impacted by coal mining residues and non-impacted estuaries, used as study controls and located nearby, with similar characteristics were analyzed. Vegetation samples were collected for analysis of biomass and maximum photochemical quantum yield of photosystem II (Fv/Fm). Water samples were collected to analyze pH, salinity, oxygen concentration and temperature. The dominant macrophyte in both locations impacted by AMD was *Schoenoplectus americanus*, and non-impacted places no pattern was found. A two-way ANOVA showed significant differences mainly for the Fv/Fm, where plants from both impacted estuaries presented lower Fv/Fm values in relation to the control estuaries, indicating possible impairment of photosynthetic activity due to the entry of the AMD. pH analysis suggests that the presence of vegetation in estuaries can function as a buffering system, increasing the pH of the water that is acidified by the mining wastes.

**Keywords:** Salt marsh, Cyperaceae, *Scirpus americanus*, Tidal marsh, River Basin

## 1. Introduction

Mineral coal is a fossil fuel that was fundamental to the economic growth of southern Brazil, and has been explored since 19<sup>th</sup> century (Zanette and Camilo, 2018). Although it is recognized as the fuel with the greatest polluting potential (Revesz and Lienke, 2016) and its burning is responsible for a large part of greenhouse gas emissions (Pandey et al., 2018), exploration and use continues to be encouraged in Brazil (Teixeira, 2022).

In a context of advancing climate change and imminent inflection points that could destabilize biosphere homeostasis (Armstrong Mc et al., 2022), identifying stressors and their impacts on the functioning of different systems is urgent. Acid mine drainage (AMD), with pH values often between 2-3 (Freitas et al., 2009; Lessmann et al., 2000; Luís et al., 2009; Prasanna et al., 2011), produces one of the most severe environmental impacts related to coal mining activity. Environmental liabilities arise from the oxidation of minerals, such as pyrite (Radovic and Schobert, 1992), present in coal sedimentary rock and which are separated during coal processing (Castilhos and Fernandes, 2011). After separation, the pyrite is discarded without adequate environmental management in areas close to mining sites, and over time, its oxidation forms large pools of an acidic aqueous waste composed of sulfur acid and ferric hydroxides, which receives the name of AMD (Iatan, 2021).

In addition to the low pH, the effluent from mining activities has high concentrations of heavy metals, and the combination of these characteristics makes it difficult to predict and treat the waste that is generated and its impacts on ecosystems (Daraz et al., 2023; Robb and Robinson, 1995). In coal mining regions, there are high concentrations of iron, manganese, sulfate and aluminum (Robb and Robinson, 1995). These metals, when present in AMD, can alter biogeochemical cycles of essential nutrients, interfering with the availability of nutrients and the geochemical distribution of metals in soil and water (Castilhos and Fernandes, 2011), compromising the health of aquatic ecosystems (Robb and Robinson, 1995).

Anthropogenic disturbances, such as those mentioned above, have the capacity to shape the vegetation of environments (Taylor, 2016), and in places acidified by AMD, there is a low diversity and richness of photosynthetic organisms, such as algae (Freitas et al., 2009; Luís et al., 2009; Varshney et al., 2015) and plants. Contact with AMD impacts the physiology and morphology of the plants, causing growth reduction or inhibition in *Phragmites australis*, *Scirpus validus* (Cyperaceae), *Typha orientalis* (Typhaceae), *Cyperus glomeratus* (Cyperaceae) (Wu et al., 2022b) and *Punica granatum* (Lythraceae) (Hajihashemi et al., 2023).

The absorption of heavy metals from AMD in the aerial and belowground structures of vascular plants is already known, and the concentration of those metals may vary depending on seasonality (Mays and Edwards, 2001). Iron accumulation is observed in *Eleocharis laeviglumis* (Cyperaceae) (Silva, 2015) and *P. granatum* (Lythraceae) (Hajihashemi et al., 2023), and together with several other elements such as copper, lead and zinc in *Typha domingensis* (Typhaceae) (Basallote et al., 2023), *Typha latifolia* (Typhaceae), *Juncus effusus* (Juncaceae) and *Scirpus cyperinus* (Cyperaceae) (Mays and Edwards, 2001). Accumulation of copper and zinc is also reported for *Spartina densiflora* (Poaceae), *Sarcocornia perennis* (Amaranthaceae) (Idaszkin et al., 2017) and *P. granatum* (Hajihashemi et al., 2023). AMD can

also reduce the photosynthetic tissue of macrophytes, as in *E. laeviglumis* (Cyperaceae) (Silva, 2015), and alter photosynthetic activity in some species, compromising its functioning (Hajihashemi et al., 2023).

AMD offers to flora the most challenge conditions to thrive (Ghosh et al., 2017), and in locations impacted by different types of drainage, vegetation diversity can be reduced by up to 80% compared to nearby locations not impacted by mining (Xu et al., 2022). The diversity of salt marshes, communities dominated by macrophytes (Schaeffer-Novelli, 1999), in estuaries impacted by high levels of heavy metals is reduced, predominantly encompassing the genus *Spartina* (Poaceae) in Argentina (Idaszkin et al., 2017) and China (Pang et al., 2017).

Different botanical families can dominate AMD impacted environments, and the pollution from distinct ores sources may influence the family colonization. In Azerbaijan AMD areas, Astraceae and Poaceae were dominant (Ghorbani et al., 2018). In Australia, *Isolepsis subtellisima* (Cyperaceae) and *Phragmites australis* (Poaceae) were dominant (Buckney and Higgs, 1998), and *Eleocharis equisetina* (Cyperaceae) colonized abandoned mining places (Lottermoser and Ashley, 2011). In Indonesia, Cyperaceae (*Cyperus* sp. and *Fimbristylis* sp.) were often found around basins and humid substrates, and Poaceae (*Paspalum* sp. and *Echinochloa* sp.) were most present among stones and boulders (Novianti et al., 2017). In USA, AMD locations were dominated by *Typha latifolia* (Typhaceae) and *Equisetum* spp (Equisetaceae) (Tarutis et al., 1992).

Generally, the same families that have the capacity to colonize and dominate impacted environments, are also used for water phytoremediation (RoyChowdhury et al., 2015), since they present high resilience and physiological features that support stressed places. The genera *Typha*, *Scirpus* (homonym to *Schoenoplectus*), *Phragmites*, *Juncus* and *Eleocharis* appear as the most commonly macrophyte used worldwide to treat different types of water pollution (Vymazal, 2013) and are also known as species that grow and dominate environments acidified by AMD, according to (Dunbabin and Bowmer, 1992). *P. australis*, *T. orientalis*, *C. glomeratus*, *S. validus*, *Iris wilsonii* and *J. effusus* are recognized as acid-tolerant plants (Wu et al., 2022a).

Considering the ability of AMD to cause variations in water quality, and distinct responses in the composition of macrophyte communities and their physio-morphological performances, the aim of this study was to investigate the impacts of AMD contamination on the composition and ecophysiology of dominant flora species of estuaries in southern Brazil, specifically in the Araranguá Drainage Basin, Urussanga Drainage Basin and D'Una Drainage Basin.

## 2. Materials and methods

### 2.1 Study site

Sampling was conducted in four estuaries located in the river basins of Araranguá, Urussanga and D'Una rivers, in the State of Santa Catarina, southern Brazil, from South to North (Table 1, Fig. 1,2). River basins impacted and not impacted by coal mining activity in Santa Catarina were selected by similarities among them, so that they could later be compared and serve as study controls. However, there are weaknesses in this method, such as the difference in the



size and the flow of the rivers selected. All of these basins are located in a geomorphological region of the coastal plain type (Rocha, 2014). Geographically entangled with the Santa Catarina Carboniferous Basin (Menezes et al., 2019), the Araranguá Drainage Basin and the Urussanga Drainage Basin are highly impacted by mining activity, according to (Castilhos and Fernandes, 2011).

Drainage Basin	City	Estuary	Point	Influence of mining activity
Araranguá (ADB)	Araranguá	Balneário Ilhas	28°53'36.16"S 49°18'1.56"O	Impacted
Urussanga (UDB)	Urussanga	Barra do Torneiro	28°48'3.77"S 49°11'7.77"O	Impacted
D'Una (DDB)	Garopaba	Barrinha da Ferrugem	28° 4'57.06"S 48°37'55.61"O	Not Impacted (Control)
		Siriú	27°58'36.40"S 48°37'45.15"O	Not Impacted (Control)

Table 1: Location of the estuary and the respective drainage basin, and influence from the coal mining exploration.

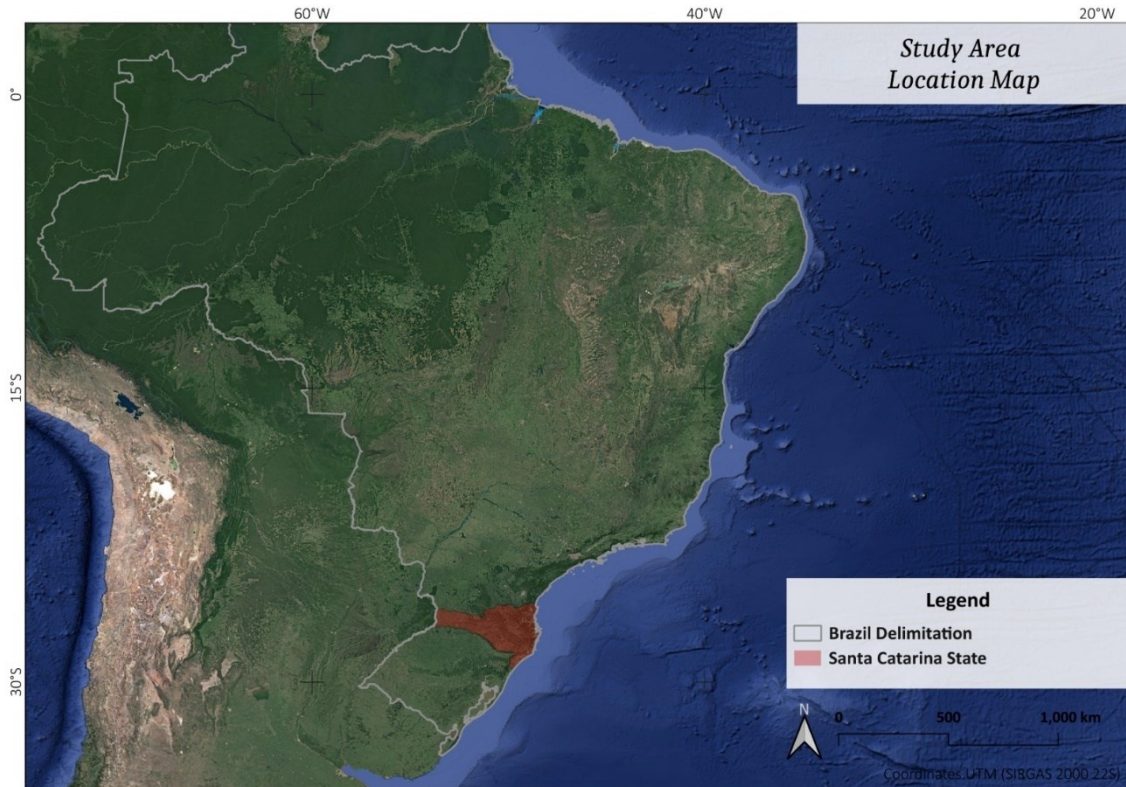


Figure 1: Location of Santa Catarina, Brazil, the study area.



Figure 2: Location of sampling points in the estuaries in the Santa Catarina State. Red pins: estuaries impacted by mining activity. White pins: estuaries non-impacted by mining activity.

The Araranguá Drainage Basin (ADB) has an area of 3,007 km<sup>2</sup> (Rocha, 2014) and currently has a high level of compromised quality, mainly caused by the entry of acidic sulfur residues from coal extraction (Rocha, 2014). The Urussanga Drainage Basin (UDB), located north in relation to the Araranguá River Basin, has an area of 620 km<sup>2</sup> (Rocha, 2014), and is also highly compromised in terms of environmental quality, especially when observing the set of polluting loads generated by coal mining (Rocha, 2014).

The D'Una Drainage Basin (DDB) has an area of 492 km<sup>2</sup>, it is located in a permanent preservation area and the majority of its right sides tributaries has good water quality conditions (Rocha, 2014). The D'Una Drainage Basin is used as a public water supply resource in the municipalities where it runs (Noldin et al., 2011), in addition to being used for crop irrigation, with a focus on rice farming, and for fishing activities (Furtado et al., 2000), not receiving effluents from mining activities. It is located northward in relation to the Araranguá and Urussanga Drainage Basins.

Therefore, among all four estuaries, we based this work in the hypothesis that the Araranguá Drainage Basin and Urussanga Drainage Basin will present lower richness and diversity of macrophyte species, as well as a greater impact on the vegetation and a lower water quality.

## 2.2 Sampling collections

During the first week of April 2022, from 04/01 to 04/02, four estuaries were sampled.

Vegetation sampling was conducted using transects, parallel to the estuary margin, and were established randomly at each sampling site in order to better represent plant communities on AMD impacted and non-impacted sites. Each sampling station was approximately 5 meters away from the other, and the sampling stations in all four estuaries were executed at similar distances upstream from the river mouth. Replicate samples were taken on the same side of the estuary at each site, as seen in the (Fig. 3), in equidistant points. The plants were collected using a shovel to a depth of 25 cm. For each sample, the area sampled was 625 cm<sup>2</sup>. The samples were cleaned with distilled water, and then packed into polyethylene bags, labeled and transported to the laboratory.

Biological and physical-chemical samplings were carried out, with replicates adapting the protocol from (Underwood, 1996). Biological and physical-chemical analyzes were performed at the Phycology Laboratory of the Federal University of Santa Catarina (LAFIC – UFSC).

Biological analyses consisted of taxonomic identification of the specimens and quantification of aerial and belowground biomass. Also, the maximum photochemical quantum yield of photosystem II (Fv/Fm) in macrophytes. *In situ*, after dark acclimation for 15 minutes, Fv/Fm was measured, using a DIVING-PAM – Heinz Walz. For the taxonomic identification were used dichotomous keys, and the identification was made primarily based on the macrophyte inflorescence. Specimens that did not have an inflorescence during the analysis were identified based on other morphological structures, such as leaves and roots. After identification, the macrophytes were separated (leaves, stem and roots) using scissors. The separated samples were oven-dried at 60 °C for 48 hours to attain a constant mass, after which the dry weights were measured.

Physical-chemical variables of the surface water, such as pH, salinity, oxygen concentration and temperature were determined using a YSI 7500 multiparameter probe and a pHmeter. pH, salinity and oxygen concentration were analyzed depending on its relationship with the presence (within the saltmarsh) or absence of vegetation (on the riverbed).

A total of 24 groups of samples were collected, 12 of which being control samples (from the non-impacted estuaries) and 12 collected in impacted areas. In each estuary, 6 samplings were carried out, 3 on the riverbed (10 meters from the bank/margin) and 3 on the saltmarshes banks, within the salt marsh vegetation (Fig. 3). The physical-chemical samples that were taken at the riverbed were collected at the same point as the macrophyte samples, but with a higher number of replicates.

Weather conditions during the collecting of the samples varied, with most data collection occurring during high tide and during the passage of a cyclone, except in Araranguá (impacted site).

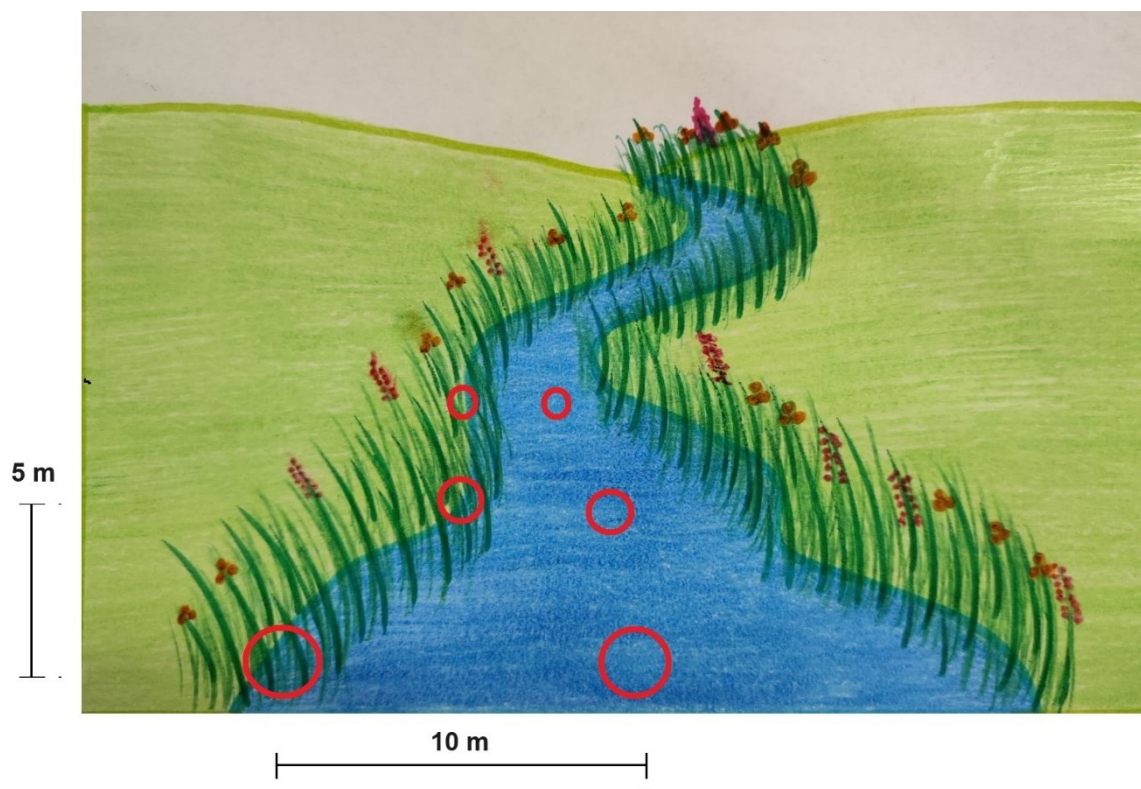


Figure 3: Scheme with the sampling design, illustrating the estuarine system with the salt marsh by the margins of the river. Red circles: sampling points.

