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WILLIAN DA SILVA OLIVEIRA

Modelagem da biorremediação da Lagoa da Conceição - Florianópolis/SC com biomassa de *Ulva ohnoi* (Ulvophyceae, Chlorophyta)

DISSERTAÇÃO DE MESTRADO

Florianópolis

Setembro 2023

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Orientador: José Bonomi Barufi

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O presente trabalho em nível de Mestrado foi avaliado e aprovado, em 06 /09/ 2023 pelos membros:

Prof .Dr. Paulo Tamaso Mioto (Membro Titular - Interno)

Universidade Federal de Santa Catarina

Profª.Drª Estela Maria Plastino (Membro Titular - Externo)

Universidade de São Paulo

Prof. Dr. Carlos Yure Barbosa Oliveira (Membro Suplente – Interno)

Uiversidade Federal de Santa Catarina

Dr. Fábio Nauer (Membro Suplente - Externo)

Instiuto de Pesquisas Ambientais/ SP

Certifico que esta é a versão original e final do trabalho de Mestrado.

Documento assinado digitalmente
Fernanda Maria Cordeiro de Oliveira
Data: 05/10/2023 10:33:08-0300
CPF: ***.315.629-**
Verifique as assinaturas em https://v.ufsc.br

Coordenação do Programa de Pós-Gradução

Biologia de Fungos, Algas e Plantas - PPGFAP

Documento assinado digitalmente
Jose Bonomi Barufi
Data: 03/10/2023 16:46:00-0300
CPF: ***.777.208-**
Verifique as assinaturas em https://v.ufsc.br

Prof. Dr. José Bonomi Barufi

Orientador

Florianópolis

Setembro 2023

“Dedico a Deus por sempre estar ao meu lado nos momentos mais difíceis desse trabalho e na vida”.

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“Pequenos passos na direção certa, podem ser o maior passo da sua vida”

“O medo de CAIR não pode ser maior que a paixão de Voar”.

RESUMO

Ulva ssp. são tolerantes às variações de salinidade e apresentam fácil aclimatação, desempenhando um papel essencial na despoluição dos ecossistemas aquáticos justamente por sua alta eficiência na absorção e acúmulo de nutrientes. Por esta razão, *Ulva* spp. tornam-se uma solução atrativa para a recuperação de áreas que sofrem impactos de problemas como a eutrofização de origem antrópica. Além de ser uma alternativa promissora para a bioeconomia azul, as algas desse gênero podem contribuir para a sustentabilidade das atividades econômicas nas áreas costeiras. Portanto, o presente estudo pretendeu desenvolver e elucidar o comportamento de *Ulva ohnoi* usando modelos preditivos de resposta de superfície. As algas foram cultivadas sob diferentes concentrações de nutrientes e níveis de salinidade, conforme previsto pelo delineamento experimental, e foram avaliadas quanto ao potencial da biomassa em absorver os nutrientes, bem como seu desempenho fotossintetizante e parâmetros bioquímicos. Este trabalho confirmou a alta eficiência de *Ulva ohnoi* na absorção de nitrogênio dissolvido no meio na forma de NH_4^+ (91,58% ± 1,28) e que a salinidade é um fator essencial na dinâmica e velocidade de absorção de amônia, principalmente em longos períodos de cultivo com disponibilidade limitada de fontes. A absorção de fósforo por *U. ohnoi*, na forma de ortofosfato ($P\text{-}PO_4^{3-}$), é revertida para o meio de cultivo quando submetido a cultivo de duas semanas de duração. Este processo é intensificado sob salinidades baixas mesmo em condições de disponibilidade do composto (29,10% ± 0,94dia^{-1}). Os modelos 3D de superfícies de resposta obtidos na análise das variáveis empregadas elucidam o desempenho de *Ulva ohnoi*, atribuindo uma correlação dinâmica entre disponibilidade de nutrientes e salinidade e o desempenho biológico da espécie. Esses modelos se mostram valiosos na aplicação de ações de biorremediação em situações problemáticas, como observado na Lagoa da Conceição – SC/Brasil. Diante do exposto por esses modelos, bem como os efeitos da distribuição salina ao longo da Lagoa, são sugeridas as regiões: Centro-Norte, Centro e Sul - como potenciais áreas de implementação de projetos de biorremediação com *Ulva ohnoi*.

Palavras-chaves

Ulva ohnoi – Biorremediação – Modelo de Superfície de Resposta – Design Experimental – Otimização - Eficiência de Absorção de Nutrientes.

ABSTRACT

Ulva ssp. are tolerant to salinity variations and exhibit easy acclimation, playing an essential role in the depollution of aquatic ecosystems precisely due to their high efficiency in absorbing and accumulating nutrients. For this reason, *Ulva* spp. becomes an attractive solution for recovering areas that suffer the impacts of problems such as the eutrophication of anthropogenic origin. In addition to being a promising alternative for the blue bioeconomy, it can contribute to the sustainability of economic activities in coastal areas. Therefore, the present study aimed to develop and elucidate the behavior of *Ulva ohnoi* using predictive surface response models. The algae were grown under different concentrations of nutrient and salinity levels, as predicted by the experimental design, and it was evaluated according to the potential of the biomass to absorb the nutrients, as well as its photosynthetic performance and biochemical parameters. Our study confirmed the high efficiency and preference of *Ulva ohnoi* in the absorption of nitrogen dissolved in the medium in the form of NH_4^+ (91.58% ± 1.28) and that salinity is an essential factor in the dynamics and speed of ammonium absorption, mainly in long periods of cultivation with limited availability of sources. The absorption of phosphorus by *U. ohnoi*, in the form of orthophosphate ($P\text{-}PO_4^{3-}$), is reverted to the culture medium when subjected to long-term cultivation. This process was more intense because of low salinity, even at conditions of availability of the compound (29.10 ± 0.94%.day^{-1}). The 3D models of response surfaces obtained in the analysis of the variables employed elucidate the behavior of *Ulva ohnoi*, attributing a dynamic correlation between nutrient availability and salinity and the biological behavior of the species. These models prove to be valuable in the application of bioremediation actions in problematic situations, as observed in Lagoa da Conceição – SC/Brazil. In view of what is exposed by these models, as well as the effects of saline distribution along the Lagoon, the following regions are suggested: Center-North, Center and South - as potential areas for the implementation of bioremediation projects with *Ulva ohnoi.*

Keywords

Ulva ohnoi – Bioremediation – Response Surface Model – Experimental Design – Optimization - Nutrient uptake efficiency.

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LISTA DE ABREVIATURAS E SIGLAS

AMTI – Integrated Multitrophic Aquaculture

ANOVA – Analysis of Variance

CCD – Central Composite Design

DOE – Experimental Design for Optimizing the Environmental

EAUE – Effective Amnonia Uptake Efficiency

EPUE – Effective Phosphate Uptake Efficiency

GR – Growth Rate

LAFIC – Phycology Laboratory

LEI – Evapoinfiltration Pond

RAUE – Relative Ammonia Uptake

Efficiency RPUE – Relative Phosphate Uptake

Efficiency RSM – Response Surface

Methodology

SES – Sistema de Esgotamento Sanitário

SUMÁRIO

1. INTRODUÇÃO - GERAL

O filo Chlorophyta abrange uma ampla diversidade de macroalgas marinhas verdes, e uma de suas classes mais notáveis é a classe Ulvophyceae, estabelecida por Mattox & Stewart em 1984. Essa classe engloba uma variedade de formas, hábitos e organização celular em suas macroalgas (Lu et al., 2006; Raven et al., 2014; Guiry & Guiry, 2021). Ulvophyceae compreende 10 ordens, sendo a ordem Ulvales uma das mais representativas em termos de número de espécies (Guiry & Guiry, 2021).

A ordem Ulvales foi originalmente criada por Blackman & Tansley (1902) para acomodar a família Ulvaceae J.V. Lamouroux ex Dumortier. Essa família é caracterizada por talos eretos, organização celular parenquimatosa, células uninucleadas com um único cloroplasto e zoósporos quadriflagelados, além de isogametas biflagelados. Os talos da ordem Ulvales podem apresentar morfologia tubular ou foliácea, distanciando-se dos talos filamentosos da ordem Ulotrichales, onde a família costumava ser classificada anteriormente (Maria & Pereira, 1998; Barata, 2004). A ordem Ulvales é composta por 22 representantes com três níveis de organização do talo: foliáceo, tubular e filamentoso.

O gênero *Ulva* desempenha um papel crucial no ecossistema marinho, apresentando funções vitais na ciclagem de nutrientes e servindo como habitat e fonte de alimento para uma ampla variedade de espécies. As espécies de *Ulva*, conhecidas como *Ulva* spp., geralmente ocupam a zona entremarés do litoral marinho, fixando-se ao substrato por meio de células rizoidais para formar um disco basal (Guiry & Guiry, 2022). Além disso, algumas espécies de *Ulva* são de vida livre e podem flutuar com as correntes marinhas (Melton et al., 2016). Essas algas verdes são frequentemente encontradas em estuários e lagunas costeiras, especialmente quando a concentração de nitrogênio e fósforo nesses ambientes aumentam (Lanari & Copertino, 2017; Netto et al., 2018).

Ulva spp. demonstram notável tolerância às variações de salinidade e possuem uma capacidade de aclimatação ao ambiente, o que resulta na formação de diferentes ecótipos ao longo de sucessivas gerações (Lobban & Harrison, 1994). Essa capacidade de adaptação está associada ao desenvolvimento de mecanismos de defesa, aumentando a disponibilidade de antioxidantes e a atividade de enzimas antioxidantes em resposta às mudanças na salinidade (Lu et al., 2006). As espécies de *Ulva* têm uma ampla gama de aplicações em diversas áreas, desde suplementos alimentares até aplicações biomédicas. Elas têm sido consideradas agentes nutracêuticos, ou seja, podem ser utilizadas como alimentos que promovem funções fisiológicas benéficas, melhorando o bem-estar e reduzindo a suscetibilidade a certas doenças, como distúrbios inflamatórios (Fournière et al., 2019), câncer (Abd-Ellatef et al., 2017) e infecções antibacterianas e virais. Os principais polissacarídeos presentes em *Ulva* spp. podem ser agrupados e designados como ulvanos (Mo'o et al., 2020).

1.1. Eutrofização e Bioprocessos associados à biomassa de *Ulva* spp.

1.1.1. Eutrofização

A adição e acúmulo de nutrientes inorgânicos e matéria orgânica em corpos d'água desencadeiam um aumento nos processos de fotossíntese e respiração de sistemas orgânicos presentes nesses ambientes. Essa condição é conhecida como eutrofização, que representa um estado de alto metabolismo no processamento de energia nos ecossistemas aquáticos (Beyers & Odum, 1994). A eutrofização é considerada uma etapa na sucessão natural desses ecossistemas, onde, ao longo do tempo, os nutrientes se acumulam e o fitoplâncton se desenvolve em maior quantidade, levando ao florescimento frequente de algas (Wetzel, 1993). Quando ocorre naturalmente, a eutrofização é um processo gradual elento, levando muitas décadas para se estabelecer.

No entanto, quando o processo é acelerado pela ação humana, ocorre um aumento desordenado na produção de biomassa, que não pode ser incorporado pelo sistema aquático na mesma velocidade, resultando em um desequilíbrio ecológico. Esse tipo de eutrofização é denominado de "eutrofização cultural" (Souza, 1993). A eutrofização cultural é causada principalmente por atividades humanas, como o lançamento de esgotos domésticos, despejo de resíduos agrícolas, poluição do ar e deposição atmosférica de materiais, além do desmatamento das áreas próximas a represas antes de seu fechamento. As principais consequências da eutrofização cultural nos ecossistemas aquáticos incluem o aumento da biomassa e da produção primária do fitoplâncton, a diminuição da diversidade de espécies, a redução da concentração de oxigênio dissolvido, a diminuição da concentração de íons, o aumento do fósforo total no sedimento e o aumento da frequência de florescimento de cianofíceas (Tundisi, 1986a).

O crescimento acelerado das espécies de *Ulva*, juntamente com sua capacidade de se adaptar a diversos ambientes e climas, e o aumento da entrada de nutrientes nas águas costeiras têm levado à ocorrência de florações dessas macroalgas em ecossistemas costeiros (Teichberg et al., 2010). Essas florações podem causar sérios problemas para o uso humano das áreas costeiras e reduzir significativamente o interesse comercial nessas regiões. A grande quantidade de *Ulva* pode se acumular na linha de costa, produzindo maus odores durante o processo de decomposição (Wilce et al.,1982), prejudicando o turismo e a pesca locais. Além disso, as florações têm efeitos drásticos sobre as comunidades naturais e as funções dos ecossistemas afetados. A decomposição da matéria orgânica das algas contribui para condições anóxicas, levando à morte de peixes e crustáceos.

1.1.2. Bioprocessos associados à produção e biomassa de *Ulva* spp.

Quando são cultivadas de forma controlada como monoculturas, as espécies de *Ulva* podem gerar uma biomassa de alta qualidade com composição bastante regular. Em média, as algas verdes possuem cerca de 11% de proteínas, 36% de carboidratos e 53% deminerais como cálcio, ferro e fósforo (Castro-González et al., 1996). Essa biomassa de *Ulva* spp. pode ser processada e seus resíduos utilizados como fertilizantes na agricultura, adicionados a rações para animais, processados como alimentos, biocombustíveis e até mesmo para a produção de produtos com aplicação farmacológica (Finkl et al., 2007).

A tecnologia de biorremediação conhecida como Aquacultura Multitrófica Integrada (AMTI) é uma abordagem ecossistêmica que combina o cultivo de animais aquáticos (como peixes e camarões), alimentados artificialmente, em associação com organismos fotossintetizantes, como por exemplo *Ulva* spp.. Essa abordagem busca criar um sistema integrado onde diferentes organismos se beneficiam mutuamente e contribuem para a sustentabilidade ambiental e econômica (Chopin et al., 2001; Troell et al., 2003; Neori et al., 2004; Buschmann et al., 2008).

No sistema de Aquacultura Multitrófica Integrada, o cultivo de espécies que são alimentadas com ração ou suplementos é combinado com o cultivo de *Ulva* spp.. Os resíduos e nutrientes produzidos pelas espécies alimentadas artificialmente são aproveitados pelas macroalgas como fonte de nutrientes, contribuindo para o seu crescimento. As *Ulva* spp., por sua vez, atuam como agentes de biorremediação, pois absorvem esses nutrientes residuais e os convertem em processos fisiológicos e metabólicos, como crescimento vegetativo e reprodutivo (Shpigel et al., 2019; Massocato et al., 2023).

Essa abordagem tem sido proposta como uma solução para mitigar os efeitos ambientais negativos da aquacultura intensiva, pois ajuda a reduzir a poluição por nutrientes nas águas costeiras. Ao utilizar as macroalgas para absorver e reciclar os nutrientes excedentes, a AMTI contribui para manter um equilíbrio mais saudável nos ecossistemas aquáticos (Copertino et al., 2009; Shpigel et al., 2019; Shahar et al., 2020).

1.2. Lagoa da Conceição - Breve histórico e a Lagoa de Evapoinfiltração (LEI)

A bacia hidrográfica da Lagoa da Conceição é considerada como uma laguna sufocada uma vez que da ocupação total da área de 82,1 km^2, o corpo lagunar representa uma área de 20,3 km^2, conectada ao mar através de um canal raso e meandrante com 2,8 km de extensão (José, 1998). Por meio desse canal, podem ocorrer trocas de volumes hídricos com o oceano, permitindo a entrada e saída de nutrientes da laguna, por exemplo. Essas limitações relacionadas à capacidade de trocas com os oceanos e alto tempo de resistência na infiltração das águas nos lençóis freáticos da laguna, são fatores que aumentam a importância de se estabelecer um controle de descargas de nutrientes neste corpo d'água.

