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SPECTRAL AND PHOTOSYNTHETIC RESPONSE OF MARINE BIOINDICATORS TO HEAVY METAL-INDUCED STRESS IN A CONTROLLED ENVIRONMENT

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RIASSUNTO

Gli ecosistemi marini, essenziali per la biodiversità e l'equilibrio ecologico globale sono sempre più minacciati dalle attività umane come scarichi industriali, deflussi agricoli e urbanizzazione, che introducono inquinanti nocivi come metalli pesanti negli ambienti marini. I metalli in traccia, tra cui il cromo, rappresentano un rischio significativo a causa della loro tossicità, persistenza e capacità di bioaccumulo. Questi inquinanti impattano sulla biodiversità marina, sulla resilienza e sulla funzionalità dell'ecosistema. A questi se ne aggiungono altri provenienti da fonti distanti, trasportati da fiumi, vento e processi marini più ampi, che lo possono ulteriormente danneggiare. Gli impatti antropogenici sono ulteriormente esasperati dai cambiamenti climatici, che possono amplificare gli effetti dell'inquinamento. Tutti questi fattori influenzano la vita marina e la sua rete trofica, sottolineando la necessità di sviluppare metodologie moderne e facilmente implementabili basate su nuove tecnologie per ottenere una comprensione sempre più accurata di questi cambiamenti. Questa tesi è servita a produrre un'indagine completa sugli effetti dei metalli pesanti sulle macroalghe e sulle fanerogame marine, con particolare enfasi sul processo fotosintetico e sulle conseguenti variazioni della loro risposta spettrale, per valutare la fattibilità del loro utilizzo per l'identificazione e la quantificazione degli impatti degli inquinanti per un'applicazione futura basata sulle nuove tecniche di telerilevamento. Tra le macroalghe brune, le specie appartenenti al genere *Cystoseira* spp. C. Agardh, 1820 (Fucales, Sargassaceae) sono rigorosamente protette dall'Allegato I della Convenzione di Berna e della Convenzione di Barcellona. Inoltre, sono incluse nella rete NATURA 2000 all'interno dell'habitat "Scogliere" (codice 1170) e riconosciute come indicatori di qualità ecologica dalla Direttiva Quadro sulle Acque dell'Unione Europea (2000/60/CE). Queste alghe brune sono essenziali per gli ecosistemi mediterranei, svolgono un ruolo chiave nelle comunità costiere sostenendo la biodiversità, le reti alimentari e promuovendo il sequestro del carbonio. Inoltre, studi recenti le hanno riconosciute come efficaci bioindicatori grazie alla loro sensibilità ai metalli pesanti, alla rilevanza ecologica e alla capacità di bioaccumulo. Questa ricerca fa parte del progetto STOPP (Strumenti e Tecniche di Osservazione della Terra in Prossimità e Persistenza), finanziato dall'ASI (Agenzia Spaziale Italiana), finalizzato allo sviluppo di nuove metodologie di monitoraggio per valutare la qualità ecologica delle comunità bentoniche negli ecosistemi marini costieri, rilevando "lunghezze d'onda target" che possono essere osservate da piattaforme remote dotate di sensori multispettrali, fornendo informazioni sugli impatti degli inquinanti chimici sull'ambiente marino.

Questa ricerca fornisce una dettagliata revisione della letteratura, che evidenzia i recenti impatti all'avanguardia dell'inquinamento da metalli pesanti su macroalghe marine e fanerogame marine, con un'attenzione specifica al loro processo fotosintetico e alla conseguente risposta spettrale.

Nella seconda fase, è stato allestito un ambiente di laboratorio per comprendere e valutare le risposte fotosintetiche e spettrali di *Cystoseira* spp. a diverse concentrazioni di cromo, fornendo una base di partenza per ulteriori studi dI telerilevamento. L'analisi della firma spettrale ha rivelato modelli di riflettanza indotti da stress e cambiamenti fisiologici significativi (alterazione dell'efficienza fotosintetica e della composizione dei pigmenti), fornendo un approccio non invasivo per rilevare i cambiamenti indotti dagli inquinanti. Le immagini iperspettrali possono essere utilizzate per rilevare lo stress del cromo in *Cystoseira* spp. su scale spaziali più grandi, il che sarà fondamentale per integrare questi risultati in programmi di monitoraggio ambientale più ampi. La ricerca è in linea con le politiche ambientali europee, come la direttiva quadro sulle acque (WFD) e la direttiva quadro sulla strategia marina (MSFD), evidenziando l'importanza di integrare la ricerca di laboratorio con le applicazioni sul campo, promuovendo l'uso di bioindicatori marini e telerilevamento per una gestione sostenibile degli ecosistemi marini sotto le attuali e crescenti pressioni antropiche.

ABSTRACT

Marine ecosystems, essential for biodiversity and global ecological balance, are increasingly threatened by human activities such as industrial discharges, agricultural runoff and urbanization, which introduce harmful pollutants such as heavy metals into marine environments. Trace metals, including chromium, pose a significant risk due to their toxicity, persistence and bioaccumulation capacity. These contaminants impact marine biodiversity, ecosystem resilience and functionality. Local pollution is compounded by that generated from “remote” sources, which can impact marine-coastal ecosystems as it is transported by rivers, wind and more generally, marine dynamics. Anthropogenic impacts are further exacerbated by climate change, which can amplify the effects of pollution. All these factors affect marine life and its trophic food web, underscoring the need to develop modern, readily deployable methodologies based on new technologies to achieve an increasingly accurate understanding of these changes.

This thesis served to produce a comprehensive investigation, by a review based on the effects of heavy metals on macroalgae and seagrasses, with particular emphasis on the photosynthetic process and the consequent variations of their spectral response, to evaluate the feasibility of their use for the identification and quantification of pollutant impacts for future application based on new remote sensing techniques. Among brown macroalgae, species belonging to the genus *Cystoseira* spp. (Fucales, Sargassaceae) are strictly protected under Annex I of both the Bern Convention and the Barcelona Convention. Additionally, they are included in the NATURA 2000 network within the "Reef" habitat (code 1170) and are recognized as ecological quality indicators under the European Water Framework Directive (2000/60/EC). These brown algae are essential to Mediterranean ecosystems, playing a key role in coastal communities by supporting biodiversity, food webs, and promoting carbon sequestration. Moreover, recent studies recognised them as effective bioindicators due to their sensitivity to heavy metals, ecological relevance and bioaccumulation capacity.

This research project has enabled the development of a comprehensive literature review to identify the key parameters used to assess the photosynthetic response of Mediterranean coastal macroalgae and seagrasses to heavy metal exposure. The bibliographic analysis led to the creation of a species/contaminant/response matrix, providing a synoptic and updated overview of the state of the art for the identification of target species in coastal marine ecosystems. The analysis of this matrix revealed that metal accumulation in marine macroalgae and seagrasses, particularly chromium (Cr), results in a decline in photosynthetic efficiency and pigment concentration, alongside an increase in antioxidant response. In the second phase, a laboratory

environment was set up to understand and evaluate the photosynthetic and spectral responses of *Cystoseira* spp. to different chromium concentrations, providing a baseline for further remote sensing studies. Spectral signature analysis revealed stress-induced reflectance patterns as well as significant physiological changes (altered photosynthetic efficiency and pigment composition), providing a non-invasive approach to detect pollutant-induced changes. Hyperspectral images can be explored for detecting chromium stress in *Cystoseira* spp. at larger spatial scales, which will be crucial for integrating these findings into broader environmental monitoring programs.

The research is in line with European environmental policies, such as the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD), highlighting the importance of integrating laboratory research with field applications, promoting the use of marine bioindicators and remote sensing for sustainable management of marine ecosystems under current increasing anthropogenic pressures.

1. INTRODUCTION

In the context of international guidelines for the protection and conservation of biodiversity in natural environments (Habitat Directive 92/43EEC), the assessment of the effects of human pressure on marine ecosystems assumes a key role. Since 2000, the European Union has introduced the use of biological communities as bioindicators in assessing the ecological status of water bodies according to the Water Framework Directive (WFD) (Butt and Hamid, 2025). Furthermore, the recent Restoration Law also emphasizes provides the urgent need to integrate bioindicator-based assessments into decision-making to assess the effectiveness of restoration actions (Swart, 2024).

Marine macroalgae and seagrasses respond differently to nutrient enrichment due to their distinct ecological and physiological characteristics, which is why they serve as indicators of direct and indirect effects, respectively (Viana et al., 2020).

Macroalgae are indicators of the direct effects of nutrient enrichment. Opportunistic species, such as Ulva or Cladophora, respond rapidly to increased nutrient availability by accelerating their growth rate and biomass accumulation. This rapid proliferation is an immediate consequence of nutrient enrichment, making macroalgae effective bioindicators of eutrophication. Excessive macroalgal growth can result in algal blooms, leading to shading, oxygen depletion, and habitat degradation (Fernández-Alías et al., 2022).

On the other hand, seagrasses primarily reflect the indirect effects of nutrient enrichment. Species such as Posidonia oceanica or Zostera marina are more sensitive to the secondary consequences of eutrophication rather than to the direct increase in nutrients. Nutrient enrichment stimulates macroalgal overgrowth, phytoplankton blooms, and epiphyte proliferation, all of which reduce light penetration. Since seagrasses rely on adequate light for photosynthesis, prolonged shading can cause declines in their biomass and coverage, ultimately making them valuable indicators of long-term eutrophication effects (Fernández-Alías et al., 2022).

Marine macroalgae and phanerogams have been recognized as crucial bioindicators, thanks to their considerable biomass, permanence, retrieval, and easy identification. Their sensitivity to pollutants in terms of response at the individual level (presence/absence, physiological response), and community level (biodiversity) makes them extremely useful elements for the classification of water bodies (Borfecchia et al., 2013).In particular, brown algae can accumulate huge amounts of heavy metals, and this makes them ideal to monitor pollution in coastal areas (Ryabushko et al, 2022). That also aligns with the EU-Marine Strategy Framework Directive (MSFD, M.S.F.D, Dir. 2008/56/EC), which recognises these algae as valuable indicators of water and ecosystem quality (Arici et al., 2019).

Heavy metals have a significant impact on photosynthetic activity in marine ecosystems (Singh et al. 2022), inhibiting photosynthesis and reducing pigment concentrations (Kondzior and Butarewicz, 2018). In recent years, numerous studies were conducted on the analysis and classification of marine spectral signatures of submerged vegetation giving information on their biophysical and physiological condition (Douay et al., 2022), as well as on the occurrence of marine pollution phenomena (Mendoza et al. 2018).

The aim of this PhD thesis, through the composition of a review and a scientific paper, is to analyse the spectral signature of selected marine plant species in response to specific pollutants. The objective was to contribute to the advancement of innovative methodologies for marine environment monitoring by identifying "target wavelengths" applicable in the future to innovative remote sensing techniques and facilitating the development of new multispectral sensors and stratospheric platforms. This research is part of the STOPP project (Strumenti e Tecniche di Osservazione della Terra in Prossimità e Persistenza), founded by ASI (Agenzia Spaziale Italiana).

1.1 ANTHROPOGENIC STRESSORS AND THEIR IMPACTS ON COASTAL MARINE ECOSYSTEMS

Marine and coastal ecosystems are vital to human well-being, offering essential services such as climate regulation, food provision, and biodiversity support (EEA, 2019; Albert et al., 2020). However, these ecosystems including seagrass beds, coral reefs, and algal forests are increasingly degraded due to anthropogenic pressures such as overexploitation, pollution, and aquaculture (IPBES, 2020). Climate-related stressors, including marine heatwaves, extreme weather events, and ocean acidification, further amplify these impacts, compromising ecosystem resilience and service provision (EEA, 2019; IPCC, 2019).

Cumulative and interactive effects of multiple stressors, especially near human population centres, exacerbate ecosystem vulnerability (Berrouet et al., 2018; Beusen et al., 2022). The "Big Five" anthropogenic threats—climate change, pollution, habitat destruction, invasive species, and overfishing—compound these challenges (Miao and Nduneseokwu, 2024). Research highlights that no fewer than five overlapping stressors affect marine ecosystems globally, necessitating integrated management approaches (Luypaert et al., 2020; Brow et al., 2022).

In response, cumulative impact assessment (CIA) methodologies have been developed to support sustainable marine governance (Fairbanks et al., 2021). International and EU policies, including the UN SDGs, the EU Water Framework Directive, and the Biodiversity Strategy 2030, emphasize the need for comprehensive risk management strategies. The decline of key ecosystem services, such as fisheries and nutrient cycling, underscores the urgency of addressing both direct and cascading stressor effects (Ali et al., 2022; Vignesh et al., 2024). A holistic understanding of these interactions is crucial for sustaining marine ecosystem functions and services.

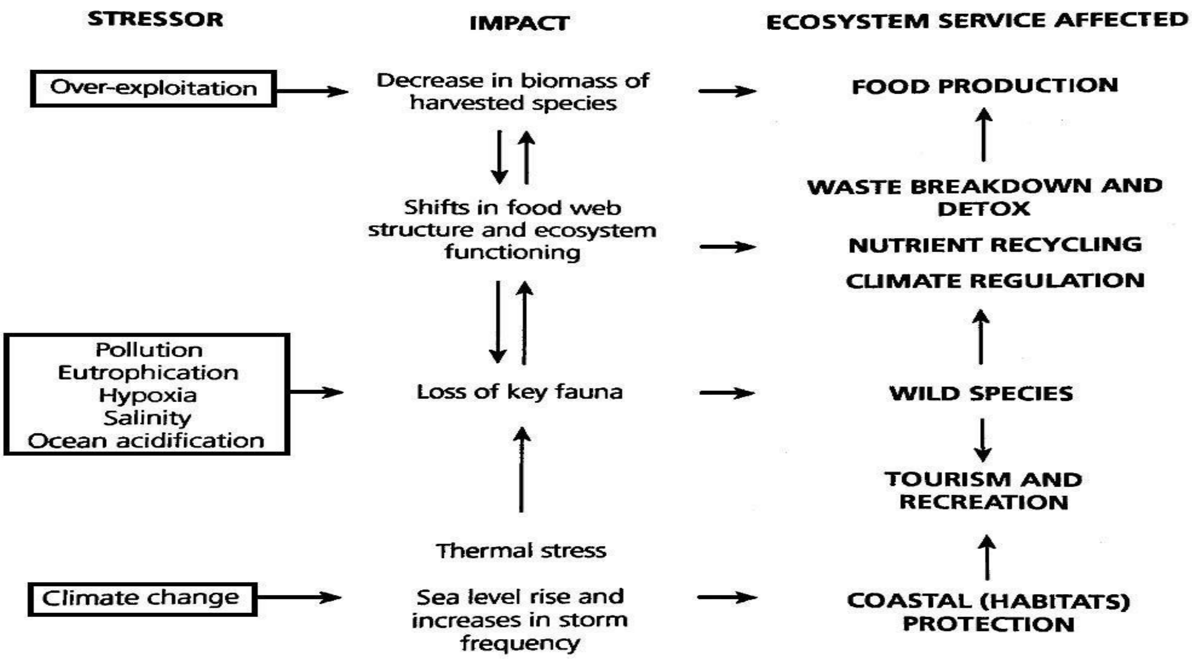

Fig. 1 Some examples of the impacts of human-induced stressors on marine ecosystem services (Austin, Z., and White, 2016).

The over-exploitation of marine resources and its impact on ecosystem services stem from both direct and indirect drivers, including policy failures, unsustainable catch quotas, and advancements in fishing technology (Linke et al., 2020). While some formally assessed fisheries have seen declines in harvest rates with recovery plans in place, unassessed fisheries-accounting for over 80% of global catch—continue to decline (Rivera-Hechem et al., 2021). This depletion affects not only target species but also non-target species and the broader marine ecosystem, particularly when keystone species are removed. For instance, the loss of Alaskan sea otters has led to the collapse of kelp forests, resulting in urchin barrens due to the lack of predation (Luypaert et al., 2020). Such disruptions in food web structures and ecosystem functions further compromise supporting and regulating services.