### 2.3 Statistical analyses

For the synthesis and integration of all descriptors and environmental variables, statistical analyses were performed using the TIBCO Statistica software, version 13.5.0.17. Univariate analyses were carried out to better interpret the data and compare the study regions, such as two-factor analysis of variance (ANOVA) followed by the Newman-Keuls *a posteriori* test, respecting the assumptions for parametric analyses. For all analyses, a 5% level of significance ( $p < 0.05$ ) was used.

For the two-way ANOVA, the two factors used to observe whether or not there was an interaction were: absence or presence of vegetation and the estuaries sampled. The dependent variables of this test were: pH, O<sub>2</sub> and salinity.

For the one-way ANOVA, the factor used to observe whether or not there was interaction with vegetation was: absence or presence of coal mining activity at the sampled points. The dependent variables of this test were: maximum photochemical quantum yield of photosystem II, aerial biomass and belowground biomass.

## 3. Results

### 3.1 Vegetation sampling

### 3.1.1 Richness variation

Five plant species were identified in the four estuaries sampled, with a predominance of the Cyperaceae family, in 3 of the 4 points analyzed (Table 2).

Drainage Basin	Estuary	Species	Family	Influence of mining activity
Araranguá	Balneário Ilhas	<i>Schoenoplectus americanus</i>	Cyperaceae	Impacted
		<i>Crinum americanum</i>	Amaryllidaceae	
Urussanga	Barra do Torneiro	<i>Schoenoplectus americanus</i>	Cyperaceae	Impacted
		<i>Crinum americanum</i>	Amaryllidaceae	
		<i>Bacopa</i> sp.	Plantaginaceae	
D'Una	Barrinha da Ferrugem	<i>Bulboschoenus maritimus</i> var. <i>robustus</i>	Cyperaceae	Not Impacted (Control)
	Siriú	<i>Paspalum vaginatum</i>	Poaceae	Not Impacted (Control)

Table 2: Specimens collected in each one of the estuaries sampled.

*S. americanus* dominated both estuarine environments of Araranguá Drainage Basin and Urussanga Drainage Basin. The Urussanga Drainage Basin, one of the places that receives mining effluents, showed the greatest species richness, totaling 3 species.

The lowest richness was observed in the D'Una Drainage Basin, in the location of Ferrugem and in Siriú, with only 1 species per region sampled.

### 3.1.2 Biomass quantification

Belowground biomass (Fig. 4a) did not show a significant difference among all sampling points ( $n = 3$ ), although Araranguá and Siriú showed a tendency towards a state of significant difference. The highest belowground biomass values were found in DDB, with the Siriú ( $n = 3$ ) point presenting 726 grams of dry biomass per plant. Aerial biomass (Fig. 4b) showed significant difference between regions polluted by AMD, with the plants from UDB showing higher biomass in comparison to ADB. In Urussanga, the aerial biomass value was 85.6 g. Aerial biomass also showed significant difference between control regions, with the Siriú point, at DDB, having higher aerial biomass in comparison with the Ferrugem point. At Siriú it was found 188 g of dry biomass per plant. At Ferrugem, it was found 42.3 g of dry biomass per plant.

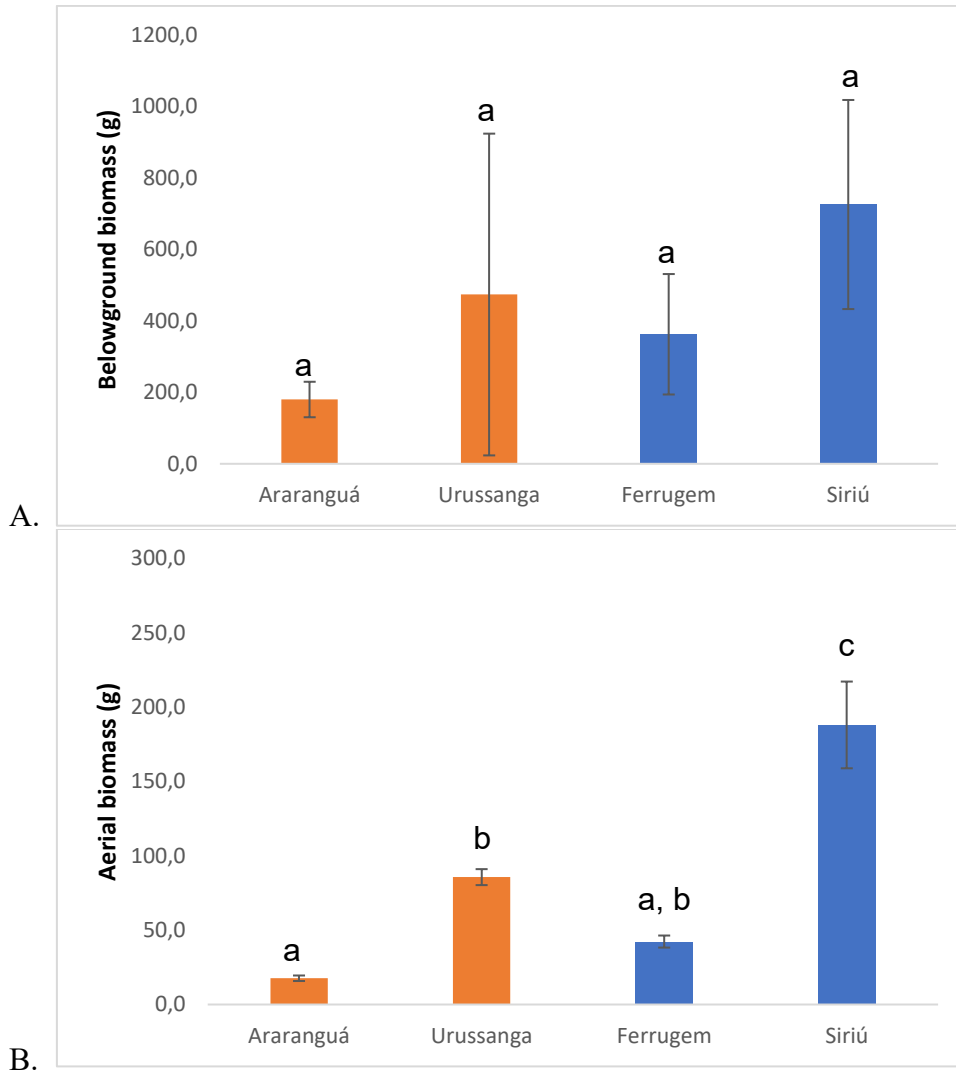


Figure 4: (a) Belowground biomass of the plants in each one of the estuaries sampled. (b) Aerial biomass of the plants in each one of the estuaries sampled (where: different letters represent significant differences related with the ANOVA and the Newman-Keuls Test).

### 3.1.3 Maximum quantum yield on PS II

The photochemical quantum yield of photosystem II showed a significant difference between all sampling points (Fig. 5a), with the flora from ABD and UDB, the locations impacted by coal mining activity, presenting the lowest  $F_v/F_m$  values. Macrophytes from UDB presented the lowest value, 0.679, and in the DDB, Siriú point, the highest value was seen, 0.876.

### 3.2 Physical-chemical parameters

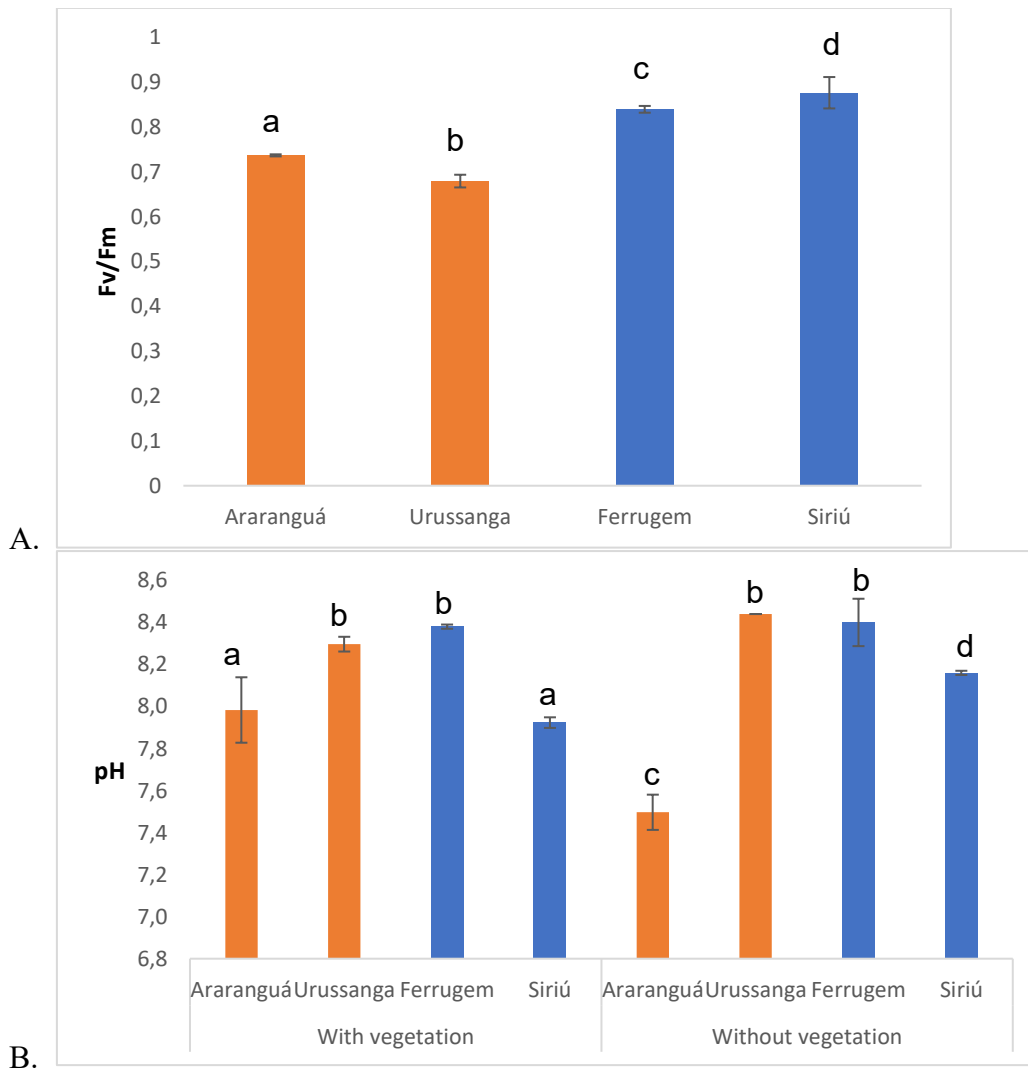
The pH of the water (Fig. 5b) showed significant differences in regions with the presence of macrophytes, in places such as ADB and DDB, Siriú point. In Araranguá the pH in the presence and absence of vegetation varied from 7.98 to 7.49, respectively. In Siriú, the pH in the presence and absence of vegetation varied from 7.92 to 8.16, respectively. The pH ranges in places with

macrophytes varied from 7.92 to 8.38. In places without macrophytes, the pH variation ranges from 7.49 to 8.44.

The dissolved oxygen in the water column (Fig. 5d) showed significant differences at UDB only. At UDB, in the presence of the macrophytes, the dissolved oxygen value was 6.7 mg/L, and in the absence of macrophytes, 8 mg/L. The lowest dissolved oxygen value was 6.5 mg/L at DDB, Ferrugem point, in the presence of macrophytes. In the absence of macrophytes, the lowest value found was 6.5 mg/L, also at Ferrugem point. Comparing places impacted by AMD with and without vegetation, there was significant difference between them, with higher values at UDB in comparison to ADB.

In relation to the salinity of the water (Fig. 5c), in places with vegetation, there were no significant differences between the points impacted by the ADM. The control points present a significant difference between them, with the lowest value found in DDB, at Siriú point, with 18.86 and the highest value also in DDB, at Ferrugem point, with 34.4. This pattern was repeated for places without vegetation.

The arithmetic average water temperature values found were: 21° C in Araranguá, 22° C in Urussanga, 22.9° C in Ferrugem and 20.3° C in Siriú.



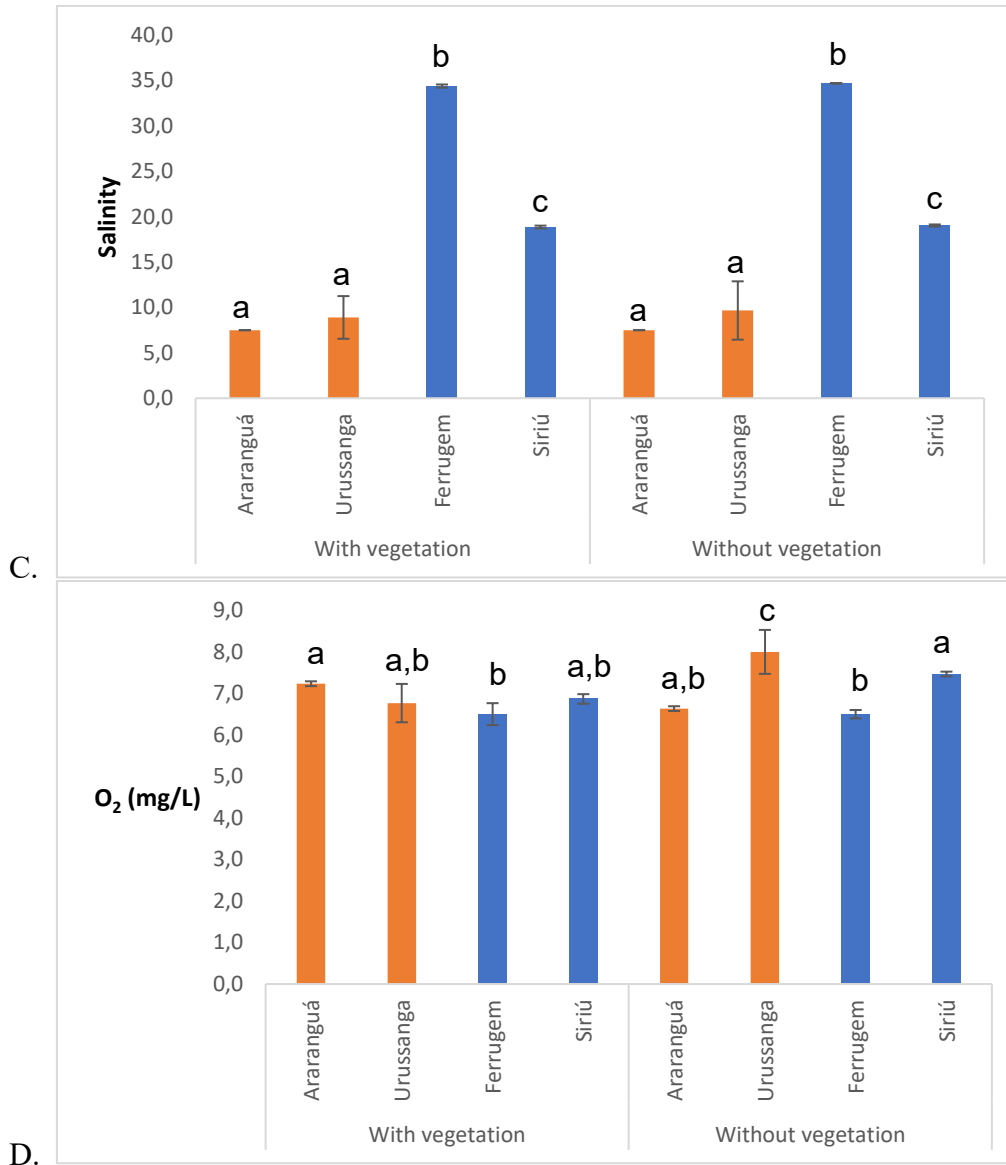


Figure 5: (a) Maximum photochemical quantum yield of PS II of the plants in each one of the estuaries sampled. (b) pH of the water, comparing regions with and without vegetation, in each one of the estuaries sampled. (c) Salinity of the water, comparing regions with and without vegetation, in each one of the estuaries sampled. (d) Dissolved oxygen of the water, comparing regions with and without vegetation, in each one of the estuaries sampled (where: different letters represent significant differences related with the ANOVA and the Newman-Keuls Test).

## 4. Discussion

### 4.1 Vegetation analysis

The flora composition was mostly dominated by the Cyperaceae family in the three drainage basins sampled. In both places impacted by coal mining activity, ADB and UDB, we found



that the salt marsh environment was dominated by *Schoenoplectus americanus*. Another species that colonized both AMD impacted places, but were seen with lower frequency, was *Crinum americanum*. In the places that are not impacted by coal mining activity, such as DDB, Cyperaceae and Poaceae family species were seen. At Ferrugem point, *Bulboschoenus maritimus* var. *robustus* (Cyperaceae) dominated the estuarine environment and at the Siriú point, *Paspalum vaginatum* (Poaceae). The higher richness of species in places impacted by AMD in comparison to places that are not impacted by coal mining activity (3 species vs. 1 species) appeared as a surprise. The aerial and belowground biomass quantification showed a tendency to higher values at the DDB, Siriú point. Photochemical quantum yield of photosystem II showed a significant difference between all sampling points, with the species from places impacted by AMD, ADB and UDB, showing the lowest values for Fv/Fm. The results found suggests that the entry of AMD into the estuarine ecosystem can cause changes in the photosynthetic activity of the macrophytes, and this may also affect the aerial biomass.

Recognition of the flora in mining regions is imperative for the preservation of biodiversity, as well as the vegetation restoration (Ghorbani et al., 2018), and the increased flora richness of estuaries impacted by acid mine drainage could be explained by the intermediate disturbance theory. According to the theory, the most biodiverse photosynthetic communities are maintained through intermediate levels of disturbance, in opposition to the idea that the greater variety of species is going to exist only in a state of equilibrium (Connell, 1978). Hypothesis about equilibrium and non-equilibrium states are not mutually exclusive, therefore into the same ecosystem may happen variations in resources that enable different species to coexist at distinct levels of equilibrium (Connell, 1978). Together with non-equilibrium states in ecosystems, the difference between points under the influence of the AMD and control points can be explained on the phenotypic and genotypic plasticity that some species have (Veldkornet et al., 2016). This mechanism allows that macrophytes deal with fluctuations, natural or anthropogenic, that occur in ecosystems (Veldkornet et al., 2016).