A fim de atender à crescente demanda populacional e suprir a necessidade de tratamento de esgoto em Florianópolis, foi implementado o Sistema de Esgotamento Sanitário (SES) Lagoa da Conceição em 1988. Esse sistema tem como objetivo coletar e tratar os esgotos gerados nas regiões próximas à Lagoa da Conceição, um corpo lagunar importante da região.

Até maio de 2022, o tratamento realizado no SES era do tipo secundário, no qual não ocorria a remoção de nutrientes, como fósforo e nitrogênio, presentes nos esgotos (Santos, 2018). O efluente tratado é encaminhado para uma lagoa de evapoinfiltração, localizada em uma área de dunas. Essa lagoa é uma estrutura especialmente projetada para permitir a evaporação do líquido tratado e a infiltração do efluente no solo. Esse processo ajuda a garantir que o efluente tratado seja adequadamente devolvido ao ambiente de forma segura e com baixo impacto ambiental. O processo de evapoinfiltração visa o retorno do efluente tratado para a natureza tanto pela infiltração na subsuperfície, onde chega ao lençol freático, como pela evaporação diretamente à atmosfera.

No dia 25 de janeiro de 2021, ocorreu o rompimento da barragem da Lagoa de evapoinfiltração (LEI) do SES na Lagoa da Conceição. Alguns aspectos como o excessivo depósito de efluentes, a sobrecarga da capacidade de infiltração no solo, e a somatória de níveis pluviométricos além do esperado para época em Florianópolis, potencializaram o derramamento e o despejo de cerca de 100.000 m^3 de fluidos represados na LEI, diretamente na Lagoa contendo componentes elutriados dissolvidos e particulados, além do comporte de água doce que comprometeu em larga escala a salinidade da Lagoa da Conceição, já impactada pela abertura do canal que a conecta com o oceano.

Assim, considerando que as espécies de *Ulva* são eficientes na remoção de nutrientes do meio aquático, o presente estudo foi experimentalmente realizado a fim de determinar as condições ótimas de desenvolvimento do bioprocesso de remediação das águas da Lagoa da Conceição impactadas pela deposição de efluentes advindos da Lagoa de Evapoinfiltração. Assim, pretendeu-se determinar as condições de salinidade e quantidade de resíduo na qual a alga desempenharia melhor suas funções fisiológicas incluindo a remoção de nutrientes da água em um determinado periodo de tempo. Isso permite que tomadores de decisão possam implementar um sistema de biorremediação mais eficiente na Lagoa da Conceição utilizando *Ulva* spp.

2. OBJETIVO GERAL

Este trabalho pretendeu determinar em laboratório as condições ótimas para biorremediação de um efluente estuarino eutrofizado por parte de *Ulva ohnoi*.

2.1. Objetivos específicos

- Determinar a absorção dos componentes elutriados ($P\text{-}PO_4^{-3}$ – $N\text{-}NH_4^{+}$) por *Ulva ohnoi* em condições com diferentes teores de elutriado e variação de salinidade.

- Analisar o crescimento e o potencial de rendimento da fotossíntese de *Ulva ohnoi* em condições de variação de salinidade e elutriados;

- Avaliar o conteúdo pigmentar e o total de carboidratos presentes na biomassa de *Ulva ohnoi* após 7 e 14 dias de cultivo sob variação de salinidade e elutriado na água.

- Determinar os modelos de superfícies de resposta das diferentes variáveis dependentes a partir de equações polinomiais, abrindo a perspectiva de predizer as condições de cultivo de *Ulva ohnoi* sob variações de salinidade e teor de elutriado na água.

3. MODELING BIOREMEDIATION OF CONTAMINATED EFFLUENTS BY *Ulva ohnoi* – A PREDICTIVE PERSPECTIVE

Authors

Oliveira, W.[1], Moreira, B.R.[2], Rörig, L.[1,2,3], Horta, P. A.[3], Treichel, H.[4], Bonomi Barufi, J.[1,2,3]

[1] Post-Graduate Program in Biology of Fungi, Algae and Plants, Phycology Laboratory, Department of Botany, Biological Sciences Center, Federal University of Santa Catarina, Florianópolis 88040-900, Santa Catarina, Brazil.

[2] Post-Graduate Program in Biotechnology and Biosciences, Phycology Laboratory, Department of Botany, Biological Sciences Center, Federal University of Santa Catarina, Florianópolis 88040-900, Santa Catarina, Brazil.

[3] Phycology Laboratory, Department of Botany, Biological Sciences Center, Federal University of Santa Catarina, Florianópolis 88040-900, Santa Catarina, Brazil.

[4] Laboratory of Microbiology and Bioprocess (LAMIBI), Department of Biological Science, Federal University of Fronteira Sul, Erechim, RS, Brazil.

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3.1. INTRODUCTION

Enhancing the accuracy and relevance of water quality management requires a thorough understanding of the primary sources and consequences of global water quality issues. This knowledge is crucial as it directly influences the overall health of the aquatic environment and the ecological system (Xu et al., 2022). The prevalence of emerging pollutants in our water supply or wastewater is constantly rising due to escalating urbanization, industrialization, and agricultural production. These emerging pollutants include contaminants that currently lack regulations, which require their monitoring or public disclosure regarding their existence in water sources. The primary origins of these emerging contaminants are domestic discharges, hospital effluents, industrial effluents, agricultural runoff, livestock and aquaculture activities, and leachate from landfills. Municipal wastewater treatment systems significantly introduce emerging contaminants into water bodies (Morin-Crini et al., 2022).

Algae play an essential role in the depollution of aquatic ecosystems due to their high efficiency in absorbing and accumulating nutrients, heavy metals, and toxic substances present in water (Le Moal et al., 2019, Massocato et al., 2022; Lekshmi et al., 2022). Therefore, algae production can be a solution for recovering coastal areas affected by eutrophication. However, it is essential to note that algae production must be sustainable, considering environmental, social, and economic aspects. Adequate planning, regulation, monitoring, and management of the waste and by-products generated by algae production are necessary to minimize environmental impacts. In summary, algae production is a promising alternative for the blue bioeconomy and can contribute to the sustainability of economic activities in coastal areas (Araújo et al., 2021).

The *Ulva* genus is composed by green macroalgae and is extensively investigated due to its potential as biofilter for dissolved nutrients in mariculture effluents. With a global distribution of 99 taxonomically recognized species (Guiry & Guiry, 2022), *Ulva* plays a crucial role in marine ecosystems, contributing to nutrient cycling and serving as

habitat and food for various species. *Ulva* spp. tolerate salinity variations and quickly acclimates, forming different ecotypes through successive generations (Lobban & Harrison, 1994). This tolerance is linked to developing defense mechanisms, which can include increment of enzymatic and non-enzymatic antioxidant capacities due to changes in salinity (Lu et al., 2006).

Nutrients are one of the most important factors for maintaining algal species growth, and the algal ability to absorb them, preferably nitrogenous compounds and phosphorus constituents, contributes to the wide ecological distribution of them algae, being found in coastal areas around the world. The preference for NH_4^+ over NO_3^- is related to the fact that ammonium is available in a reduced state, facilitating its absorption. For phosphorus, macroalgae mainly absorb orthophosphate (PO_4^{3-}), which are utilized for the synthesis of nucleic acids and amino acids (Lobban & Harrison, 1994).

Salinity is widely recognized as one of the main environmental factors affecting *Ulva* development's ecophysiology. Research related to the growth of macroalgae of the genus *Ulva*, together with the variation in salinity in the environment, show that the reduction in salinity levels has a negative impact on the growth and nutrient absorption capacity of these organisms. These studies suggest that a salinity decrease negatively affects *Ulva*'s physiological performance and metabolism, limiting their ability to fully develop under low salinity conditions (Fong et al., 1996, De Oliveira et al., 2016).

Experimental design and response surface methodology (RSM) are valuable statistical methodologies that aid in identifying and optimizing the factors that influence a specific process. These techniques are beneficial when the goal is to reduce the number of experiments required while still achieving meaningful results (Aydar, 2018). The study by Ba & Boyaci (2007) addressed recent research on biochemical and chemical optimization, using response surface methodology (RSM) as a predictive source to investigate the effects of independent factors. The study also highlighted the importance of the choices and applications

of these parameters in the interpretation and association of the results obtained.

An ecological disaster was recorded on January 25, 2021, in Florianopolis, Santa Catarina (Brazil). The dam of the artificial evapo-infiltration lagoon (LEI) that receives treated effluents from the municipal sanitary regulation company was disrupted, introducing about 5.08 tons of sediment containing high amount of nitrogenous and phosphate compounds to the aquatic ecosystem of Lagoa da Conceição. The evapo-infiltration process aims to return the treated effluent to nature both by infiltration into the subsurface, where it reaches the water table, and by evaporation directly into the atmosphere (CASAN, 2021a). Lagoa da Conceição reservoir is connected to the ocean and presents a salinity range variation from 22 to 34. Moreover, some species of *Ulva* usually can be found in the lagoon, potentially causing blooms in this waterbody (Fonseca, 2006; Torres et al., 2014).

Then, this study aimed to determine the optimal responses after a response surface modelling experiment considering *Ulva ohnoi* against the variable conditions found after the disaster at the LEI of Lagoa da Conceição, considering the algal capacity of nutrients removal, as well as the algal growth, photosynthesis and biochemical responses.

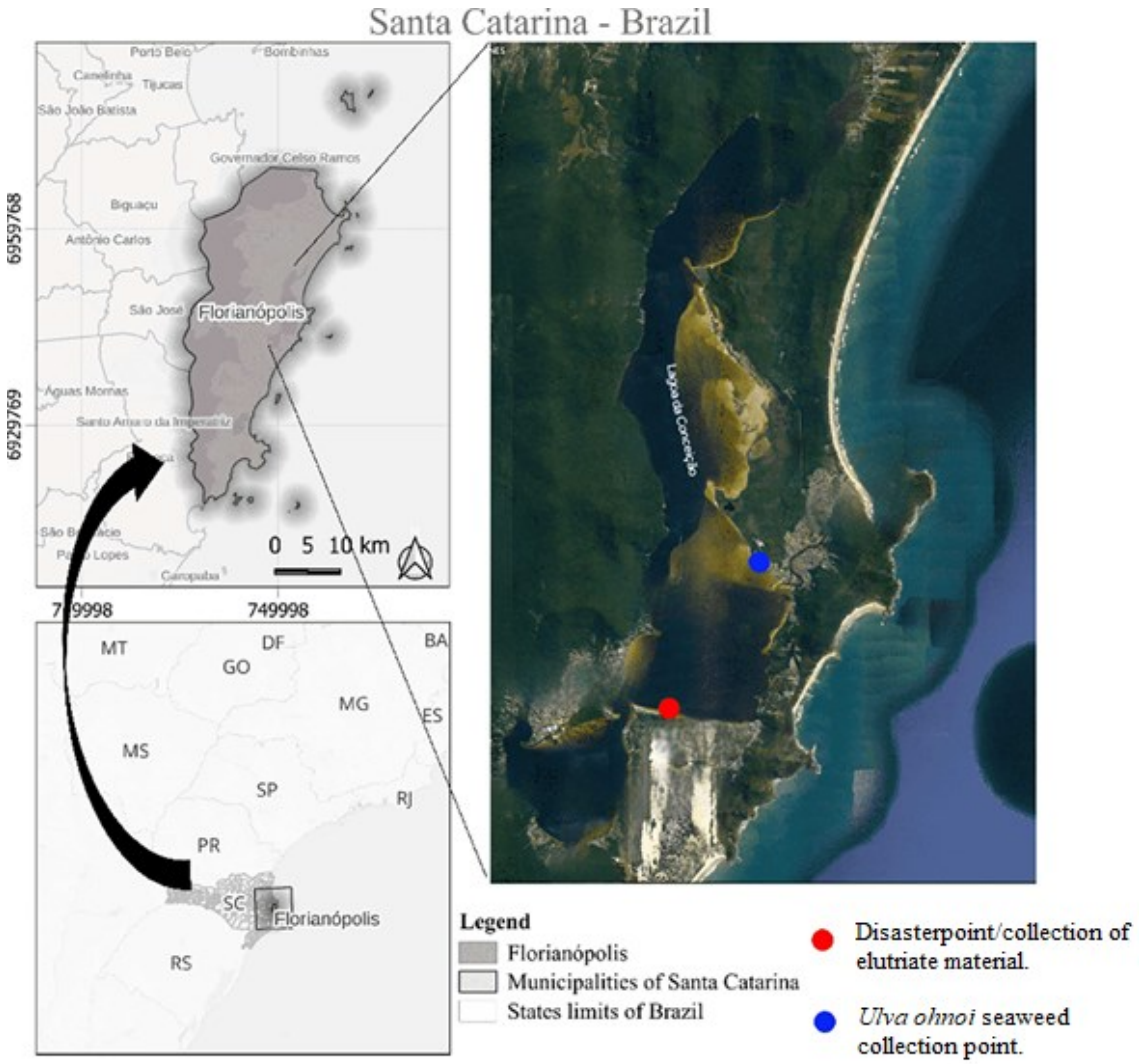
3.2. MATERIAL AND METHODS

3.2.1. Collection of algae material and effluent.

The *Ulva* species used in this study was collected from the artificial lagoon at the Elpídio Beltrame mariculture station located in Florianópolis, Santa Catarina, Brazil (27°354.64 S; 48°26 31.10 W). The effluent was collected from the Lagoa da Conceição (Figure 1), resulting from the rupture of the evapo-infiltration dam belonging to the city's sanitary treatment system. The algae and effluent were transported to the Phycology Laboratory of the Federal University of Santa Catarina (LAFIC–UFSC), where the experiments were conducted. The effluent underwent a vacuum filtration treatment with a porosity barrier of 0.25 μM to eliminate residues with a solid aspect and high density.

The filtration was performed to separate possible decomposed elements that could influence the parameters evaluated. Henceforth, the effluent will be referred to as the elutriate. The study material consisted of cultivated biological samples of *Ulva* spp. identified in work published by Salvi et al. (2021), from now on referred to as *Ulva ohnoi.*

Figure 1 – Sampling area of study in Lagoa da Conceição, Florianópolis, where samples were collected, considering the place of disruption of the evapo-infiltration reservoir and elutriate sampling (in red), and the place where the macroalgal samples of *Ulva ohnoi* were taken (in blue).



3.2.2. Experimental Design for optimizing the environmental factors (DOE) and response surface models (RSM).

In this study, we used a Design of Experiments (DOE) methodology to evaluate the effects of the two independent factors, salinity and elutriate concentration, on the cultivation of *Ulva ohnoi* to analyze bioremediation, algal physiology, and biochemical parameters. The experimental conditions were determined by a Central Composite Design (CCD) coupled to Response Surface Methodology (RSM), using the software Statistica 7®. The parameters and the levels to study the abiotic effects on *Ulva ohnoi* were: elutriate (20, 60, and 100%), salinity (15, 22.5, and 30), and the addition of a central point (60% of elutriate and 22 of salinity), with three replicates.

Eleven response variables of *Ulva ohnoi* were evaluated using the RSM, as follows: growth rate (% day^{-1}), effective ammonia uptake efficiency (EAUE = %. day^{-1} DW), relative ammonia uptake efficiency (RAUE = %. day^{-1}), effective phosphate uptake efficiency (EPUE = % day^{-1} DW), relative phosphate uptake efficiency (RPUE = %. day^{-1}), rate phosphate increment in treatments (%.day^{-1}), chlorophyll *a*, *b* (mg.g.$^{-1}$ DW), total carotenoids (mg.g.$^{-1}$ DW), total soluble carbohydrates (mg.g.$^{-1}$DW), and total insoluble carbohydrates (mg.g.$^{-1}$ DW).