One of the most tangible consequences of over-exploitation is the decline in marine food supply. In the UK, fishery-derived food provision has reached its lowest level in a century, with a 94% reduction in landings per unit of fishing power over 118 years (Rogers et al., 2022). Additionally, eutrophication—driven by excessive nitrogen inputs from fossil fuel combustion and synthetic fertilizers—leads to algal blooms, shifts in food web structures, and oxygen depletion (Luypaert et al., 2020; Marx et al., 2023). The most severe impact of eutrophication arises from hypoxic conditions resulting from microbial decomposition, which significantly disrupts ecosystem services (Akinnawo, 2023).

Addressing these challenges requires a comprehensive approach that considers cumulative impacts, integrates sustainable management strategies, and enhances policy frameworks to prevent further ecosystem degradation.

Human activities introduce a wide range of pollutants into marine environments through marine dumping and land-based runoff (Ayyam et al., 2019). Persistent organic pollutants (POPs) are particularly concerning due to their long half-lives, persistence, and biomagnification through trophic levels (Luypaert et al., 2020; Lv et al., 2020). While their species-level effects are documented, their broader ecosystem-level impacts remain poorly understood. However, they are known to cause mortality and sublethal effects in marine organisms, potentially affecting humans through seafood consumption (Babaei et al., 2022). These disruptions likely impair ecosystem function and compromise multiple ecosystem services in areas with high pollution levels.

Ocean acidification, driven by increasing atmospheric CO_2 absorption, lowers seawater pH and reduces carbonate ion availability, negatively affecting calcifying organisms such as corals and shellfish (Rial et al., 2019; Luypaert et al., 2020). Declining coral calcification rates threaten reef-associated biodiversity and the ecosystem services they provide, including fisheries, aquaculture, and tourism (Gentry et al., 2020).

Climate change further exacerbates marine ecosystem stress through rising sea levels, increased storm frequency, and shifts in fish and shellfish distributions, impacting food production and fisheries (McHenry et al., 2019). Coastal habitats, including seagrass meadows, kelp forests, and wetlands, play crucial roles in coastal protection and as nurseries for commercially important species (Williams et al., 2022). Their degradation due to intensified storms and sea-level rise poses significant threats to biodiversity, fisheries, and recreation-based ecosystem services.

Addressing these interconnected stressors requires comprehensive mitigation strategies to safeguard marine ecosystem services and ensure their long-term sustainability.

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1.2 GENERAL CHARACTERISTICS OF MARINE BIOINDICATORS

Biological monitoring, or biomonitoring, can be defined as the systematic use of biological responses to assess changes in the environment, with the aim of establishing a quality control program (Sumudumali and Jayawardana, 2021). Individual organisms and entire ecosystems are influenced by biotic and abiotic stress factors, thus the ability to respond to stressors is a key characteristic of all living systems (Mulenga et al., 2024). A bioindicator is an organism (or part of an organism, or a community of organisms) that contains information about the quality of the environment (Nalumenya et al., 2024). Conversely, a biomonitor is an organism (or part of an organism, or a community of organisms) that contains information about the quantitative aspects of environmental quality. While a biomonitor is always a bioindicator, a bioindicator does not necessarily meet the requirements of a biomonitor. Since the 1960s, the use of bioindicators has increased in the study of both aquatic and terrestrial environments. However, the level and spatio-temporal scale of the indicator's application must be proportionate to the reference scale of the pollution event, the environment, and the natural communities under investigation (Shan et al., 2022). Species with moderate tolerance to environmental variability effectively indicate environmental conditions, while rare species with narrow tolerances are often too sensitive to environmental changes or are encountered too rarely to reflect the overall biotic response. Similarly, ubiquitous species with very broad tolerances are less sensitive to environmental changes that disrupt the rest of the community (Misya and Prasetya, 2023). The use of bioindicators to assess the environmental impacts of chemical pollutants over time differs fundamentally from traditional chemical analyses, which rely on direct measurements of pollutant concentrations in air, water, or soil (e.g., heavy metals, persistent organic pollutants, and pesticides). The latter method only characterizes the conditions at the time of sampling. In contrast, bioindicators provide insight into contamination over the lifespan or residence time of an organism within a given system, with the ability to reveal both direct and indirect biotic effects of pollutants. However, a limitation of using bioindicators is that populations of indicator species may be influenced by factors other than disturbance or stress (e.g., disease, parasitism, competition, predation), which can confound the response to environmental changes (Marcogliese, 2023).

Biomonitoring can be conducted at different levels of the biological scale. At the organism level, alterations induced by a contaminant in the structure or function at the molecular or cellular components level are considered, which can be easily highlighted and quantified. These alterations are called biomarkers, defined as measurable biological parameters at the sub-organism level (genetic, enzymatic, physiological, morphological), where changes indicate environmental influences in general and pollutant action in particular, both qualitatively and quantitatively. A good bioindicator organism should meet the following criteria (Manzoor et al., 2021):

· sublethal responses within the concentration range under study;
· known dose-response relationship for one or more biomarkers;
· sedentary or low-mobility habits;
· easily found and identified;
· broad spatial distribution;
· sufficiently large to allow manipulation.

The introduction of new substances into the environment through human activity and the release of potentially harmful substances (xenobiotics, many radionuclides) in greater quantities than in the past (heavy metals, natural radionuclides) has had effects on organisms and communities. These new factors have added to the effects of natural systems, or they themselves act in combination, resulting in the "tolerance level" of organisms being surpassed in their ability to resist or adapt (Thakur et al., 2025).

When biomonitoring is conducted at the population or community level, data on presence/absence and/or abundance are collected and used to calculate environmental quality indices. In order to meet the requirements of the latest European regulations, which assign a fundamental role to the biological matrix alongside the physical and chemical ones in assessing the ecological status of ecosystems, specific ecological quality indices for different trophic levels of the ecosystem (primary producers, consumers of various orders, macrobenthic communities, macrophytes, and fish communities) have been developed in recent years.

Biomonitoring studies can be divided into two main categories: active biomonitoring and passive biomonitoring. Active bioindication involves selecting specific species to be used as bioindicators, which are bred in the laboratory under standardized conditions and then introduced into the study area for a defined period of time, after which they are analyzed to study the reactions provoked and the substances present in the organism. In passive biomonitoring, organisms naturally present in the ecosystem are studied (directly and/or with chemical tissue analysis).

Bioaccumulation refers to the irreversible accumulation of a substance/element in living organisms through any route (respiration, ingestion of food, contact) and is used as a parameter to estimate the contamination of organisms (Shah (Ed.)., 2021). The bioaccumulation of toxic substances can occur through direct absorption from the environment in which the organism lives (water), through tissues or organs, and is defined as bioconcentration (Szynkowska et al., 2018). The bioconcentration factor (BCF) (Krivokapić, 2021) is the ratio between the concentration of the element in the organism and that in the water, which may vary depending on the substance and species. In this type of bioaccumulation, the concentrations of the substance in the organism become progressively higher than those in the surrounding environment. Substances can also accumulate through biomagnification (Miller et al., 2020).

Organizations such as the World Conservation Union and the International Union for Conservation of Nature actively promote the utilization of bioindicators to facilitate biomonitoring and evaluate human impacts on the environment. Plants, in particular, are highly sensitive tools for predicting and recognizing environmental stressors (Walsh et al., 2024). The increasing industrialization and urbanization have exacerbated water contamination and pollution, necessitating effective monitoring strategies (Yadav et al., 2023). Marine plants, for instance, are invaluable for predicting the state of oceanic environments as they are immobile and rapidly equilibrate with their surroundings (Ioanna et al., 2023).

The efficacy of a bioindicator organism depends on several characteristics, including its sensitivity to specific environmental stressors, broad geographical distribution, ease of sampling, and ecological relevance (Greenfield, 2022). Key traits of marine bioindicators include:

(a) Sensitivity to Stressors: bioindicators display measurable responses to environmental changes, such as shifts in community composition, physiological stress, or reproductive success (Chen, M., et al., 2024).

(b) Trophic Representation: Marine bioindicators represent diverse trophic levels, from primary producers like phytoplankton to apex predators such as cetaceans, providing ecosystem-wide insights into environmental changes (Ismail et al., 2023).

(c) Bioaccumulation and Biomagnification: Certain bioindicators accumulate contaminants such as heavy metals and organic pollutants, reflecting the bioavailable contaminants in their habitats (Edo et al., 2024).

Marine bioindicators are integral to environmental monitoring programs, offering early warning signals of ecosystem degradation (Carnevali et al., 2020). Their application is prominent in biomonitoring frameworks like the Water Framework Directive (WFD) of the European Union, which emphasizes the ecological assessment using biological quality elements (O'Brien et al., 2021). For instance, the *Posidonia oceanica* Rapid Easy Index (PREI) evaluates seagrass meadows as indicators of coastal water quality (Oprandi et al., 2021). Similarly, benthic macroinvertebrates are widely used to assess sediment quality in estuarine and coastal environments (Thrush et al., 2021).

Understanding the distribution and characteristics of marine bioindicators is fundamental for marine conservation and management. Integrating bioindicator data into environmental monitoring programs enables scientists and policymakers to devise robust strategies to mitigate anthropogenic impacts and ensure the sustainability of marine ecosystems.

1.3 MACROALGAE IN BIOMONITORING MARINE POLLUTION

According to Ajagbe et al. (2020), biomonitors are organisms mostly used for the quantitative determination of contaminants in an ecosystem. There are different methodological approaches of biological monitoring of a marine ecosystem. However, the approach based on the bioaccumulation capacity of some chemical, like the trace metals, is counted among the most vital (Kolarova and Napiórkowski, 2021). This bioaccumulation capacity in the marine environment is particularly present among macroalgae, gastropods, bivalves, polychaetes and some crustaceans. These organisms can therefore be used as bioindicators of trace metals especially when a direct correlation exists between their internal concentration of these metals and the environmental levels (Phillips, 2018). The chemical analysis of the tissues of these organisms represents a method for measuring the bioavailability of the trace metals in the marine environment over time, given their remarkable bioconcentration capacity (Berezina et al., 2023). Comparing the levels of metals in the organisms collected at different sites is a very useful way to gather information on the biologically available levels of these metal contaminants in that environment. Heavy metals are natural constituents of the environment, mostly in trace forms and not harmful at that level. However, when highly concentrated, they become harmful in the environment. Since the heavy metals cannot be eliminated from the water body, they settle at the bottom and get released into the water column by water currents and other activities that cause sediment resuspension. This pose risks of contamination for the marine biota. Metal bioaccumulation in marine algae is a function of the total enrichment in metal by a specific algal organism as well as the bioavailable metal forms that are more easily integrated by the organism (Ketife et al., 2020). Early studies have found a linear correlation between zinc concentrations in *Ulva Lactuca* L, 1753 and *Laminaria digitata* (Huds) Lamouroux, 1813 and mean concentrations of zinc in the surrounding water (Circuncisão et al, 2018). Boundir et al. (2019) showed an existing correlation of 0.98 for copper and 0.97 for lead between seawater and a number of algal species (*Blidingia* Kylin, 1947, *Enteromorpha* Link, 1820 and *Fucus* Linnaeus, 1753). Luoma (2018) had opined that the level of suspended metals present in an ambient water is a function of the type and quantity of sediments, and only partially dependent on climatic and hydrographic processes. Algae only bind free metal ions, the concentration of which would depend on the nature of suspended particulate matter formed by organic and inorganic compounds. Studies reveal that at an early stage, algae accumulate metals on their external surface through a reversible physico-chemical uptake process, followed by a process regulated by cellular metabolism. It is widely accepted that the metal uptake through cellular membranes of the macroalgae is mainly due to diffusion rather than an active transport mechanism. These algae can also produce organic complexes which can affect the uptake of metals to varying degrees. In particular, copper and mercury have a strong tendency to form organic complexes when compared to other heavy metals, although both coastal and estuary organic complexes contain several ligands presenting different. stability constants (Bonanno et al., 2020). The macroalgae in saline environment require ion exchange mechanisms to selectively absorb essential metal ions such as calcium and potassium. The complexes formed by these marine macroalgae are in form of jelly polysaccharides and can present on the external cellular walls of the algae a defense mechanism against other organisms, increase the elasticity of the cellular wall and consequently give the algae an adaptive ability against wave action (Ciancia et al., 2020). Temperature and salinity play an important role in the bioaccumulation of heavy metals by marine algae. For instance, *Ulva rigida* C. Agardh, 1823 accumulates large amounts of Fe, Zn and Cd when salinity is low (Jacobsen et al., 2023). Among the most studied microalgae, there is the species *Chlorella* M. Beijerinck, 1890 which has remarkable bioaccumulation capacities of a wide range of metal cations like copper and nickel, cadmium and zinc, lead, and rare earth elements (REEs) (Kang et al., 2019). It is important to note that the bioavailability of a pollutant determines its true ecotoxicological value. Its speciation-oxidation status and reactivity are much more relevant than its accumulation level in the water body.

1.4 *Cystoseira* spp. AS AN EFFECTIVE BIOINDICATOR TO ASSESS HEAVY METAL POLLUTION

Macroalgae are critical components of marine ecosystems and have gained significant attention as bioindicators in environmental science (D'Archino and Piazzi, 2021). Their ability to respond to anthropogenic pressures and their widespread distribution and ecological relevance makes them ideal for monitoring changes in marine environments (Luypaert et al., 2020). Among marine bioindicators, the genus *Cystoseira* spp. stands out due to its sensitivity to environmental changes, wide distribution, and ecological importance (Sumudumali et al., 2021). *Cystoseira* species, as perennial brown macroalgae in the order Fucales, are foundational to coastal ecosystems (D'Archino et al., 2021). Found predominantly in the Mediterranean Sea and North Atlantic Ocean, Cystoseira species contribute significantly to marine biodiversity and ecosystem functions (Fehér et al., 2023). Species from this genus form complex three-dimensional forest structures that provide shelter and habitat for many other marine species (Lochhead and Hedley, 2022).

Fig. 2 *Cystoseira* spp. (*from Bouafif et al., 2018)*

The sensitivity of *Cystoseira* species to environmental stresses makes them reliable bioindicators for assessing marine ecosystem health (Butt and Hamid, 2025). Their responses to pollution, nutrient enrichment and climate change are particularly informative (Hoppit and Schmidt, 2022). *Cystoseira* species show physiological and population-level changes in response to:

Chemical pollutants and heavy metals, such as chromium, mercury, lead, and cadmium, serve as measurable indicators of pollution levels (Pagana et al., 2024). Oil spills and urban runoff negatively impact *Cystoseira* spp. by reducing their density and photosynthetic efficiency (Balzan et al., 2020). Additionally, microplastic pollutants adhere to algal surfaces, disrupting light absorption and interfering with photosynthetic processes (Li et al., 2020). Excessive nutrient input from agricultural runoff leads to eutrophication, where fast-growing opportunistic algae outcompete *Cystoseira* spp., reducing light availability and altering community composition by replacing *Cystoseira* spp. with less sensitive species (Smith et al., 2023; Bernal-Ibáñez et al., 2024). Beyond pollution-related stressors, *Cystoseira* species also serve as effective indicators of climate change, as rising sea temperatures impact their reproductive cycles and cause shifts in their distribution (Monserrat et al., 2022).

Technological innovations in biomonitoring have enabled the integration of modern technologies, revolutionizing the monitoring of *Cystoseira* spp. forests (Pan et al., 2018). Emerging approaches include: remote sensing using high-resolution satellite imagery, enabling large-scale mapping of algal forests of this and many other species (Nijland et al., 2019); DNA Barcoding using molecular techniques that improve species identification and genetic diversity assessments (Antil et al., 2023); Machine learning algorithms, such as Random Forest, have been used to analyze environmental data and predict *Cystoseira* spp. responses to various stressors (Fabbrizzi et al., 2020). In conclusion, incorporating *Cystoseira* spp. into global monitoring frameworks is a vital step toward sustainable ocean management and is indispensable for understanding and managing marine ecosystem health (Smith, 2023).