From the point of view of groups observed in the study area, the Cyperaceae family is recognized for being cosmopolitan, and can be easily found in floodable aquatic environments around the world, such as salt marshes and mangroves (Judd et al., 2009). Although physical, chemical and biological features of AMD places do not support the growth of many plants, there are particular species and families present on these substrates (Novianti et al., 2017). The botanic family Cyperaceae is one of the most known for bear AMD impacts (Buckney and Higgs, 1998; Lottermoser and Ashley, 2011; Novianti et al., 2017), and is also used in phytoremediation treatments (RoyChowdhury et al., 2015; Vymazal, 2013). Our results showed that in the estuaries impacted by AMD in Southern Brazil there was dominance by the Cyperaceae family, but future studies are needed since the number of replicates in this study was low and may not correspond to the real environmental pattern of phytodominance.

Photosynthetic organisms from salt marshes have developed a variety of physiological adaptations to survive and grow in these stressed ecosystems (Batty, 1999), but physiological, biochemical and morphological processes are negatively affected due to heavy metals and low pH present in AMD areas (Daraz et al., 2023). The reduction or limitation on the growth, which was partially seen in the biomass quantification, especially in lower values in aerial biomass data for AMD places, is related to the concentration of AMD, and even acid-tolerant species have different rates to support AMD concentrations. The genus *Scirpus* can be tolerant to low concentrations of heavy metals (Wu et al., 2022b). In a study about *Scirpus*

*cyperinus*, differences at the level of population were found in the aerial biomass parameters. One population had significantly higher growth than the other population of the same species, when growing in AMD (Demchik and Garbutt, 1999), and this information may explain why *Schoenoplectus americanus* showed a tendency to have a higher aerial biomass in Urussanga than in Araranguá, both regions impacted by coal mining activity. However, other factors may explain the variation in the biomass values, which may be related to the large variation of salinity in the estuaries. And it was not necessarily the AMD that determined the differences in species and biomass values in these specific points, but also the salinity. Although, more studies need to be carried out for this to be truly confirmed.

The photosynthetic process is significantly affected by heavy metals from AMD in vascular plants, causing changes in the light-harvesting protein complexes (Ghosh et al., 2017), and reducing the photosynthetic activity (Hajihashemi et al., 2023). In circumstances of environmental impact, the differences imposed by vegetation can be very large, and this type of behavior described above allows us to hypothesize that in AMD impacted areas, the quantum efficiency of PSII is going to be affected. We can speculate that the vegetation has part of its physiological system compromised by the entry of pollutants from AMD, observing the data acquired in this study, where the macrophytes from both AMD impacted places have lower Fv/Fm values in comparison to both control places. However, the response obtained by the macrophyte communities may also refer to potential natural and anthropogenic factors in the adjacent regions.

#### 4.2 Physical-chemical parameters

The presence or the absence of macrophytes influenced the pH of the water in AMD impacted places, such as ADB, but also in control places, such as DDB, at Siriú point. In Araranguá, the presence of plants can increase the pH in 0.49. In Siriú, the pH increases in places without vegetation, and one of the explanations may be related to the different species that colonize those places. In general, a tendency that was noted in our study is that places with macrophytes have lower pH variation gradient, suggesting that the presence of vegetation in estuaries may act as a buffering system, increasing the pH of the water that is polluted by AMD.

Although the water acidity influenced by AMD can not be fully proven in our study for the chosen sampling points, previous studies showed the impact of AMD in the ADB (Couceiro and Schettini, 2010; Silvestrini and D'Aquino, 2020) and UDB (Schnack et al., 2018). The estuarine region, as it is highly dynamic and has great variability in physical-chemical variables (Silvestrini and D'Aquino, 2020), may be buffering the acid effluent that is released into the rivers, by the action of the carbonate system. Our results may also be related to salinity, represented by the carbonate system, and also by distance from the AMD sources.

The dissolved oxygen data showed significant differences comparing regions with and without vegetation only at the UDB, which had the highest values. Those data were collected in the presence of a diatom bloom, which was identified from the brown foam, which indicates the presence of fucoxanthin, and what can be seen in the graph may be the interference of the high oxygen production by the phytoplankton.

Salinity of the water showed significant difference only in the control points, and the vegetation does not seem to have an impact on it. The higher salinity values that were seen in DDB, at Ferrugem e Siriú, may also be explained by the presence of high waves and the strong entry of the tides into the estuary during the sampling procedure.

Estuaries are dynamic environments that can, over time, serve as the final destination of anthropogenic substances resulting from human activities that occur throughout the river basin, such as AMD (Silvestrini and D'Aquino, 2020). Salt marshes serve as important providers of ecosystem services, improving water quality and mitigating the AMD effects (Dean et al., 2013), and wide environmental fluctuations in abiotic parameters, such as salinity and oxygen concentration in the water column, are characteristics of the estuarine region (Pinto-Coelho and Havens, 2014). Low pH alone has profound effects on the survival, growth of organisms and their interaction in aquatic environments (Lessmann et al., 2000), but vegetation, in addition to physically stabilize the soil, can also act as a kind of chemical stabilizer, according to the pH data obtained in the places with the presence or the absence of macrophytes in this study. It was observed that in places where vegetation is present, the pH fluctuation is smaller.

Species as *Juncus effusus* (Juncaceae) and *Phragmites australis* (Poaceae) have the ability to retain metal contamination and reduce heavy metal flow towards the sea. Also, *J. effusus* and *P. australis* are able to increase the pH from 2.7 to 5.5 in AMD impacted areas, resulting in a long-term improvement in the acidified areas, providing an efficient system to mitigate AMD (Dean et al., 2013). Therefore, analyzing the pH data in this study, we can suppose that the same effect may occur in the macrophytes from the Cyperaceae family from the estuaries sampled. The physiological activity of those plants present in environments impacted by AMD may have the ability to increase the pH of the water and mitigate the AMD impacts in direction to the sea.

According to (Silvestrini and D'Aquino, 2020), in the Araranguá Drainage Basin, the pH always remains close to 8 near the river mouth, corroborating with the values seen in the data acquired by this study for areas with vegetation. Towards the source of the river, far from the estuary and closer to the effluent generation sites, the pH values vary from 5.5 (Silvestrini and D'Aquino, 2020) to near 3 in value (Couceiro and Schettini, 2010). As the sampling sites move away from the places where the AMD is released, an increase in the pH is expected, due to the dissolution of the effluent throughout the river basin (Silvestrini and D'Aquino, 2020). In the Urussanga Drainage Basin, the pH remains close to 6.5 near the river mouth, but values around 4 were already seen in some studies (Schnack et al., 2018). The pH data collected for Urussanga were higher in this study in comparison to (Schnack et al., 2018), and one of the possible explanations may be the diatom bloom that was occurring at the sampling point. At last, there was difficulty in finding reliable previous data on pH buoyancy at D'Una Drainage Basin to serve as a comparative.

The regions close to the estuary mouth are dominated by marine processes and have high salinity values (Couceiro and Schettini, 2010). Salinity, which interfered with the bioavailability of metals, fluctuates according to the cycle of rising and falling tides (Pinto-Coelho and Havens, 2014). The salinity and oxygen concentration in the water column observed in this study are expected for estuaries, since saline intrusion into the estuarine environment will alter salinity and oxygen levels, as well as the movement of the salt wedge towards the ocean in the following cycle. However, short-term meteorological phenomena can increase the

gradient and do not necessarily represent the environment most of the time, which was another limitation of this study. This great variability that was sampled makes the process of obtaining and interpret the results obtained difficult. And variables that are expected to change with the entering of the tides into the estuary channel are: water salinity, oxygen concentration and pH.

Additionally, the “tongue” of drainage does not necessarily reach the river mouth, where the sampling points were, which highlights the fragility of the sampling scheme. This fragility was also reflected in the data sampling of pH and oxygen. However, towards the river, into the river basins, it is possible to observe the impact of the acid mine drainage (Couceiro and Schettini, 2010; Schnack et al., 2018; Silvestrini and D’Aquino, 2020).

## 5. Conclusions

The results for the species found in the AMD impacted places in Southern Brazil are consistent with earlier studies, which showed Cyperaceae as one of the most important botanical families as having acceptable tolerance for AMD (Buckney and Higgs, 1998; Lottermoser and Ashley, 2011; Novianti et al., 2017), and the highest degree of stress in the Fv/Fm value seen in species from Araranguá e Urussanga was expected, since both places are impacted by the input of heavy metals and low pH in the water. The ability of plants to survive in acidified environments, even reducing its photosynthetic capacity (Hajihashemi et al., 2023), is an interesting characteristic not only to be used in remediation strategies (Robb and Robinson, 1995), but also in the protection of salt marsh areas, since they are also rich in biodiversity. The presence of vegetation, in addition to remedying pollutants and reducing water toxicity, also provides more oxygen to the water column and habitat for other organisms to the trophic web.

Acid mine drainage has emerged as a significant worldwide issue, given its effects on the environments and living organisms (Daraz et al., 2023), and the macrophytes of estuarine environments impacted by coal mining in southern Brazil, in terms of physico-chemical features, especially pH, appears to perform the function of a chemical buffer, minimizing pH fluctuations. However, by maintaining a more stable pH, an important physiological activity ends up being compromised, as can be observed through the performance of photosynthetic activity, which is reduced in places with the presence of AMD in relation to control places. But more studies on the effect are necessary, especially closer to the place where AMD enter the respective river basins.

Without adherence to serious public policies of a socio-environmental nature in relation to the use of renewable energy sources in Brazil, the tendency for regions such as Araranguá and Urussanga is that the vegetation and all organisms in the estuarine zone continue to suffer from the input of pollutants from the exploration and use of coal in the system.

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## CAPÍTULO 2

(Este capítulo foi apresentado de forma mais sucinta no I Congresso Internacional: Obsolescência do Carvão Mineral, Energias Sustentáveis e Transição Justa, em Setembro/22: <https://arayara.org/wp-content/uploads/2022/11/Pre-lancamento-dos-resultados-do-I-Congresso-Internacional-Obsolescencia-do-Carvao-Mineral-Energias-Sustentaveis-e-Transicao-justa.pdf>, e foi formatado de acordo com os padrões do evento).

### **O impacto da drenagem ácida de mina (DAM) e de diferentes tratamentos de efluente ácido em macrófitas aquáticas**

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**Palavras-chave:** Organismos fotossintetizantes. Mitigação. Mineração de carvão.

### **1. INTRODUÇÃO E ENQUADRAMENTO TEÓRICO**

A drenagem ácida de mina (DAM) é um dos impactos ambientais mais graves que podem ocorrer em regiões influenciadas pela mineração de carvão, e surge como resultado do processo de extração do mineral. Quando o carvão é extraído do solo, minerais como a pirita ( $\text{FeS}_2$ ), podem aparecer associados à rocha sedimentar (RADOVIC, 1998). Após a extração, a pirita e o carvão são desassociados por métodos de separação física (CASTILHOS; FERNANDES, 2011), e, como a pirita não possui valor econômico interessante para as mineradoras, é rejeitada sem manejo adequado em

extensas áreas abertas próximas ao local da mineração.

À céu aberto, a pirita reage com o oxigênio presente no ar atmosférico e com a água, presente na umidade do ar e na chuva, e inicia um processo de oxidação lenta, que é finalizado com a formação de ácido sulfúrico. Com o passar do tempo, são formadas grandes piscinas alaranjadas de rejeito ácido nas áreas onde houve rejeito de pirita. A este resíduo aquoso, dá-se o nome de drenagem ácida de mina. O ácido sulfúrico, presente no efluente, então penetra no solo das regiões que possuem estes depósitos piritosos e também escoam até chegar em lagos, córregos e rios. Quando este rejeito penetra no solo ou na água, possui a capacidade de mudar o pH destes ambientes, acidificando-os. Além do baixo pH, os rejeitos ácidos da mineração também carregam consigo altas concentrações de metais pesados.

A combinação de valores baixos de pH e altas concentrações de metais pesados na água pode matar a vida aquática e dissolver minerais e nutrientes essenciais para o ciclo de vida de plantas (RADOVIC, 1998), como visto em análises toxicológicas (NETTO *et al.*, 2013; CHAMORRO *et al.*, 2017). Além de também aumentar os índices de mortalidade de peixes e moluscos, fazendo com que haja grande perda de biodiversidade local e comprometimento da saúde dos ecossistemas impactados por esta atividade antrópica.

O carvão mineral é um combustível fóssil que desperta interesse econômico inconsequente e criminoso, considerando o atual cenário de avanço das mudanças climáticas e da preocupante crise da diversidade biológica. Encontrado principalmente nos estados da região Sul do Brasil (UFRGS, 2000), movimentou cerca de 462 milhões de dólares no setor de importação do minério em 2020, de acordo com o Instituto Brasileiro de Mineração (IBRAM). Pode ser utilizado em usinas termoelétricas para a queima e geração de energia ou como matéria-prima para fabricar aço em siderúrgicas (BELOLLI *et al.*, 2002), e é indiscutivelmente reconhecido como o combustível com maior potencial poluente (REVESZ; LIENKE, 2016).

Embora atualmente já existam alternativas energéticas sustentáveis, o Brasil, à revelia de acordos internacionais e do acumulado de conhecimento científico produzido ao longo das últimas décadas, continua a incentivar a geração de energia hidrelétrica, que, direta ou indiretamente, incentiva também a mineração do carvão (BRASIL, 2022; UNIÃO NACIONAL DA BIOENERGIA, 2022), principalmente em momentos de estiagem, onde recorre-se ao uso de termoelétricas para o abastecimento da população

(ROSA, 2007).

O presente trabalho teve como objetivo avaliar o impacto da drenagem ácida de mina e de possíveis soluções no crescimento de macrófitas aquáticas, organismos fundamentais para a manutenção da produção primária e saúde dos ambientes aquáticos. Bem como, avaliar o efeito do tratamento com o filtro de conchas de ostras associado ao crescimento de macrófitas.

## 2. MATERIAL E MÉTODOS

### 2.1 Área de estudo

As Bacias Hidrográficas do Extremo Sul de Santa Catarina são consideradas altamente impactadas pela atividade carbonífera, de acordo com o CETEM (2001 *apud* CASTILHOS; FERNANDES, 2011). Em Urussanga e em Siderópolis foram coletadas amostras de água durante os dias 07 e 08/10/21, cujo valor de pH era 3 a 19,5° C. A água foi mantida em tanques fechados no laboratório até o momento de desenvolvimento do experimento. O presente experimento foi realizado de 16/02/22 a 21/02/22.

#### 2.2.1 Desenho experimental

Com a água coletada, foram feitos 5 grupos de amostras com 15 frascos de vidro cada, totalizando 75 frascos. Em cada grupo foram feitas 5 réplicas de 3 espécies de macrófitas (*Elodea* sp., *Lemna* sp. e *Azolla* sp.), que foram coletadas a partir da manutenção de espécimes que ocorre na estufa do Departamento de Botânica da Universidade Federal de Santa Catarina. As macrófitas escolhidas, após serem retiradas dos tanques da estufa, foram secas em papel toalha e pesadas em balança de precisão. Em cada um dos frascos de vidro, foi inoculado 200 mL de solução, a depender do tipo de tratamento (drenagem de locais diferentes e diferentes métodos de tratamento da drenagem), e as macrófitas foram inseridas nos frascos. Todas as amostras ficaram sob fotoperíodo de 24 horas e os frascos foram colocados em disposição aleatória. Analisou-se a taxa de crescimento ao passar de 120 horas. Para o cálculo da taxa de crescimento, as macrófitas foram pesadas antes da inserção nos frascos e após o fim do experimento. Assim, o cálculo da taxa de crescimento deu-se por:  $(\text{peso final} - \text{peso inicial}) / (\text{peso}$

inicial). Foram inoculadas, em média, 35 g de *Azolla* sp., 8 g de *Lemna* sp. e 524 g de *Elodea* sp. no início do experimento. A diferença de peso se deu em função da morfologia de cada uma das espécies.

### 2.2.2 Tratamentos

No grupo **Controle**, estão amostras de água do Rio Maior, localizado em Urussanga. No Rio Maior é dito que não existe poluição pela atividade mineradora (CARDOSO, 2013). Entretanto, observa-se no fundo das amostras de água a formação de precipitados férricos, evidenciando potenciais bocas de minas ilegais carregando material até o rio (Figura 1).

O grupo **DAM M** foi utilizado também como grupo de controle. Nele, estão amostras de água do Rio Carvão, localizado em Urussanga. É um rio conhecido por ser poluído pela atividade mineradora (Figura 1).

O grupo **DAM F** possui água de região de DAM não tratada. Nele, estão amostras de água da Língua do Dragão, localizado em Siderópolis. É um local conhecido por ser poluído pela atividade mineradora (Figura 1).

O grupo **DAM T** possui água de região de DAM tratada com filtro de conchas de ostras. Nele, estão amostras de água da Língua do Dragão (Figura 1).

O grupo **DAM E** possui água de região de DAM tratada com filtro de conchas de ostras e também com inoculação de esgoto doméstico, em uma proporção de 70% de água tratada com filtro de conchas de ostras e 30% de esgoto. Nele, estão amostras de água da Língua do Dragão (Figura 1).

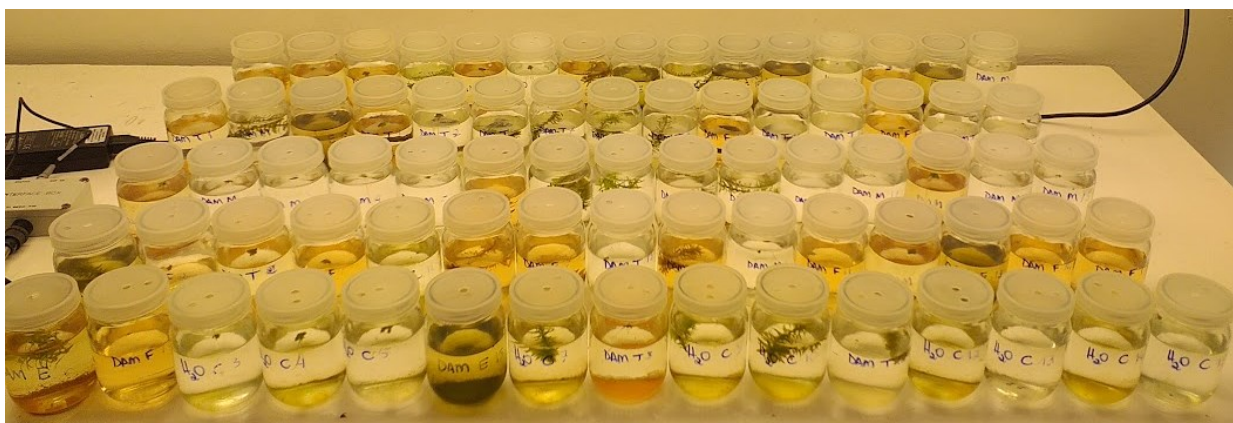


Figura 1: Distribuição dos tratamentos experimentais.