DOE enables the systematic and efficient optimization of processes, reducing the number of assays needed in an experiment while achieving optimal results. The experimental design used included two factors at three levels (-1, 0, and +1) and three repetitions of the central composite point: Elutriate (20%, 60%, and 100%) and Salinity (15, 22.5, and 30).

Through CCD (Central Composite Design) analysis, the independent variables main, interaction, and quadratic effects were optimized and predicted in surface models with equations (**Table S2**) designed for further studies involving such factors. They are widely

used as they are relatively efficient concerning the number of runs required. In general, a k -factor CCD requires: (1) $2k^2$ factorial or apex points; (2) $2k^2$ axial or star points; and (3) number of center points (usually between three and five). Therefore, for two factors and only one central (minimum) point, we have 22+22+1=9 points. The distance of the axial points from the center will be given by α. The choice of the α value depends on several factors and some properties of axial designs. Therefore, the equation second order model was:

$$y=\beta 0+j=1k\beta jxj+i<j\beta ijxixj+j=1k\beta jjx2j+...;$$

Where y is the dependent variable to be evaluated, $\beta 0$, βj, βij, and βjj correspond to the regression coefficients, xi and xj to the independent variables, and e to the error. βjj represents quadratic or second-order effects. This model is called a second-order response surface (Rodrigues & Iemma, 2014).

Response surface models are mathematical equations describing the relation between two or more independent and dependent variables. These models are used to identify optimal conditions for a process or system and to understand the system's behavior under different conditions.

3.2.3. Experimental Set

The cultivation experiment was conducted to reproduce nine treatments, containing three replicates for each. The samples of *Ulva ohnoi* collected in the artificial settling lagoon were cleaned and placed in an acclimation environment, which consisted of 500 mL Erlermeyers flasks containing culture medium (autoclaved seawater) enriched with 8 $mL.L^{-1}$ Von Stoch solution (Von Stosch & Drebes, 1964) for seven days with a photoperiod of 12/12 hours (light/dark), at 25 ± 1°C, salinity of 35, aeration 15:15 minutes, under irradiance of 120 ± 10 μmols.photons.m^{-2}.s^{-1}. After the acclimation period, three portions of biomass of approximately 3 cm each were sectioned, totaling 0.2 g of fresh material per

treatment. The algae were placed in 400 mL flasks and submitted to the combinations proposed by the CCD, varying salinity and elutriate availability. Then, the ratio biomass/volume was 0.05 $g.mL^{-1}$, the other culture conditions remained the same as the adaptation period.

The experiment was carried out over fourteen days, and the measurements and ample collection to determine the response variables were performed at the beginning, after 7 and 14 experimental days. Algal biomass was sampled and frozen to biochemical procedures detailed below. After removing biomass on the seventh experimental day, the residual biomass was fitted with the resting water volume to the same original ratio (0.05 $g.mL^{-1}$).

3.2.4. Biofiltration capacity

To evaluate the nutrient removal performance of the algae, water samples were recollected from each treatment at the end of each proposed period (initial, after 7, and 14 days) for analysis of $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$. Initial values were obtained through the quantitative measurements of the samples recollected in the field. Aliquots of 50 mL of each treatment per analysis period were collected and stored at -20°C in polyethylene flasks for further analysis using the light spectrophotometry technique, according to Grasshoff & Johannsen (1972).

A standard curve was prepared according to the applied methodology of Grasshoff & Johannsen (1972) to calculate residual nutrients on the treatments. Nutrient ammonium uptake efficiency (RAUE/EAUE) and nutrient phosphate uptake efficiency (RPUE/EPUE) were calculated as follows:

$$\text{RAUE/EAUE (\%); RPUE/EPUE (\%)} = 100 - [(C_{t+1} \times 100) / Ct],$$

Ct represents the initial concentration of nutrients, and Ct+1 represents the concentration after the proposed period. The systemic analysis of the absorption capacity of nitrogen (NUA) compounds (specifically $N\text{-}NH_4^+$) and phosphate (NUP) compounds

(Orthophosphate-PO_4^{-3}) was basedon determining the initial concentration of the collected filtered elutriated substrate, with the gradual analyses established in the proposed experimental framework. The sample development chosen, in the form of a percentage of relative and effective efficiency, was based on a mathematical analysis of the micromolar values (μM) discovered and translated into an absorption rate system.

3.2.5. Relative algal growth rate

The relative growth rate (%.day^{-1}) of *Ulva ohnoi* was calculated using the formula described by Lignell & Pedersen (1989):

$$GR\ (\%\ day^{-1}) = [(Wt / Wi)^{1=t} - 1] \times 100,$$

Where Wi = initial wet weight, Wt = wet weight after t days, and t = days of cultivation. The biomass growth rates were calculated at the end of the seventh and fourteenth experimental days.

3.2.6. Photosynthetic performance

The photosynthetic performance was evaluated when recollecting the biomass samples in the field and during the experimental time (at days 1 and 7) in the different treatments. Measurements consisted of saturation pulses with a Diving PAM (Walz, Effeltrich®, Germany) connected to a Win-Control software. The algae samples were submitted to the saturation pulse in the dark photoperiod, allowing the measurement of chlorophyll *a* fluorescence variation. Then, the maximum quantum yield of PSII F_v/F_m was determined by the equation:

$$F_v/F_m = (F_m - F_0) / F_m$$

Where F_0 is the initial fluorescence of PSII; F_m is the maximum fluorescence of PSII after a saturating light pulse (0.4 s, approx. 9000 μmol photons m^{-2} s^{-1}), and F_v is the variable fluorescence corresponding to the difference between F_m and F_0.

3.2.7. Analysis of pigments and total carbohydrates

Chlorophyll *a*, chlorophyll *b*, and total carotenoids were extracted following the adapted methodology of Lichtenthaler & Buschmann (1987). For pigment quantification, 10- 15 mg of algae biomass from each treatment at different experimental times were ground in liquid nitrogen and then lyophilized.

Through methanolic extraction (MeOH) and the use of scanning wavelength reading with the aid of the spectrophotometer (Global Analyser-GTA-97®, Brazil), the corresponding values were obtained for each wavelength with the application of the correlation equations proposed in the methodology of Sumanta et al. (2014). Pigment concentration results are expressed as in $mg.g^{-1}$.DW of the respective pigment compounds.

For the analysis of total carbohydrates, the protocol stipulated by Ubreit et al. (1957) was used, where the resting pellet of pigments extraction was utilized to obtain an aqueous fractionation with the washes of this substrate for the total soluble carbohydrate reaction and the residue treated with digestion enzymes for the measurement of total non-soluble carbohydrates.

3.2.8. Statistical analyses

A multifactorial analysis of variance (ANOVA) was performed to evaluate the statistical significance of the abiotic factors on *Ulva ohnoi* cultivation, considering elutriate and salinity as independent factors. The significance level was set at p-value < 0.05. Newman-Keuls post-hoc test was used to compare the significant differences among the treatments. Statistica 7® was used to design the experiment through a central composite design associated with the response surface methodology.

The two-way analysis of variance (ANOVA) was conducted to determine the

effectos of the factors elutriate concentration and salinity. The significance level was set at p- value < 0.05, meaning that any results with a p-value lower than 0.05 were considered statistically significant. After conducting the ANOVA test, the post hoc Newman-Keuls test was applied to the means multiple comparison, establishing the differences among the treatments which were significantly influenced by the experimental independent variables.

3.3. RESULTS

3.3.1. Response surface models

In this study, response surface models were used to explain the behavior of dependent variables according to elutriate concentration and salinity. With these models, it was possible to predict the variable behaviors in different conditions of these factors. The analysis of the variance of responses (**Table S2**) allowed to detect the presence of significant influence of the two factors (elutriate and salinity and their interaction) against the responses found from the alga and promoted the validation of the presented models.

All the dependent variables were adjusted to a second-order response surface model and their respective equations are presented in Table 1.

Table 1 - Equations obtained through the response surface models of *Ulva ohnoi*, according to the studied variables (z, response variable, x or y elutriate / salinity values) and their respective values of R^2 and adjusted R^2.

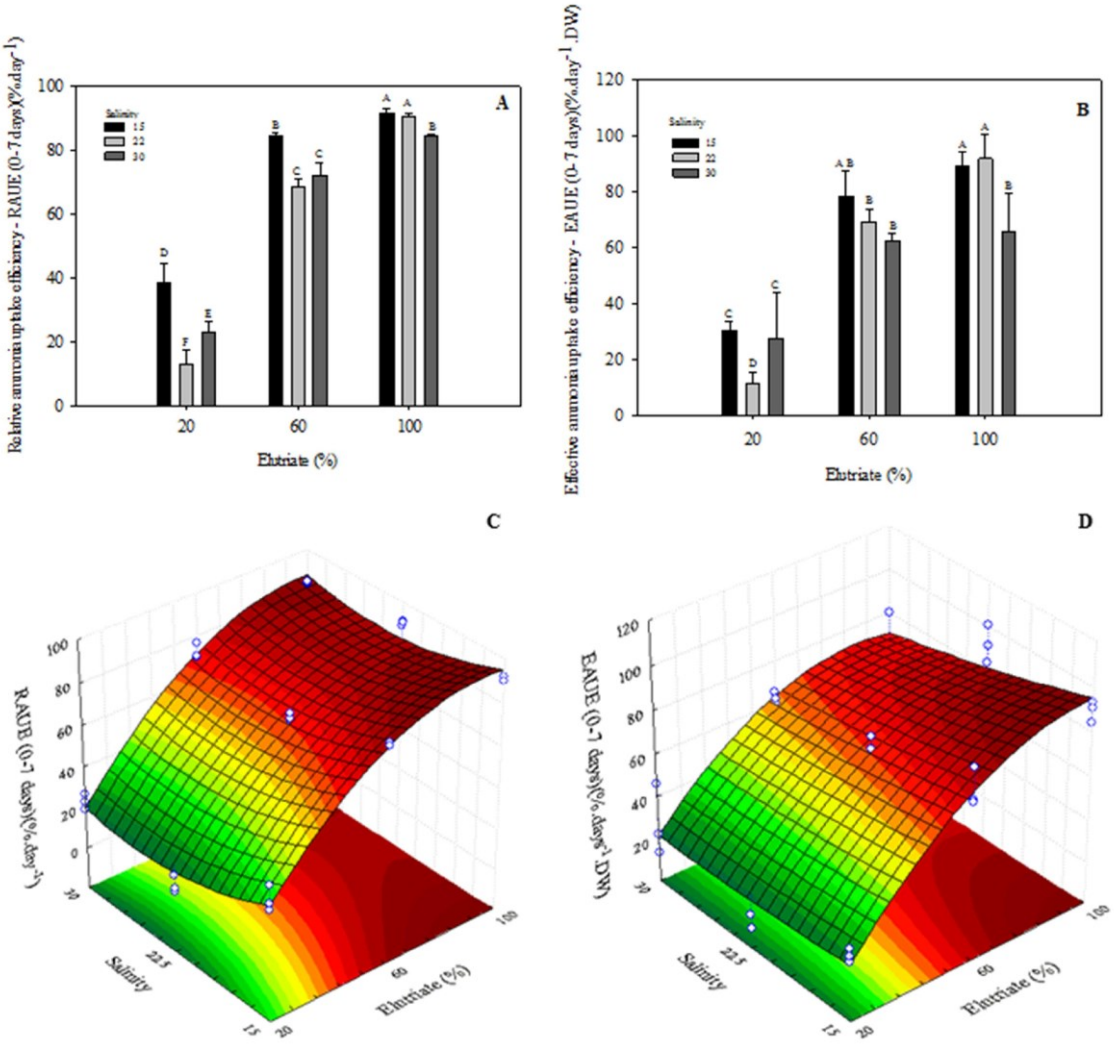
Variable	Descriptive Equation of Surface Models	R^2	R^2 adjs
Relative phosphate uptake efficiency (0-7 days)	z=82.43+32.36*x-22.63*x^2-3.26*y+6.28*y^2+.91*x*y+0	0.975	0.97
Effective phosphate uptake efficiency (0-7 days)	z=82.10+29.68*x-21.75*x^2-5.09*y-1.48*y^2-6.73*x*y+0	0.879	0.853
Relative amnonia uptake efficiency (0-7 days)	z=69.20+31.97*x-18.02*x^2-5.85*y+8.52*y^2+2.10*x*y+0	0.972	0.966
Effective amnonia uptake efficiency (0-7 days)	z=69.13+29.54*x-17.34*x^2-7.11*y+1.41*y^2-5.14*x*y+0	0.886	0.862
Relative amnonia uptake efficiency (7-14 days)	z=21.99+25.31*x-7.05*x^2+12.998*y-6.18*y^2-.83*x*y+0	0.735	0.68
Effective amnonia uptake efficiency (7-14 days)	z=52.65+47.87*x-26.29*x^2+10.27*y-24.39*y^2+13.81*x*y+0	0.536	0.439
Chlorophyll a (7- 14 days)	z=108.18-25.44*x+67.46*x^2-102.08*y+113.42*y^2+34.91*x*y+0	0.587	0.501
Chlorophyll b (7- 14 days)	z=82.00-21.06*x+82.18*x^2-100.96*y+117.33*y^2+52.47*x*y+0	0.622	0.543
Total Carotenoids (7- 14 days)	z=89.70-54.16*x+69.70*x^2-93.71*y+102.55*y^2+106.54*x*y+0	0.665	0.595
Total Carbohydrates Insolubles (0-7 days)	z=28.92+1.13*x-1.79*x^2-2.80*y-4.53*y^2+.49*x*y+0	0.571	0.482
Total Carbohydrates Solubles (7- 14 days)	z=97.36-24.41*x-15.40*x^2-11.73*y+18.24*y^2+45.02*x*y+0	0.563	0.472
Total Carbohydrates Insolubles (7- 14 days)	z=31.18-5.672*x-1.77*x^2-6.88*y+14.01*y^2+17.76*x*y+0	0.712	0.652
Rate phosphate increment in treatments (7- 14 days)	z=23.01-4.12*x-2.03*x^2-13.66*y-8.99*y^2-4.39*x*y+0	0.746	0.693
Relative Fv/Fm (7 days)	z=.62+.057*x-.010*x^2-.043*y-.012*y^2+.04*x*y+0	0.642	0.568

3.3.2. Biofiltration capacity

3.3.2.1. Effective and relative uptake ammonia efficiency 0-7 days

According to the results obtained by the two-way ANOVA (Table S1), for the evaluation of the relative and effective rates of ammoniacal nitrogen (NH_4^+) uptake, the treatments were significantly influenced by the interaction of both variables, elutriate and salinity ($p < 0.05$). Regarding the relative uptake rate of ammonia nitrogen (RAUE %.day^{-1}), the treatments containing 100% elutriate associate to the salinity of 15 and 22 showed the highest potential to uptake ammonia (90.54% ± 1.12 and 91.58% ± 1.28, respectively). The treatments containing 20% elutriate showed the lowest ammonia uptake rates (Figure 2A). The effective ammonia uptake efficiency exhibited the same pattern of the relative absorption (Figure 2B), with the highest rates of absorption in the treatments containing 100% of elutriate associated to 15 and 22 of salinity.

Figure 2 – Uptake of ammoniacal nitrogen by *Ulva ohnoi* for 0-7 days of treatment, considering the independent factors in the study, elutriate and salinity. **A, B:** Relative and effective efficiency of uptake. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test; **C-D:** Response surface graph associated with relative and effective uptake of ammoniacal nitrogen.