1.5 INNOVATIVE TOOLS TO MAP MARINE BIOINDICATORS AND THEIR POTENTIAL RESPONSES TO ANTHROPOGENIC POLLUTANTS

Long-term marine monitoring has demonstrated high value for informed management decisions addressed to reduce the impacts of human activities and pressures. Therefore, maintenance of these long-term monitoring networks is critical, and relies on the development of cost-effective implementation approaches (Bazairi et al., 2023) and methods (Hyvärinen et al., 2021). These methods can be either traditional or rely on novel technologies, with a broad range of sampling, observation and analytical techniques (Bayley, 2019), such as: instruments (e.g. buoys to deploy sensors, physico-chemical sensors, remotely operated vehicles, autonomous vehicles, biosensors, biologgers, passive sampling), molecular approaches (e.g. metabarcoding of eDNA), acoustic devices (e.g. multibeam), flow cytometry, imaging, remote sensing (optical, thermal and radar images from airborne, including drones, and satellite sensors), artificial substrata, etc.

Traditional methods are often time consuming, and costly, which typically translates into pronounced limitations of the spatio-temporal resolution and coverage that is required by policymakers, stakeholders and decision-makers (Mack et al., 2020). To improve cost-efficiency, reproducibility and spatio-temporal resolution, novel methods are being proposed, i.e. as proof-of-concept, or already operational, but with little application in the marine realm, using five basic criteria (Mack et al., 2020): (i) technology readiness level of seven or higher; (ii) comprehensive and stand alone techniques; (iii) filling a gap in the current monitoring frameworks; (iv) novel and not in general use; and (v) evaluated as cost-efficient in terms of their cost-benefit ratio (including investment costs, monitoring costs, reliability, environmental impact, added value, limitations, required expertise).

For novel methodologies to prove their usefulness and value to monitor and assess marine ecosystem status and health of coasts and seas, they need to score well on relevant criteria, as those proposed by Mack et al., (2020) and in addition be properly communicated to end-users, especially to stakeholders as management agencies, who will be using data generated by such innovative tools for improve management actions (Seys et al., 2022). Some marine organizations have proposed step-by-step tailored guides for an accurate and efficient communication (Seeyave et al., 2017). Usually, this communication requires (i) identifying the target audience (e.g. end-users of monitoring and assessment methods, policymakers, decision-makers, managers); (ii) break down the communication objectives into relevant messages for each target audience, starting with those with highest priority (e.g. those deciding on monitoring and assessment infrastructures); (iii) for each target, decide the most appropriate channel to communicate (e.g., hard copy, digital, in-person, etc.); and (iv) build the communication implementation plan, including the objectives, target audience, tasks and methods, timing and key performance indicators (Seeyave et al., 2017).

Research programmes on monitoring and assessment, based on individual and unconnected projects, can lead to research fragmentation, wasting resources through duplication, dispersion and overlap (Elliott et al., 2020). To minimize these deficits, the Horizon Europe programme offers an instrument for collaboration among “sister” projects (i.e. those under the same call or addressing close topics), facilitating synergies in their outcomes, communication, stakeholders' participation and increase of knowledge.

However, the spectral signatures of macrophytes and seagrasses are affected by the effect of the water column, therefore; it is necessary to use specific correction algorithms for each water condition. Spectral discrimination of aquatic plants is mainly based on the spectral characteristics of the pigments they contain, within the visible wavelengths that penetrate the water column and can be reflected to the sensor (Mu et al., 2023). Unlike the terrestrial environment, wavelengths over 680 nm are significantly attenuated by pure water (Hu et al., 2018). The changes in the pigment content can also determine variations in the reflectance intensity of the three main algal groups (Chlorophyceae, Rhodophyceae and Phaeophyceae) (Davies et al., 2023). An interesting study is the work of Pham et al. (2019), which produced a library of the spectral signatures of Submerged Aquatic Vegetation (SAV) to gather optical data of macroalgae (red, green, and brown), seagrasses, and sediment characteristics in coastal waters to support the selection of spectral bands and bandwidths for different environmental conditions such as clear water and high turbidity water bodies and to choose suitable satellite sensors for different benthos.

In recent years, European environmental policy has supported the development of Copernicus, the Earth observation component of the European Union's Space program. In particular, the Copernicus Marine Service (or Copernicus Marine Environment Monitoring Service), which provides integrated information from satellites, in situ observations and models on the state of the ocean, making available important datasets to monitor changes and trends on a global and regional scale. In particular, the Copernicus program is served by a set of dedicated satellites (the Sentinel family) with seven Sentinel satellites in orbit, of four different types and contributing missions (existing commercial and public satellites) and it is expected a constellation of almost 20 more satellites in orbit before 2030. Different remote sensing platforms have their own capabilities for mapping and monitoring water pollution of different types, characteristics, and concentrations (Hafeez et al., 2018). According to the platforms here we consider the following categories. Satellite-based remote sensing images and techniques are advantageous as they provide uniform data with common acquisition and recording schemes, covering extensive and remote areas for marine monitoring. Numerous satellite remote sensing missions allow us to obtain ocean color, sea surface temperature and sea surface salinity data for the open sea. The first remote sensing operating systems (such as CZCS, SeaWiFS, MODIS, MERIS, VIIRS) were equipped with average spatial resolution sensors for data acquisition in the open sea with a spatial resolution ranging from kilometers to hundreds of meters.

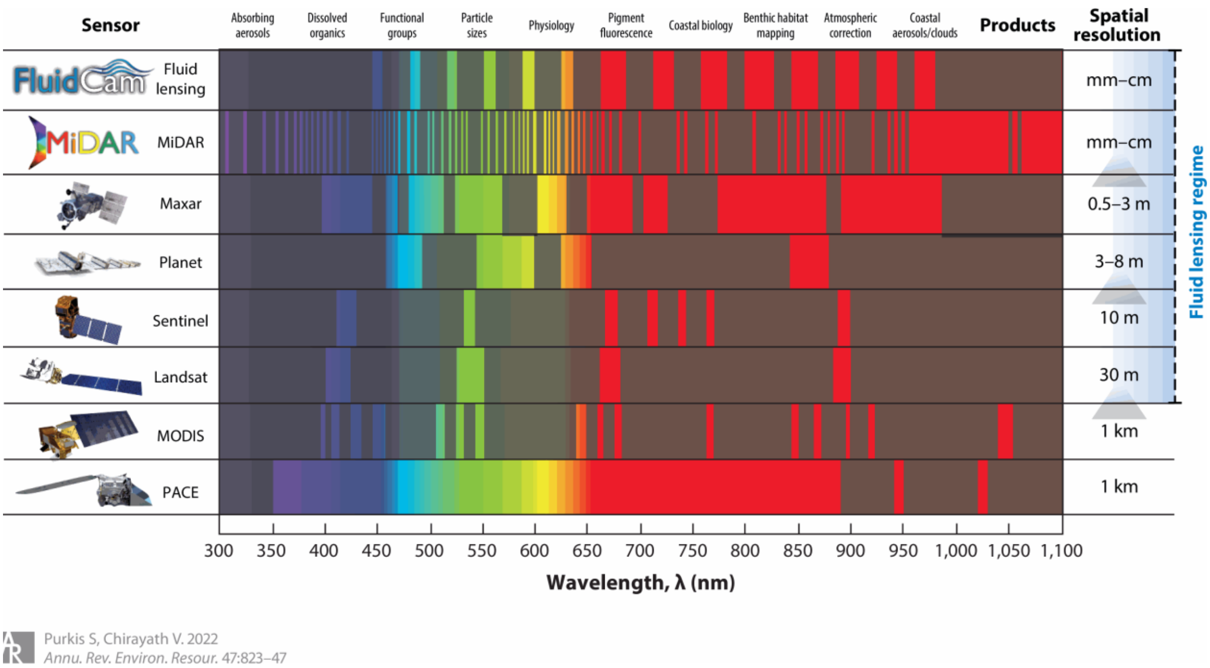

Fig. 3 Spectral coverage and spatial resolution of optical ocean biosphere remote sensing systems (*from Purkis et al., 2022).*

Airborne data, including aerial photographs and videography, have been widely used in seagrass research (Komatsu et al., 2020). Both passive (photographs, multispectral and hyperspectral data) and LiDAR data can be obtained from airborne platforms. Different types (colour, false colour near-infrared, and black and white) of aerial photographs can also be used for seagrass mapping in coastal areas with varying depths. Veettil et al. (2020) provide an overview of seagrass aerial remote sensing research methods, highlighting advantages and limitations. Aerial photography or airborne imagery acquired from a human-crewed aircraft have been used for mapping marine bioindicators since the 60s. Planes can fly at lower altitudes than satellites, providing high-resolution images and detailed data on seabed characteristics. In recent years numerous works were reported in many aspects of coastal research, such as beach erosion control, coral reefs and marine habitats management, oil spill pollution, heavy metal detection. Airborne platforms are commonly used for the acquisition of hyperspectral images (e.g. AISA+, CASI-2, HyMap, Ocean PHILLS, PRISM), and these have been increasingly used for: mapping the extent of seagrasses (Kutser et al., 2020); species level discrimination; estimation of biomass and productivity; as well as other factors including water column depth, water quality, bottom types (and spectral separation between seagrasses and other bottom substrates such as algae (Baek and Kim, 2024).

The use of Unmanned Aerial Vehicles (UAVs) for marine ecological research has been growing rapidly in recent years with an unprecedented development for missions dedicated to the monitoring of seagrasses, mangroves, saltmarshes, coral reef and many other applications (Mahrad et al., 2020). Most of these vehicles are drones, unmanned aircraft that can reach difficult locations to be monitored through traditional methods. Notable advantages of using UAVs compared to aircraft, ships or satellite platforms, are low operational costs, high operational flexibility and high spatial resolution (Aasen, 2018). Drones can be equipped with weight sensors with multispectral, hyperspectral and LiDAR systems on UAVs (Manfreda et al., 2018). Good results were obtained with the combination of multispectral satellite images with data taken from UAVs producing high quality maps of seagrass meadow. Recent advances have also combined AUVs technologies with Autonomous Surface Vehicles (ASVs), considered promising approaches in the marine science community (Rende et al. 2020). An immediate advantage is related to the collection of ground-truth data (optic and acoustic) with single-beam echosounders and images from underwater photogrammetry cameras, which ensures very high accuracy in very shallow waters (Carlson et al., 2019). This approach could represent, in the future, a replacement of scuba diver seagrass investigations and field campaigns. Moreover, Yuan, S., et al., 2023 suggested a 3D representation that can improve the classification, visualization, and mapping of coastal morphology in high resolution, using UAV images. Furthermore, they provided a protocol for UAV aerial survey that can improve data acquisition quality, in a timely, efficient manner that represents an effective methodology for coastal and marine survey.

2. AIMS OF THE THESIS

This thesis, through the writing of a review and a scientific article, presents the results of three years of doctoral research aimed at analyzing the spectral signature of target marine plant species in response to specific pollutants. The goal was to contribute to the development of innovative methodologies for monitoring the marine environment by identifying "target wavelengths" that could be used for remote sensing techniques, enabling new multispectral sensors and stratospheric platforms. This research is part of the STOPP project (Strumenti e Tecniche di Osservazione della Terra in Prossimità e Persistenza), funded by ASI (Agenzia Spaziale Italiana).

- The Review:

- The first phase of the research involved the creation of a bibliographic collection, selecting Google Scholar as the database and defining specific keywords (Seagrasses, Macroalgae, Photosynthesis, Trace Metal Pollution, Mediterranean Sea).
- The second phase focused on selecting publications based on search criteria, considering only studies published within the last ten years (2014–2024).
- The third phase involved compiling tables summarizing the selected results based on their relevance to the PhD research. The data were organized into five columns: (References & Authors, Species, Studied Elements, Parameters, Responses).

- The Scientific Article

- First, a literature review was conducted to identify the target species suitable for our experiment, leveraging the selection process from the review.
- Next, the experiment was designed by comparing it with existing studies most relevant to our research objectives.
- The experiment was then set up, incorporating necessary modifications and adaptations based on multiple trials until comparable results were obtained.
- Finally, the results were analyzed to determine whether the initial research objectives were met-specifically, identifying variations in the spectral signature of the selected species in response to the chosen pollutant.

3. Review

Title: Adverse effects of trace metal pollution on marine macroalgae and seagrasses: a focus on impacts to photosynthetic activity.

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Keywords:

Brown Algae, Seagrasses, Spectral Signatures, Chromium, Reflectance, Irradiance, Mediterranean Sea, Heavy metal, Marine Pollutants, Trace Metals.

3.1 Abstract

Coastal areas host ecosystems which provide fundamental ecosystem services. However, these areas are densely populated and often impacted by human activities, leading to pollution. Climate change exacerbates the effects of human-induced pollution, posing an additional threat to marine ecosystems.

The benthic habitat hosts biocenoses capable of recording the effects of pollution events and could respond with changes in their composition and structure. Macroalgae and seagrasses are fundamental to marine food webs, providing resources and serving as habitats for diverse animal and plant species. They play a vital role in global oxygen production, carbon and nutrient cycling. Marine macroalgae and seagrass species are recognized as excellent bioindicators of bioavailable trace elements in the marine environment, as they can accumulate these elements from surrounding sources, such as seawater and sediments, at concentrations several times higher than their environmental levels.

We examined scientific studies from the past decade (2014–2024) that investigate the impact of trace metal pollution on marine macroalgae and seagrasses, with a specific focus on its effects on the photosynthetic process.

3.2 Introduction

Trace metal pollution is a major threat to marine ecosystems, with deleterious effects on a wide range of organisms, including macroalgae and marine phanerogams. (Bonanno and Orlando-Bonaca, 2018) These metals, resulting from anthropogenic activities such as industry, agriculture and waste disposal, can accumulate in marine environments, altering fundamental biological processes.

(Sonone et al., 2020). In particular, the photosynthetic activity of macroalgae and marine phanerogams is particularly vulnerable to the toxic effects of trace metals, with significant consequences for the primary productivity and health of coastal ecosystems (Renzi et al., 2014). This review aims of this review to provide a comprehensive overview of the adverse effects of trace metal pollution on the photosynthesis of marine macroalgae and seagrasses (Sánchez-Quiles et al., 2017). We will analyse the mechanisms of toxicity, the physiological and biochemical responses of affected organisms, and the ecological implications of these alterations (Di Giulio et al., 2020). Furthermore, we will discuss future perspectives for research in this field, with a focus on the use of advanced technologies such as remote sensing and spectral signature studies to monitor and assess trace metal pollution in marine ecosystems (Lovynska et al., 2024). Remote sensing techniques offer significant potential for non-invasive, large-scale monitoring of the effects of pollution (Fascista, 2022). Through the analysis of spectral signatures, changes in the health of macroalgae and marine seagrasses can be detected, providing valuable data for the management and conservation of marine ecosystems (D'Archino, and Piazzi, 2021). This review aims to highlight the importance of such innovative approaches and to promote further research to address the challenges posed by trace metal pollution (Vardhan et al., 2019).

3.3 Trace metals in the marine environment

Among the existing inorganic pollutants, trace metals are considered a serious threat due to their toxicity to marine life and persistence in the marine environment. Trace metals have both natural and anthropogenic origins (Zhang et al., 2019; Bonamano et al., 2021). The principal natural source of trace metals is from crustal material that is either weathered on and eroded from the Earth's surface or released into the atmosphere by volcanic activity (Gautam et al., 2016). Furthermore, there are a multitude of anthropogenic sources of trace metals: rapid industrialization, urbanization and increased human activity have resulted in an increased quantity of trace metals accumulated in air, water, soil, and sediment as described in several works (Bastami et al., 2014; Gautam et al., 2016; Bello et al., 2023).