Nos grupos DAM T e DAM E, as amostras de água foram tratadas em um filtro de conchas de ostras (AMARAL, 2023), com o objetivo de aumentar o pH da solução. O filtro foi montado em um cano de PVC para o volume de 1 L de solução. Antes de ser montado, as conchas foram lavadas com água comum, e, após a lavagem, trituradas. Dentro do cano colocam-se as conchas trituradas até o topo, para que o carbonato seja exposto às amostras. As amostras foram passadas de 20-30 vezes no filtro. Após a passagem pelo filtro de ostras, a solução passa por uma segunda filtração apenas com papel filtro para diminuir os metais precipitados.

Assim como a filtração pelo filtro de conchas de ostras, a inoculação de esgoto doméstico tinha como objetivo aumentar o pH da solução acidificada, uma vez que o esgoto utilizado possuía característica alcalina.

### 3. RESULTADOS E DISCUSSÃO

Após a análise estatística de variância (ANOVA), observou-se que a taxa de crescimento relativo das macrófitas (Fig. 2) mostra que *Elodea* sp. responde de forma negativa à todos os tratamentos ao longo de 120 horas, mesmo no tratamento Controle. A taxa de crescimento negativa pode ser interpretada como perda de biomassa vegetal.

*Azolla* sp. sobrevive nos tratamentos Controle, DAM T e DAM E ao longo de 120 horas, não existindo uma diferença significativa entre os tratamentos (Fig. 2). Nos outros tratamentos é possível observar perda de biomassa vegetal. Os tratamentos DAM T e DAM E passaram por filtração em filtro de conchas de ostras e por filtração em filtro de conchas de ostras com inoculação de esgoto, respectivamente.

*Lemna* sp. sobrevive nos tratamentos Controle, DAM T e DAM E (Fig. 2), mostrando diferenças significativas nas amostras que passaram por tratamentos com filtro de conchas de ostras e filtração em filtro de conchas de ostras com inoculação de esgoto, como os tratamentos DAM T e DAM E, respectivamente. Nos outros tratamentos é possível observar perda de biomassa vegetal. A macrófita mostra desenvolvimento mais positivo nos tratamentos DAM T e DAM E do que no tratamento Controle, o que evidencia a importância do tratamento do efluente para o desenvolvimento e crescimento do organismo.

Em relação ao pH, nos grupos DAM F, DAM T e DAM E, o pH antes dos tratamentos variou de 2,02 a 3. Após a passagem pelo filtro de conchas de ostras o pH dos tratamentos DAM T e DAM E variou de 5,05 a 5,72.

O tratamento com conchas de ostras e inoculação de esgoto doméstico mostra-se eficaz para minimizar o impacto da DAM, visto que induz a redução da toxicidade dos efluentes ácidos, de acordo com a observação do crescimento de *Azolla* sp. e *Lemna* sp.. Pode-se notar esta diferença observando os grupos DAM F e DAM M, onde as amostras não passaram por nenhum tipo de tratamento e houve perda de biomassa vegetal. A perda de biomassa evidencia os riscos destes efluentes para a biodiversidade, uma vez que, além do impacto da toxicidade, deve-se considerar que o comprometimento do desempenho fisiológico destes organismos irá limitar as bases das teias tróficas, impactando diferentes seres vivos e níveis tróficos.

Os resultados aqui descritos reforçam que métodos de tratamentos alternativos, como a adição de filtragem utilizando conchas de ostras (GIMENEZ, 2017), possuem o potencial de elevar o pH de rejeitos poluentes e mitigar os efeitos da DAM, tornando ambientes impactados novamente “habitáveis” para diferentes espécies, como *Azolla* sp. e *Lemna* sp., por exemplo.

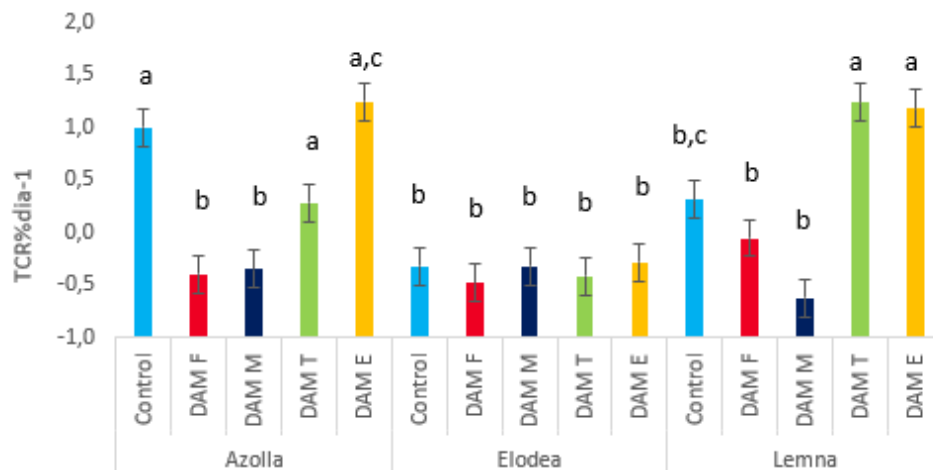


Figura 2.:Taxa de crescimento relativo, comparação entre tratamentos e espécies analisadas.

#### 4. CONSIDERAÇÕES FINAIS

Sabendo que o rejeito ácido da mineração do carvão possui a capacidade de causar variações nas características físico-químicas da água, e estas variações causam mudanças no funcionamento de organismos fotossintetizantes, observadas através da

perda de desempenho fisiológico em determinadas espécies, este trabalho evidencia o quanto a drenagem ácida de mina pode ser tóxica para macrófitas aquáticas e que a utilização de tratamentos adequados possuem o potencial de diminuir a toxicidade da água. Ademais, organismos que sobrevivem à DAM podem sofrer com bioacumulação de metais pesados e consequente transferência de poluentes ao longo da teia trófica, causando também desdobramentos para a saúde humana.

Olhando para a rica biodiversidade brasileira e seu funcionamento, podemos encontrar e desenvolver formas de mitigar, minimizar ou mesmo de anular os impactos ambientais que a mineração de carvão produz a partir de soluções inspiradas na natureza. Associada ao banimento de combustíveis fósseis, e ao desenvolvimento de fontes alternativas e sustentáveis de energia, ações de mitigação podem compor uma economia que seja a base da transição ecológica para termos um planeta mais saudável com uma sociedade mais resiliente.

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### CAPÍTULO 3

(Este capítulo apresenta a base de um artigo em preparação que será submetido à publicação, e formatado de acordo com os padrões do período *Aquatic Botany*).

#### **Marine heatwaves and acid mine drainage effects in saltmarshes**

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**Abstract:** The increase of the frequency and intensity of marine heatwaves events affects the photophysiological performance and survival of photosynthetic organisms, leading to mass death and population decline, aggravating the effect of marine pollution. With changes in climate patterns being continuously stimulated by human activities, and assuming these changes occur at a rate too fast for species to adapt, it is important to know the functioning of species that have a greater capacity to thrive in adverse situations. Currently, coastal ecosystems in southern Brazil are experiencing high levels of effluents from coal mining activities, and the combined effect of anthropogenic activities and marine heatwaves may be lethal to plant communities. Therefore, it is necessary to investigate the interaction between chemical pollutants such as AMD and marine heatwaves and their response in the flora of coastal areas. For this purpose, an mesocosm experiment was performed. *Spartina alterniflora* from Costeira do Pirajubaé Mangrove, Santa Catarina Island, was cultivated for 24 days in different solutions of AMD and the induction of a marine heatwave. We analyzed the maximum photochemical quantum yield of photosystem II (Fv/Fm), chlorophyll-a and chlorophyll-b, aerial and belowground biomass and number of shoots in *S. alterniflora*. Water samples were collected to analyze pH, salinity, oxygen concentration, temperature and nutrients (N and P). Carbonate content of sediment was measured too. A two-way ANOVA showed significant difference for the Fv/Fm at the 12<sup>th</sup> day, where plants presented lower values of Fv/Fm when in interaction with solutions with high content of AMD, but at the end of the experiment there was recovery in the Fv/Fm activity, and the photosynthetic pigments were not impacted by the AMD nor by the simulated marine heatwave. There was also the development of new biomass, appearing as new shoots. At the end of the experiment, *S. alterniflora* presented itself as a resilient and resistant species for the period of time evaluated.

**Keywords:** Poaceae, Resilience, Acidification, Tidal Marsh.

## 1. Introduction

Two hundred years after the beginning of the Industrial Revolution, Planet Earth finds itself different in its environmental and climatic configuration (Elias, 2018). With the introduction of a socioeconomic model that encourages the unsustainable exploitation of natural resources for profit, the financial development of several countries was based on the extraction and use of fossil fuels (Wagner, 2021). Mineral coal, in addition to having the greatest polluting potential (Revesz and Lienke, 2016), is also one of the most exploited around the world (Karunathilake and Witharana, 2023). In Brazil, despite the compromises to reduce emission and the pledges to foster energetical transition, its exploration and use continue to be subsidized (Teixeira, 2022). Every day, tons of carbon dioxide, sulfur oxides and metals are released into the atmosphere during the combustion of coal (Breeze, 2015), rising emission of greenhouse gases, intensifying climate change (Rosenfeld and Feng, 2011). Concomitantly, thousands of liters of acidic aqueous waste are produced, such as acid mine drainage (AMD), which end up being released into terrestrial and aquatic ecosystems, and threatening the survival of fauna and flora in those places (Ochieng et al., 2010).

Climate collapse appears to be imminent, and the relationship between the unrestrained exploitation of fossil fuels, the intensification of the greenhouse effect, and the rise in average temperatures of the oceans and atmosphere becomes clear (Hartmann, 2016). Climate changes, which occur now at an accelerated rate and are induced anthropogenically, lead to the occurrence of heatwaves in the ocean that have longer duration and greater frequency than ever (Areco et al., 2021). According to the Intergovernmental Panel on Climate Change (IPCC), heatwaves are expected to increase in frequency and intensity around the world (Traboni et al., 2018). Currently, all ocean and land surface temperature records have been broken, with ocean warming considered one of the most dangerous signs of global climate change (Traboni et al., 2018). According to IPCC, since the Industrial Revolution there has been an increase of 1.2° C and an increase of 0.2° C per decade is expected for the atmosphere and ocean if no change in relation to the use of fossil fuels is made (Allen et al., 2022). The year 2023 was the warmer of human history, with records above the 1.5° C targeted in Paris in 2015, resulting in extreme events more frequent and intense, such as the heat waves (Hansen et al., 2023).

The increase in the number of heatwaves in the ocean affects the photophysiological performance and survival of primary producers (Areco et al., 2021; Traboni et al., 2018), leading to mass death of shoots and population decline (McDonald et al., 2023; Traboni et al., 2018). The duration of these events affects the severity of their effects, as observed in a wide range of photosynthetic organisms (Traboni et al., 2018). With changes in micro and mesoscale climate patterns being continuously stimulated by human activities, and assuming these changes occur at a rate too fast for species to adapt, functional extinction is likely to occur (Traboni et al., 2018). Depending on these probabilities, in a future scenario, a change in physiological behavior in plants is expected.

In this sense, it is extremely important to know the functioning of species that have greater adaptive capacity to thrive in adverse situations, which can help to remedy anthropogenic changes, as they can be allies throughout the creation of preservation areas and in environmental recovery treatments. Currently, coastal aquatic ecosystems in southern Brazil are experiencing

high levels of effluents from coal mining activities (Castilhos and Fernandes, 2011; Rocha, 2014) and other pollutants (e.g. sewage), leading to a scenario of high environmental and ecological degradation (Areco et al., 2021). The release of liabilities from the exploitation of fossil fuels, such as AMD, associated with other anthropogenic activities, influences the fragmentation of habitats and the regression of vegetated regions, with losses of biodiversity becoming even more frequent and exaggerated by the greater frequency of extreme climatic events (McDonald et al., 2023). Acidification caused by low pH values from AMD, as well as high concentrations of heavy metals (Daraz et al., 2023; Robb and Robinson, 1995), may influence the reduction of biodiversity (Xu et al., 2022), the physiology and morphology of the plants (Hajihashemi et al., 2023; Silva, 2015; Wu et al., 2022b), and losses of plant communities have been attributed to the combined effect of anthropogenic activities and global climate change (Traboni et al., 2018). Heatwaves and chemical pollutants, such as AMD, acting as combined stressors are a challenge for the adaptability of photosynthetic organisms. Controlled experiments suggests that anthropogenic stress also reduces the resilience of photosynthetic organisms, and that shoots are more likely to die during the occurrence of extreme events (McDonald et al., 2023).

*S. alterniflora* is a native species to the American continent (Lee, 2001). In Brazil, it can be found from the South to the North region, in the saltmarsh and mangrove ecosystems, having inherent resilience characteristics, such as supporting a wide gradient of temperature, salinity and tidal inundation range (Craft et al., 2008; Schaeffer-Novelli, 1999). Saltmarshes and mangroves play a major role in maintaining the balance in the carbon cycle, and are known as “blue carbon ecosystems”, but all of these environments are threatened by human activities and their impacts, and once of the ecosystems is degraded or destroyed, their carbon stores are released as carbon dioxide, contributing to climate change (IUCN, 2023). Currently, there are few studies that focus on the impacts of AMD and their interaction with global climate change, and their response in the water quality of affected aquatic ecosystems (Anawar, 2013).

In this scenario, of increasing global temperatures and greater volumes of pollutants being released into ecosystems, this work was developed. In this study, through a medium-term mesocosm experiment, we analyzed the physiological response of *Spartina alterniflora* when exposed to a simulated marine heatwave and different concentrations of pollutants from coal mining activity. We hypothesize that the combination of stressors will be lethal for the species, reducing concentrations of chlorophyll-a, chlorophyll-b and photochemical maximum quantum yield of photosystem II. As well, the interaction between stressors will produce a more powerful effect in the plant’s response than just one stressor alone. Specific objectives include determining water quality and sediment characteristics. We want to clarify whether, for *S. alterniflora*, the combination of stressors is enough to threaten its survival, and we hope that our experiment can provide new information about the behavior of this species, in situations that may occur more frequently in the future.

## **2. Materials and methods**

### **2.1 Study site**

The Costeira do Pirajubaé Mangrove is located in the southwestern portion of Santa Catarina Island, southern Brazil (27°38'52'' S 48°32'12'' O). A portion of this marine-coastal ecosystem is part of the Pirajubaé Extractive Reserve (RESEX), which has around 1.712 hectares (Instituto Chico Mendes de Biodiversidade, 2023) and is protected by federal law, from which several families derive their livelihood through the collection of molluscs (*Anomalocardia* sp.) (Araújo, 1993), and is considered the best-preserved mangrove on the Santa Catarina Island (Vale et al., 2018). The Costeira do Pirajubaé Mangrove is intertwined with the Tavares River Basin (Diederichsen, 2014), which flows and enters into the South Bay of Santa Catarina Island (Araújo, 1993; Vale et al., 2018). The Tavares River itself is 9 km long (Diederichsen, 2014), and together with its tributaries, they form the second largest hydrographic basin on the Santa Catarina Island (Trindade, 2009), the Tavares River Basin, with 48.36 km<sup>2</sup> (Araújo, 1993). One of its main tributaries is the Pirajubaé River (Schneider, 2014).

Although a considerable portion of the largest mangrove of the Santa Catarina Island (Araújo, 1993) has been protected by the government since the 1990s (Instituto Chico Mendes de Biodiversidade, 2023), the result of anthropogenic activities distributed throughout the Costeira do Pirajubaé Mangrove has been observed since 1970 (Diederichsen, 2014; Vale et al., 2018), ranging from episodes involving mineral oil spills (Ministério Público de Santa Catarina, 2021), the entry of untreated storm sewage from adjacent areas, to the presence of plastic (Araújo, 1993; Silva, 2017). Pollution contaminated the soil, water and influenced the livelihood of families who depend on molluscs extractive activities, which are made in a sustainable way (Ministério Público de Santa Catarina, 2021). In these episodes of environmental contamination, shellfish and animals collected in the region were prevented from being consumed and sold (Ministério Público de Santa Catarina, 2021).

## 2.2 Sampling procedure

The communities of *Spartina alterniflora* (Poaceae) and sediment used in this work were collected in March, 2023 in Costeira do Pirajubaé Mangrove, outside RESEX area, with the help of shovels at a depth of around 30 cm. Plant samples were collected, including their rhizome and roots and associated clams. Sampling was carried out in the marginal portion of the mangrove. The plant and sediment samples were collected in conjunction and immediately transferred to plastic pots, and the pots were transferred to experimental trays, which were flooded by salt water. The trays had a capacity of 34 liters of water. The vegetation-sediment system was acclimatized for approximately 2 weeks at a room temperature in a greenhouse before the beginning of the experiment. The experiment was conducted at the Phycology Laboratory (LAFIC) of the Federal University of Santa Catarina.

## 2.3 Experimental Setup

The experiment was conducted for 24 days in April 2023, simulating the entry of acid mine drainage combined with a simulated marine heatwave event. The vegetation-sediment system was acclimatized for 2 weeks at greenhouse's average temperature, about 21° C (INMET, 2023), before the beginning of the experiment. The eight treatments used included: 1) a control, with no marine heatwave or AMD (24° C); 2) exposure to low AMD concentration

(10%) (24° C); 3) exposure to medium AMD concentration (20%) (24° C); 4) exposure to high AMD concentration (40%) (24° C); 5) a control, with heatwave and without AMD (28° C); 6) exposure to heat and low AMD concentration (10%) (28° C); 7) exposure to heat and medium AMD concentration (20%) (28° C); 8) exposure to heat and high AMD concentration (40%) (28° C) (Fig.1).