The surface model responses allow to identify the optimal condition to the response evaluated. ANOVA results (**Table S2**) indicated that, for both models (Figure 2C and 2D),

ammonia uptake by *Ulva ohnoi* was enhanced with the increase of elutriate concentrations, with the highest uptakes found when there were more elutriate. The linear effects of salinity were also observed, however the effect of elutriate concentration were more determining on ammonia uptake.

3.3.2.2. Effective and relative uptake ammonia efficiency 7-14 days

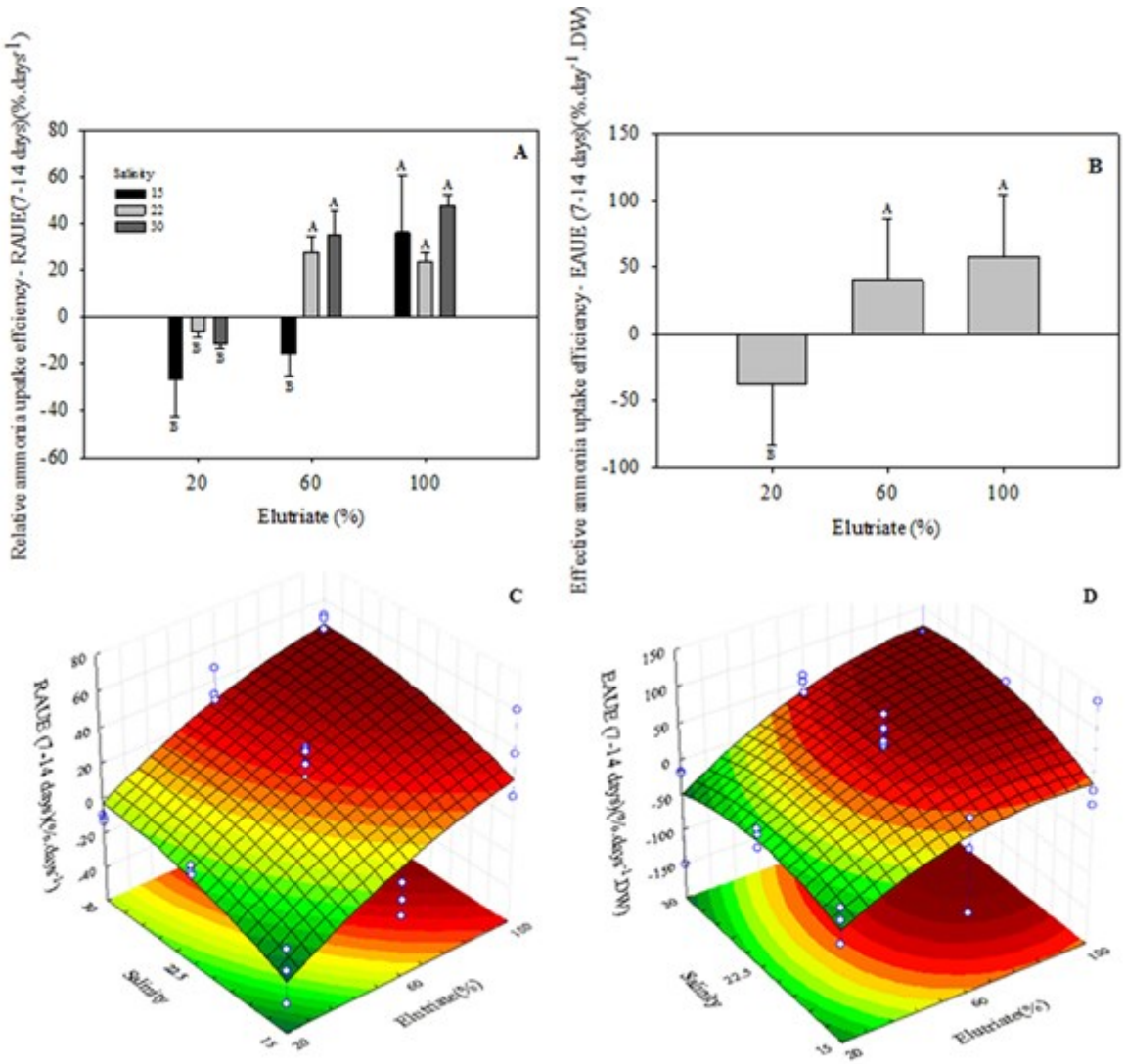
After 14 days of uninterrupted treatment and without replacement of the ammoniacal nitrogen sources, the relative effective absorption rates were analyzed following the same statistical parameters performed for the samples of 7 days of experimentation, and the results are presented in Figure 3.

At the second week on the experimental time, elutriate and salinity statistically influenced relative ammonia uptake efficiency, meanwhile only elutriate affected effective uptake rate efficiency (ANOVA – Table S1, $p < 0.05$).

The treatments that contained higher concentrations of elutriate, 60 and 100%, presented positive values for the relative uptake rates (Figure 3A), showing a response of capture and absorption of available ammonia in the medium (18.89% ± 22.37 and 36.13% ± 16.25). However, treatments with 20%, regardless of the studied elutriate, presented negative mean values concerning the absorption rate (-14.49% ± 12.27). For effective uptake rates (Figure 3B), *Ulva ohnoi* absorbed more ammonia levels on treatments with 60% and 100% than those with 20%.

Figure 3 - Uptake of ammoniacal nitrogen by *Ulva ohnoi* for 7-14 days of treatment, considering the independent factors in the study, elutriate and salinity. **A:** Relative efficiency of uptake ammoniacal nitrogen. **B:** Effective uptake of ammoniacal nitrogen, with salinity as a significant factor in the response; Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars

indicate statistical differences, after a posteriori Student-Newman Keuls test; **C-D:** Response surface graph associated with relative and effective uptake of ammonic nitrogen.



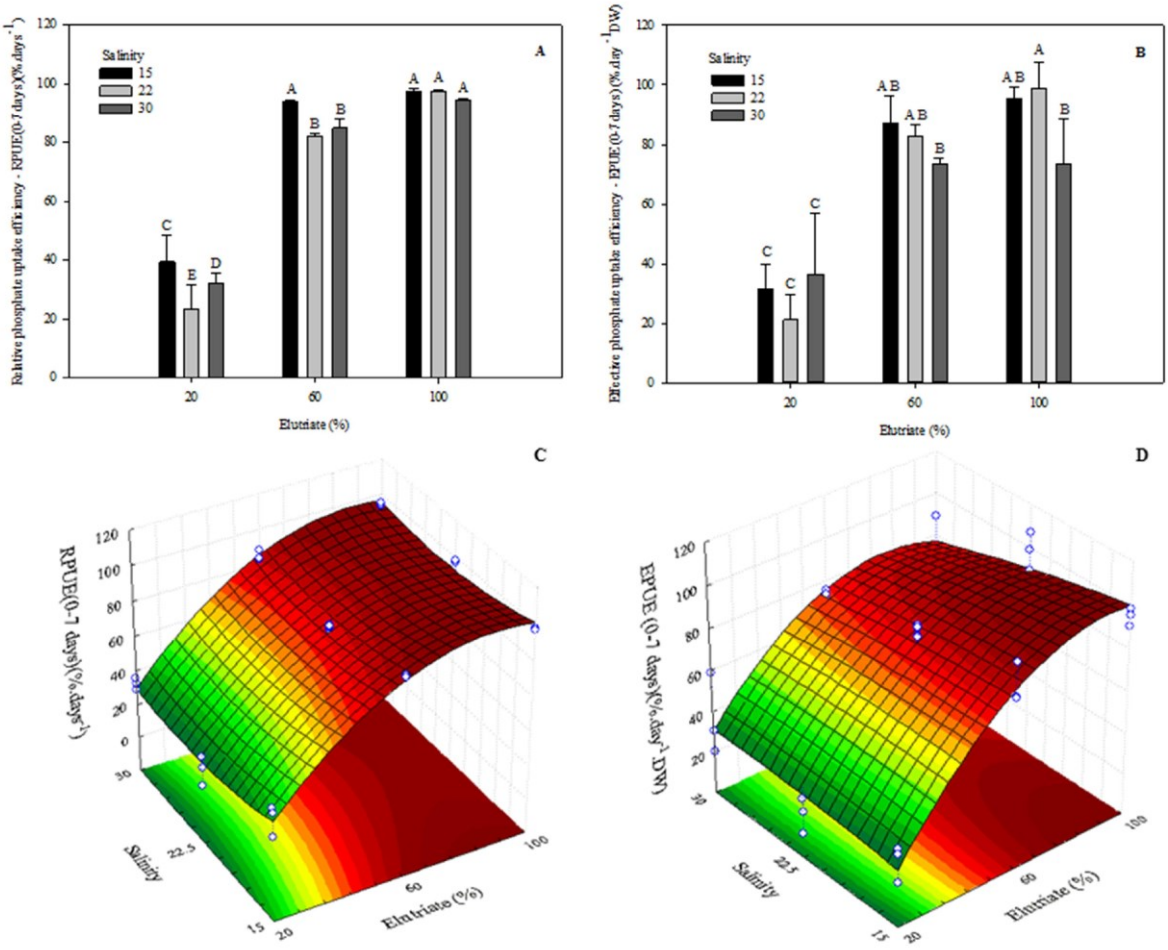
The surface response model associated with the relative efficiency of ammonia nitrogen absorption from 7 to 14 days showed that increasing of elutriate concentration and salinity influenced independently the ammonia uptake (Figure 3C). The increasing of elutriate also positively affected effective ammonia uptake by *Ulva ohnoi* (Figure 3D), meanwhile salinity did not affect this biological response (ANOVA – **Table S2**).

3.3.2.3. Relative and Effective uptake phosphate efficiency 0-7 days

The results obtained with ANOVA analysis (**Table S1**) of relative and effective phosphate uptake efficiency in 7 days of treatment indicated that salinity and elutriate significatively influenced those rates. Higher relative phosphate uptake rates were obtained in treatments with 60% and 100% elutriate concentrations when compared to treatments containing 20% of elutriate (Figure 4A). The lowest values of phosphate uptake were found in treatments containing 20% elutriate and salinity equivalent to 22 and 30 (23.23% ± 8.32; 32.29% ± 3.46.day^{-1} DW). As observed for relative uptake efficiency, the effective phosphate uptake rate of *Ulva ohnoi* at the first week of the experiment, the higher elutriate concentrations (60% and 100%) stimulated phosphate uptake (Figure 4B).

The surface response model of relative phosphate uptake efficiency indicated that the increase of elutriate concentration enhanced phosphate uptake, being the highest values found on treatments with 100% elutriate (Figure 4C, ANOVA – **Table S2**). For effective phosphate uptake efficiency, ANOVA analysis (Table S2) evidenced the interaction between elutriate concentration and salinity. The RSM indicated that increasing elutriate concentration and reducing salinity, there was a tendency of improvement of phosphate uptake rates (Figure 4D).

Figure 4 - Uptake of phosphate by *Ulva ohnoi* for 0-7 days of treatment, considering the independent factors in the study, elutriate and salinity **A-B:** Relative and Effective efficiency of uptake phosphate. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test; **C-D:** Response surface graph associated with relative and effective uptake of phosphate.

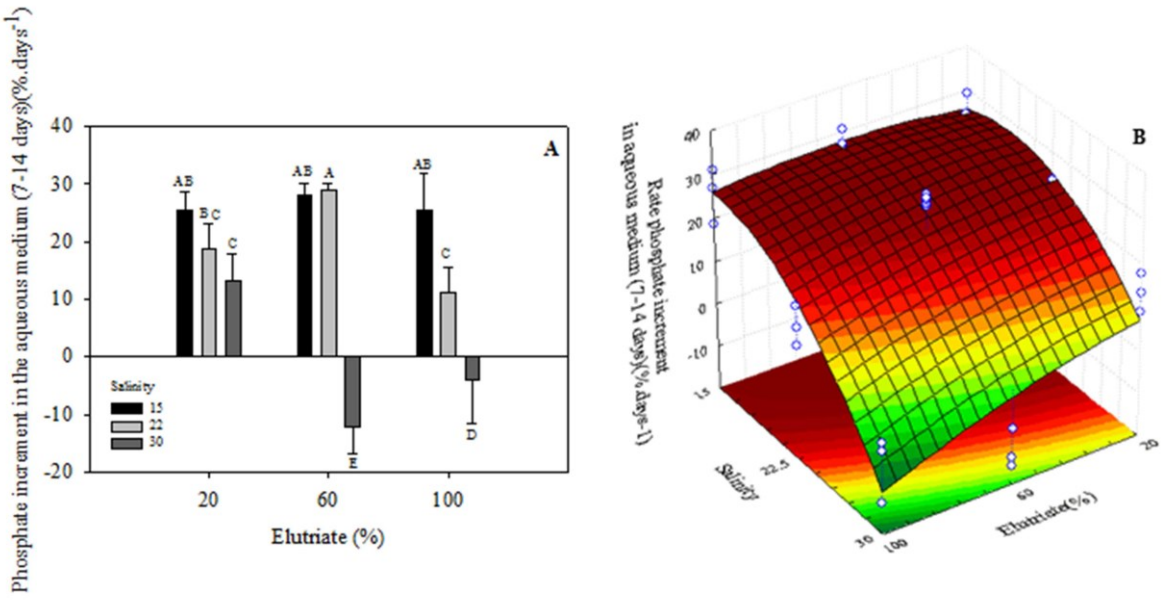


3.3.2.4. Incremental rate of phosphate returns in the aqueous medium in the period 7-14 days

During the second week of the experiment, *Ulva ohnoi* showed a reversal of the

uptake of phosphate assimilated into the aqueous medium of specific treatments. It means, instead of the expected decreasing phosphate in the water, there was an increment of the nutrient in the seawater after the period, indicating that possibly the algal released them to the media. Thus, to elucidate the behavior of the treatments under the observed conditions, the quantification of phosphate increment was performed (Figure 5).

Figure 5 – Phosphate increment in the seawater after cultivating *Ulva ohnoi* for 7-14 days of treatment, considering the independent factors in the study, elutriate and salinity. **A:** Rate of increase of phosphate in the aqueous medium of the treatments over the period 7-14 days. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test;. **B:** Response surface graph associated with a rate of phosphate increase.



The ANOVA analysis (**Table S1**) showed that the interaction between salinity and elutriate significatively affected the rate of phosphate increment in the aqueous medium. It

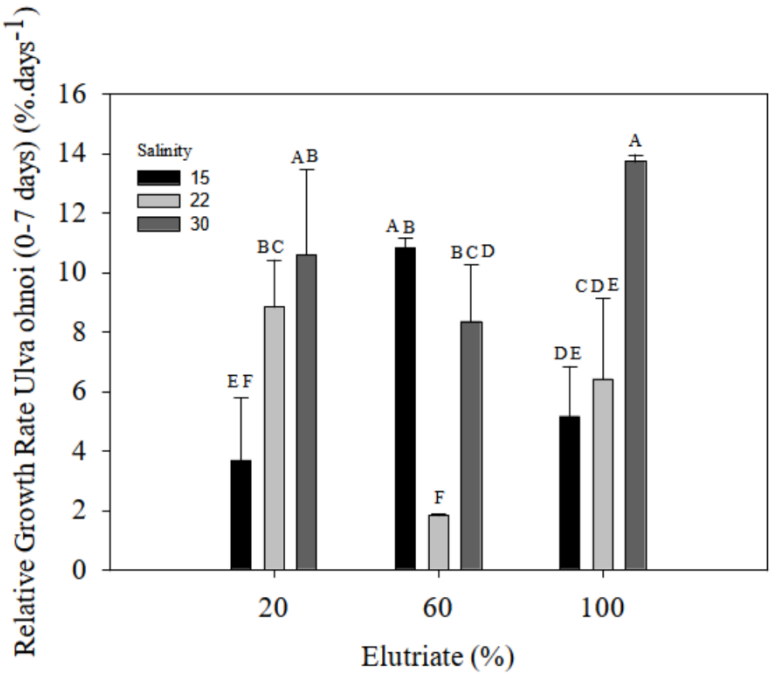
was observed that algae cultivated in all treatments, except treatments containing salinity equivalent to 30 and 60/100% of elutriate, stopped to absorb phosphate and started to release phosphate into the culture medium (Figure 5A).