The major anthropogenic source of trace metals is from mining and smelting. Considering these two major anthropogenic inputs, metals are released in the ionic form in the riverine environment and/or into the atmosphere, where, following the atmospheric dynamics, would fall onto the ground or directly into the sea. More in detail, mining activity releases metals to the fluvial environment as tailings and to the atmosphere as metal-enriched dust. Moreover, smelting releases metals to the atmosphere because of high temperature refining processes (Adnan et al., 2022; Zhang et al., 2022). Other important anthropogenic sources of trace metals include fossil-fuel combustion, municipal waste incineration, cement production, phosphate mining and the use of commercial fertilizers and pesticides (Nieder and Benbi, 2024).

Trace metals released from natural and anthropogenic sources are then transported to the marine environment by different pathways including river basin runoff, atmospheric deposition and discharge of sewage (Gautam et al., 2016; Cimboláková et al., 2019). It is widely shown in the international literature that affinity exist between the finer grain size of sediment and the chemical species present in the water column: potentially chemical pollutant, such as trace metals, tend to bond with the pelitic sediment particles ($x < 63$ micron), and then they follow the sediment fate (Bonamano et al., 2021). As a result, the final receptors of pollutants in the sea are the depositional areas of the pelitic fraction which are internationally considered as the main "archive" of pollutants reflecting the pollution levels in the water column.

The processes implied in the element-particle association are listed below (Qasem et al., 2021):

- ionic exchange, precipitation or hydrophobic interaction with the particle’s surface;
- co-precipitation with iron and manganese hydroxide coatings;
- complexation with organic substances that are linked to or aggregated with the particles;
- inclusion in mineral grid, organisms, fecal pellets;
- flocculation of organic and inorganic substances during the freshwater-salt water mixing.

After that, metals would follow the depositional destiny of the particles to which they are associated, settling down on the seabed in low-energy environment, which are considered as basins rich in pollutants (Fig.1).

Once sedimented, metals can be resuspended in the water column through physical and chemical alteration or remain in the sediment. In both cases they will interact with marine organisms.

Research on metal contamination in marine environment is fundamental to protect the health status marine ecosystems, since trace metals can exponentially accumulate at all levels of the trophic chain through bioaccumulation and biomagnification (Domingos et al., 2015; Brezonik et al., 2020; Knezovich, 2024).

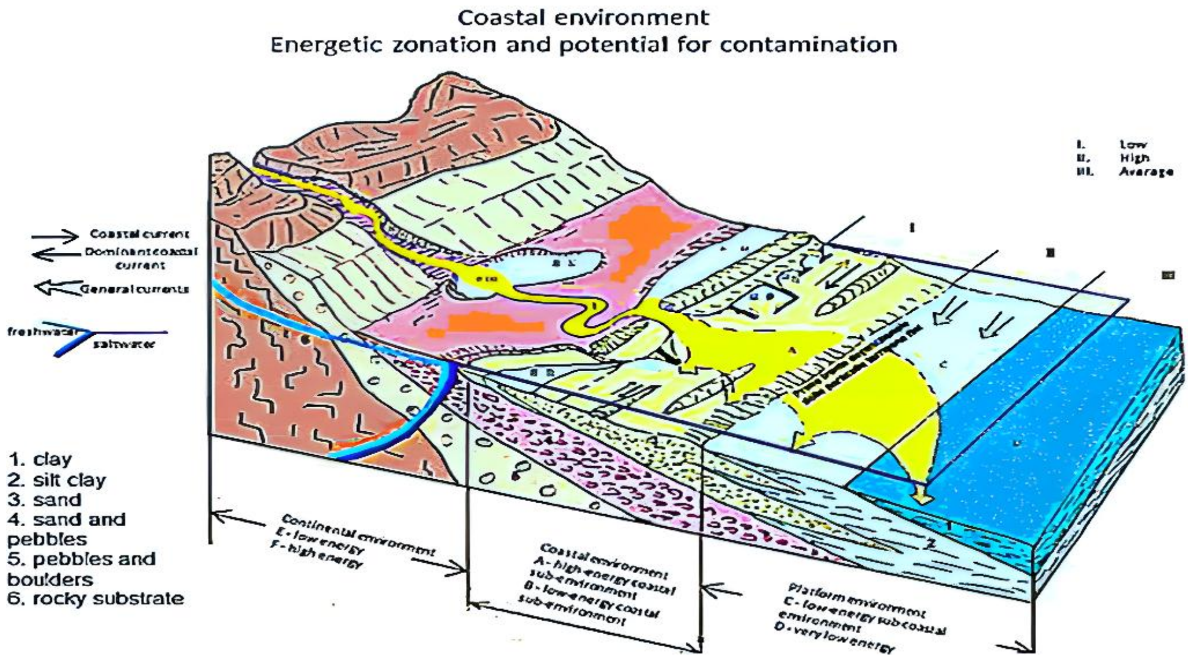

Fig.1 Potential contamination in the marine environment (Modificated from Brondi et al., 2008).

3.4 Seagrasses and macroalgae as bioindicators of trace metal pollution in the marine environment

Several metals are essential for marine organisms at very low concentrations, but at higher doses most of them are toxic (Langston, 2018).

A growing interest of scientific community has been addressed to the analysis of the effects of trace metals on marine life thus large number of scientific surveys focused on the speciation of metals, their toxicity, accumulation, biomagnification, bioindication, migration, removal, phytoremediation, and biomonitoring have been conducted during the last decades (Tarish et al., 2024). In this context a particular attention has been addressed to marine macroalgae and seagrasses, considered excellent bioindicators of the bioavailable trace elements in the marine environment, as they accumulate elements from the surrounding matrices (seawater, sediments) several times their environmental levels. In addition, they are ideal bioindicators as they are sessile, have a widespread distribution over the Mediterranean coastal areas, are available all year long, and easy to sample (Richir and Gobert, 2016).

Moreover, seagrasses and macroalgae are at the basis of complex food webs providing e.g. grazing and detrital food, habitats for numerous animal and plant species, as well as up to 50% of the estimated net amount of global oxygen (Macreadie et al., 2017). They are fundamental also to global carbon and nutrient cycling, providing many ecosystem services (Scanu et al., 2022). Focusing on their accumulation capacity, seagrasses can reflect significantly the levels of trace elements present in sediments, whereas macroalgae species are more sensitive to spatial and temporal oscillations in seawater element concentrations, since they do not have root systems, thus intake of trace elements from sediments is supposed to be lower than in seagrasses (Bonanno and Orlando- Bonaca, 2018).

For what concern seagrasses, leaves are considered as effective short-term bioindicators able to provide accurate information on the presence of trace elements in the marine environment over short time periods, e.g. months (Boutahar et al., 2019). On the contrary, permanent organs such as roots and rhizomes of seagrasses, whose element concentrations can reflect multiyear inputs of trace elements, seem more appropriate in case of long-term biomonitoring, but with the limitation that these organs are usually less sensitive to element variations in the environment compared to leaves (Kaiser et al., 2014). Genotype and environment variation in elemental composition of spring wheat flag leaves. Agronomy Journal, 106(1), 324- 336.).

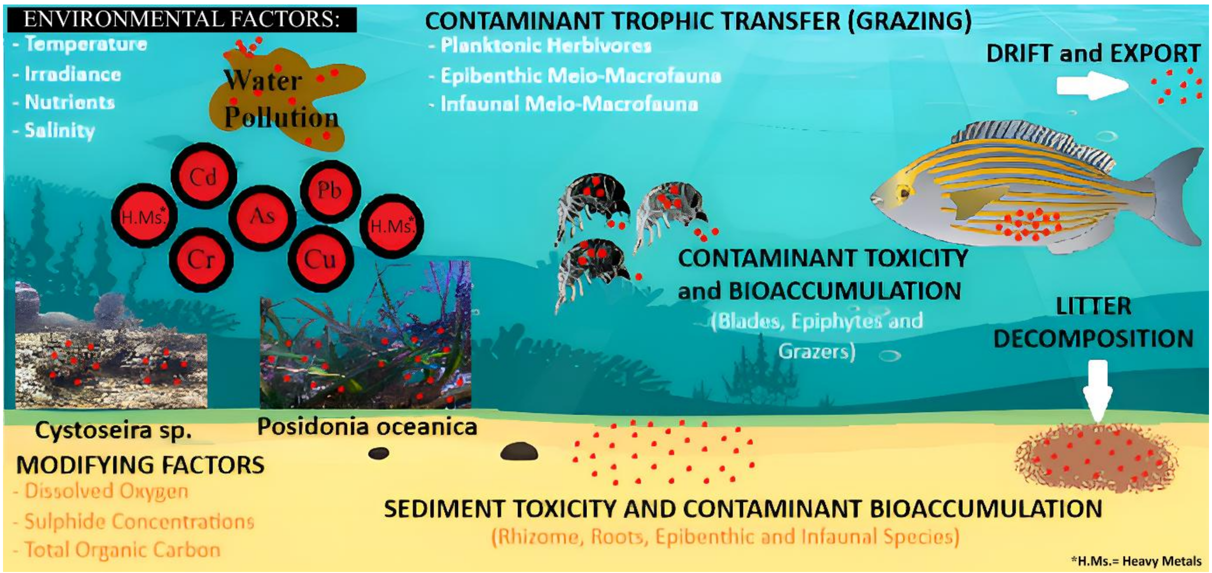

Fig.2 Major toxicity pathways of anthropogenic contaminants taken as example (Trace Metals): bioaccumulation and trophic level shifts associated with seagrass beds and algal forests.

The levels of trace elements in sediments related to seagrass and macroalgal ecosystems are on average three orders of magnitude greater than the levels in marine water. The comparable levels of trace elements in seawater related to seagrass and macroalgal ecosystems, suggest that both ecosystems are subjected to the same intensity of element inputs from the water column (Bonanno and Orlando-Bonaca, 2018).

Regarding seasonal variations, the levels of trace elements vary seasonally in seagrasses, with higher concentrations in the dormant period than in the growing season (Bonanno and Orlando-Bonaca, 2017). Seasonal variability of trace element levels is more controversial for macroalgae, since some studies report that seasonal variations of element concentrations occur regularly in macroalgal species (Benfares et al., 2015), while other papers did not report significant variations over time in algal tissues (García-Seoane et al., 2021).

3.5 Trace metals uptake

The toxicity of trace metals largely depends on their bioavailability, that is, the mechanisms of uptake through cell membranes, intracellular distribution, and binding to cellular macromolecules (Zhao et al., 2016).

Metals are not accessible to plants in their elemental forms (valence state of 0). On the contrary, they are available only in solution; hence, only metal ions play a role in biological systems (Jomova et al., 2022). This implies that metal availability is strongly dependent on environmental conditions, such as pH, redox and organic content of the considered compartment (Zhao et al., 2016).

The accumulation of a trace element is achieved through mechanisms of absorption, desorption and translocation from one compartment to the next (Zakaria et al., 2021). The absorption seems to occur in three phases: a passive accumulation phase within the membrane's free space, a diffusion phase across the plasmalemma into the protoplasm, and an active accumulation within the cell through chelating proteins (Ghori et al., 2019). Following its absorption, the pollutant particle could be:

- trapped in a neutral fraction (metal-proteins for the metals), and then stored in a compartment of the organism (Piergentili, 2016);
- fixed due to its affinity to a fixation site within the organism;
- metabolized by biotransformation enzymes that increase the water solubility of toxic substances and thus facilitate their natural elimination. (Somu et al., 2022) Biotransformation enzymes increase the water solubility of toxic substances and thus facilitate their natural elimination (Kebamo et al., 2015**).**

Assimilation of trace elements among rooted aquatic plants occurs through two pathways: uptake by leaves from the water column or by roots from interstitial water (Glibert et al., 2019).

Different strategies to cope with an excessive metal concentration has been observed, particularly in seagrasses. Generally, it has been observed in the seagrasses an “exclusion” strategy that consists in the accumulation of metals in the roots and in the organs far from the leaves, which are the site of the photosynthesis (Rahman et al., 2024). On the contrary, in *Posidonia oceanica* (L.) Delile, 1813 and *Cymodocea nodosa* (Ucria) Ascherson, 1870 was observed a “removal” strategy in which the plant accumulates metals in the leaves, which fall in the leaves-turnover season (Bonanno and Raccuia, 2018; Bonanno et al., 2020). Also, the upper parts of the seagrasses presented generally higher levels of metals than the underground ones; attributed to the higher metabolic activity of these tissues (Lee et al., 2023). In addition, trace metal concentrations in leaf decrease in the following order: leaf tip>whole leaf>leaf basal tissue. (Palm et al., 2017). This trend is consistent with the observation that the seagrass leaves grow by formation of new tissue at the base. Therefore, the tip becomes the older part of the leaf, hence the tissue with a longer exposure to metal loads in the ambient habitat. However, many authors pointed out that there are specific pathways of accumulation for each trace metal. For example, Pb and Cr are more concentrated in the rhizomes than in the leaves according to Zhu et al., (2018). Also, Watson, M., et al., (2015) found that Cr resulted more concentrated in sheets, while other trace metals are preferentially accumulated in blades, in agreement with previous studies (Zhang et al., 2023). This therefore leads to the hypothesis of a preferential uptake of Cd, Co, Hg, Ni and Pb from the water column to the photosynthetic tissue. Furthermore, the different behaviour of Cr suggests another uptake and distribution route for this element.

3.6 Trace metals toxicity

Although trace metals are characterized by different mobility, solubility thus potential toxicity, in general toxicity arises when metals non-specifically bond with coordination sites of important biological molecules such as enzymes and other proteins, thereby altering normal metabolic functions. Regarding seagrasses and macroalgae, they produce the following effects (Singh et al., 2015; Petsas and Vagi, 2017; Ahmad et al., 2017):

- block functional groups of proteins (direct binding to the sulfhydryl groups (-SH) disrupting their structure and function, and thus making them inactive);
- displace and/or substitute essential metals from specific binding sites that lead to a collapse of function;
- induce conformational changes;
- denature enzymes and disrupt cells and organelle integrity

Toxicity can also be related to oxidative stress induced in living cells either by increasing concentrations of reactive oxygen species (ROS) or by reducing cellular antioxidant capacity (Lushchak, 2014): molecular oxygen is unreactive with organic molecules because it has two unpaired valence shell electrons in outer shell. However, when activated through reduction it forms ROS such as superoxide radical, singlet oxygen, hydrogen peroxide, hydroxyl radical and finally water. ROS are toxic because they interact rapidly with biological molecules (proteins, lipids, DNA) which can result into cell death via apoptosis or necrosis (Bertini et al., 2019; Juan et al., 2021), involving consequences in cell growth and in metabolism. Trace metal toxicity is also known to affect reproduction, interfere with cell permeability, cause the loss of K ions, affect protein synthesis and degradation, and cause oxidation and lipid peroxidation (Mukherjee et al., 2022).

Among the cited toxic effects, trace metals strongly interfere with the photosynthetic process (Rai et al., 2016) by inhibiting the chlorophyll biosynthesis and the activity of the enzymes involved in the photosynthesis (Zhang et al., 2020). Since it represents a fundament, here it follows a focus on the interference of trace metals on photosynthesis.

3.7 Materials and Methods

The first phase of this research involved the creation of a bibliographic collection, selecting Google Scholar as the database and defining specific keywords (Seagrasses, Macroalgae, Photosynthesis, Trace Metal Pollution, Mediterranean Sea). In the second phase, publications were filtered based on search criteria, considering only studies published within the last ten years (2014–2024).

Based on this bibliographic analysis, we developed a species/contaminant/response matrix, providing a comprehensive and updated overview of the current state of knowledge. This matrix identifies the selection of target species among seagrasses and macroalgae for use in experimental research, facilitating a more effective assessment of their responses to trace metal pollution.