To determine the temperature used in this study as a marine heatwave (28° C) in relation with the control temperature (24° C), climate events from May 1981 to June 2022 were used to calculate the time series of daily average sea surface temperature from the National Oceanic and Atmospheric Administration (NOAA, 2023) for the chosen study area. The heatwave events duration, intensity and threshold temperature were based on (Hobday et al., 2016). The future intensity of marine heatwaves was simulated, based on projections of an increase of 2 to 4° C until the year 2100 (Oliver et al., 2019) as simulated by Dias *et al.* (2024, under submission).

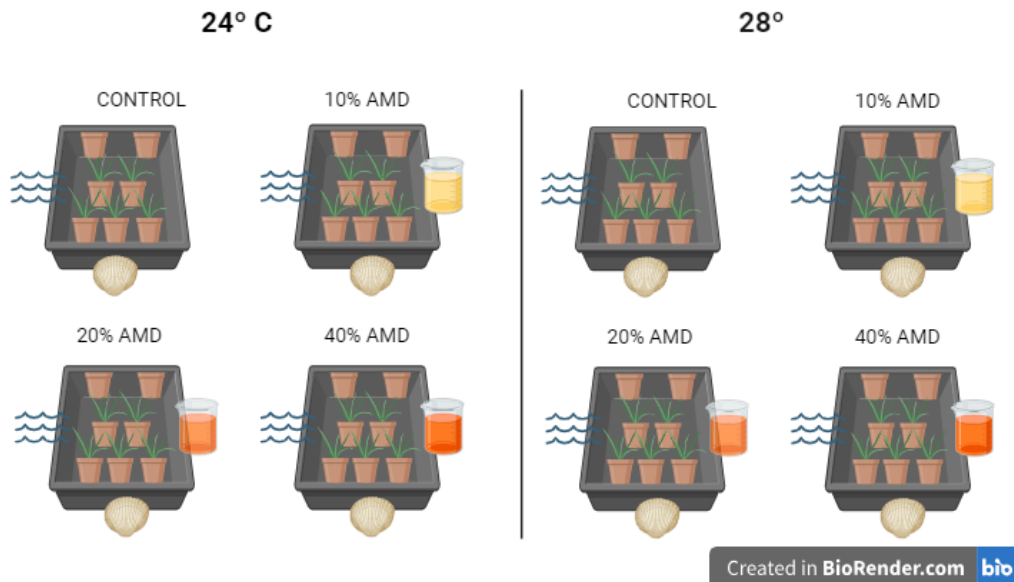


Fig.1: Experimental design. (Created with BioRender.com).

Control treatments were solutions containing 20 L of seawater and 14 L of fresh water. 10% AMD treatments were solutions that contain 20 L of seawater, 10 L of fresh water and 4 L of AMD. 20% AMD treatments were solutions that contain 20 L of seawater, 7 L of fresh water and 7 liters of AMD. 40% AMD treatments were solutions that contain 20 L of seawater and 14 L of AMD. The AMD used in these solutions was collected in a freshwater region, in the Araranguá Drainage Basin, at Língua do Dragão (Leão and Krebs, 2017), in March 2023. The effluent concentration chosen for the treatments was based on the amount of waste available for the experiment. Other features of the AMD used are listed below (Table 1).

Characterization of the AMD and the treatments	AMD (raw)	Control (0% AMD)	10% AMD	20% AMD	40% AMD
pH	2.59	7.47	3.7	3.18	3.02
O <sub>2</sub> (mg/L)	10.46	8.09	7.36	6.5	7.67
Temperature (°C)	23	22.9	23.5	23.3	24.1
Salinity	2.6	21	22	22	25

Table 1: Solutions used in the treatments. The solutions and its dilutions were characterized before the experiment and resulted in the general conditions above.

In each tray, 5 plastic pots were placed with *S. alterniflora* and sediment, and 2 plastic pots with only sediment, totaling 7 pots per tray. A tide simulation system was developed inside the trays, regulated by a timer, with a period of 6 hours of high tide (when the trays were flooded by the solutions mentioned above), and 6 hours of low tide. Whenever necessary, the trays were filled again using the same initial solution until reaching a volume of 34 liters of water per tray. This procedure had to be carried out due to higher volume of evaporation of the trays that were under the heatwave, so that the system could always remain with similar salinity values. The controlled variables were temperature and salinity. The irradiation was based on the natural light that radiated into the greenhouse. The temperature was controlled by a chiller, in treatments at lower temperatures, and heaters in heatwave simulation treatments. All treatments had digital temperature controllers. At the end of the acclimatization period and the beginning of the experiment, the tide simulation began and was maintained until the end of the experiment. The experimental design prioritized the replication of plants ( $n = 5$ ) as their ecophysiological behavior was the focus of the present study (Oksanen, 2001; Colegrave and Ruxton, 2018).

## 2.4 Vegetation analyses

The photochemical maximum quantum yield of photosystem II (Fv/Fm) was determined during the acclimation period and throughout the experiment, on alternate days, three times a day (morning/afternoon/night). To obtain these data, after dark acclimation for 15 minutes, Fv/Fm was measured using a DIVING-PAM – Heinz Walz. Saturation pulses were always applied in the same plants, which were tagged for identification.

During the 12<sup>th</sup> and 24<sup>th</sup> day, samples of leaves of *S. alterniflora* were collected and frozen (-80 °C) in order to quantify the chlorophyll-a and chlorophyll-b content, following the methodology proposed by (Ritchie, 2006). Acetone was used as a solvent and this analysis was performed in triplicates ( $n = 3$ ). The data was processed in the MetaSpec Pro software.

At the end of the experiment, the number of new shoots that appeared in the pots of the different treatments was counted. After counting, the shoots were fixed in formaldehyde and acetic acid for further studies of heavy metal accumulation.



The macrophytes were then separated (leaves, stem and roots) using scissors. The separated samples were oven-dried at 60 °C for 48 hours to attain a constant mass, after which the dry weights were measured, to calculate aerial and belowground biomass.

## 2.5 Water and soil analyses

Physical-chemical variables of water, such as pH, salinity, oxygen concentration and temperature were collected three times a day (morning/afternoon/night) throughout the entire experiment using a YSI 7500 multiparameter probe and a portable pHmeter.

At the beginning and at the end of the experiment, water samples were collected and frozen (-80 °C) for nutrient analysis. Nitrite, nitrate and phosphate were analyzed following the methods described by (Grasshoff et al., 1999) using a Double Beam Spectrophotometer U-2900 – Hitachi. This analysis was performed in triplicates ( $n = 3$ ) in the Laboratório de Oceanografia Química of the Federal University of Santa Catarina.

Sediment samples were collected in order to analyze the presence of carbonate in the soil and their relation with the presence or the absence of plants. Samples were taken at the end of the experiment and were frozen (-80 °C). The samples were processed according to (Dean, 1974). This analysis was performed in triplicates ( $n = 3$ ).

## 2.6 Statistical analysis

For the synthesis and integration of all descriptors and environmental variables, statistical analyses were performed using the TIBCO Statistica software, version 13.5.0.17. Univariate analyses were carried out to better interpret the data and compare the different treatments, such as two-factor analysis of variance (ANOVA) followed by the Newman-Keuls *a posteriori* test, respecting the assumptions for parametric analyses. For all analyses, a 5% level of significance ( $p < 0.05$ ) was used.

For the two-way ANOVA the two factors used to observe whether or not there was an interaction between factors were: concentration of AMD and temperature (presence or absence of heatwave). The dependent variables of this test were: chlorophyll-a, chlorophyll-b and Fv/Fm, nutrients concentration (nitrite, nitrate, phosphate) and carbonate contents in the soil.

After data normality and homogeneity tests, linear regression between the dependent variables (chlorophyll-a, chlorophyll-b, Fv/Fm, aerial biomass, belowground biomass, number of shoots, nitrite, nitrate and phosphate) and independent variables (pH, temperature, salinity, oxygen concentration) was made from the sum of the graph area of the temperature, pH, salinity and oxygen values throughout the entire experiment. With the values obtained in the linear regression, Pearson correlation was performed between dependent variables.

For the PERMANOVA the two factors used to observe whether or not there was interaction between factors were: concentration of AMD and temperature (presence or absence of

heatwave). PERMANOVA analyses were performed using the software PRIMER 6.0 and a 5% level of significance ( $p < 0.05$ ) was used. This analysis was followed by the Monte Carlo *a posteriori* test and was based on (Zhang et al., 2019).

### 3 Results

#### 3.1 *Spartina alterniflora* analyzes

##### 3.1.1 Photosynthetic pigments

The exposure of *Spartina alterniflora* to acid mine drainage and the simulated marine heatwave did not generate significant differences in chlorophyll-a and chlorophyll-b contents in the end of the experiment (Table 2). Chlorophyll-a and chlorophyll-b analyses for the 12<sup>th</sup> day were also made (Table 3, Supplementary material Fig. S1, S2).

Effect	Univariate Results for Each DV (Spreadsheet11) Sigma-restricted parameterization Effective hypothesis decomposition												
	Degr. of Freedom	Chl-a SS	Chl-a MS	Chl-a F	Chl-a p	Chl-b SS	Chl-b MS	Chl-b F	Chl-b p	Fv/Fm SS	Fv/Fm MS	Fv/Fm F	Fv/Fm p
Intercept	1	85.01913	85.01913	275.3127	0.000000	8.179607	8.179607	371.7761	0.000000	16.04589	16.04589	64151.49	0.000000
AMD	3	1.25202	0.41734	1.3515	0.293129	0.066105	0.022035	1.0015	0.417593	0.00190	0.00063	2.53	0.094152
Temperature	1	0.02297	0.02297	0.0744	0.788564	0.029434	0.029434	1.3378	0.264397	0.00003	0.00003	0.13	0.722537
AMD*Temperature	3	2.29355	0.76452	2.4757	0.098726	0.175976	0.058659	2.6661	0.082932	0.00048	0.00016	0.64	0.600186
Error	16	4.94095	0.30881			0.352023	0.022001			0.00400	0.00025		
Total	23	8.50949				0.623538				0.00641			

Table 2: Two-way ANOVA (AMD x Marine Heatwave) at 24<sup>th</sup> day. Test for Chl-a and Chl-b content and Fv/Fm activity in the treatments.

Effect	Univariate Results for Each DV (Spreadsheet4) Sigma-restricted parameterization Effective hypothesis decomposition												
	Degr. of Freedom	Chl-a SS	Chl-a MS	Chl-a F	Chl-a p	Chl-b SS	Chl-b MS	Chl-b F	Chl-b p	Fv/Fm SS	Fv/Fm MS	Fv/Fm F	Fv/Fm p
Intercept	1	67.69632	67.69632	181.0421	0.000000	6.613828	6.613828	201.4668	0.000000	19.10218	19.10218	93394.92	0.000000
AMD	3	0.68900	0.22967	0.6142	0.615667	0.051897	0.017299	0.5269	0.670072	0.00255	0.00085	4.16	0.023411
Temperature	1	0.27011	0.27011	0.7224	0.407909	0.041317	0.041317	1.2586	0.278475	0.00008	0.00008	0.39	0.543426
AMD*Temperature	3	0.52993	0.17664	0.4724	0.705775	0.060994	0.020331	0.6193	0.612582	0.00087	0.00029	1.42	0.272407
Error	16	5.98281	0.37393			0.525254	0.032828			0.00327	0.00020		
Total	23	7.47185				0.679462				0.00678			

Table 3: Two-way ANOVA (AMD x Marine Heatwave) at 12<sup>th</sup> day. Test for Chl-a and Chl-b content and Fv/Fm activity in the treatments.

At the 24<sup>th</sup> day, chlorophyll-a (Chl-a) values varied from 1.37 to 2.60 mg/L (Fig. 4), and chlorophyll-b varied from 0.43 to 0.75 mg/L. The higher concentration of Chl-a was seen in the MHW treatment and 0% AMD and MHW, and the higher difference was seen in 0% AMD. The higher concentration of Chl-b was seen in the MHW treatment with 0% AMD, and the higher difference was seen in 0% AMD (Fig. 5).

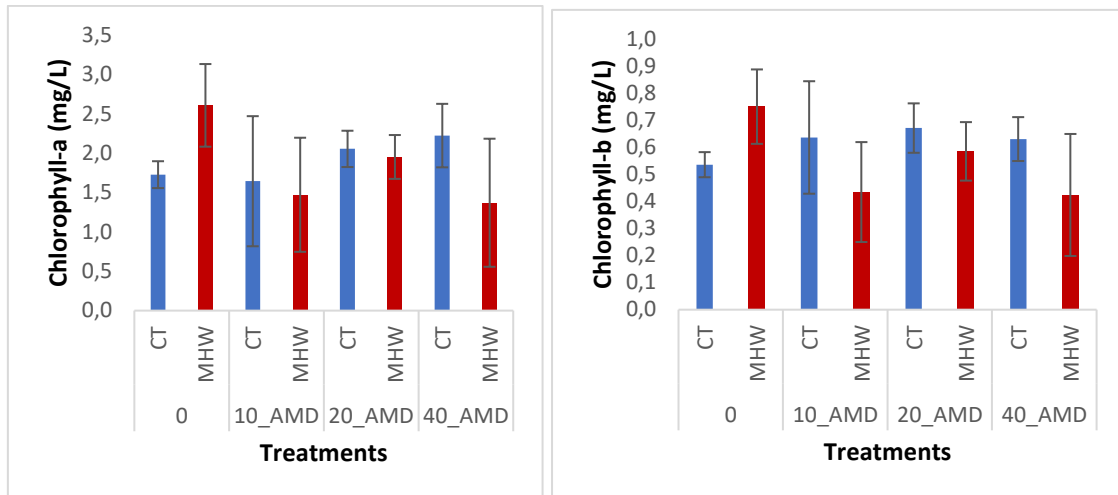


Fig. 4 and 5: Concentration of Chl-a and Chl-b at 24th day, in mg/L. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_ AMD = 10% AMD, 20\_ AMD = 20% AMD, 40\_ AMD = 40% AMD.

### 3.1.2 Chlorophyll fluorescence

The exposure of *Spartina alterniflora* to acid mine drainage and the simulated marine heatwave did not generate significant differences in the Fv/Fm at the end of the experiment (Table 2). Chlorophyll fluorescence analysis for the 12<sup>th</sup> day were also made (Table 3).

At the 24<sup>th</sup> day, Fv/Fm varied from 0.799 to 0.829. The higher value of Fv/Fm was seen in the CT treatment and 10% AMD, and the higher difference was seen in 0% AMD (Fig. 6).

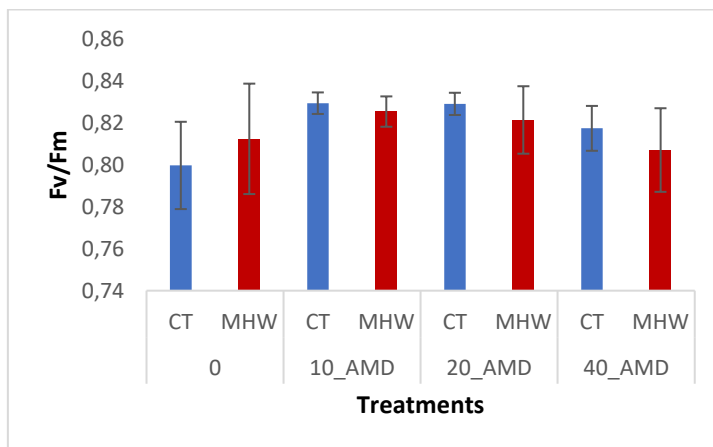


Fig. 6: Fv/Fm activity at the 24th day. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_ AMD = 10% AMD, 20\_ AMD = 20% AMD, 40\_ AMD = 40% AMD.

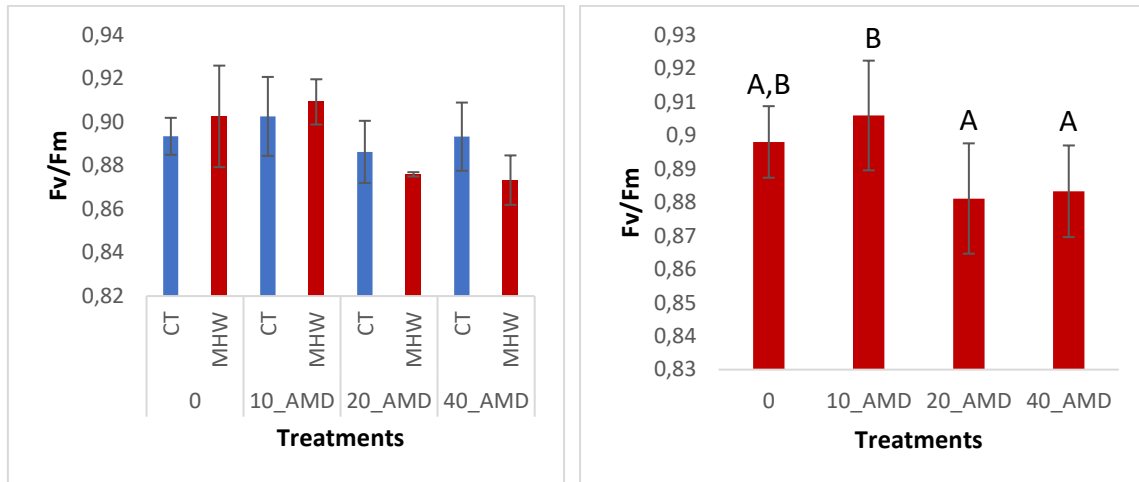


Fig. 7 and 8: Fv/Fm activity at the 24th day. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_AMD = 10% AMD, 20\_AMD = 20% AMD, 40\_AMD = 40% AMD (where: different letters represent significant differences related with the ANOVA and Newman-Keuls Test).

At the 12<sup>th</sup> day, Fv/Fm showed significant difference between treatments under the effect of the AMD only. The treatments with 0 and 10% of AMD and CT presented the higher values of Fv/Fm, 0.898 and 0.906, respectively, according to the Newman-Keuls Test (Fig. 7 and 8).

### 3.1.3 Biomass and shoots quantification

Aerial and belowground biomass and number of shoots were quantified not considering the replicates, therefore the values in the (Table 4) are a sum of all the replicates within treatment. These values were obtained at the 24<sup>th</sup> day.

The highest dry aerial biomass values were seen in the treatments with 20 and 40% AMD and the simulated marine heatwave, 68 and 72 g, respectively. The highest dry belowground biomass values were seen in the treatments with 0% AMD and CT, 465 g and 10% AMD and simulated marine heatwave, 395 g.