The surface model suggested a behavior of reversion of phosphate to the aqueous medium by the algae considering elutriate and salinity, independently. It is possible to notice that lower values of salinity favored phosphate reversal to culture medium, instead of being absorbed (Figure 5B).

3.3.2.5. Relative growth rate

To evaluate vegetative growth rates of *Ulva ohnoi*, only the data obtained in the first week of experiment were analyzed. It was not possible to calculate growth rates of the second week because algal biomass losses were observed.

Figure 6 - Relative Growth Rate of *Ulva ohnoi* (%. $days^{-1}$) in the period of 0-7 days, cultivated under treatments with salinity variation rate (15, 22, and 30) and percentage of elutriate available in aqueous medium (20, 60, and 100%). Data are mean ± standard deviation, n=3. When significant differences weere indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test.

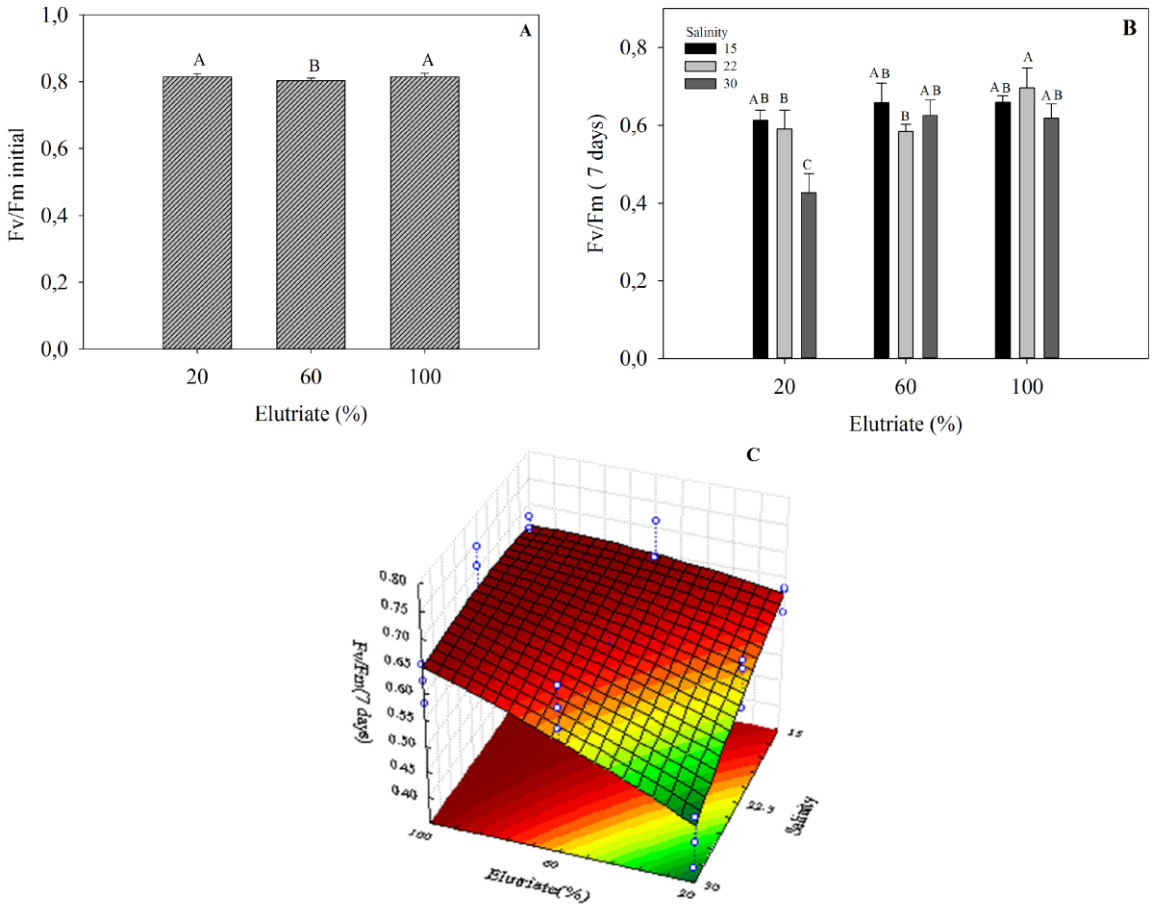


ANOVA analysis showed that salinity and elutriate significantly influenced vegetative growth rates in the period of 0-7 days for *Ulva ohnoi* (**Table S1**). The treatments with 100% elutriate and salinity of 30 in the culture medium were the ones that presented the highest growth rates, 13.72 ± 0.23 %.day^{-1} (Figure 6). Moreover, the treatments associated with 60% elutriate and salinity of 22 were the ones that presented the lowest growth of 1.83 ± 0.03 %.day^{-1}. The growth rates data were not significantly fitted with any type of model.

3.3.2.6. Photosynthetic performance

Maximum quantum yield (F_v/F_m) of *Ulva ohnoi* in the different treatments at the first and seventh experimental days are presented in Figure 7.

Figure 7 – Photosynthesis potential (maximum quantum yield, F_v/F_m) of *Ulva ohnoi* for the beginning and after 7 days of experiment, treated with different conditions of elutriate and salinity. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test. **A:** F_v/F_m associated with elutriate variation for the initial period; **B:** F_v/F_m associated with variation of elutriate and salinity factors after 7days; **C:** Surface response model described to photosynthetic response after 7 days.



ANOVA analysis showed a significant difference between the treatments influenced only by elutriate (p-value < 0.05, **Table S1**). The treatments containing 20% and 100% elutriate showed photosynthetic responses with values of 0.81 ± 0.01 and 0.81 ± 0.10of F_v/F_m, respectively, and the treatment with 60% elutriate obtained the response with an average of 0.80 ± 0.01 F_v/F_m (Figure 7A).

After 7 days, the interaction between elutriate and salinity significantly influenced the treatments (p-value < 0.05, Table S1), resulting in a reduction in the photosynthetic activity for all treatments compared to their initial values. The treatments show no significant difference between them over 7 days for F_v/F_m (Figure 7B). On the other hand, the treatment containing 20% elutriate and salinity 30 showed the most significant reduction on F_v/F_m (0.42 ± 0.04).

The surface model of photosynthetic activity suggests that lower concentrations of elutriate associated with high levels of salinity negatively affect the photosynthetic response (Figure 7C).

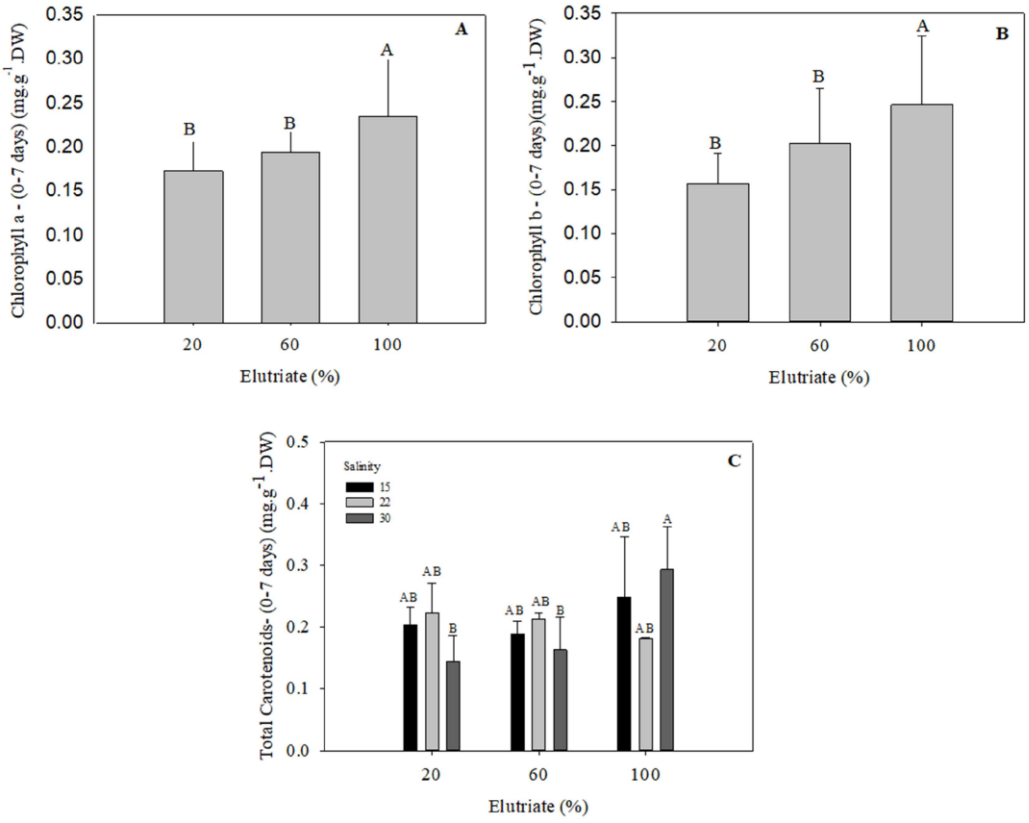
3.3.2.7. Pigments and total carbohydrate

3.3.2.7.1. Pigments

Pigment contents of *Ulva ohnoi* regarding chlorophyll *a*, chlorophyll *b*, and total carotenoids levels after 7 experimental days are presented in the Figure 8. Elutriate was the unique factor that influenced chlorophyll *a* and chlorophyll *b* synthesis (ANOVA- **Table S1**, p-value < 0.05). Treatments with a concentration of 100% showed the highest values of both pigments, chlorophyll *a* and *b* (0.23 ± 0.06 mg. g^{-1} DW and 0.24 ± 0.07 mg. g^{-1} DW, respectively) (Figure 8A and 8B).

The interaction between salinity and elutriate was significant in total carotenoids production, with its highest value found in treatments containing 100% of elutriate associated with salinity 30 (0.29 ± 0.06 mg. g^{-1} DW) (Figure 8C).

Figure 8 – Pigment contents of *Ulva ohnoi* (mg. g^{-1} DW) after 7 days of experiment, treated with different conditions of elutriate and salinity. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test. **A**: Chlorophyll *a*; **B**: Chlorophyll *b*; **C**: Total carotenoids.

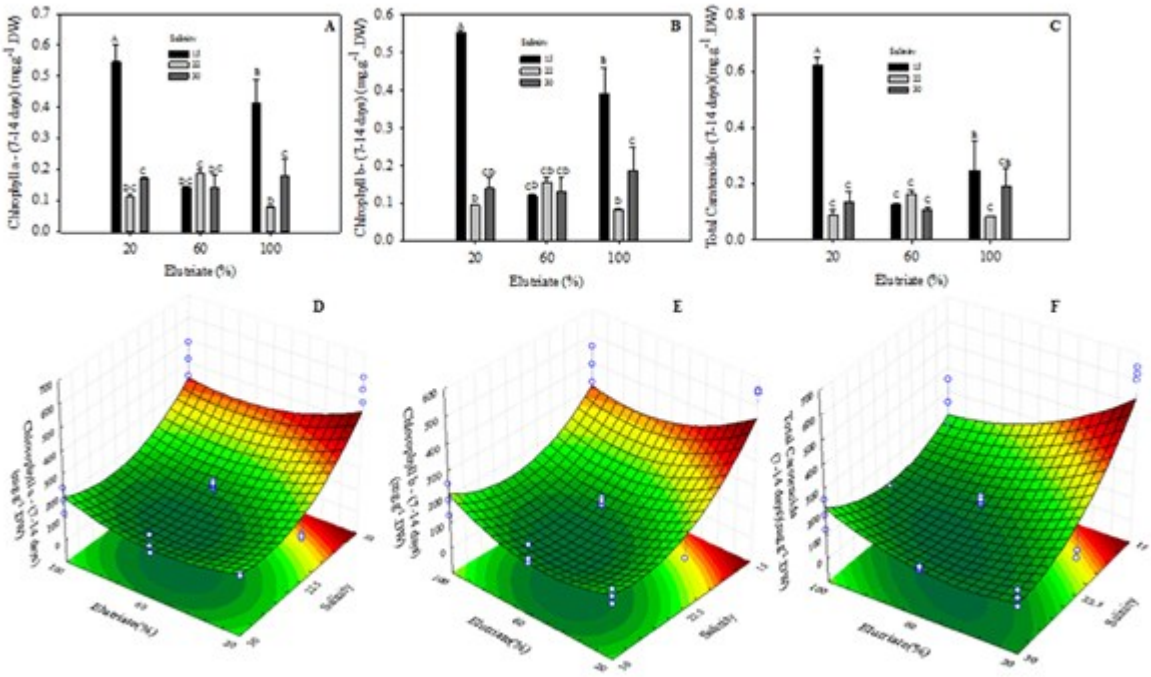


After 14 days of cultivation, the production of all pigments evaluated in *Ulva ohnoi* biomass was significantly affected by the interaction between salinity and elutriate (ANOVA – Table S1, p-value < 0.05). The highest chlorophyll *a*, *b* and carotenoids production, represented in Figure 9A, 9B and 9C, were obtained in treatments with 20% elutriate and 15 of salinity (chlorophyll *a* = 0.546 ± 0.055 mg.g^{-1}DW; chlorophyll *b* = 0.553 ± 0.005 mg.g^{-1}DW; total carotenoids = 0.621 ± 0.025 mg.g^{-1}DW).

The response models obtained for chlorophyll *a* and chlorophyll *b* exhibited the same pattern. Both pigment's productions were influenced by elutriate and salinity,

independently (**Table S2**). The optimal photosynthetic pigments production was found when *Ulva ohnoi* was associated with low salinity and low availability of elutriate (Figures 9D, 9E and 9F).