REFERENCE	SPECIES	STUDIED ELEMENTS	PARAMETERS	RESPONSES
Bonanno, G. and Di Martino,V. (2017). Trace element compartmentation in the seagrass Posidonia Oceanica and biomonitoring applications. Marine Pollution Bulletin, 116 (1-2), 196-203.	*Posidonia oceanica*	As, Cd, Cr, Cu, Hg, Ni, Pb, Zn	Metal concentration, translocatopn factor (TFs) and bioconcentration factor (BCFs)	Major uptake from, the sediment and major concentration of Ni and Zi in leaves.
Bonanno G. and Raccuia, S. A. (2018). Comparative assessment of trace element accumulation and bioindication in seagrasses Posidonia Oceanica, Cymodocea nodosa and Halophila stiupulacea. Marine Pollution Bulletin, 131, 260-266	*Posidonia oceanica, Cymodocea nodosa, Halophila stipulacea*	Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn	Metal concentration, translocation factor (TFs) and bioconcentration factor (BCFs)	Major uptake from, the sediment and major concentration of Cu, Mn, Zn in leaves.

Bonanno G., Borg, J.A. and Di Martino, V. (2017). Levels of heavy metals in wetland and marine vascular plants and their biomonitoring potential: a comparative assessment. Science of the Total Environment, 576, 796-806.	*Posidonia oceanica, Cymodocea nodosa*	As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn	Metal concentration, translocation factor (TFs) and bioconcentration factor (BCFs)	Major uptake from, the sediment and major concentration of Cu, and Zn in roots and leaves.
Zhao, J.S., Zhang, Q., Liu, J., Zhang, P.D. and Li, W.T. (2016). Effects of copper enrichment on survival, growth and pfotosynthetic pigment of seedlings and young plants of the eelgrass *Zostera marina*. Marine Biology Research, 12(7), 695- 705.	*Zostera marina*	Cu,	Pigments analysis and leaf growth analysis	Major concentration of Cu leads to decrease of pigments and growth.
Bonanno, G., & Raccuia, S. A. (2018). Seagrass Halophila stipulacea: capacity of accumulation and biomonitoring of trace elements. Science of the Total Environment, 633, 257-263.	*Halophila stipulacea*	As,Cd,Cr,Cu,Hg,Mn,Ni,Pb,Zn	Metal concentration and translocation factor (TFs) and bioconcentration factor (BCFs)*	-major concentration of Cu, Zn and Mn. -major uptake from sediment
Bonanno, G. & Di Martino, V. (2016).	*Cymodocea nodoosa*	As,Cd,Cr,Cu,Hg,Ni,Pb,Zn	Metal concentration and	-major uptake from sediment

Seagrass Cymodocea nodosa as a trace element biomonitor: bioaccumulation patterns and biomonitoring uses. Journal of Geochemical Exploration,169,43-49.			translocation factor (TFs) and bioconcentration factor (BCFs	-major concentration of Ni e Zn
Bonanno, G., Veneziano, V., Raccuia, S. A., & Orlando-Bonaca, M. (2020). Seagrass Cymodocea nodosa and seaweed Ulva lactuca as tools for trace element biomonitoring. A comparative study. Marine PollutionBulletin, 161, 111743.	*Cymodocea nodosa,* *Ulva lactuca*	Cd, Cr, Cu, Ni, Pb, Zn	Metal concentration	Cymodocea absorbes more than Ulva
Celis-Plá, P. S., Brown, M. T., Santillá n-Sarmiento, A., Korbee, N., Sáez, C. A., & Figueroa, F. L. (2018). Eco-physiological and metabolic responses to interactive exposure to nutrient sand copper excess in the brown macroalga *Cystoseira tamariscifolia.*	*Cystoseira tamariscifolia*	Cu	Chla fluorescence; Pigments content; Fenolic compounds and antioxidants; Cu accumulation.	antioxidant response with increasing In Cu concentration; increase in pigments content with high Cu concentration

Marine pollution bulletin, 128,214-222.				
Wickramasinghe, W. A. D., Mubiana, V. K., &Blust, R. (2017). The effects of heavy metal concentration on bio-accumulation, productivity and pigment content of two species of marine macroalgae. Sri Lanka Journal of Aquatic Sciences, 22(1).	*Fucus vesciculosus* *Ulva lactuca*	Cu, Cd, Pb	Primary productivity measure Pigments concentration	Decrease in pigments with increasing concentration of metals -decrease in primary productivity

Tab.1 Species/contaminant/response matrix of metal toxicity reported for seagrasses and macroalgae

3.8 Interference of trace metals on photosynthesis

There are important studies that have deepened the knowledge on the toxic effects of trace metals on photosynthesis (Kumar et al., 2014; Petsas and Vagi, M. C., 2017; Souri et at., 2019). Generally, trace metals can have direct effects on reactions in the dark and light and indirect effects which result in the reduction of the content of photosynthetic pigments, including chlorophylls and carotenoids, and of the functionality of the stomata. They can also affect the structure of the chloroplast membrane, disturb the perception of light and oxygen-evolving complexes, inhibit photosystems and constituents of the electron transport chain and block the pentose phosphate reduction cycle (Foyer and Harbinson, 2019; Singh et al., 2015). They can also cause the inhibition of enzymes important for photosynthetic pathways, for example the inhibition of NADP-oxidoreductase by Cadmium, Zinc, Silver and Copper (Aljerf and AlMasri, 2018). Finally, it is reported that many trace metals influence the photosynthetic activity of algae through bleaching processes which have been correlated with their tendency to generate ROS which can attack thylakoid lipids and initiate biochemical oxidation reactions which destroy membranes and damage the pigment-protein structural complexes.

Cause and effect relationships of specific chemicals to different types of target species are easily studied by the conduction of single species laboratory-controlled experiments. The various methodologies of single species bioassays differ slightly in design but basically, they utilize a uni-algal population of an available, easily cultivated, and sensitive algal test species (Verma, et al., 2021).

Photosynthetic activity is considered as a significant effect parameter of a variety of toxicants on algae related to physiological and morphological variations. Information about changes in the efficiency of photosynthesis can be acquired by measuring the yield of Chl-α-fluorescence (Kumar et al., 2014; Qian et al., 2018). Chl-α-fluorescence is a physical signal defined as the radiative energy evolved from de- exciting Chl-α-molecules (λ= 690 nm for PSII, λ= 740 nm for PSI), used as a rapid, non- intrusive, and highly sensitive bioindicator of algal stress in response to different chemicals in recent years (Kumar et al., 2014; Petsas and Vagi, 2017).

Among the different fluorescence techniques, Pulse Amplitude Modulation (PAM) fluorometry has been demonstrated as a rapid, non-invasive, reliable, economically feasible, time-saving, and accurate technique, well suited for investigating changes in photochemical efficiency of aquatic algae (Melo-Santos et al., 2024). In the last years many authors provide new interesting insights on the effectiveness of Chl-α-fluorescence parameters employed in assessing the effects of toxicants both in macroalgae and seagrasses (Madonia et al., 2021; Menone and Pérez, 2023).

Although many works deal with the effect of pollutants on the photosynthetic process, the response to increasing levels of trace metals in terms of variation of spectral reflectance signature is not fully investigated. Such variations could be detected by remote sensing allowing to quickly identify the occurrence of marine pollution phenomena. The results of these research activities will provide new important insights for the scientific community and environmental decision makers. Moreover, Bonanno and Orlando-Bonaga, (2018) highlighted that some of the most toxic elements (e.g. As, Cr, Hg) are not sufficiently investigated in marine plant organisms and the sensitivity of most response parameters is unknown in seagrasses and macroalgae, needing further detailed research on the real effects of toxic elements on both groups of marine organisms.

3.9 Spectral signatures of Macroalgae and Seagrasses

In recent years, numerous studies have been conducted on the analysis and classification of spectral signatures of submerged vegetation, demonstrating that remote sensing systems can provide detailed maps of benthic species and/or habitats, as well as information on the biophysical and physiological conditions of macrophytes and seagrasses (Kotta et al., 2014; Douay et al., 2022) and monitor marine pollution phenomena. However, the spectral signatures of macrophytes and seagrasses are influenced by the effect of the water column, so specific correction algorithms must be used for each water condition. Spectral discrimination of aquatic plants is mainly based on the spectral characteristics of the pigments they contain, within the visible wavelengths that penetrate the water column and can be reflected back to the sensor (Rowan and Kalacska, 2021). In contrast to the terrestrial environment, wavelengths above 680 nm are significantly attenuated by pure water (Lee et al., 2015). Variations in pigment content can also determine variations in the reflectance intensity of the three main algal groups (Chlorophyceae, Rhodophyceae and Phaeophyceae) (Wu et al., 2015). An interesting study is the work of Hoang et al. (2016), who produced a submerged aquatic vegetation (SAV) spectral signature library to collect optical data of macroalgae (red, green and brown), seagrasses and sediment characteristics in coastal waters, in order to support the selection of spectral bands and bandwidths for different environmental conditions, such as clear water and high turbidity water bodies, and to choose satellite sensors suitable for different benthos.

Twenty-two species of submerged coastal aquatic plants, including red, green, brown macroalgae and seagrasses, were collected at Cape Peron, Western Australia, to measure their spectral reflectance in situ.

Comparison of the measured spectral reflectance value of the SAV groups (Fig. 3) and the associated substrate revealed significant differences in the reflectance spectra characterizing red, green, brown macroalgae and phanerogams: In particular:

- Red macroalgae are characterized by peaks in the red spectral regions (580 nm) and strong absorption features in the blue and green spectral regions (Fig. 3a).

- Green macroalgae exhibit common absorption features with peaks in the blue spectral regions (450 nm) and maximize the spectral reflectance in the green spectral regions (550 nm) (Fig. 3b).

- Brown macroalgae exhibit strong spectral absorption peaks in the blue and green spectral regions (400-550 nm). The spectral reflectance peaks were observed between 600 and 650 nm (Fig. 3c).

- P.oceanica sp. exhibited maximum spectral absorption in the blue and green spectral regions (450-550 nm) and maximum reflectance in the red spectral region (600-650 nm), while Amphibolis antarctica exhibited spectral absorption in the blue spectral regions (400-500 nm) and a peak spectral reflectance in the green spectral region (550 nm) (Fig. 3d).

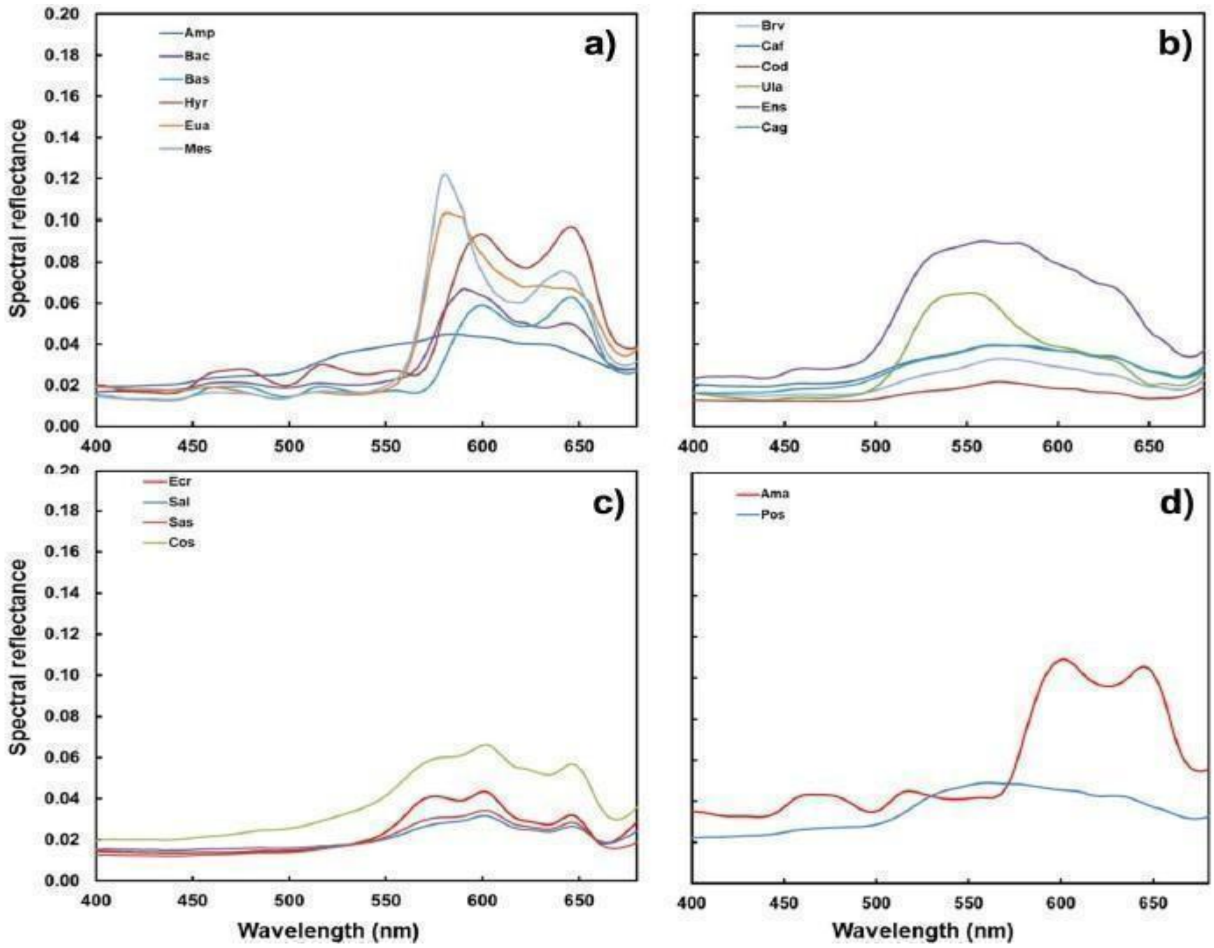

Fig.3. Spectral reflectance divided into groups: a) red algae; b) green algae; c) brown algae; d) seagrasses. (from Hoanget al., 2016).

To assess the effects of pollutant damage on the in vivo optical properties of macroalgae, applicable to laboratory and remote systems, May (2000) exposed the green macroalga E. intestinalis to selected trace metals (copper and zinc), triazine herbicides (lrgarol1051eatrazine) and a series of alcohols (n=1-9) in the laboratory. Each pollutant, except zinc, had a significant effect ($P<0.05$) on the in vivo spectral properties at the range of concentrations used. The results reported in Fig. 4 indicate that the technique has the potential to identify pollutant-affected algal systems, as the signatures obtained appear to indicate whether the change is due to structure or pigments.