Aerial and belowground biomass values can be related to the seasonality of the plant, which is sometimes associated with greater incorporation of biomass in the roots, sometimes in the leaves. The biological characteristics of the species also influence the differences between biomass values, and are not necessarily linked to the study variables. Although there was decrease in belowground biomass with MHW and 20% AMD, in comparison with other treatments with higher values of AMD.

Treatments	N.o of Shoots	Biomass	
		Aerial biomass (g)	Belowground biomass (g)

Control (0% AMD/24° C)	6	40	465
10% AMD/24° C	9	57	307
20% AMD/24° C	12	58	322
40% AMD/24° C	10	49	354
Control (0% AMD/28° C)	5	49	375
10% AMD/28° C	8	41	395
20% AMD/28° C	8	68	222
40% AMD/28° C	4	72	350

Table 4: Sum of the number of shoots, aerial biomass and belowground biomass in all the treatments at the 24<sup>th</sup> day.

### 3.2 Water and soil analyses

#### 3.2.1 Carbonate in the sediment

The interaction between the acid mine drainage and the simulated marine heatwave did not produce significant differences in the carbonate concentration at the end of the experiment (Table 5). The carbonate concentration in the sediment was analyzed based on the pots with the presence of *S. alterniflora*.

Effect	Univariate Tests of Significance for CARBONATO (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	89,19121	1	89,19121	222,3420	0,000000
DAM	0,35316	3	0,11772	0,2935	0,829527
HEAT WAVE	0,03341	1	0,03341	0,0833	0,776586
DAM*HEAT WAVE	1,94406	3	0,64802	1,6154	0,225175
Error	6,41831	16	0,40114		

Table 5: Two-way ANOVA (AMD x Marine Heatwave) at 24<sup>th</sup> day. Test for carbonate concentration in the treatments.

At the 24<sup>th</sup> day, carbonate concentration varied from 1.52 to 2.47 g (Fig. 9), and in all treatments there was not significant difference. The higher concentration of carbonate and the higher differences were seen in the control temperature and 40% of AMD.

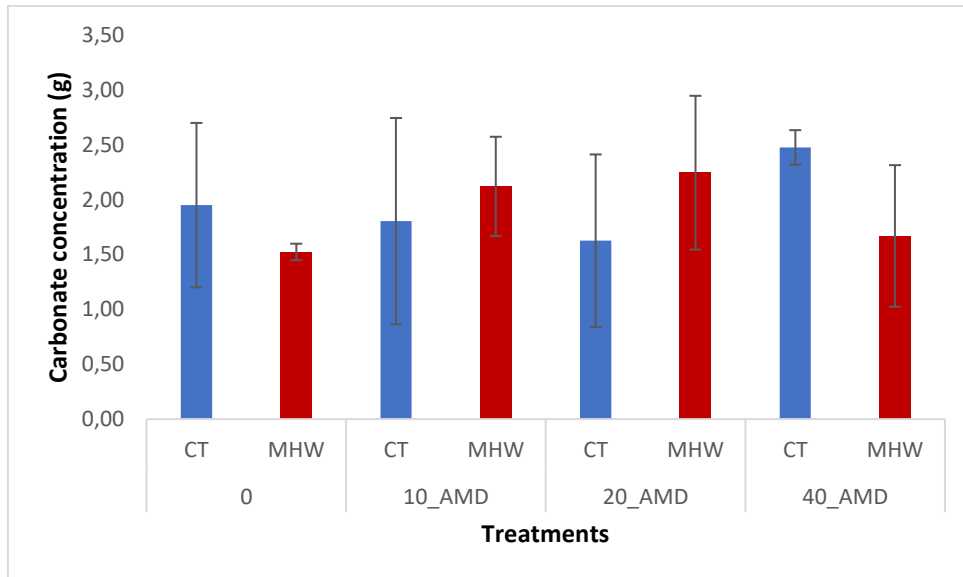


Fig. 9: Concentration of carbonate (g) at 24<sup>th</sup> day. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_AMD = 10% AMD, 20\_AMD = 20% AMD, 40\_AMD = 40% AMD.

### 3.2.2 Phosphate in the water

The exposure of AMD and the induction of a marine heatwave showed significant difference in the phosphate concentration in the water column. Under the effect of the AMD and the higher temperature, but not the interaction between them, there were significant difference at the end of the experiment (Table 6, Supplementary material, Table S1, S2). Phosphate concentration analyses for the 1<sup>st</sup> day were also made (Supplementary material, Table S3, S4, S5, Fig. S3, S4).

Effect	Degr. of Freedom	Univariate Results for Each DV (Spreadsheet7) Sigma-restricted parameterization Effective hypothesis decomposition			
		Phosphate SS	Phosphate MS	Phosphate F	Phosphate p
Intercept	1	452767,5	452767,5	165,8746	0,000000
Temperature	1	39728,8	39728,8	14,5549	0,001523
AMD	3	78269,5	26089,8	9,5582	0,000746
Temperature*AMD	3	6486,5	2162,2	0,7921	0,515915
Error	16	43673,2	2729,6		
Total	23	168158,0			

Table 6: Two-way ANOVA (AMD x Marine Heatwave) at 24<sup>th</sup> day. Test for phosphate concentration.

At the end of the experiment, the phosphate concentration varied from 54.9 to 274.9  $\mu\text{M}$ . (Fig. 10). The higher concentration of phosphate was seen in the control treatments with 0% AMD, and the higher differences was seen at 10% AMD treatment.

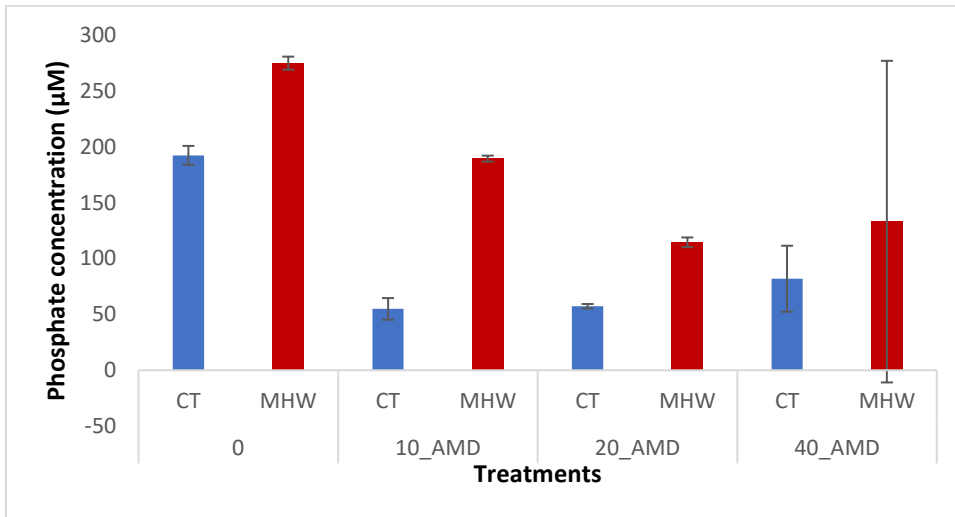


Fig. 10: Concentration of phosphate at 24<sup>th</sup> day. Overall raw data graph. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_ AMD = 10% AMD, 20\_ AMD = 20% AMD, 40\_ AMD = 40% AMD.

Under the effect of the AMD, there was a significant difference in the concentration of phosphate in the water column. Under the effect of the marine heatwave, there was also significant difference in the concentration of phosphate.

The highest phosphate concentration was seen at 0% AMD, 233.7 µM (Fig. 11). Under the influence of the marine heatwave, the highest value was 178 µM at MHW (Fig. 12).

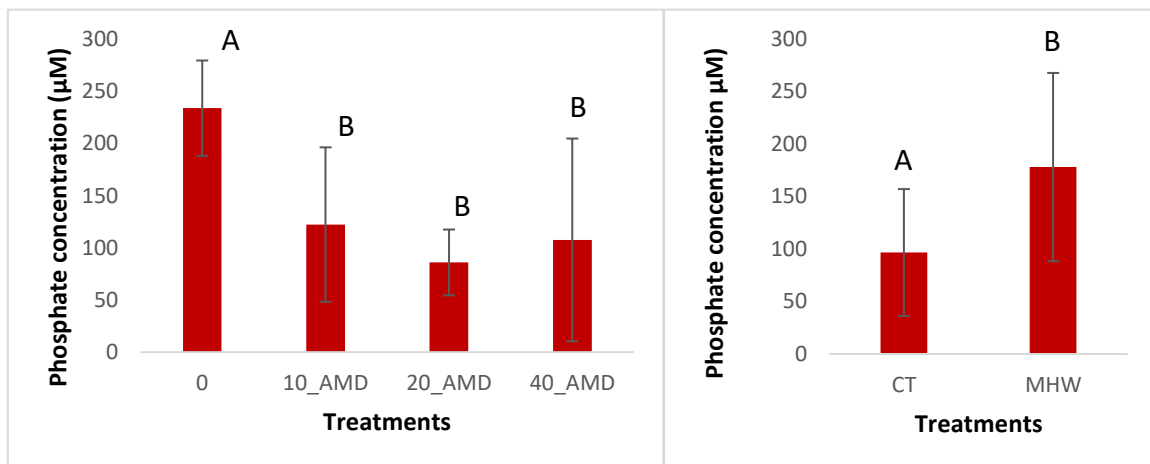


Fig. 11 and 12: Concentration of phosphate at 24<sup>th</sup> day. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_ AMD = 10% AMD, 20\_ AMD = 20% AMD, 40\_ AMD = 40% AMD (where: different letters represent significant differences related with the ANOVA and Newman-Keuls Test).

### 3.2.3 Nitrate + Nitrite in the water

The exposure of AMD and the induction of a marine heatwave showed significant difference in the nitrate+nitrite concentration in the water column, under the combined effect of the factors at the end of the experiment (Table 7, Supplementary material, Table S7).

Nitrate+nitrite concentration analysis for the 1<sup>st</sup> day were also made (Supplementary material, Table S6, Fig. S5).

Effect	Univariate Results for Each DV (Spreadsheet16) Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of Freedom	Nitrate SS	-Nitrate MS	Nitrate F	-Nitrate p
Intercept	1	562705	562705.3	362382.2	0,00
AMD	3	1119778	373259,5	240379,1	0,00
Temperature	1	284947	284947,5	183506,2	0,00
AMD*Temperature	3	1007832	335944,0	216347,9	0,00
Error	16	25	1,6		
Total	23	2412583			

Table 7: Two-way ANOVA (AMD x Marine Heatwave) at 24<sup>th</sup> day for nitrate concentration.

Under the combined effect of factors, there was significant difference between the concentration of nitrate in the water column between treatments for the 24<sup>th</sup> day of experiment. The highest nitrate concentration was seen at 0% AMD and CT, where the value was 990  $\mu\text{M}$ , and the lowest were seen in the 20% AMD and CT, where the value was 4.2  $\mu\text{M}$ .

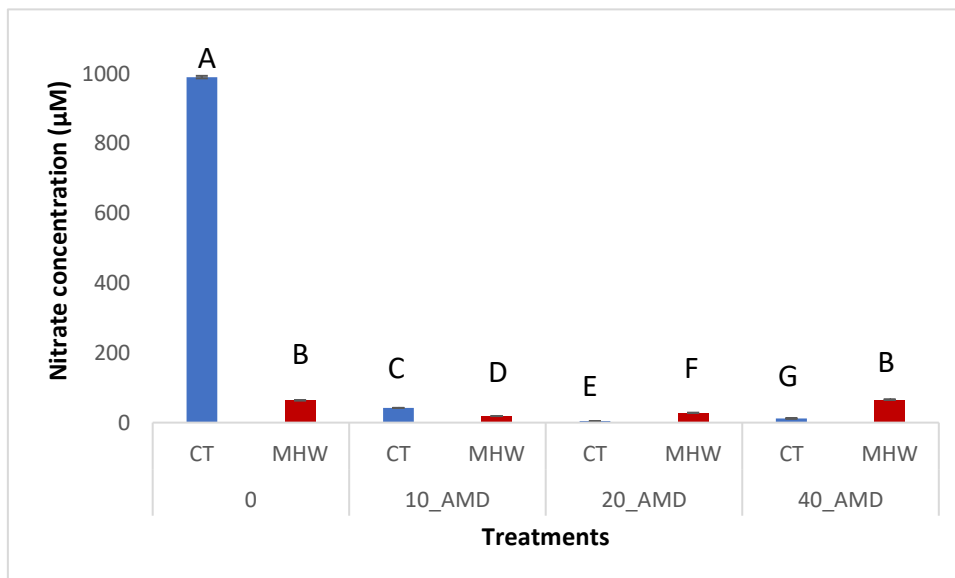


Fig. 13: Concentration of nitrate at 24<sup>th</sup> day. Graph according Newman-Keuls Test for the interaction between AMD and MHW factors. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_ AMD = 10% AMD, 20\_ AMD = 20% AMD, 40\_ AMD = 40% AMD (where: different letters represent significant differences related with the ANOVA and Newman-Keuls Test).

### 3.3 Pearson's correlation



At the 24th day, the correlation was strongly positive for the relation between the chlorophyll-a and chlorophyll-b, between the number of new shoots and the Fv/Fm. The correlation was strongly negative between the belowground biomass and the aerial biomass, between phosphate and the number of shoots, and between and between nitrate and the Fv/Fm (Table 8). It was also made Pearson's correlation comparing the 12 and the 24<sup>th</sup> day for Chl-a, Chl-b and Fv/Fm variables (Supplementary material, Table S8).

Variable	Correlations (Spreadsheet1)									
	CHL-A	CHL-B	FV/FM	AERIAL BIOMASS	BELOWGROUND BIOMASS	SHOOTS	NO3	NO2	PO4	
CHL-A	1,0000	<b>.8840</b>	,0178	-,1802	-,0999	,1712	-,1543	-,0312	,2585	
	p=---	<b>p=.004</b>	p=.967	p=.669	p=.814	p=.685	p=.715	p=.941	p=.536	
CHL-B	<b>.8840</b>	1,0000	,2500	-,0868	-,2269	,3454	-,1728	-,1989	-,0125	
	<b>p=.004</b>	p=---	p=.550	p=.838	p=.589	p=.402	p=.682	p=.637	p=.977	
FV/FM	,0178	,2500	1,0000	,1371	-,5947	<b>.7573</b>	<b>-.7115</b>	-,4768	-,5567	
	p=.967	p=.550	p=---	p=.746	p=.120	<b>p=.030</b>	<b>p=.048</b>	p=.232	p=.152	
AERIAL BIOMASS	-,1802	-,0868	,1371	1,0000	-,7553	-,0895	-,4705	,4794	-,4474	
	p=.669	p=.838	p=.746	p=---	<b>p=.030</b>	p=.833	p=.239	p=.229	p=.266	
BELOWGROUND BIOMASS	-,0999	-,2269	-,5947	-,7553	1,0000	-,3458	,6708	,0433	,5413	
	p=.814	p=.589	p=.120	<b>p=.030</b>	p=---	p=.401	p=.069	p=.919	p=.166	
SHOOTS	,1712	,3454	<b>.7573</b>	-,0895	-,3458	1,0000	-,3243	<b>-.7387</b>	<b>-.7113</b>	
	p=.685	p=.402	<b>p=.030</b>	p=.833	p=.401	p=---	p=.433	<b>p=.036</b>	<b>p=.048</b>	
NO3	-,1543	-,1728	<b>-.7115</b>	-,4705	,6708	-,3243	1,0000	-,2060	,3226	
	p=.715	p=.682	<b>p=.048</b>	p=.239	p=.069	p=.433	p=---	p=.625	p=.436	
NO2	-,0312	-,1989	-,4768	,4794	,0433	<b>-.7387</b>	-,2060	1,0000	,3659	
	p=.941	p=.637	p=.232	p=.229	p=.919	<b>p=.036</b>	p=.625	p=---	p=.373	
PO4	,2585	-,0125	-,5567	-,4474	,5413	<b>-.7113</b>	,3226	,3659	1,0000	
	p=.536	p=.977	p=.152	p=.266	p=.166	<b>p=.048</b>	p=.436	p=.373	p=---	

Table 8: Pearson's correlation at 24<sup>th</sup> day.

The linear regression between the pH and the biomass (Fig 14A, 14B) showed the differences between aerial and belowground biomass, corroborating the Pearson's correlation.

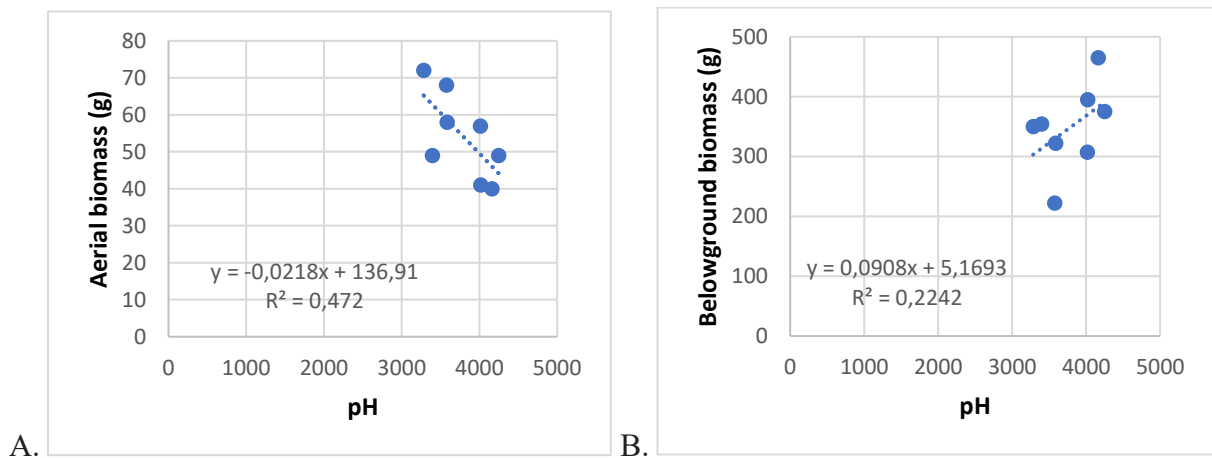


Fig. 14: Linear regression between the pH and the: A) aerial biomass (g), in all the treatments; B) belowground biomass in all the treatments.