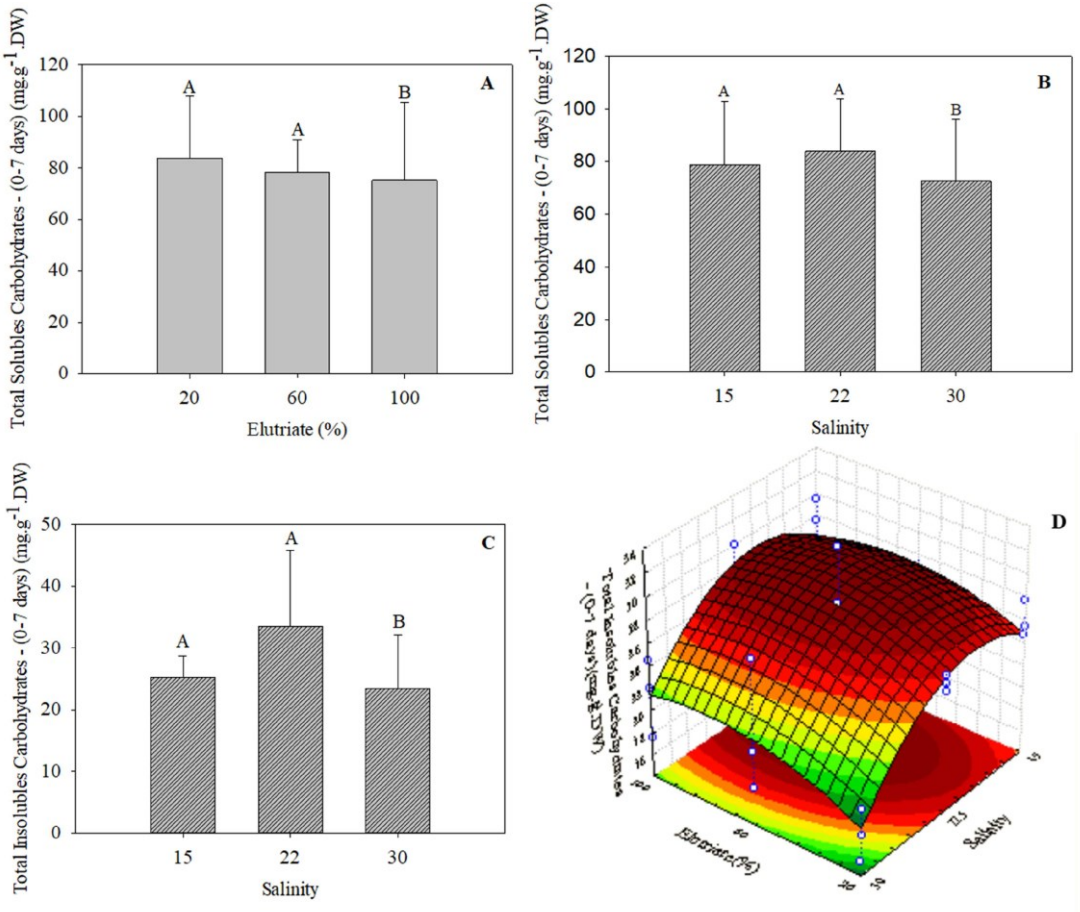
Figure 9 - Pigment contents of *Ulva ohnoi* (mg. g^{-1} DW) after 14 days of experiment, treated with different conditions of elutriate and salinity. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test. **A:** chlorophyll *a*; **B:** chlorophyll *b*; **C:** Total carotenoids; **D-E-F:** Response surface models associated with each variable, respectively.



3.3.2.7.2. Total Carbohydrates

Soluble carbohydrates and total insoluble carbohydrates of *Ulva ohnoi* after 7 days of cultivation with elutriate and salinity variation are showed in Figure 10.

Figure 10 - Total carbohydrates of *Ulva ohnoi* (mg. g-1 DW) after 7 days of experiment, treated with different conditions of elutriate and salinity. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test **A**: Total soluble carbohydrates associated with the elutriated factor; **B**: Total soluble carbohydrates related to the salinity factor; **C**: Total insoluble carbohydrates associated with the salinity factor; **D**: Surface response model of insoluble carbohydrates associated with the seven-day treatment period.



When analyzed separately, the total soluble carbohydrate content was affected by salinity and elutriate, independently (ANOVA- **Table S1**, p-value < 0.05). Figure 10A evidenced that treatments with 20% and 60% elutriate concentrations, presented the highest levels of soluble carbohydrates (81.54 ± 12.16 mg. g^{-1} DW and 77.60 ± 21.15 mg. g^{-1}

DW). In the salinity 15 and 22 (Figure 10B), where it was found the highest production values of soluble carbohydrates (78.73 ± 23.86 mg. g^{-1} DW and 83.90 ± .71 mg. g^{-1} DW).

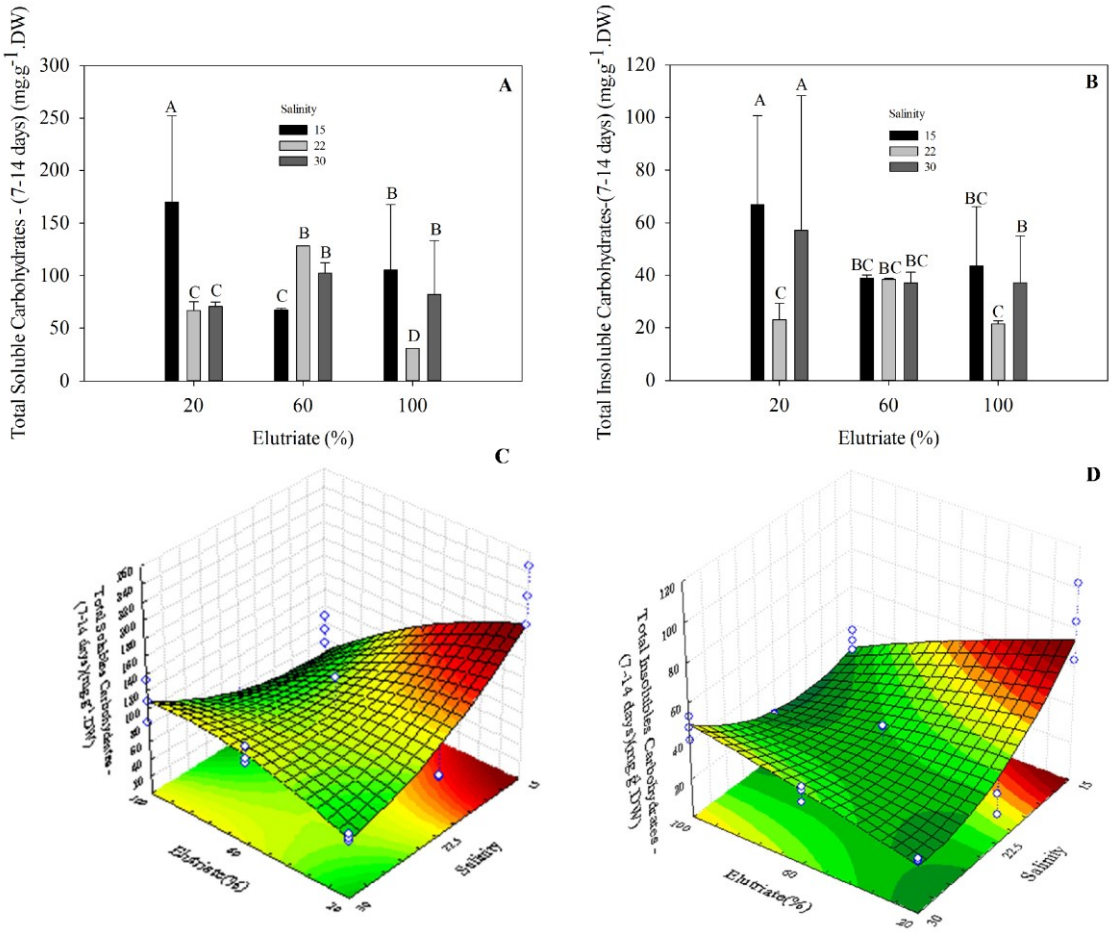
Only salinity factor showed to be significant (ANOVA-**Table S1**, $p < 0.05$) in the results of total insoluble carbohydrates. The highest content of insoluble carbohydrates (Figure 10C) was obtained in treatments with salinity 22 and 15 (28.03 ± 3.68 and 26.00 ± 1.87 mg.g^{-1} DW). The response model for production of total insoluble carbohydrates after seven days of the experiment suggests that median salinity trigger the best *Ulva* responses in the production of insoluble carbohydrates (Figure 10D).

Total soluble and insoluble carbohydrates production during period comprising 7-14 days of experimentation were modulated by the significant interaction between elutriate and salinity (ANOVA- **Table S1**, $p < 0.05$). The values found for 20% elutriate and salinity 15 were the most expressive among those studied (170 ± 81.75 mg. g^{-1} DW). In comparison, the treatment with 100% elutriate and salinity 22 obtained the smallest value (30.84 ± 0.09 mg. g^{-1} DW) (Figure 11A).

In the case of total insoluble carbohydrates, treatments with 20% elutriate and salinity 15 produced the highest values (66.87 ± 33.86 mg. g^{-1} DW). However, the treatments with 100% of elutriate and salinity at 22 resulted in the lowest assigned values (21.47 ± 1.17 mg. g^{-1} DW) (Figure 11B).

Considering the surface response model for insoluble carbohydrate production at 14 days of experiment, the pattern has changed when compared to the same response after 7 days. At the second week of experiment, the interaction of elutriate and salinity affected both soluble and insoluble carbohydrate levels (ANOVA - **Table S2**, $p < 0.05$), being the optimal condition to the production of these molecules the combination of low salinity values and low elutriate concentrations (Figure 11C and 11D).

Figure 11 - Total carbohydrates of *Ulva ohnoi* (mg. g^{-1} DW) after 14 days of experiment, treated with different conditions of elutriate and salinity. Data are mean ± standard deviation, n=3. When significant differences were indicated by ANOVA ($p<0.05$), different letters over the bars indicate statistical differences, after a posteriori Student-Newman Keuls test. **A:** Total soluble carbohydrates associated with the elutriated and the salinity factors; **B:** Total insoluble carbohydrates associated with the elutriated and the salinity factors; **C-D:** Response surface models associated with each variable respectively.



3.4. DISCUSSION

3.4.1. Effective and relative uptake ammonia efficiency

Ulva ohnoi's capacity to absorb nitrogen in the ammoniacal form ($N\text{-}NH_4^+$) was verified in this study, confirming the species' efficiency in removing nitrogen from wastewater. As observed, there is a high interaction effect between nitrogen availability and salinity, affecting ammonia absorption. Bews et al. (2021) found the negative influence of salinity on the physiological responses of *Ulva lactuca* when it is cultivated at low saline content. However, these effects were mitigated when associated with a preferred nitrogen source. Studies carried out by Arévalo et al. (2007), Henriques et al. (2017), and Brundu and Chindris (2018) have already highlighted the high efficiency in the removal and assimilation of nutrients by *Ulva* spp. and their high demand for nitrogenous compounds. They suggested an absorption rate of around 40–90% removal efficiency and the nutritional requirement based on nitrogenous compounds that are 4-6 times higher than any other seaweed species. Our results corroborate current literature, where was observed that the highest absorption rates and assimilation values in μM of ammonia in the outlined treatments (Figure 2) indicate that the cultures with 100% nutritional composition with NH_4^+ presented efficient responses around 91%. Moreover, precisely this higher absorption association is related to the high NH_4^+ content available in the medium, even in low salinity conditions, which goes against the assumptions of Bews et al. (2021). While treatment with lower availability of ammonia and lower saline composition result in late or limited absorption, a weakened efficiency was observed compared to the other treatments in the same period. This observation is explained by the preference of *U. ohnoi* for nitrogen sources arising from the presence of NH_4^+, to the detriment of sources arising from NO_3^- (L'Helguen et al., 2008; Glibert et al., 2014).

Glibert et al. (2016) and Shahar and Guttman (2020) showed that the preference for nitrogen in the form of NH_4^+ is considered adequate due in part to the lower energy parameters for cellular uptake, where NH_4^+ is more accessible for passive transport across the cell membrane than NO_3^-. In this way, observing high rates of $NH4^+$ absorption is feasible in view of the high nutritional availability required. This preference is possibly related to *Ulva* species directly metabolizing NH_4^+ to form amino acids using the Glutamine-Synthetase-Glutamine: 2-Oxoglutarate-amidotransferase GS-GOGAT) pathway.

For the period between 7 and 14 experimental days, it was possible to verify a change in the proposed scenario. The behavior of absorption and affinity of the seaweed for NH_4^+ was maintained even in conditions of low nutrient availability. For treatments with a high initial content of nitrogenous components, rates were sustained in absorption. However, it is possible to observe a decrease in absorption efficiency in general, so the treatments that previously showed an absorption rate of around 91% have now been reduced to 40% efficiency for the residual μM amount. Brundu and Chindris (2018), observed the same behavior: high absorption efficiency and assimilation of nitrogenous components, followed by a consistent drop in absorption rates after ten days of cultivation.

However, for all treatments characterized by a 20% nitrogenous constitution based on NH_4^+, regardless of salinity, an increase in ammonia concentration in the culture medium was observed over the 14 experimental days. This was explained by other authors (Krom and Neori, 1989; Krom et al., 1995; and Shpigel et al., 2009) who described the proliferation of ammonifying surface bacteria over a long period of cultivation (> 10 days). This proliferation is based on obtaining the optimal conditions for growth and development and on the variation of dissociated nutritional components and abiotic factors in the culture medium, which would trigger an external source of NH_4^+ production.

3.4.2. Uptake phosphate efficiency

When compared to the initial values discovered in the medium's availability during the experimental period, which consisted of the first seven days, the results obtained for the values associated with *Ulva ohnoi*'s orthophosphate (RPUE/EPUE: PO_4^{3-}) absorption rates were positive, as shown in Figure 4. Runcie et al. (2004) found that macroalgae's P content depends mainly on its absorption and storage capacity, varying over time according to availability in the growth medium. In addition, algae absorb phosphorus from the environment, mainly in the form of inorganic phosphate or orthophosphate (Pi). The level of phosphate absorption by macroalgae is generally saturable (Wallentinus, 1984). Therefore, Harrison et al. (1989) and Berges et al. (1994) described as being a rectangular hyperbole model that describes the dynamics of absorption is that a single carrier becomes saturated in a few high doses of concentration, many times above that found in the analysis of sea water or even in eutrophic estuaries.

The ability of an alga to absorb phosphorus-rich compounds is partially dependent on the action of extracellular phosphatases (Lee, 2000; Hernández et al., 2002), whereas the commonly observed phosphate absorption process is driven by one or more active phosphate transport systems (Raghothama & Karthikeyan, 2005). Studies promoted by Wallentinus (1984), Hurd and Dring (1990) and Runcie et al. (2004) suggested that the P uptake saturation in most macroalgae is around ~3.5 μM. These previous studies are in agreement with the efficiency of orthophosphate absorption by *Ulva ohnoi* in the first seven days. Nutritional availability is very relevant to absorption efficiency. Thus, the behavior of

phosphate absorption was more evident in the treatments with the highest composition (100% and 60% nutritional) and the median-low salinities (22 and 15), which were associated with a better effective response.

In contrast to absorption, the efflux of P from algae into the growth medium has been observed and claimed to be constant and unaffected by quantities of phosphorus that are available externally (Istvánovics & Herodek, 1995 and Lean and White, 1983). This is explained by the passive phosphate absorption by macroalgae, due to the negative electric potential flow through the plasmalemma. Therefore, external phosphate levels (micromolar concentrations) are usually far below internal cellular concentration (mM).

Given the above, the events observed for the second experimental phosphate plot (7–14 days) explain the algal need to conduct all phosphate concentrate accumulated during the initial absorption to an intermediate efflux, returning phosphate not metabolized and assimilated in biosynthetic pathways. In this case, salinity was an essential factor in the speed and intensity of the phosphate efflux into the medium. This scenario warns us about the time associated with the corresponding variables in the phosphate absorption and assimilation pathway since the efficiency dynamics are not modulated by the quantification of the available phosphate compound but by the need for the alga to metabolize it. Therefore, factors such as salinity can minimize the adverse effects of reversion of absorption but not prevent it from happening.

3.4.3. Relative growth rate

Growth rates for *Ulva* ssp. are strongly variable according to the conditions where the species are cultivated. Abiotic and biotic factors associated with the physiology of macroalgae influence their vegetative and/or germination growth dynamics (Choi et al., 2010, Brundu and Chindris, 2018), and Massocato et al., (2022), among others, stipulated that the growth range of *Ulva* species varies around 12.1–18.4% Day^{-1} for maximum growth potential under favorable conditions. Our study corroborates the observed assumptions that, in the situation of treatment with 100% elutriate and salinity 30, we obtained a growth rate of 13.72 ± 0.23% Day^{-1}. At the second period of experiments, growth rates were not calculated because of macroalgal thalli biomass loss. This could be explained by some stressing condition for the algae, maybe absence of nutrients.

3.4.4. Photosynthetic performance

Photosynthetic responses of *Ulva ohnoi* after 7 days of cultivation were not significantly reduced. *Ulva prolifera* can withstand various salinities while maintaining its photosynthetic activity (Xiao et al., 2016). Alternatively, growth occurs at a rate of 10.6 – 16.7%.day^{-1}.