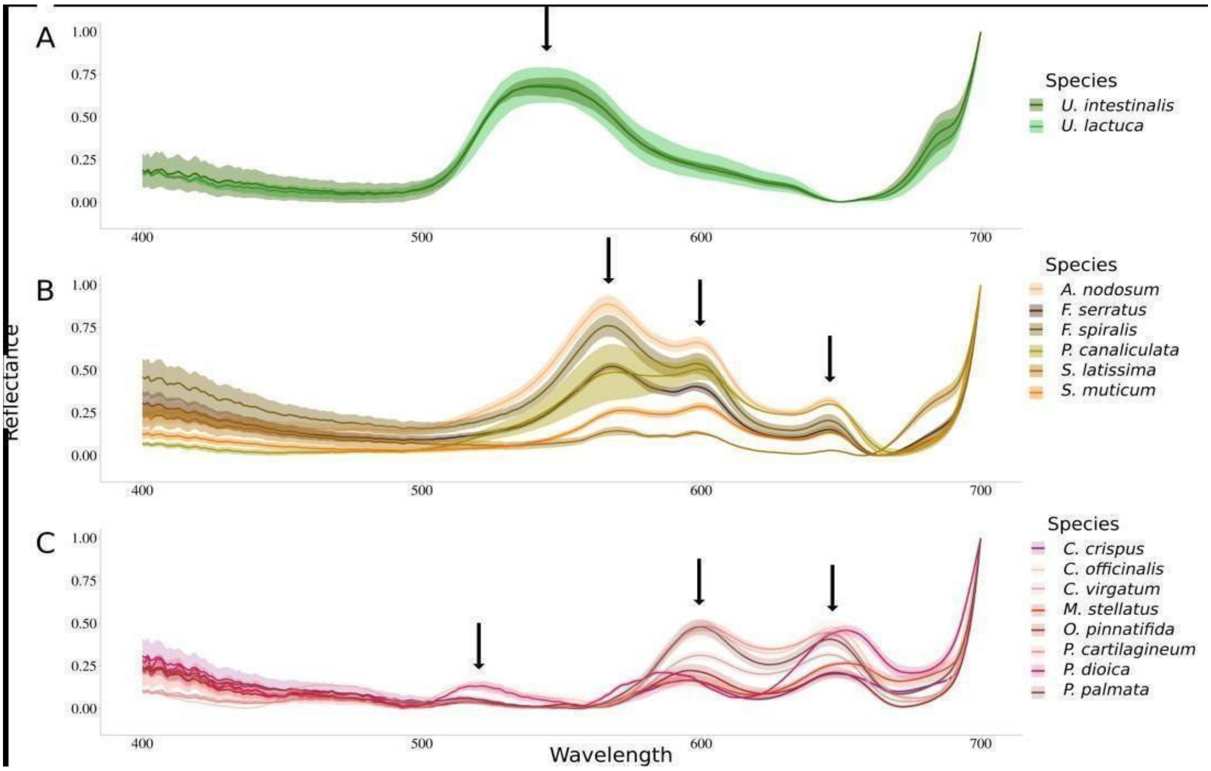

Fig.4. Normalized reflectance spectra measured for sixteen species of green, brown and red macroalgae (from Douay et al., 2022).

Fig. 4 also shows the normalized reflectance spectra of sixteen species of green, brown and red macroalgae measured by Douay et al. (2022). Green algae are characterized by a single large reflectance peak at 545 nm, while brown algae showed three reflectance peaks, including a brown algae-specific peak located at 570 nm. The other two reflectance peaks were also detected in red algae and are located at 600 nm and 645 nm, respectively. Red algae are characterized by three reflectance peaks, the two common to brown algae and one specific to this group detected at 515 nm.

3.10 Conclusion and future perspectives

Trace metal pollution poses a significant threat to marine ecosystems, adversely affecting the photosynthesis of macroalgae and marine phanerogams (Boutahar et al., 2021). These organisms, critical to the primary productivity and health of coastal ecosystems, are particularly vulnerable to the toxic effects of trace metals (Tripathi et al., 2021). Our review highlighted the mechanisms of toxicity, the physiological and biochemical responses of affected organisms, and the ecological implications of these alterations (Regoli and Giuliani,2014).

Trace metals, such as chromium, cadmium, lead and mercury, can interfere with photosynthetic processes through various mechanisms (Roychoudhury and Chakraborty, 2020), including substitution of essential ions in photosynthetic reaction centres, generation of reactive oxygen species (ROS) and inhibition of key enzymes involved in photosynthesis (Pospíšil, 2016). These effects can lead to reduced photosynthetic efficiency, decreased growth and, in extreme cases, death of organisms (Sharma et al., 2020).

Looking ahead, it is essential to develop and implement innovative methods to monitor and assess trace metal pollution in marine ecosystems (Langston, 2018). Advanced technologies, such as remote sensing and spectral signature analysis, offer powerful tools for non-invasive and large-scale monitoring of pollution effects (Alexan and Ianovici, 2024). Remote sensing enables the collection of large-scale, real-time data, making it possible to quickly identify affected areas and monitor changes over time (Cao et al., 2024).

Spectral signature analysis can detect specific changes in the reflectance of macroalgae and marine seagrasses, indicative of physiological stress caused by the accumulation of trace metals (Mcilwaine et al., 2019). These changes can be correlated with laboratory measurements to validate the results and improve our understanding of toxicity mechanisms (Mcilwaine et al., 2019).

In addition to remote sensing and spectral signature analysis, several other innovative methods can be used to study the effects of trace metal pollution on marine macroalgae and seagrasses (Bat et al., 2021):

1. Omics techniques: The use of genomics, proteomics and metabolomics techniques can provide a detailed understanding of the molecular responses of organisms to trace metal pollution (Singh et al., 2020). These approaches can identify changes in genetic, protein and metabolic profiles, providing a comprehensive view of toxicity mechanisms (Liu et al., 2022).

2. Biosensors: Biosensors based on macroalgae, and marine seagrasses can be developed to detect trace metals in the marine environment in real-time (Liu et al., 2023). These devices exploit the biochemical properties of organisms to provide fast and accurate measurements (Homaei et al., 2016).

3. Ecological Modelling: The use of ecological models can help predict the long-term effects of trace metal pollution on marine ecosystems (Eismann et al., 2020). These models can integrate environmental, biological and chemical data to simulate future scenarios and assess the effectiveness of mitigation strategies (Abatan et al., 2024).

4. Sediment Bacterial Communities: Analysis of bacterial communities in sediments can provide valuable information on the accumulation and distribution of trace metals (Lu and Lu, 2014). The characteristics of bacterial communities can be used as biomarkers to assess pollution risk and manage pollution control (Dudchik et al., 2020).

5. Advanced Imaging Techniques: The use of advanced imaging techniques, such as electron microscopy and X-ray spectroscopy, can reveal the subcellular distribution of trace metals within the cells of macroalgae and marine seagrasses (Goldstein et al., 2017). These methods can help to better understand the mechanisms of accumulation and toxicity (Jaishankar et al., 2014).

Furthermore, integrating these technologies with traditional monitoring approaches, such as chemical and biological analyses, can significantly improve our ability to detect and understand the effects of trace metal pollution (Sahu and Basti, 2021).

Promoting further research in this field is crucial to developing effective mitigation and conservation strategies, ensuring the protection and sustainability of marine ecosystems for future generations (Kennish, 2022).

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4. Article

Title:

Analysis of spectral and photosynthetic response variations of *Cystoseira* spp. exposed to Chromium

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4.1 Abstract

This study explores the physiological and spectral responses of different species belonging the genus *Cystoseira* to varying concentrations of chromium (Cr) in controlled experimental conditions. The research highlights the potential of *Cystoseira* spp. as a bioindicator for monitoring heavy metal pollution in marine ecosystems. Experiments were conducted in tanks with increasing chromium concentrations (0, 0.1, 1, and 10 μmol l^{-1}), analyzing changes in reflectance, photosynthetic efficiency, and pigment composition over 14 days. Results showed dose- and time-dependent effects, with higher chromium levels causing significant photosynthetic inhibition, oxidative stress, and chlorophyll degradation. Spectral analyses identified key wavelengths (550 nm and 710 nm) correlating with chromium-induced stress, supporting their use in remote sensing for environmental monitoring. The study underscores the importance of integrating field validation and remote sensing for large-scale monitoring of marine ecosystems impacted by heavy metal contamination. These findings contribute to conservation strategies and the development of innovative tools for assessing the ecological quality of coastal waters.

4.2 Introduction

The increasing pressures exerted by human activities on marine ecosystems have highlighted the urgent need for comprehensive conservation strategies (Roberts et al., 2017). These ecosystems provide essential ecological, economic, and social benefits but are severely impacted by pollution, climate change, overexploitation of resources, and habitat destruction (Ford et al., 2022). In response, international initiatives, such as the United Nations Convention on Biological Diversity (CBD) and the European Union's Marine Strategy Framework Directive (MSFD), emphasise the importance of monitoring and protecting marine biodiversity (Boiral and Heras-Saizarbitoria, 2017).

A key approach to assessing marine ecosystem health is using bioindicators-species or communities whose responses to environmental changes provide valuable insights into ecological conditions (Parmar et al., 2016). Among marine bioindicators, macroalgae, particularly species within the *Cystoseira* genus, have proven highly effective due to their sensitivity to environmental stressors (Pagana et al., 2024). These brown algae are foundational species in Mediterranean and Atlantic coastal ecosystems, where they form complex habitats that support a wide range of marine organisms (Mancuso et al., 2024; Lipej et al., 2023). However, their populations are in decline due to anthropogenic pressures such as pollution, habitat degradation, and climate change (Mancuso et al., 2018).

Cystoseira species are particularly valuable in biomonitoring programs due to their ability to bioaccumulate contaminants, including heavy metals introduced into marine environments through industrial and urban discharges (Boundir et al., 2019). Heavy metal pollution poses serious risks to marine life, disrupting physiological processes and threatening the long-term survival of sensitive species (Ali et al., 2019; Chernova and Shulkin, 2019). Studies have documented significant declines in *Cystoseira* populations in industrialized coastal areas, underscoring the urgent need for effective pollution mitigation strategies (Smith et al., 2023). Traditional monitoring methods, such as in situ surveys and laboratory analyses, provide essential data but are often labour-intensive and limited in scope (Gianni and Mangialajo, 2016). Recent advances in remote sensing technologies, including satellite imagery and hyperspectral sensing, offer new opportunities to study and map *Cystoseira* distribution across large spatial and temporal scales (Gutierres et al., 2016; Tait et al., 2019). Integrating remote sensing with ground-based validation enhances assessment accuracy, facilitating the development of targeted conservation measures (Chang et al., 2015).

The conservation of *Cystoseira* species is vital for maintaining the ecological integrity of coastal waters. These algae contribute to nutrient cycling and habitat provision, supporting marine biodiversity and local economies dependent on fisheries and tourism (Mancuso et al., 2024; Cotas et al., 2023). Protecting these habitats is both an ecological necessity and a socio-economic priority, particularly in regions where human well-being is closely linked to marine ecosystem health (Spalding et al., 2014). By combining traditional biomonitoring approaches with emerging technologies, researchers can develop more effective strategies to safeguard *Cystoseira* populations and the ecosystems they support.

4.3 The main objective of this study

To evaluate the variation of the spectral signature of *Cystoseira* spp. subjected in tanks to the exposure of the pollutant chromium at different concentrations to highlight substantial alterations for potential future applications in remote sensing.

4.4 Study Area

The coastal marine area of Civitavecchia (Lazio, Italy) is located in the northern Tyrrhenian Sea and falls in the physiographic unit M. Argentario-Capo Linaro (Fig. 1). The sediments in the study area come mainly from the Mignone river and secondarily from other minor rivers, but these influence the marine sedimentation only locally and during the phases of high flood (Romão et al., 2024). The Mignone river basin (500 km^2) is located between the Tyrrhenian margin (to the west) and the Pleistocene volcanic reliefs of the Sabatini and Cimino-Vicano mountains. Among the minor rivers that provide local contributions, the most important is the Marangone basin. Its delta is located south of the port of Civitavecchia. Although modest in size (only 23 km^2), the Marangone stream has abandoned in its basin mines of pyrite, marcasite and galena, as well as deposits of marcasite slag and small galena pits (Kreidie et al., 2011), which strongly influence the contributions of some trace metals in the coastal area.

The Civitavecchia area hosts the largest energy production site in Europe, as well as one of the most important ports for cruise traffic in the Mediterranean Sea (Gobbi et al., 2020). The recent history of Civitavecchia (i.e. post-war) is marked by various industrial activities that have continuously influenced the concentrations of trace metals in all environmental compartments, particularly in the sedimentary matrix (Luciani, 2023).

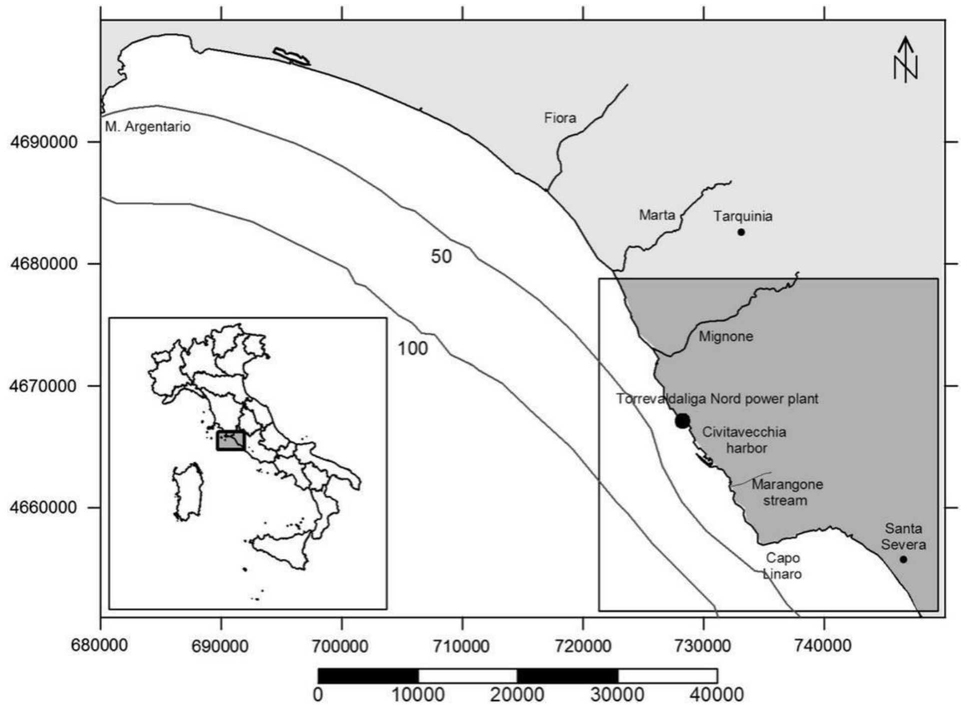

Fig. 1. Map of Civitavecchia coast highlighting rivers, towns, industrial sites, bathymetry and study area location.

The geomorphologic and anthropic characteristics of the coastal area of Civitavecchia determine a geochemical context of the environmental matrices that appears complex, given the overlap between the presence of geochemical provinces in particular of AS and Hg and the decades-long presence of industrial activities with polluting inputs of various trace metals, including those referable both to coal-fired power generation activities and others referable to maritime activities and cruise traffic. Over the years, several studies have been conducted both in the sediment matrix and in some bio-indicators in order to assess both natural and anthropogenic enrichments. Trace metal concentrations in marine sediments follow the order Mn > Pb > Cr > Ni > As > Cu. The concentrations of Pb, Cr, Ni and Cu present an increasing gradient coast - wide up to the bathymetric of - 50 m, while Mn and As present maximum concentrations in two hotspots coinciding with the mouths of the Mignone and Marangone rivers, supporting the hypothesis of the main geochemical nature of these enrichments (Piazzolla et al., 2015).

The potential ecological Risk Index (RI), described by Hakanson, (1980) shows a spatial distribution (Fig. 2) in which a severe toxicity hotspot is found in the area between the port of Civitavecchia and the Marangone Delta and a moderate toxicity level in the coastal area between Capo Linaro and the mouth of the Mignone river (Piazzolla et al., 2015).