### 3.4 Variation of temperature, salinity, pH and dissolved oxygen

Although temperature (Table 9) and salinity (Table 10) have been controlled variables since the beginning of the experiment, there were fluctuations throughout the collection of the data, in function of the experiment design and structure.

The highest levels of dissolved oxygen (Table 11) in the water column were seen in the 0% AMD and CT, where the highest value was 8.7 mg/L. The lowest value was 1.2 mg/L at 10% AMD and MHW. These values were observed even with the systems in aeration.

The pH variation (Table 12) in the treatments with the highest content of AMD, 40%, showed a similar pattern of increasing values throughout the entire experiment. The biggest pH value was 8.06 at the 10% AMD and CT and the lowest was 2.88 at 40% AMD and MHW.

Non-metric multidimensional scaling showed the relation between pH, dissolved oxygen, temperature and salinity throughout the entire experiment in all the treatments (Fig. 15)

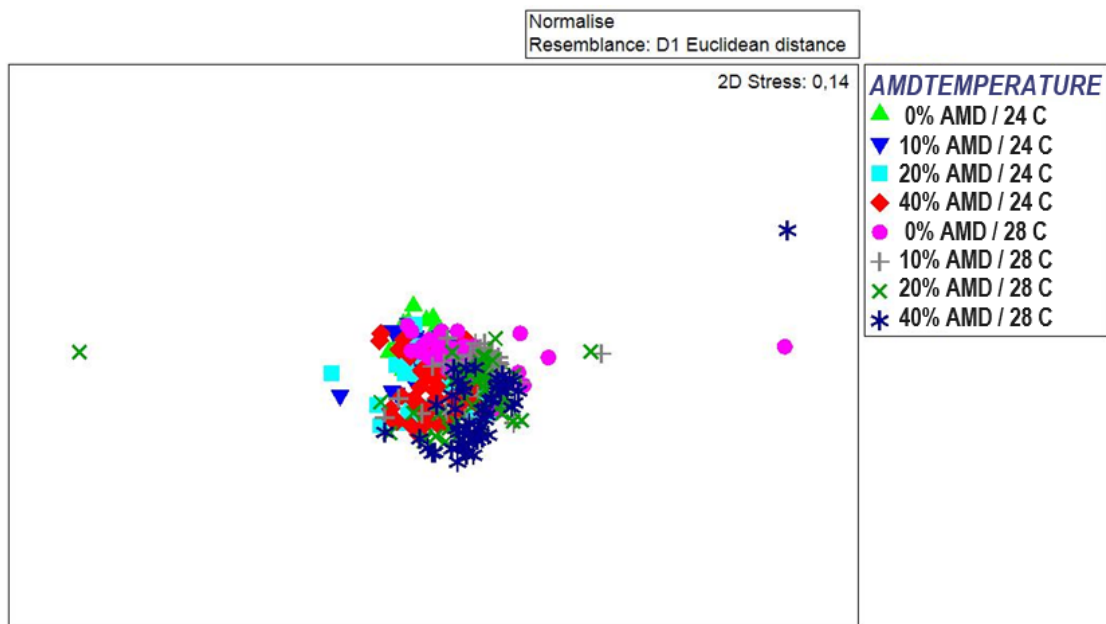


Fig. 15: Non-metric multidimensional scaling (NMDS) visualizes the dissimilarities and the corresponding Euclidean distances between treatments. Each point corresponds to one of the eight treatments. Permutational multivariate analysis of variance (PERMANOVA,  $n = 9999$ ) further showed that the AMD and the simulated marine heatwave are significantly different, but not the interaction between them (Table 13).

Time (Days)	CT 0% AMD	CT 10% AMD	CT 20% AMD	CT 40% AMD	MHW 0% AMD	MHW 10% AMD	MHW 20% AMD	MHW 40% AMD
1	23.5 ± 2.10	23.7 ± 2.07	23.8 ± 2.24	23.9 ± 2.27	25.6 ± 3.71	25.5 ± 3.22	25.0 ± 3.09	25.4 ± 3.07
10	26.1 ± 1.22	26.0 ± 1.08	26.8 ± 1.48	25.8 ± 1.00	28.4 ± 0.94	30.7 ± 0.32	29.3 ± 0.81	29.1 ± 1.94
24	24.0 ± 2.03	24.3 ± 1.74	21.1 ± 3.43	22.7 ± 2.46	34.8 ± 3.95	30.2 ± 2.59	30.6 ± 2.12	30.6 ± 2.05

Table 9: Temperature variable at 1<sup>st</sup>, 10<sup>th</sup> and 24<sup>th</sup> day in all treatments. Arithmetic average and standard deviation.

Time (Days)	CT 0% AMD	CT 10% AMD	CT 20% AMD	CT 40% AMD	MHW 0% AMD	MHW 10% AMD	MHW 20% AMD	MHW 40% AMD
1	23.0 ± 0.26	22.4 ± 0.21	22.1 ± 0.16	25.3 ± 0.12	23.5 ± 0.26	24.2 ± 0.20	23.6 ± 0.33	24.3 ± 0.21
10	24.0 ± 0.28	23.4 ± 0.04	23.9 ± 0.08	23.3 ± 0.21	25.5 ± 0.20	27.5 ± 0.20	26.8 ± 0.24	27.0 ± 0.54
24	22.5 ± 0.04	22.9 ± 1.57	22.7 ± 1.60	25.1 ± 1.90	27.4 ± 7.91	26.0 ± 5.55	25.9 ± 5.28	31.9 ± 6.84

Table 10: Salinity variable at 1<sup>st</sup>, 10<sup>th</sup> and 24<sup>th</sup> day in all treatments. Arithmetic average and standard deviation.

Time (Days)	CT 0% AMD	CT 10% AMD	CT 20% AMD	CT 40% AMD	MHW 0% AMD	MHW 10% AMD	MHW 20% AMD	MHW 40% AMD
1	5.43 ± 0.33	6.52 ± 0.17	6.11 ± 0.48	6.06 ± 0.22	5.77 ± 0.50	6.27 ± 0.45	5.91 ± 0.70	5.32 ± 0.51
10	4.53 ± 0.64	5.58 ± 0.51	4.37 ± 1.20	6.30 ± 1.26	5.74 ± 0.44	5.06 ± 0.24	5.07 ± 0.94	4.04 ± 0.39
24	6.65 ± 0.98	6.54 ± 0.67	5.73 ± 1.28	6.33 ± 0.53	5.01 ± 0.97	5.85 ± 0.45	5.50 ± 0.65	4.96 ± 0.58

Table 11: Oxygen concentration (mg/L) at 1<sup>st</sup>, 10<sup>th</sup> and 24<sup>th</sup> day in all treatments. Arithmetic average and standard deviation.

Time (Days)	CT 0% AMD	CT 10% AMD	CT 20% AMD	CT 40% AMD	MHW 0% AMD	MHW 10% AMD	MHW 20% AMD	MHW 40% AMD
1	7.28 ± 0.23	5.10 ± 0.32	3.88 ± 0.11	4.29 ± 0.19	7.48 ± 0.09	4.69 ± 0.08	3.57 ± 0.34	2.93 ± 0.03
10	7.35 ± 0.06	7.80 ± 0.20	7.44 ± 0.07	3.89 ± 0.28	7.83 ± 0.14	7.74 ± 0.14	7.63 ± 0.12	3.76 ± 0.17
24	7.53 ± 0.26	7.20 ± 0.19	5.33 ± 1.26	7.04 ± 0.22	7.66 ± 0.08	7.17 ± 0.29	5.36 ± 1.27	7.02 ± 0.33

Table 12: pH variable at 1<sup>st</sup>, 10<sup>th</sup> and 24<sup>th</sup> day in all treatments. Arithmetic average and standard deviation.

Source	df	SS	MS	Pseudo-F	P (Perm)	perms	P (Monte Carlo)
AMD	3	534.38	178.13	11.458	0.0001	9948	0.0001
Temperature	1	2260.4	2260.4	145.41	0.0001	9937	0.0001
AMD x Temperature	3	80.895	26.965	1.7346	0.1045	9939	0.1061
Res	408	6342.6	15.546				
Total	415	9218.4					

Table 13: PERMANOVA results table for interaction between AMD and Temperature (where – df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation; P (perm); Perms: number of permutations; AMD: Acid Mine Drainage); p-values based on 9999 permutations. Pairwise test on Groups showed the significant differences between and withing Groups (Supplementary Material, Table S9, S10, S11).

#### 4 Discussion

Our results showed that the plant-sediment system presented resilience and resistance to most of the descriptors used. As observed for other regions and species, the genus *Spartina* is reinforced as a taxon with strong adaptability, which supports exposure to different pollutants and their interactions with climate change (Syed et al., 2021; Yuan and Shi, 2009). The reduction in belowground biomass observed in the treatment with 20% AMD and simulated marine heatwave, alert us to the damage caused by the interaction between global and local stressors already observed by other authors (Crosby et al., 2017; Watson et al., 2016), and which need to be better explored in future experiments.

#### 4.1 Vegetation analyses

*Spartina alterniflora* showed significant difference in the Fv/Fm activity at the 12<sup>th</sup> day, with a recovery at the end of the experiment. Mao et al. (2023) observed adaptability of the photosystem of our target species regarding the variabilities of intertidal regions. The species presented resilience to different xenobiotics, in part explained by its capacity to provide detoxification mechanisms, preserving the photosynthetic apparatus (Cavé-Radat et al., 2018). In a review study, the genus *Spartina* shows itself as tolerant to the contact with different heavy metals, which are presented in the AMD, but the general physiological impacts of heavy metals in *Spartina* sp. are not widely explored yet (Redondo-Gómez, 2013). According to (Naranjo et al., 2008), the Fv/Fm values decrease with the increase of heavy metals for *Spartina densiflora*. The work also suggests that *S. densiflora* has a high tolerance for heavy metals, since all the plants studied survived (Redondo-Gómez, 2013). This information may explain the behavior of *S. alterniflora* in this study, the recovery in the Fv/Fm activity and the development of new shoots, with all the plants surviving, and may also suggest high tolerance for other species of the genus. However, Fv/Fm activity should be integrated with other parameters, such as concentration of chlorophyll, in order to better understand the entire health conditions of the plants (Biber et al., 2009).

The concentrations of chlorophyll-a and chlorophyll-b were preserved during the 24 days of experiment, not showing significant difference with the AMD nor the induced marine heatwave. In a study analyzing the combined effect of period tidal inundation and the input of heavy metals, it was seen that *Spartina alterniflora* was tolerant to low and medium concentrations of heavy metals, showing decrease in the Chl-a content only at high heavy metals input (Sun et al., 2018). This pattern corroborates our results observed with 40% of AMD as a medium content of pollutant with potential exposition to 204 mg/L of Fe, 7 mg/L of Mn and 1,2 mg/L of Zn, as main metals (Odisi, 2023). In (Sun et al., 2018), the decrease in the Chl-a concentration of *S. alterniflora* was accompanied with the decrease in the photosynthetic activity, but the data differ in our study.

Therefore, the genus *Spartina* can have their productivity affected by several factors; such as: tidal range, soil waterlogging, ionic toxicity and nutrient availability (Panitz, 1992), but these parameters need to be studied with care, since they are also inherent to ecosystems such as mangroves and saltmarshes, who have abiotic factors varying in a wide fluctuation range (Craft et al., 2008). Despite the resistance and resilience of the evaluated population of *S. alterniflora*, global warming and anthropogenic activities can generate negative environmental impacts in different experimental models and species (Mvungi and Pillay,

2019), and further evaluation considering different experimental designs and populations should be considered.

According to (Curado et al., 2010), *Spartina densiflora* is even able to germinate in sediments polluted with high levels of heavy metals and extremely low pH values, such as 2, but germination occurs at a slower pace. The presence of specific heavy metals may inhibit or alter the germination dynamics of the species. Aerial and belowground rates of the seeds decrease in more acidic sediments from highly polluted areas, and root development was clearly affected in acidic sediments even when aerial growth was not (Curado et al., 2010). The same pattern was seen in our study, with the negative correlation between aerial and belowground biomass, and one of the answers may be the content of heavy metals acting in the physiology of the plants. Also, the roots of *S. densiflora* could act as a “barrier” to the uptake or transport of metals, since the concentrations of heavy metals in its tissues are not much greater than those measured in the tissues of *Spartina maritima* (Cambrollé et al., 2008). Additionally, the increase in aerial biomass and the decrease in belowground biomass related to the content of nitrogen in the sediment are linked to different saltmarsh functions (Penk et al., 2020). According to (Penk et al., 2020), more aboveground biomass leads to an environment with more availability in organic matter for the trophic web, and lower belowground biomass is related to higher susceptibility to erosion in salt marshes, leading to potentially dangerous situations for the environment.

Changes in the growth patterns related to more than one stressor at a time, such as marine heatwaves, may indicate some kind of plasticity in flora species, in response to environmental variables, such as temperature and light (Mvungi and Pillay, 2019). The data collected in this study also show similar patterns, since *S. alterniflora* survived in contact with the AMD and the marine heatwave but showed changes in the growth process throughout the biomass content.

## 4.2 Physical-chemical parameters in the water and in the sediment

*Spartina alterniflora* did not influence the concentration of carbonate in the sediment at the end of the experiment. Carbonate storages in the sediment are a vital ecosystem service, but anthropogenic activities, such as mining, can lead to carbon loss in these reservoirs and the diminishment of coastal ecosystems importance as carbon sink locations (Ontl and Schulte, 2012), altering the carbon cycling in the nature. The destruction of coastal ecosystems, such as mangroves and saltmarshes, contributes significantly to rising atmospheric carbon dioxide levels linked to climate change (Ontl and Schulte, 2012). Also, changes in the carbonate content of the sediment can also influence in the diversity of plant species (Teres et al., 2019).

For *Spartina alterniflora* the plant-sediment system participates in the retention of heavy metals, and the availability of those compounds varies significantly with the water inundation time (Sun et al., 2018). In (Sun et al., 2018), 12 h inundation system caused the highest retention of heavy metals in *S. alterniflora*, but in our study it was used a 6 h inundation time system, which exposed the plants and the sediment to 204 mg/L of Fe, 7 mg/L of Mn and 1.2 mg/L of Zn (Odisi, 2023). Periodic tidal inundation can determine the physical and chemical properties of sediment, which influences the speciation and bioavailability of heavy metals

that have different toxicological effects on plants (Sun et al., 2018), and the increase in the atmospheric and oceanic temperature due to climate change will modify the chemistry of pollutants, changing their toxicities potential (Anawar, 2013), therefore they can become lethal to some flora species.

According to (Cambrollé et al., 2008), *S. alterniflora* can be used in phytostabilization for treatments of the soil and also for immobilization of higher concentrations of heavy metals, preventing the translocation of metals from rhizosediment to the roots. Nowadays, treatments with different plant species', can change the concentration of heavy metals in the water, and the concentration can decrease significantly compared with their initial values over medium periods of time (Sun et al., 2018).

Mining activities can cause soil-sediment disturbance, leading to nutrient leaching (Ontl and Schulte, 2012), and global climate change is predicted to increase chemical reaction rates of pollutants and oxidation of minerals such as pyrite (Anawar, 2013), present in the AMD from coal mining activity. At the end of our study the acid mine drainage and the simulated marine heatwave influenced the concentration of all nutrients analyzed. The concentration of phosphate was higher in the treatments with 0% AMD and the simulated marine heatwave, and the concentration of nitrate was higher in the treatments with 0% AMD and the control temperature. For the nitrite, the higher concentration was seen in the treatment with simulated marine heatwave and 40% AMD. Both stressors caused impact in the uptake of the nutrients in our study, and modifications in the concentration of nutrients or in the nutrients ratio (which can increase or decrease) are expected for the future in the face of climate change, although the processes and the dynamics in the real environment are extremely complex (Statham, 2012).

For *Spartina alterniflora* the plant-sediment system acted in order to restore the equilibrium of the pH throughout the entire experiment, which increased in all treatments, and of one the possible explanations may be the composition of the soil, which was full of seashells, therefore, carbonate. At the end of the experiment, treatments with 40% AMD which initially had pH values as 2.93-4.29 go to 7.02-7.04, matching the pH values from the control treatments, with 0% AMD. According to (Santos et al., 2021), the iron oxides from the oxidation of the pyrite, present in the AMD, can trigger chemical reactions that result in the production of alkalinity from the degradation of organic matter. Therefore, the burial of pyrite and other ferric compounds results in long-term carbon sequestration in the sediment through the production of alkalinity (Santos et al., 2021). Primary producers sequester and sinks carbon in the soil through the photosynthesis (Ontl and Schulte, 2012), but they can also generate alkalinity in blue carbon ecosystems, by transporting part of the oxygen produced to the roots and the rhizomes (Santos, 2021), and this is an important mechanism, since the acidification imposed by effluents from fossil fuels activity can inhibit photosynthesis in the cells of primary producers (Pfanzen et al., 1986). And the removal of CO<sub>2</sub> through photosynthesis by macrophyte communities in salt marshes and mangroves can create a detectable effect on pH of the water (Santos, 2021).

Climate change will have direct impacts on water resources and geochemical reactions of liabilities from mining sites (Anawar, 2013), and new climate patterns associated to global warming and the more frequent occurrence of extreme conditions, such as droughts and floodings will also change the volume of pollutants that enter in different ecosystems

(Anawar, 2013; Nordstrom, 2009). The heavy metals concentration of the acid drainage increases during summer dry periods and during droughts, and it is predicted to be magnified with changes in the weather pattern caused by climate change (Nordstrom, 2009). Therefore, the quality of the water and the effluents that comes from coal mining places is expected to be worse in the future with the advent of global climate change, if no remediation treatment takes place (Nordstrom, 2009), and with more extreme events happening, suddenly larger concentrations of mining wastes full of heavy metals and low pH will enter in different ecosystems and increase danger to aquatic life, such as plants (Anawar, 2013).

In the future it will be necessary to consider the occurrence of more extreme conditions for mitigation and remediation treatments (Anawar, 2013), which will become more expensive because the capacity of the treatments (e.g., revegetation), that will have to be enlarged or increased in size (Nordstrom, 2009). Mining sites with inadequate design and management will be more vulnerable to impacts of climate change, and the adoption of adaptative measures will be more effective, economically and environmentally, to confront the impacts of climate change in those places (Anawar, 2013).