On the seventh day of cultivation, when we highlight an integrative fluctuation between salinity and nutritious quantity, it can show that high salinity (30) combined with low nutritional availability causes an adverse reaction in photosynthetic potential, which might point us a possible photoinhibition that occur when an alga is exposed to oxidative stress. It could not evaluate the algal photosynthetic behavior during the days that followed, most likely due to senescence and biomass loss brought on by nutritional and oxidative stress because alternative sources were provided throughout the process.

3.4.5. Biochemical responses of *Ulva ohnoi* to salinity and elutriate variation

3.4.5.1. Chlorophyll *a*, chlorophyll *b* and total carotenoids

Nitrogen source and availability were related to higher production of chlorophyll *a* and chlorophyll *b* by *Ulva ohnoi* during the first seven days of the experiment. As a result, the higher nitrogen availability, which means treatments with 100% > 60% > 20%, the higher the production of nitrogenous pigments. However, the combined effect of salinity and elutriate was seen in the output of total carotenoids. Thus, the variation in nitrogen source availability directly affects the production of pigments and total proteins, as observed by (Xiao et al., 2016). Considering those facts, it was possible to observe the gradual increment of chlorophyll *a* and *b* production while increasing the availability of elutriate in the treatments in the first 7 days.

In the biosynthesis of total carotenoids, the increasing was caused by both salinity and nutrient availability in the medium. Eismann et al. (2020) published a compilation of several studies related to the yield of total carotenoids, in which they reported that the variation in productive output is wide and can extend from 0.005–800 mg.g-1 of a compound and that salinity variability influences their biosynthetic composition.

It was feasible to confirm biochemical alterations based on the data obtained in the second experimental period (7-14 days). As a result, after 14 days of cultivation, both elutriate and salinity influence pigment biosynthesis. The responses to the physiological stress induced by Ulva ohnoi could be one plausible explanation for such facts. As a result, as reported by Lu et al. (2006), Luo and Liu (2010), and Samanta et al. (2019), salinity and elutriate may have triggered an accumulation of intracellular reactive oxygen compounds (ROS), activating algae metabolism and antioxidant defense enzymes, promoting a variation of defense responses.

During the 14 days, there was a significant increase in the production of pigment components, primarily those linked with treatments with 20% elutriate and salinity 15. This

subject is examined by Luo and Liu (2011), who concluded that physiological stress caused by prolonged exposure to a hyposaline environment increased the concentration of pigments in *Ulva prolifera*. Thus, it is possible to point out that the same phenomenon is observed in our experiment, and precisely the treatments that present this configuration showed the highest pigment production. Finally, the responses to high levels of salinity would be more linked to the species diversity used in the experiment or its growth circumstances, combined with environmental factors. According to Zheng et al. (2019), the concentration of nitrogen available in the crop affects the responses to salt.

3.4.5.2. Total carbohydrates

The results obtained by observing the total carbohydrate production indicate in the first seven days a continuous productivity (average of 80 mg. g^{-1}DW for total soluble carbohydrates and 25 mg. g^{-1}. DW for total insoluble carbohydrates) and that the statistical differences observed so far are quite subtle in relation to the experimental treatments. For example, changes in nitrogen and phosphate concentrations cause a coordinated modification of various metabolic pathways, resulting in the reallocation of cellular components such as carbohydrates, lipids, and proteins (Hockin et al., 2012; Li et al., 2012).

What was seen for the 14-day cultivation period was precisely the dynamics discovered by Pinchetti et al. (1998) and Kumari et al. (2014), namely a decline in soluble and insoluble carbohydrate productivity with increased nutrients. This supports our experimental findings that total carbohydrate contents increased over time while nitrogenous component availability decreased, particularly for values associated with low salinity of 15 (Total Soluble Carbohydrates: 213.29 ± 33.02 mg.g.$^{-1}$ DW; Total Insoluble Carbohydrates:82.79 ± 19.66 mg.g.$^{-1}$ DW). This increase may be linked to a saline and/or

nutritional stress response, where carbohydrate storage is driven to form an energy reserve to meet metabolic needs. In some environmental stress situations where excessive production of reactive oxygen species (ROS) occurs in cells, carbohydrates can be optimized as antioxidants, aiding in antioxidant processes.

3.4.6. Surface Response Modeling

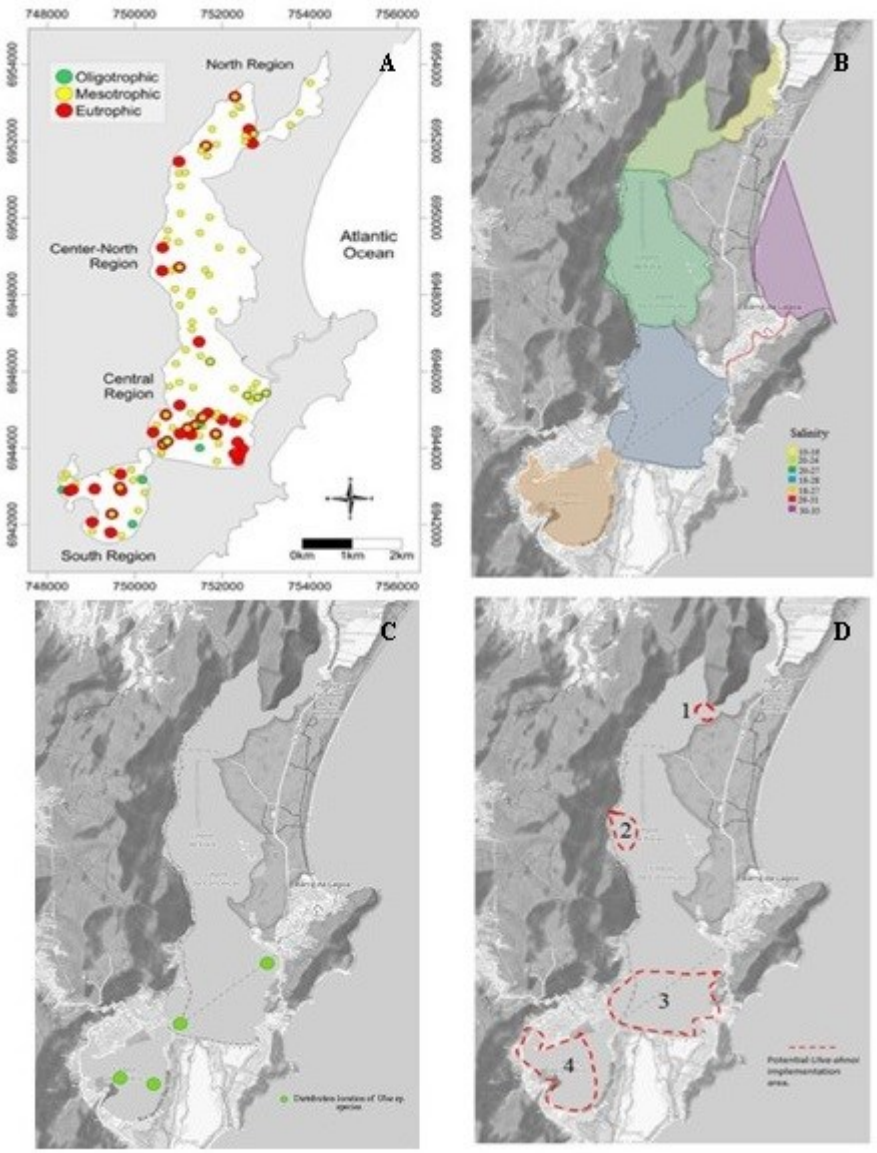
Surface response modeling obtained for the variables under study was conclusive in a descriptive and predictive manner, elucidating the key to observation and innovation in this work in the face of the exposed problem in a complementary and unique way. Fabre et al. (2021), Ferreira et al. (2021) and Ferreira et al. (2023) have already shown examples of modeling applied to the study of *Ulva* spp. explaining the biosynthesis of compounds of interest or dynamism of physiological response used to variations of factors such as luminosity, pH, nutrition, etc. The response surfaces shown by models are estimated response contours, and the overall character of the system emerges because of a fitted model rather than the underlying behavioral structure (Borror et al., 2002). As a result of the validations acquired through R^2, we can determine how much our modulated approximation in factorial replies explains the dynamics of the variable under consideration.

As a significant contribution of this study, by using the validated models, it is possible to understand how the algae could respond against variations of salinity and elutriate availability in the environment, indicating potential areas in the Lagoa da Conceição to the installation of a bioremediation plant system containing *Ulva ohnoi*, which can effectively remove nutrients over a period of one week.

3.4.7. Practical consequences for bioremediation with *Ulva ohnoi*

The response surface models allowed an optimized view of the bioremediation potential of *Ulva ohnoi* considering the variability of the eutrophic environment of Lagoa da Conceição regarding its salinity levels and the nitrogen availability in the form of ammonia and phosphate. With these results, it was possible to identify and map the potential areas where the implementation of *Ulva* would be more efficient in order to grow and remove potential contaminants, consequently reducing the eutrophication process. The characteristics of Lagoa da Conceição as well the possible locations to implement a bioremediation system are presented in Figure 12.

Figure 12 − Scenarios of potential bioremediation in Lagoa da Conceição, SC, Brazil**. A**: Trophic conditions of the lagoon, indicating Oligotrophic, Mesotrophic and Eutrophic areas. (Cury Silva et al. 2016); **B**: lagoon areas according to surface salinity; **C:** *Ulva* spp. distribution recorded to Lagoa da Conceição (Böker-Torres et al. 2010), green dots correspond to the delimited regions that present Ulvas species in Lagoa da Conceição. **D**: Potential areas of bioremediation of *Ulva ohnoi* according to the proposed surface models in our study, combining optimal conditions of salinity and elutriate/nutrient availability.



The behavior of ammonia absorption by Ulva ohnoi is linked to its linear and inversely proportional interaction with salinity, as observed by the results presented in the surface response models (Figures 2E and 2F). In this sense, the delimited potential regions (Figure 8D, zones 1, 2, 3 and 4) would refer to the best location for implementing Ulva for 7 days, since the salinity oscillation of 10–28 would comprise the range of greatest potential for the nutrient. absorption by Ulva, in an environment with high potential for eutrophication (Figure 8A). However, for periods longer than 7 days of cultivation, only areas with higher salinities (greater than 22) associated with eutrophic environments would be effective, as already shown in figure 6D, attenuating the influence associated with the blooms locations of Ulva (Figure 8C).

In this context, zone 1 (Figure 8D) would not include such efficiency potential since this location has salinity levels lower than those indicated as optimal conditions and there is no high incidence of blooms of Ulva sp. in the locality. Predictive models suggest a similar situation for phosphate absorption, indicating that, for the initial 7 days of cultivation, the higher the salinity and elutriate concentration, the greater the absorption potential. Therefore, zones 3 and 4, representing the Center-North, Center-South and South regions of Lagoa, are potential environments for implementing the cultivation of Ulva ohnoi since they are already regions with descriptions of the appearance of blooms of *Ulva* sp.(Figure 8C).

However, for the model suggested for the period of 7 to 14 days, it predicts that the efficiency and impairment of *Ulva's* ability to capture phosphate from the environment are only positive for higher salinity conditions (salinity greater than 30), so that the previously selected zones (zones 3 and 4) have low potential for long exposure times. Therefore, long-term cultivation, aiming to capture phosphate from eutrophic regions, is not recommended.

3.5. CONCLUSION

The results obtained by the present work allowed, through response surface modeling, delimitation of potential areas for the introduction of *Ulva ohnoi* in a bioremediation process in Lagoa da Conceição / SC. The ammonia absorption process is effective in the initial 7 days at high concentrations of elutriate and with median-low salinities (90.54 -91.59%.day^{-1}). However, for continuity of ammonia uptake potential over a long period (7-14 days), only in regions where salinity is high (greater than 22), the process still is efficient.

The behavior associated with phosphate absorption in the 7 days of cultivation, demonstrated similarity with the absorption rates found in ammonia, obtaining an average of 93.78-97.5%, for salinities 15 and 22. However, the use of *Ulva ohnoi* for long periods of cultivation with deprivation of elutriate is not advised, since the results obtained elucidated that only the highest concentrations associated with the highest salinities remained in the process of phosphate absorption. In the others, a reversal and availability of phosphate assimilated by *Ulva* was observed again in the medium.

Therefore, the areas determined for cultivation of *Ulva ohnoi*, would be the same shared in the process of absorption of ammonia and phosphate in the initial 7 days, thus having an optimization in the process of bioremediation.

4. CONCLUSÃO-GERAL

Ulva ohnoi foi eficiente no processo de remoção de NH_4^+, como demonstrado pela taxa de absorção mais rápida quando o meio de cultura continha uma alta concentração de NH_4^+. Essa eficiência se deve à facilidade com que o NH_4^+ pode ser transportado ativamente através da membrana celular, resultando em altas taxas de absorção quando a disponibilidade nutricional é alta. Além disso, a eficiência de absorçãode fosfato (EPUE: PO_4^{3-}) foi afetada pela disponibilidade de nutrientes e salinidade. Essas variáveis influenciaram a geração de pigmentos, principalmente clorofilas *a* e *b* e carotenoides totais.

Em termos de taxa de crescimento, *Ulva ohnoi* revelou-se sensível às variações de salinidade, embora não houvesse uma causa conclusiva para o comportamento exibido em determinados tratamentos. Pesquisas anteriores, no entanto, sugerem que a salinidade tem um impacto substancial na capacidade de proliferação das algas.

A resposta fotossintetizante das algas foi estável durante os primeiros 7 dias de crescimento, mas houve uma mudança bioquímica após 14 dias, indicando sinalização para processos de fotoinibição como resultado de um possível estresse oxidativo.

A síntese de pigmentos e carboidratos totais também foi afetada pela disponibilidade de nitrogênio e salinidade, demonstrando que a alga está se aclimatando ao seu ambiente. A produção total de carboidratos de *Ulva ohnoi* foi regulada pela disponibilidade de nutrientes e salinidade ao longo do tempo. A resposta da alga às circunstâncias ambientais pode ter resultado em um aumento na síntese de carboidratos, o que pode estar relacionado à resposta ao estresse salino e/ou nutricional, bem como ao papel dos carboidratos como antioxidantes na defesa contra o estresse oxidativo.

Delinear modelos detalhados de resposta de superfície aplicados às condições reais do ecossistema possibilita pontuar especificações de resposta com base na dinâmica preditiva observada, bem como auxiliar numa abordagem eficiente nas respostas de biorremediação;. Também permite o monitoramento de efeitos causados por eventos cíclicos, associados a disponibilidade de NH_4^+ e PO_4^{3-}, que desencadeiam processos de eutrofização, e uma dinâmica com viés biotecnológica da fabricação de biocompostos de valor comercial, bem como uma visão preditiva integrada das reações ligadas aos componentes abordados neste trabalho. Assim, este trabalho indica aos tomadores de decisão os locais da Lagoa da Conceição nos quais uma plantas de biorremediação para remoção de nutrientes nesse espaço com *Ulva ohnoi* poderiam ser instaladas.

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6. SUPPLEMENTARY MATERIAL

Table S1 - Two-way ANOVA Statistical Table, significant difference analysis of dependent factors: Effective and Relative Efficiency (EPUE/RPUE) and (EAUE/RAUE), Pigments, Total Carbohydrates (Soluble and Insoluble), Growth rate, Phosphate increment rate, with post hoc Newman-Keuls with $p < 0.05$.