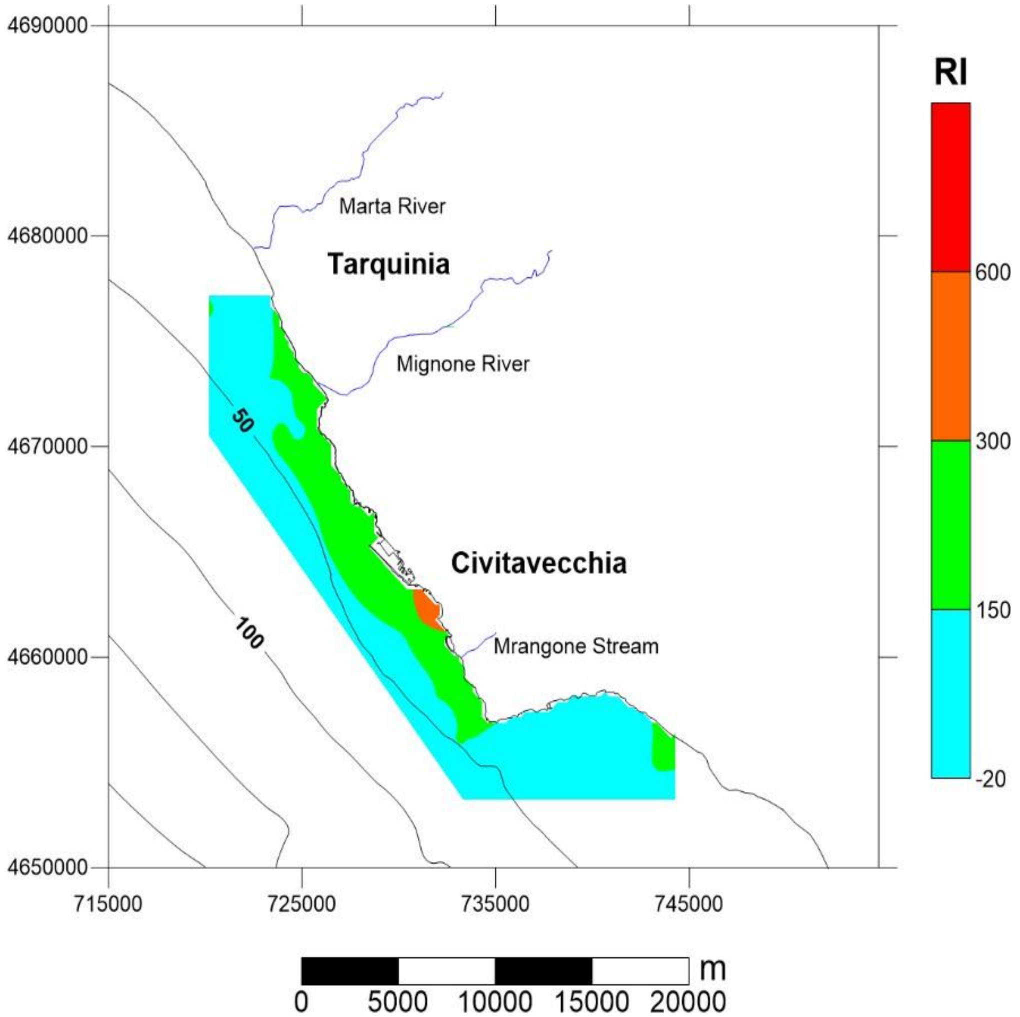

Fig. 2. Potential Ecological RI in the coastal area of Civitavecchia (Piazzolla et al. 2015)

A field survey was conducted to identify the presence and extent of *Cystoseira* spp. in the coastal area of Civitavecchia from 24 to 26 January 2023. In total, 4 coastal areas were investigated (fig.3).

Here we report a brief description of each area and the sampling activities carried out for the analysis of Cr in *Cystoseira* spp. samples and seawater in the area selected for the collection of samples to be exposed to the experimental enrichment assays.

Fig. 3. Investigated areas of *Cystoseira* spp. visual census monitoring

Area 1

The area is located approximately 1 km southeast of the port. The coastline is characterised by hard substrates, mainly of anthropogenic nature. In this area, *Cystoseira* spp. was absent. Algal communities were mainly represented by coralline algae (*Ellisolandia elongata* (J. Ellis & Solander) K. R. Hind & G. W. Saunders, 2013, *Jania* sp. J. V. Lamouroux, 1812), brown algae *Dictyota dichotoma* (Hudson) J. V. Lamour, 1809 and red algae *Asparagopsis armata* Harvey, 1855.

Area 2

In the area located approximately 2 km southeast of the port of Civitavecchia, on the southern side (42.07061 N, 11.80758 E) *Cystoseira* species were also absent here.

Area 3

In the area located about 5 km south-east of the Port of Civitavecchia, surveys were conducted along transects parallel to the coast, inspecting the natural bedrock and the outcropping (Roman) anthropogenic structures. At the site 'Molo L' (42.05034 N, 11.81955 E) the presence of *Cystoseira* spp. was confirmed. Along the external side of the structure (about 30 m long) a band of *Cystoseira* spp. is present, more abundant. On the boulders north of the pier, *Cystoseira* spp. is present with a reduced coverage.

A hundred metres further south, at the site 'Peschiera della villa romano' (42.04882 N, 11.81942 E), isolated patches of *Cystoseira* spp. were observed.

Fig. 4 *Cystoseira* spp. detected within Area 3

In correspondence with the areas indicated in Fig. 4 replicates of *Cystoseira* spp. (500 g for each sample) and seawater samples (250 ml for each sample) were collected and immediately analyzed for the detection of Cr concentrations. Additional samples of *Posidonia oceanica* were collected in proximity to the *Cystoseira* spp. sampling sites for the same set of analyses. Laboratory analyses were performed according to the EPA method 7199:1996 for total Cr. These results confirm the suitability of the identified areas for the collection of *Cystoseira* spp. thalli to be subjected to laboratory Cr enrichment, to exclude the effects on the spectral and photosynthetic response due to a natural photophysiological adaptation to high levels of Cr.

Area 4

This is an area about 7.5 km south of the port. As in the previous area, monitoring was conducted along transects parallel to the coast. Here too, the presence of *Cystoseira* spp. was not observed in the stretch of coast south of the site 'Le Due Palafitte'.

4.5 Sampling site “Area 3”

Thalli used for the experiment were collected at 20-50 cm depths from the coastal area indicated in figure 5, in which the presence of *Cystoseira* spp. was detected during previous campaigns. In addition, to exclude the effects on the spectral and photosynthetic response due to a natural photo-physiological adaptation to high Cr levels, n.3 replicates of *Cystoseira* spp. (500 g for each sample) and seawater samples (250 ml for each sample) were collected and immediately analysed for the detection of Cr concentrations according to the EPA 7199:1996 method for Total Cr and UNI EN ISO 15587-1 2002. The analysis confirms the suitability of the identified areas for the collection of *Cystoseira* spp. thalli to be subjected to Cr laboratory enrichment. Once at laboratory, thalli were acclimatized to the culture conditions in aerated seawater collected at the sampling site to ensure a standard starting point for experiments at 21-22 °C. The quantity of *Cystoseira* spp. to be taken for each tank is 200 g, considering the quantity of biological material needed for chemical analysis.

Fig. 5 Sampling site of *Cystoseira* spp. 'Molo L' Roman structure, Civitavecchia SUD (42.05034 N, 11.81955 E)

4.6 Materials and Methods

The experimental protocol was developed in accordance with the methodological references reported in (Malea et al., 2021; Falace et al., 2018; D3.7 Progetto ASI-STOPP; Douay et al., 2022).

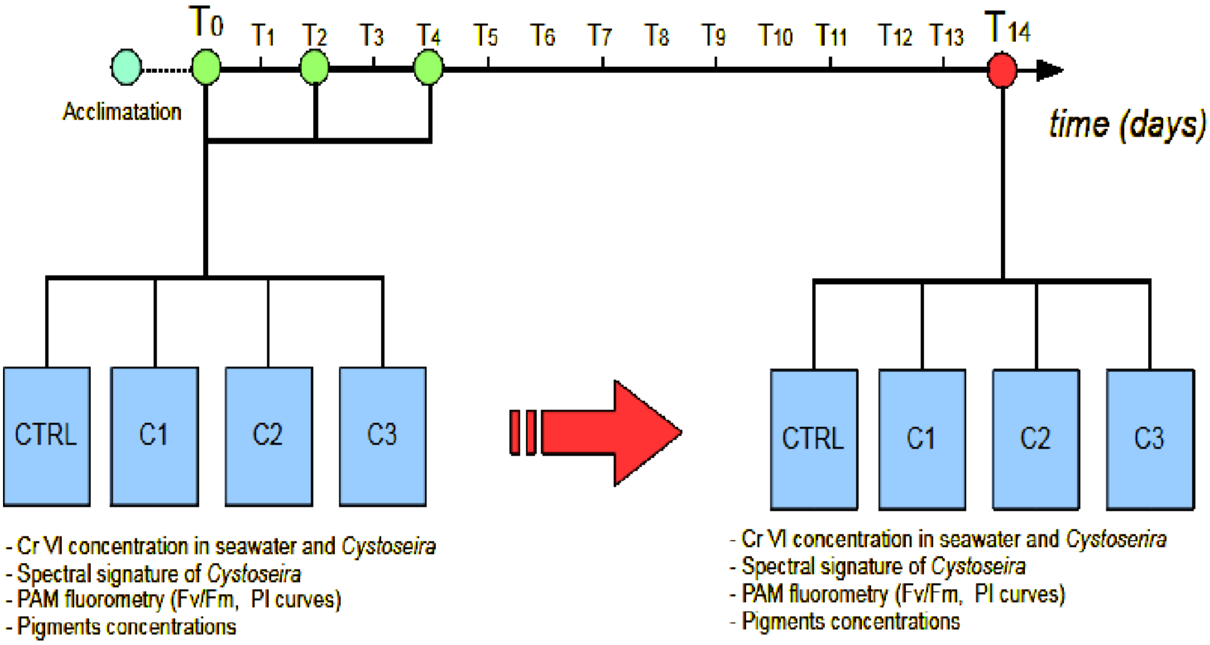

Fig. 6. Experimental design protocol

The experimental design consisted of 4 tanks (4L): 1 control tank and 3 tanks containing 3 concentrations of Cr: 0.1, 1 and 10 µmol/L (Baumann et al., 2009) (fig. 6). Cr concentrations obtained from potassium chromate K_2CrO_4 Puriss grade chemicals (Sigma- Aldrich), diluted with seawater.

Macroalgal cultures were maintained at a temperature range of 18-21 °C and a light regime of 120-125 µmol photon m^{-2} s^{-1}. The experiment lasted 14 days, with measurements taken every two days during the first week and a final measurement at the end of the experiment (day 14).

At the beginning and end of the experiment, chromium analyses were performed on Cystoseira spp. and seawater by the analysis laboratory S.C.A. Servizi Chimici Ambientali s.r.l.

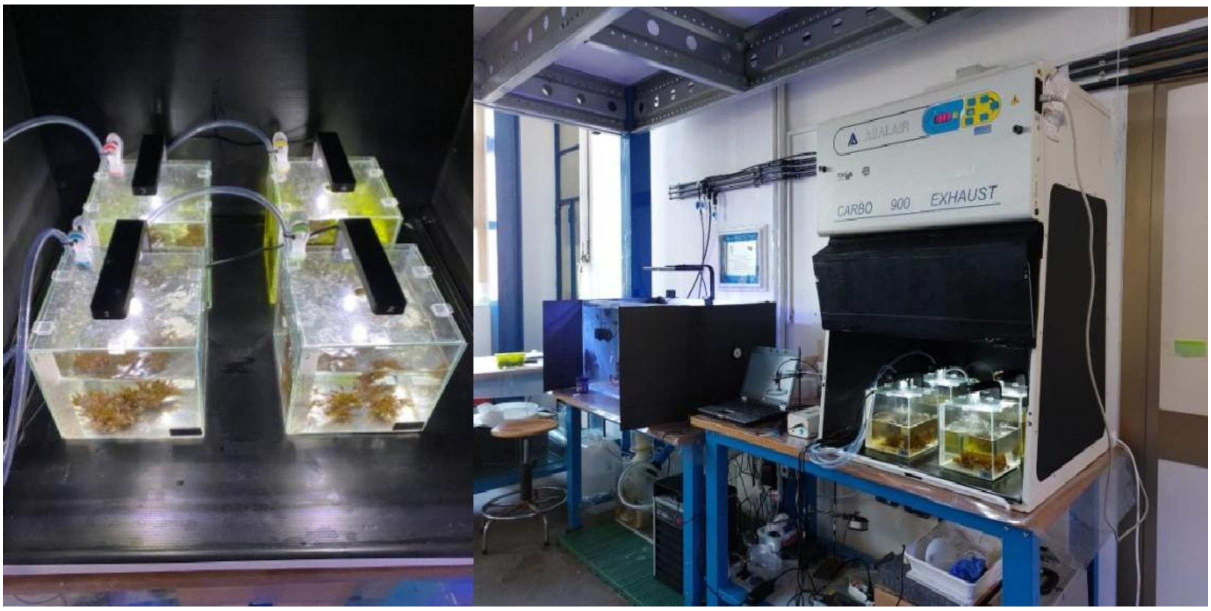

Fig. 7. Experimental setup in the darkened hood with *Cystoseira* spp. Exposed to different concentrations of chromium

Each measurement cycle included the following types of measurement in 3 replicates for each tank (fig. 7):

1. Spectrometric measurements were performed under clear sky conditions using the UV-VIS Stellarnet 2000 EPP spectrometer, equipped with fiber optics and a cosine corrector diffuser. The acquired parameters out of water were:

- water leaving spectral radiance (with the fiber optic orthogonally pointed to the surface of tank) obtained by using as incident light a halogen and a xenon lamp;

- reference incident radiance on a spectralon diffuser surface (with the same geometry used for the measurement of the water leaving radiance) of the light source;

- in air reflectance of *Cystoseira* spp. samples exposed to solar light during clear sky conditions (see Douay et al., 2022).

2. Fluorimetric and photosynthetic measurement by using the Junior PAM fluorometer (Waltz) according to Baumann et al., (2009) and Madonia et al., (2021):

A) Measurement of Fv/Fm (Maximum Quantum Yield of PSII)

- Dark adaptation: 15–20 minutes (using leaf clips or keeping samples in a dark chamber).

- Saturation pulse: ~8,000 µmol photons m^{-2} s^{-1} for 0.8 seconds.

- Fluorescence recording:

  - Fo (initial fluorescence) recorded using a weak measuring light (~0.05 µmol photons m^{-2} s^{-1}).

  - Fm (maximum fluorescence) measured with the saturation pulse.

- Calculation: $Fv/Fm = (Fm - Fo)/Fm$

B) Rapid Light Curves (RLCs)

- Number of light steps: 8–10 steps.

- Duration of each step: 10–20 seconds.

- Actinic light intensity intervals: typically between 0 and 1,000 µmol photons m^{-2} s^{-1}, distributed logarithmically or linearly (e.g., 0, 10, 20, 50, 100, 200, 400, 600, 800, 1,000 µmol photons m^{-2} s^{-1}).

- Saturation pulse: ~8,000 µmol photons m^{-2} s^{-1} for 0.8 seconds at each step.

- Data recording:

  - $Y(II) = (Fm' - F)/Fm'$, where F is the steady-state fluorescence and Fm' is the maximum fluorescence under actinic light.

  - ETR (Electron Transport Rate) can be calculated if the actinic light wavelength and absorption coefficient are known.

3. The spectrophotometric analysis of photosynthetic pigments was conducted following the methodology described by Stengel and Connan (2015). This method allows for the quantification of chlorophylls and carotenoids extracted from *Cystoseira* samples.

A) Sample Preparation and Pigment Extraction

1. Fresh algal samples were collected and immediately stored in darkness at -80°C until further processing to prevent pigment degradation.

2. Samples were homogenized in 90% acetone using a mortar and pestle under cold conditions to minimize pigment oxidation.

3. The homogenized samples were centrifuged at 4000 x g for 10 minutes at 4°C to separate the supernatant containing the extracted pigments.

4. The supernatant was carefully transferred to a clean tube, avoiding contamination with debris.

B) Spectrophotometric Measurements

1. The absorbance of the pigment extract was measured using a UV-Vis spectrophotometer.

2. Readings were taken at specific wavelengths corresponding to pigment absorption peaks:

- Chlorophyll a: 664 nm (corrected with 750 nm)
- Chlorophyll b: 647 nm (corrected with 750 nm)
- Chlorophyll c: 630 nm (corrected with 750 nm)
- Chlorophyll d: 696 nm (corrected with 750 nm)
- TotalChls = Chl-a+Chl-b+Chl-c+Chl-d

C) A blank measurement was performed using 90% acetone to calibrate the spectrophotometer.