Our results showed that, communities of *Spartina alterniflora* are resilient and resistant to stressors such as liabilities from coal mining activity and simulated marine heatwaves, for short periods of time. Although, for some species of *Spartina* (*S. maritima*), the combination of higher temperatures and higher concentrations of carbon dioxide can cause injuries (Mateos-Naranjo et al., 2021). Therefore, recognize how blue carbon ecosystems can help us to confront the climate crisis that is imminent, throughout the physiology and the resilience of their species, and the functioning of the biogeochemical dynamics of coastal environments, is important in order to help to preserve those regions, ecosystem services and marine protected areas in order to fight against climate change.

## 6. Final considerations

The results of our work suggests that, for *Spartina alterniflora*, chemical pollutants such as AMD and simulated marine heatwaves, for a period of 24 days, are not extremely dangerous or destructive for the ecophysiology of the species. We support this affirmation based on the acquired and analyzed data, the maintenance in the photosynthetic activity, the maintenance in the photosynthetic pigments content and the development of the new shoots in all the treatments. Our data also present the importance of conservation of mangrove areas in order to maintain the pH of the water and the carbon cycle stable and at equilibrium.

Therefore, understand which species are more resilient in face to the imminent climate change scenario, and how they impact biogeochemical dynamics in these environments is extremely important. Primary producers can help us through remediation, preservation and conservation strategies. In a future scenario of increase in atmospheric and oceanic temperatures, with the appearing of marine heatwaves, and higher volumes of pollutants being released in coastal ecosystems, *S. alterniflora* is one of the flora species that has a great chance to survive, since the combination of the AMD with the marine heatwave was not lethal for the species.

Coastal blue carbon ecosystems, such as mangroves and saltmarshes, are some of the most carbon rich ecosystems on Earth, and are vital to mitigating the impacts of climate change.

They are also critical to maintain the coastal biodiversity and to help in the climate adaptation process, protecting millions of people globally from the impacts of storms, coastal flooding and erosion (IUCN, 2023). It is necessary now to increase conservation efforts in these environments, including remaining areas of mangroves and salt marshes as priorities throughout the world.

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### 3 DISCUSSÃO GERAL E CONSIDERAÇÕES FINAIS

Macrófitas de marismas e manguezais compartilham características fisiológicas e morfológicas de grande amplitude de resistência à diferentes variáveis ambientais, como temperatura, salinidade e variação de maré. Entretanto, estes organismos fotossintetizantes podem responder de forma diferente à estressores químicos, como a drenagem ácida de mina (DAM).

Espécies como *Schoenoplectus americanus* dominam ambientes estuarinos impactados pela mineração de carvão no Sul do Brasil, como em Araranguá e Urussanga, e embora a vegetação consiga resistir em contato com o poluente e até completar seus ciclos vitais, dando espaço a novas gerações através do crescimento de suas estruturas reprodutivas, parte de sua atividade fotossintética é comprometida pela entrada do efluente ácido (CAPÍTULO 1). O comprometimento da saúde da espécie, analisado através da redução da atividade fotossintética, pode ser observado através de menores taxa de rendimento quântico, quando comparadas com plantas de regiões que não recebem o efluente da mineração de carvão.

Em experimentos laboratoriais controlados, *Spartina alterniflora* também consegue resistir à entrada da drenagem ácida de mina, dando espaço à novos indivíduos através do aparecimento de brotos em seus cultivos, porém, assim como com *S. americanus*, parte de sua atividade fotossintética é afetada pela entrada do resíduo ácido. Entretanto, após um breve período de tempo, a espécie consegue recuperar o funcionamento do fotossistema, mostrando características de resiliência e resistência frente ao contato com determinadas concentrações do poluente, combinado ou não com a simulação de onda de calor marinha (CAPÍTULO 3).

Embora espécies como *S. americanus* e *S. alterniflora* sejam nativas de marismas e manguezais ao longo de toda a costa do Brasil, e possuam intrinsecamente características fisiomorfológicas de plasticidade e resiliência à flutuação de fatores abióticos inerentes a estes ambientes, nem todas as macrófitas presentes nestes ecossistemas conseguem resistir e continuar seus ciclos de vida com a entrada da drenagem ácida de mina. *Elodea* sp., em experimentos laboratoriais controlados, possui sua taxa de crescimento relativo comprometida, demonstrando perda de biomassa ao interagir com a drenagem ácida de mina, podendo ser compreendida como uma espécie sensível para a interação com a DAM. Macrófitas como *Lemna* sp. e *Azolla* sp. possuem maior resistência ao entrar em contato com o poluente, principalmente após o tratamento do resíduo ácido com base em soluções sustentáveis, onde os dados mostram a recuperação das taxas de crescimento relativo, com ganho de biomassa (CAPÍTULO 2).

A presença de comunidades fotossintetizantes de *Schoenoplectus americanus* em marismas no Sul do Brasil, bem como o funcionamento fisiológico de *Spartina alterniflora* em manguezais, também se mostra importante na manutenção do equilíbrio de parâmetros físico-químicos, como o pH da água. A presença de *S. americanus* possui a capacidade de minimizar a flutuação do pH da água em locais impactados pela atividade mineradora, agindo como um estabilizador químico (CAPÍTULO 1). A vegetação de marismas e manguezais age, portanto, não apenas como estabilizador físico do solo, mas também como estabilizador químico, e talvez a rápida estabilização do pH da água que ocorre *in situ* (CAPÍTULO 1) e em experimentos controlados (CAPÍTULO 3), é o que faz com que as plantas consigam resistir à ação do poluente.

O fortalecimento de políticas públicas para restauração, conservação e preservação de bacias hidrográficas e ecossistemas estuarinos já impactados pela atividade mineradora, como marismas no Sul do Brasil, faz-se urgente. Aliado à movimentações socioambientais e políticas em busca de uma transição energética justa para formas limpas de se produzir energia, onde não mais seja necessária a utilização de combustíveis fósseis e a geração de rejeitos que tanto poluem diferentes ecossistemas. Ações conjuntas entre tomadores de decisão e sociedade são necessárias para evitar o maior comprometimento da saúde de ambientes estuarinos que estão sendo impactados, bem como reduzir a perda de biodiversidade de espécies mais sensíveis e a perda dos serviços ecossistêmicos que estes organismos proporcionam através da mitigação de efeitos negativos dos poluentes oriundos da mineração de carvão.



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OCEANOGRAFIA

Victória Silvestre Corrêa

### **APÊNDICE – CAPÍTULO 3**

**AVALIAÇÃO DO IMPACTO DA DRENAGEM ÁCIDA DE MINA (DAM) EM  
ORGANISMOS FOTOSSINTETIZANTES DE ESTUÁRIOS DE SANTA CATARINA**

Florianópolis – SC

2024

## LISTA DE TABELAS

Newman-Keuls test; variable Phosphate (Spreadsheet7) Homogenous Groups, alpha = ,05000 Error: Between MS = 2729,6, df = 16,000				
Cell No.	AMD	Phosphate Mean	1	2
3	20	85,9425	****	
4	40	107,5341	****	
2	10	122,2223	****	
1	0	233,7053		****

**Table S1:** Newman-Keuls Test for phosphate concentration at 24<sup>th</sup> day for AMD factor.

Newman-Keuls test; variable Phosphate (Spreadsheet7) Homogenous Groups, alpha = ,05000 Error: Between MS = 2729,6, df = 16,000				
Cell No.	Temperature	Phosphate Mean	1	2
1	24	96,6649	****	
2	32	178,0372		****

**Table S2:** Newman-Keuls Test for phosphate concentration at 24<sup>th</sup> day for Marine Heatwave/Temperature factor.

Univariate Results for Each DV (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition					
Effect	Degr. of Freedom	Phosphate SS	Phosphate MS	Phosphate F	Phosphate p
Intercept	1	38905,39	38905,39	297,3953	0,000000
AMD	3	4689,86	1563,29	11,9499	0,000234
Temperature	1	616,29	616,29	4,7109	0,045366
AMD*Temperature	3	426,85	142,28	1,0876	0,382702
Error	16	2093,13	130,82		
Total	23	7826,12			

**Table S3:** Two-way ANOVA (AMD x Marine Heatwave) for phosphate concentration at 1<sup>st</sup> day.

Newman-Keuls test; variable Phosphate (Spreadsheet1) Homogenous Groups, alpha = ,05000 Error: Between MS = 130,82, df = 16,000				
Cell No.	AMD	Phosphate Mean	1	2
3	20	24,10540	****	
2	10	29,17279	****	
4	40	50,25031		****
1	0	57,52094		****

**Table S4:** Newman-Keuls Test for phosphate concentration at 1<sup>st</sup> day for AMD factor.

Newman-Keuls test; variable Phosphate (Spreadsheet1) Homogenous Groups, alpha = ,05000 Error: Between MS = 130,82, df = 16,000				
Cell No.	Temperature	Phosphate Mean	1	2
2	32	35,19496	****	
1	24	45,32977		****

**Table S5:** Newman-Keuls Test for phosphate concentration at 1<sup>st</sup> day for Marine Heatwave/Temperature factor.

Newman-Keuls test; variable Nitrate (Spreadsheet12) Homogenous Groups, alpha = ,05000 Error: Between MS = ,00296, df = 16,000									
Cell No.	AMD	Temperature	Nitrite+Nitrate Mean	1	2	3	4	5	6
8	40	32	3,72584	****					
6	20	32	3,77055	****					
4	10	32	3,81211	****					
5	20	24	4,35242		****				
2	0	32	6,44260			****			
3	10	24	7,44071				****		
7	40	24	18,27231					****	
1	0	24	62,18566						****

**Table S6:** Newman-Keuls Test for nitrate+nitrite consumption at 1<sup>st</sup> day for the interaction between AMD and Marine Heatwave/Temperature factor.

Newman-Keuls test; variable Nitrate (Spreadsheet16) Homogenous Groups, alpha = ,05000 Error: Between MS = 1,5528, df = 16,000										
Cell No.	AMD	Temperature	Nitrite+Nitrate Mean	1	2	3	4	5	6	7
5	20	24	4,2693		****					
7	40	24	11,9322			****				
4	10	32	18,8304				****			
6	20	32	28,0397					****		
3	10	24	42,0499						****	
2	0	32	63,8867	****						
8	40	32	65,8776	****						
1	0	24	990,0825							****

**Table S7:** Newman-Keuls Test for nitrate+nitrite concentration at 24<sup>th</sup> day for the interaction between AMD and MHW factors.

Correlations (Spreadsheet8) Marked correlations are significant at p < ,05000 N=48 (Casewise deletion of missing data)			
Variable	Chl-a	Chl-b	FvFm
Chl-a	1,0000	,9269	-,1941
	p= ---	p=0,00	p=,186
Chl-b	,9269	1,0000	-,1598
	p=0,00	p= ---	p=,278
FvFm	-,1941	-,1598	1,0000
	p=,186	p=,278	p= ---

**Table S8:** Pearson’s correlation comparing the 12<sup>th</sup> and the 24<sup>th</sup> day.

<b>Groups</b>	<b>t</b>	<b>P (Perm)</b>	<b>perms</b>	<b>P (Monte Carlo)</b>
24,28	12,058	0,0001	9950	0,0001

**Table S9:** Pairwise test on Groups, showing the significant difference between the control treatment and the marine heatwave treatment. There is significant difference between groups.

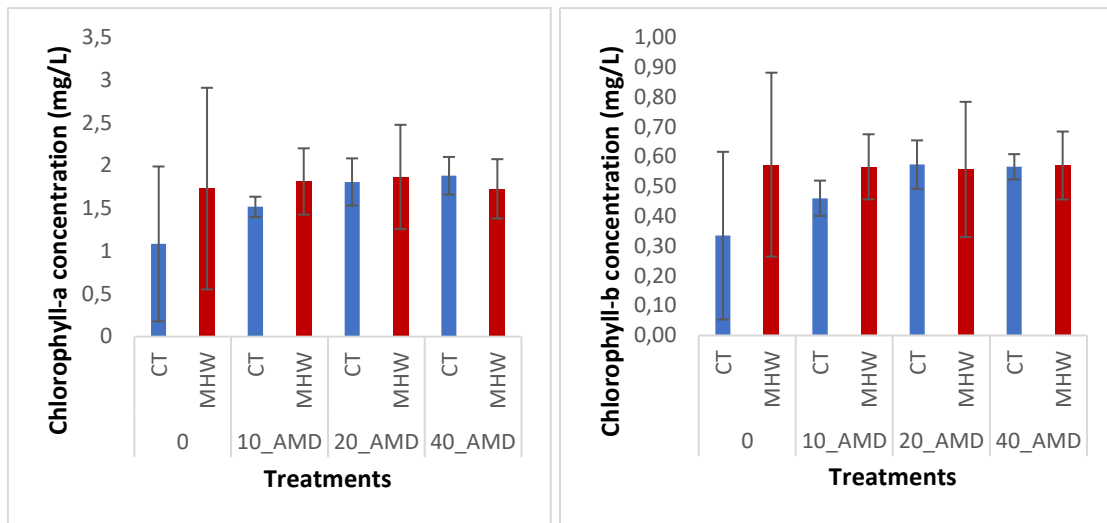
<b>Groups</b>	<b>t</b>	<b>P (Perm)</b>	<b>perms</b>	<b>P (Monte Carlo)</b>
0,10	1,9928	0,0105	9947	0,0122
0,20	4,8849	0,0001	9935	0,0001
0,40	7,8571	0,0001	9959	0,0001
10,20	3,5978	0,0001	9950	0,0001
10,40	6,3479	0,0001	9956	0,0001
20,40	3,7581	0,0001	9954	0,0001

**Table S10:** Pairwise test on Groups, showing the significant difference between the control treatment and AMD treatments. There is significant difference between all the groups, except the groups with 0% and 10% of AMD.

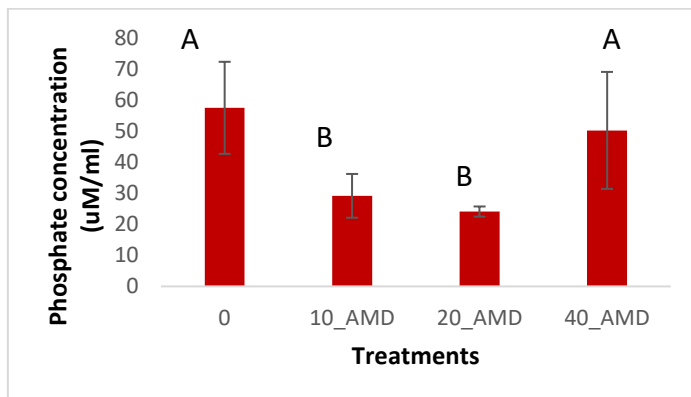
<b>Average Distance</b>	<b>0</b>	<b>10</b>	<b>20</b>	<b>40</b>
0	2,0014	0,0105	0,0001	0,0001
10	2,1864	2,2803	0,0001	0,0001
20	2,6108	2,5901	2,6022	0,0001
40	2,8835	2,7717	2,7109	2,4795

**Table S11:** Pairwise test on Groups, showing the average distance between groups.

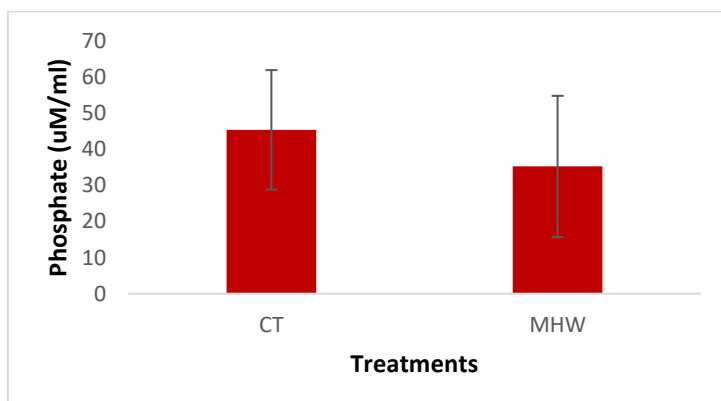




**Fig. S1 and S2:** Chl-a and Chl-b concentration at 12th day.

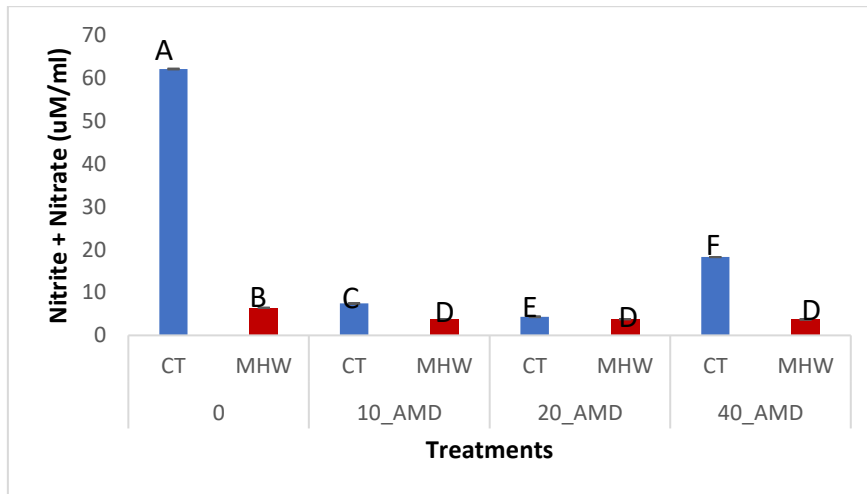


**Fig. S3:** Phosphate concentration at 1<sup>st</sup> day for AMD factor. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_AMD = 10% AMD, 20\_AMD = 20% AMD, 40\_AMD = 40% AMD (where: different letters represent significant differences related with the ANOVA and Newman-Keuls Test).



**Fig. S4:** Phosphate concentration for Marine Heatwave/Temperature factor at 1<sup>st</sup> day.

Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_ AMD = 10% AMD, 20\_ AMD = 20% AMD, 40\_ AMD = 40% AMD (where: different letters represent significant differences related with the ANOVA and Newman-Keuls Test).



**Fig. S5:** Concentration of nitrate+nitrite at 1<sup>st</sup> day for the interaction between AMD and MHW factors. Treatments: CT = Control Temperature. MHW = Marine Heatwave. 0 = 0% AMD, 10\_ AMD = 10% AMD, 20\_ AMD = 20% AMD, 40\_ AMD = 40% AMD (where: different letters represent significant differences related with the ANOVA and Newman-Keuls Test).