Pigments						
			Experiment (0-7 days)			
Chlorophyll a						
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	1,161255	1,161255	720,6271	0,000000
	Elutriate	2	0,017551	0,008776	5,4458	**0,012437**
	Salinity	2	0,007823	0,003911	2,4272	0,112640
	Elutriate*Salinity	4	0,005626	0,001406	0,8728	0,496689
	Error	21	0,033840	0,001611		
	Total	29	0,064768			
Chlorophyll b						
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	1,182558	1,182558	373,8185	0,000000
	Elutriate	2	0,036475	0,018237	5,7650	**0,010100**
	Salinity	2	0,015684	0,007842	2,4790	0,108007
	Elutriate*Salinity	4	0,019562	0,004890	1,5459	0,225462
	Error	21	0,066433	0,003163		
	Total	29	0,136998			
Carotenids						
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	1,226129	1,226129	561,2925	0,000000
	Elutriate	2	0,016427	0,008213	3,7599	**0,040203**
	Salinity	2	0,000887	0,000443	0,2030	0,817889
	Elutriate*Salinity	4	0,033501	0,008375	3,8340	**0,017166**
	Error	21	0,045874	0,002184		
	Total	29	0,095000			
			Experiment (7-14 days)			
Chlorophyll a						
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	1,383845	1,383845	1025,264	0,000000
	Elutriate	2	0,070943	0,035472	26,280	**0,000002**
	Salinity	2	0,319258	0,159629	118,266	**0,00**

	Elutriate*Salinity	4	0,237260	0,059315	43,945	**0,00**
	Error	21	0,028345	0,001350		
	Total	29	0,608572			

Chlorophyll b

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	1,222708	1,222708	1157,213	0,000000
	Elutriate	2	0,084959	0,042480	40,204	**0,000000**
	Salinity	2	0,319454	0,159727	151,171	**0,000000**
	Elutriate*Salinity	4	0,251118	0,062779	59,417	**0,000000**
	Error	21	0,022189	0,001057		
	Total	29	0,635648			

Carotenids

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	1,101452	1,101452	649,9321	0,000000
	Elutriate	2	0,112997	0,056498	33,3380	**0,000000**
	Salinity	2	0,267446	0,133723	78,9057	**0,000000**
	Elutriate*Salinity	4	0,339576	0,084894	50,0933	**0,000000**
	Error	21	0,035589	0,001695		
	Total	29	0,713892			

Experiment (0- 7 days)

Nutrient Source	Effective phosphate uptake efficiency (EPUE %.day-1 DW)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	146529,7	146529,7	8773,159	0,000000
	Elutriate	2	22364,3	11182,1	669,507	**0,000000**
PO_4^{3-}	**Salinity**	2	442,5	221,3	13,247	**0,000190**
	Elutriate*Salinity	4	232,3	58,1	3,478	**0,024935**
	Error	21	350,7	16,7		
	Total	29	23257,3			
	Relative phosphate uptake efficiency (RPUE %.day-1)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	126666,4	126666,4	1319,970	0,000000
PO_4^{3-}	**Elutriate**	2	19007,7	9503,8	99,038	**0,000000**
	Salinity	2	478,0	239,0	2,490	0,107021
	Elutriate*Salinity	4	1329,8	332,5	3,464	**0,025293**
	Error	21	2015,2	96,0		
	Total	29	23171,0			
	Effective amnonia uptake efficiency (EAUE %.day-1 DW)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	113233,7	113233,7	12043,21	0,000000

$N\text{-}NH_4^+$	**Elutriate**	2	20634,6	10317,3	1097,32	**0,000000**
	Salinity	2	1083,7	541,8	57,63	**0,000000**
	Elutriate*Salinity	4	476,6	119,1	12,67	**0,000021**
	Error	21	197,4	9,4		
	Total	29	22176,1			

Relative amnonia absorption efficiency (RAUE %.day-1.DW)

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	97807,82	97807,82	1419,518	0,000000
$N\text{-}NH_4^+$	**Elutriate**	2	17730,26	8865,13	128,663	**0,000000**
	Salinity	2	924,97	462,49	6,712	**0,005574**
	Elutriate*Salinity	4	1317,72	329,43	4,781	**0,006699**
	Error	21	1446,95	68,90		
	Total	29	21505,28			

Experiment (7-14 days)

Effective amnonia uptake efficiency (EAUE %.day-1 DW)

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	4477,10	4477,095	40,41525	0,000003
	Elutriate	2	11710,66	5855,330	52,85674	**0,000000**
$N\text{-}NH_4^+$	**Salinity**	2	3161,43	1580,715	14,26929	**0,000122**
	Elutriate*Salinity	4	3190,11	797,527	7,19937	**0,000817**
	Error	21	2326,32	110,777		
	Total	29	20830,44			

Relative amnonia absorption efficiency (RAUE %.day-1.DW)

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	8836,3	8836,32	4,77217	0,040404
$N\text{-}NH_4^+$	**Elutriate**	2	44470,6	22235,28	12,00844	**0,000333**
	Salinity	2	4743,1	2371,53	1,28078	0,298649
	Elutriate*Salinity	4	11844,8	2961,21	1,59924	0,211572
	Error	21	38884,4	1851,64		
	Total	29	104498,1			

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	6424,560	6424,560	347,7421	0,000000
Increments Rate of PO_4^{3-}	**Elutriate**	2	305,911	152,955	8,2790	**0,002233**
	Salinity	2	3688,393	1844,197	99,8208	**0,000000**
	Elutriate*Salinity	4	1391,057	347,764	18,8234	**0,000001**
	Error	21	387,976	18,475		
	Total	29	6101,732			

	Experiment (Initial)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
Fv/Fm (Relative units)	Intercept	1	18,80909	18,80909	240248,5	0,000000
	Elutriate	2	0,00062	0,00031	4,0	**0,034001**
	Salinity	2	0,00008	0,00004	0,5	0,598194
	Elutriate*Salinity	4	0,00046	0,00012	1,5	0,244813
	Error	21	0,00164	0,00008		
	Total	29	0,00306			

	Experiment (7 days)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
Fv/Fm (Relative units)	Intercept	1	10,56659	10,56659	7623,423	0,000000
	Elutriate	2	0,06266	0,03133	22,602	**0,000006**
	Salinity	2	0,03779	0,01890	13,633	**0,000160**
	Elutriate*Salinity	4	0,04892	0,01223	8,823	**0,000242**
	Error	21	0,02911	0,00139		
	Total	29	0,17356			

	Experimental (0-7 days)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
Vegetative Growth Rate *Ulva ohnoi*	Intercept	1	1701,134	1701,134	619,1256	0,000000
	Elutriate	2	10,171	5,086	1,8509	0,181819
	Salinity	2	146,055	73,028	26,5783	**0,000002**
	Elutriate*Salinity	4	214,585	53,646	19,5245	**0,000001**
	Error	21	57,700	2,748		
	Total	29	494,994			

	Experimental (0-7 days)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
Total Soluble Carbohydrates	Intercept	1	151397,3	151397,3	872,2548	0,000000
	Elutriate	2	1553,7	776,9	4,4759	**0,024037**
	Salinity	2	1960,8	980,4	5,6485	**0,010892**
	Elutriate*Salinity	4	1421,9	355,5	2,0481	0,124225
	Error	21	3645,0	173,6		
	Total	29	9250,3			

	Experimental (0-7 days)					
	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
	Intercept	1	17292,51	17292,51	1676,255	0,000000
	Elutriate	2	36,79	18,39	1,783	0,192649

Total Insoluble Carbohydrates	**Salinity**	2	258,79	129,39	12,543	**0,000261**
	Elutriate*Salinity	4	54,11	13,53	1,311	0,298146
	Error	21	216,64	10,32		
	Total	29	625,02			

Experimental (7-14 days)

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
Total Soluble Carbohydrates	Intercept	1	262650,0	262650,0	1318,512	0,000000
	Elutriate	2	10907,3	5453,6	27,377	**0,000001**
	Salinity	2	7947,8	3973,9	19,949	**0,000014**
	Elutriate*Salinity	4	51900,7	12975,2	65,136	**0,000000**
	Error	21	4183,2	199,2		
	Total	29	72714,5			

Experimental (7-14 days)

	Effect	Deg. Freedom	SS	MS	F	**p < 0,05**
Total Insoluble Carbohydrates	Intercept	1	42460,96	42460,96	865,3373	0,000000
	Elutriate	2	581,91	290,96	5,9296	**0,009087**
	Salinity	2	2670,02	1335,01	27,2070	**0,000001**
	Elutriate*Salinity	4	5420,93	1355,23	27,6191	**0,000000**
	Error	21	1030,44	49,07		
	Total	29	9257,86			

Table S2 - Two-way ANOVA statistical table for obtaining response surface models: effective and relative efficiency (EPUE/RPUE) and (EAUE/RAUE), pigments, total carbohydrates (soluble and insoluble), Maximum photosynthetic rate, and increment rate of phosphate, with $p<0.05$.

Relative phosphate uptake efficiency (0-7 days)

	SS	df	MS	F	p
(1)Elutriate(L)	18852,95	1	18852,95	789,3332	**0,000000**
Elutriate(Q)	3584,98	1	3584,98	150,0958	**0,000000**
(2)Salinity(L)	190,89	1	190,89	7,9922	**0,009324**
Salinity(Q)	276,31	1	276,31	11,5684	**0,002350**
1L by 2L	9,86	1	9,86	0,4129	0,526614

Error	573,23	24	23,88		
Total SS	23257,31	29			

Effective phosphate uptake efficiency (0-7 days)

	SS	df	MS	F	p
(1)Elutriate(L)	15859,21	1	15859,21	135,8479	**0,000000**
Elutriate(Q)	3312,83	1	3312,83	28,3773	**0,000018**
(2)Salinity(L)	466,12	1	466,12	3,9927	**0,057149**
Salinity(Q)	15,36	1	15,36	0,1316	0,719962
1L by 2L	543,19	1	543,19	4,6529	**0,041231**
Error	2801,82	24	116,74		
Total SS	23170,97	29			

Rate phosphate increment in treatments (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	305,910	1	305,910	4,74284	**0,039478**
Elutriate(Q)	28,741	1	28,741	0,44560	0,510800
(2)Salinity(L)	3361,382	1	3361,382	52,11487	**0,000000**
Salinity(Q)	565,947	1	565,947	8,77445	**0,006788**
1L by 2L	231,046	1	231,046	3,58213	0,070526
Error	1547,987	24	64,499		
Total SS	6101,732	29			

Relative amnonia uptake efficiency (0-7 days)

	SS	df	MS	F	p
(1)Elutriate(L)	18392,91	1	18392,91	710,9176	**0,000000**
Elutriate(Q)	2273,42	1	2273,42	87,8716	**0,000000**
(2)Salinity(L)	616,61	1	616,61	23,8331	**0,000056**
Salinity(Q)	508,20	1	508,20	19,6429	**0,000176**
1L by 2L	53,10	1	53,10	2,0523	0,164879
Error	620,93	24	25,87		
Total SS	22176,11	29			

Effective amnonia uptake efficiency (0-7 days)

	SS	df	MS	F	p
(1)Elutriate(L)	15708,45	1	15708,45	154,0571	**0,000000**
Elutriate(Q)	2105,37	1	2105,37	20,6479	**0,000132**
(2)Salinity(L)	910,93	1	910,93	8,9338	**0,006371**
Salinity(Q)	13,84	1	13,84	0,1358	0,715747
1L by 2L	317,51	1	317,51	3,1139	0,090353
Error	2447,16	24	101,97		
Total SS	21505,28	29			

Relative Fv/Fm (7 days)

	SS	df	MS	F	p
(1)Elutriate(L)	0,059469	1	0,059469	23,02919	**0,000069**
Elutriate(Q)	0,000697	1	0,000697	0,26978	0,608236
(2)Salinity(L)	0,034071	1	0,034071	13,19395	**0,001326**
Salinity(Q)	0,000963	1	0,000963	0,37273	0,547257
1L by 2L	0,016051	1	0,016051	6,21564	**0,019957**
Error	0,061976	24	0,002582		
Total SS	0,173555	29			

Relative amnonia uptake efficiency (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	11535,46	1	11535,46	50,26238	**0,000000**
Elutriate(Q)	348,09	1	348,09	1,51670	0,230051
(2)Salinity(L)	3041,19	1	3041,19	13,25111	**0,001301**
Salinity(Q)	267,14	1	267,14	1,16397	0,291367
1L by 2L	8,32	1	8,32	0,03624	0,850613
Error	5508,12	24	229,50		
Total SS	20830,44	29			

Effective amnonia uptake efficiency (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	41071,4	1	41071,41	20,34969	**0,000144**
Elutriate(Q)	4840,3	1	4840,29	2,39822	0,134560
(2)Salinity(L)	1897,2	1	1897,22	0,94002	0,341943
Salinity(Q)	4163,5	1	4163,53	2,06291	0,163830
1L by 2L	2290,5	1	2290,47	1,13486	0,297341
Error	48438,8	24	2018,28		
Total SS	104498,1	29			

Chlorophyll a (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	11650,9	1	11650,9	1,11411	0,301701
Elutriate(Q)	31858,9	1	31858,9	3,04647	0,093705
(2)Salinity(L)	187561,4	1	187561,4	17,93539	**0,000291**
Salinity(Q)	90048,9	1	90048,9	8,61084	**0,007248**
1L by 2L	14622,1	1	14622,1	1,39823	0,248604
Error	250982,8	24	10457,6		
Total SS	608572,0	29			

Chlorophyll b (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	7980,9	1	7980,9	0,79719	0,380801
Elutriate(Q)	47279,9	1	47279,9	4,72261	**0,039865**
(2)Salinity(L)	183468,8	1	183468,8	18,32602	**0,000259**
Salinity(Q)	96365,4	1	96365,4	9,62558	**0,004858**
1L by 2L	33032,9	1	33032,9	3,29954	0,081811
Error	240273,2	24	10011,4		

Total SS	635647,9	29			

Total Carotenoids (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	52796,0	1	52796,0	5,30247	**0,030273**
Elutriate(Q)	34006,3	1	34006,3	3,41536	0,076953
(2)Salinity(L)	158078,5	1	158078,5	15,87631	**0,000548**
Salinity(Q)	73616,2	1	73616,2	7,39350	**0,011970**
1L by 2L	136200,5	1	136200,5	13,67904	**0,001124**
Error	238965,0	24	9956,9		
Total SS	713891,9	29			

Total Carbohydrates Insolubles (0-7 days)

	SS	df	MS	F	p
(1)Elutriate(L)	22,9494	1	22,9494	2,05606	0,164503
Elutriate(Q)	22,3525	1	22,3525	2,00258	0,169877
(2)Salinity(L)	141,0805	1	141,0805	12,63957	**0,001607**
Salinity(Q)	143,7075	1	143,7075	12,87493	**0,001481**
1L by 2L	2,8658	1	2,8658	0,25675	0,616984
Error	267,8835	24	11,1618		
Total SS	625,0157	29			

Total Carbohydrates Solubles (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	10727,83	1	10727,83	8,10720	**0,008893**
Elutriate(Q)	1657,20	1	1657,20	1,25238	0,274175
(2)Salinity(L)	2476,06	1	2476,06	1,87120	0,184010
Salinity(Q)	2329,19	1	2329,19	1,76021	0,197085
1L by 2L	24326,02	1	24326,02	18,38358	**0,000254**
Error	31757,93	24	1323,25		
Total SS	72714,53	29			

Total Carbohydrates Insolubles (7- 14 days)

	SS	df	MS	F	p
(1)Elutriate(L)	579,203	1	579,203	5,21788	**0,031496**
Elutriate(Q)	21,990	1	21,990	0,19810	0,660245
(2)Salinity(L)	851,074	1	851,074	7,66710	**0,010670**
Salinity(Q)	1373,926	1	1373,926	12,37733	0,001761
1L by 2L	3787,293	1	3787,293	34,11869	**0,000005**
Error	2664,083	24	111,003		
Total SS	9257,859	29			