Calculation of Pigment Concentrations:

The concentration of each pigment was calculated using the specific extinction coefficients provided by Stengel and Connan (2015):

- Chl-a = 11.85A664 – 1.54A630
- Chl-b = 20.13A647 – 5.03A664
- Chl-c = 24.52A630 – 2.99A6645
- Chl-d = 21.24A696 – 3.32A664
- TotalChls = Chl-a+Chl-b+Chl-c+Chl-d

where A represents the absorbance at the respective wavelengths.

Quality Control and Replicates

- Each measurement was conducted in triplicate to ensure reproducibility.
- Pigment stability was monitored by minimizing exposure to light and heat throughout the process.
- Results were expressed as μg pigment per g fresh weight of algal tissue.

Data distribution was initially modelled by using the Shapiro-Wilk test (Shapiro and Wilk,1965); this preliminary test indicated that the data did not follow a normal distribution, therefore for further analyses a non-parametric model, the Kruskal-Wallis test (Kruskal and Wallis, 1952), was used. This latter test provides an effective statistical tool for comparing the features characterizing multiple samples and testing whether they fit with the same distribution. Furthermore, Wilcoxon-Mann-Whitney test (Fagerland and Sandvik, 2009) was used as two-sample test.

4.7 Results

The spectra reported in Figure 8 effectively show the optical responses of *Cystoseira* spp. under different experimental conditions at different Cr concentrations. In the left column, the irradiance curves are presented for four experimental conditions: the red line (CTRL) corresponds to the control condition with 0 μmol Cr, the blue line (C1) represents

0.1 μmol Cr, the green line (C2) corresponds to 1 μmol Cr, and the black line (C3) represents 10 μmol Cr. Each plot captures the optical response of *Cystoseira* spp. at specific time points of the experiment: 0 h, 48 h, 96 h, and 14 days. The y-axis of these plots is expressed in units of photon flux density (μmol photons m^{-2} s^{-1}), while the x-axis expresses the wavelength in nm.

In the right column, reflectance curves are shown for the same experimental conditions and time steps. The $R(\lambda)$ on the y-axis describes how the incident light interacts with the algal surface, providing valuable information on the structural and pigment changes that occur over time.

The spectra are characterized by two key features that are noteworthy in the context of benthic vegetation and optical properties of brown algae. First, there is a prominent peak at 570 nm, which is indicative of accessory pigments such as fucoxanthin, a pigment unique to brown algae. Fucoxanthin plays a critical role in photosynthetic efficiency by absorbing light in the blue-green range, ensuring optimal energy capture under specific environmental conditions.

Second, the spectra reveal a pronounced peak at 710 nm, which corresponds to the red-edge effect, a well-documented phenomenon in algal studies. This effect marks the sharp transition between the strong absorption of chlorophyll in the visible red region (about 680 nm) and the high reflectance in the near-infrared (NIR) region (~710 nm). In benthic vegetation such as *Cystoseira* spp., this feature reflects the internal structure of the algae and its physiological responses to stress or environmental changes, including chromium exposure.

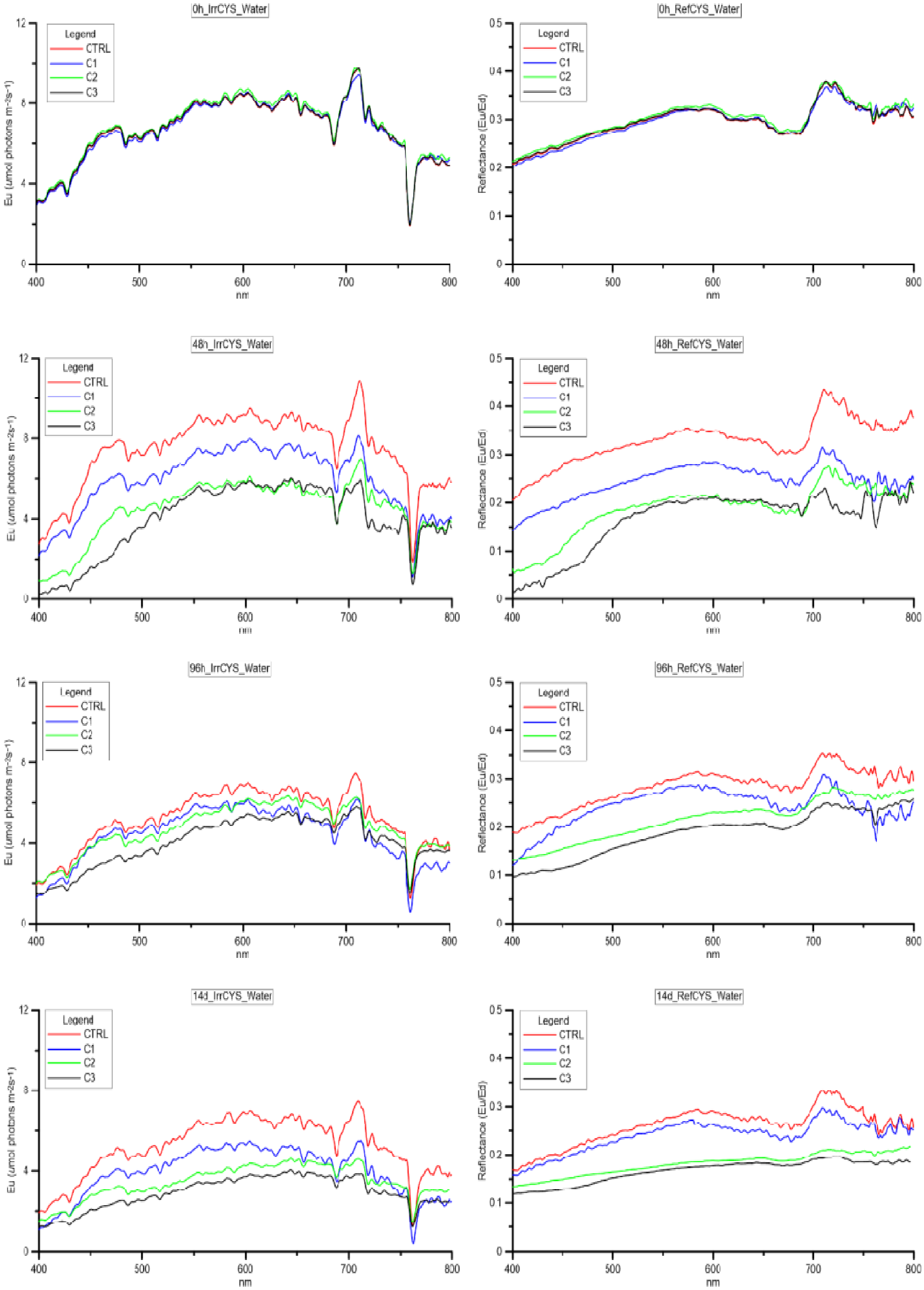

Fig. 8. The figure is divided into two columns: the left column represents the irradiance measurements, while the right column illustrates the reflectance curves of *Cystoseira* spp. during the experiments with Chromium

The graph (Fig. 9) represents the normalized spectral reflectance response of a *Cystoseira* spp. sample over a wavelength range from 400 to 800 nm after 14 days of exposure to chromium.

The y-axis indicates the normalized reflectance value, where the difference in reflectance between the control (Rctrl) and the sample with the highest chromium concentration (RC3) has been divided by the maximum value of this difference, ensuring that the data are dimensionless and that the values are between 0 and 1. The x-axis corresponds to the wavelength of light in nanometers (nm), which extends from the visible spectrum to the near-infrared region.

The graph shows characteristic spectral features, such as a large increase in reflectance between 500 and 600 nm, followed by a sharp peak near 700 nm. These features correspond to specific absorption or reflectance characteristics of the pigments or chlorophyll fluorescence in the sample. These reflectance data contain small oscillations, which indicate potential noise or natural variability in the optical properties of the sample. By normalizing the reflectance, the plot highlights relative spectral differences without being influenced by absolute quantities, making it suitable for comparative analysis.

After 14 days, the difference between the control and the C3 tank, with the highest Cr concentration, reached the maximum value, above all for the spectral region higher than 710nm that confirming the usefulness of this wavelengths also for marine mesolittoral vegetation monitoring.

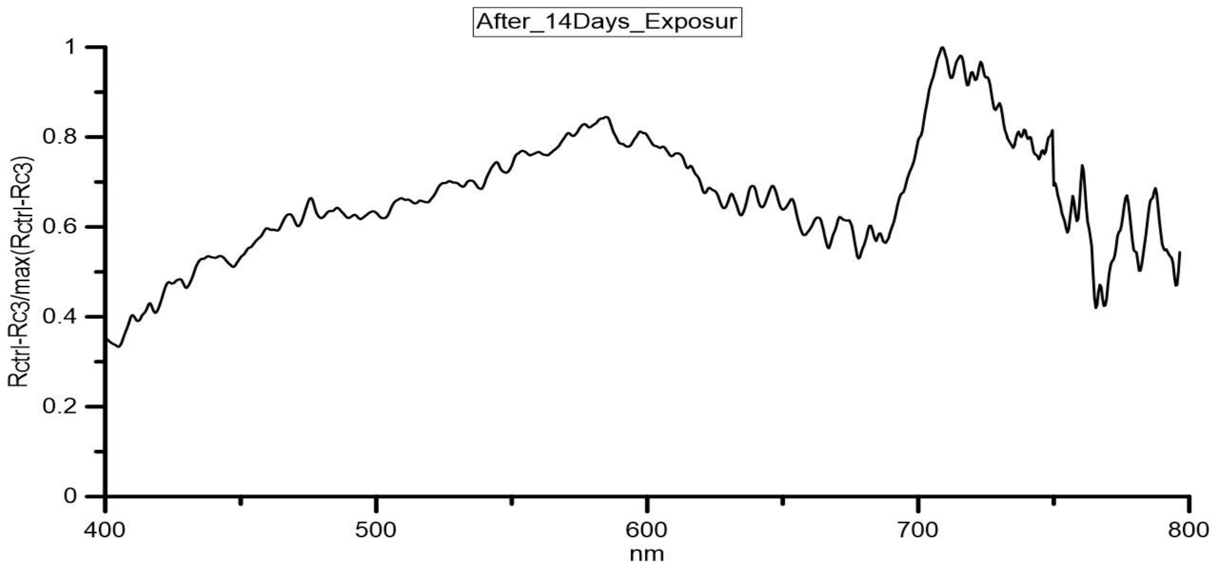

Fig. 9 *Cystoseira* spp. Rctrl-Rc3/max (Rctrl-Rc3) after 14 days of exposure to chromium.

The photosynthetic efficiency (P-E) curves presented in Fig. 15 provide a detailed assessment of the light-dependent photosynthetic performance of *Cystoseira* spp. under different experimental conditions. These curves plot the relationship between photosynthetically active radiation (PAR, μmol photons m^{-2} s^{-1}) on the x-axis, representing the range of light wavelengths available for photosynthesis (400– 700 nm), and electron transport rate (ETR, μmol e^{-} m^{-2} s^{-1}) on the y-axis, an indicator of the photosynthetic electron flow through photosystem II (PSII).

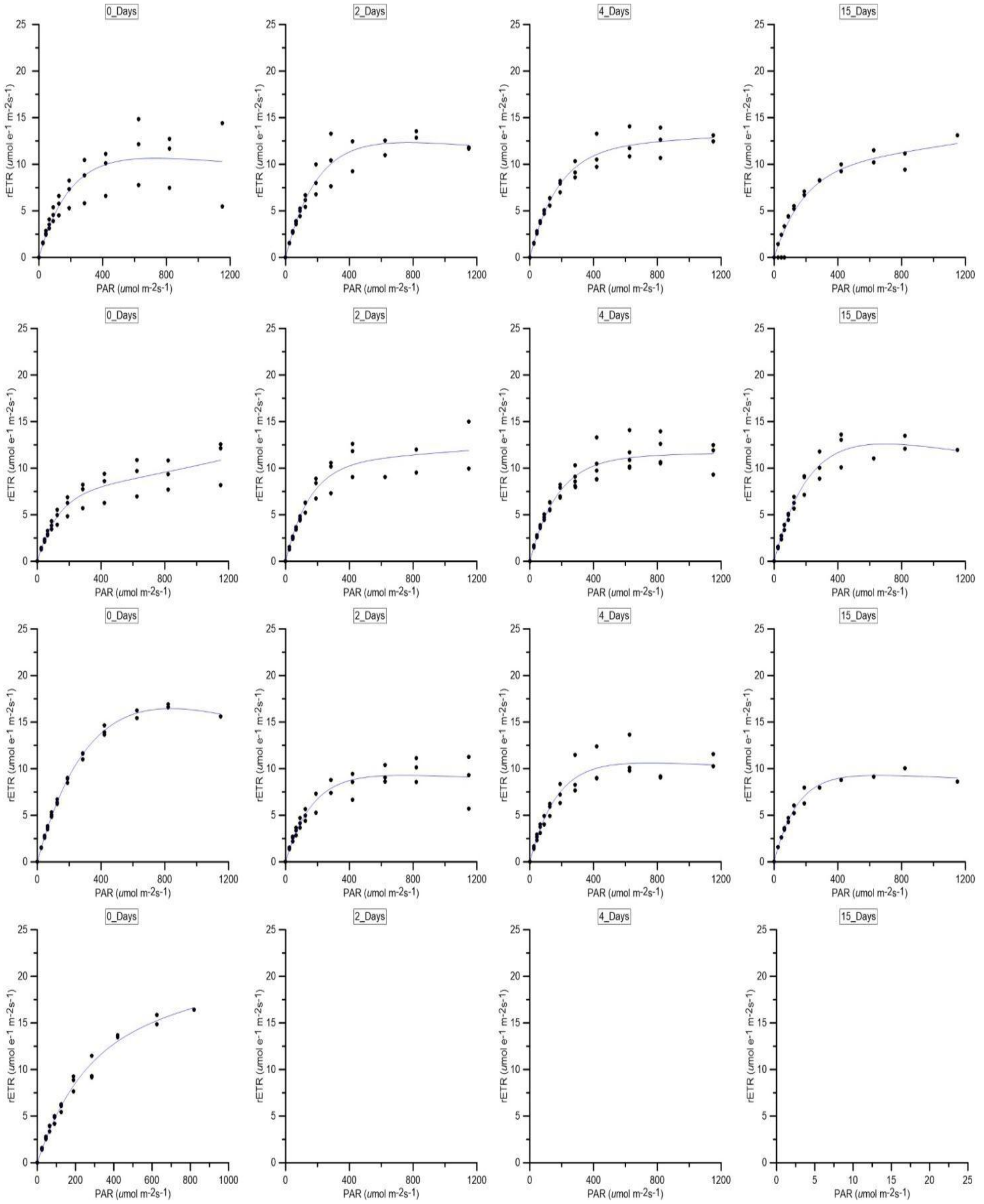

Fig. 10. ***Cystoseira*** **spp. RLCS before and after the exposure to Chromium (*PAM* measurements*)*.**

To verify the influence of Cr concentrations on the algae over time, and the presence of statistically significant differences among the data, statistical tests (Gower's distance PERMANOVA) were conducted considering the Cr concentrations in the tanks water and in the algae thalli, the bioaccumulation factor (BAF), the concentrations of photosynthetic pigments (chla, chlb, chlc, and chld) in the *Cystoseira* spp. thalli, the variable fluorescence/maximum fluorescence in shaded (Fv/Fm) leaves indicates variations in the photonic efficiency of photosystem II (PSII) (Adams et al., 1995) (Table 1) and the Electron Transport Rate (ETR) (Fani and Lazzara, 2006) (Fig. 10).

Table 1. *Cystoseira* spp. spectral signature before and after the exposure to Chromium.

ID	chl a (µg/ml)	chl b (µg/ml)	chl c (µg/ml)	chl d (µg/ml)	Totals Chls (µg/ml)	Fv/Fm
CTRL_0	8,217	0,054	1,055	0,223	9,437	0,685
CTRL_2days	9,673	0,168	1,391	0,228	11,327	0,655
CTRL_4days	12,980	0,206	1,565	0,249	14,820	0,698
CTRL_14days	8,731	0,268	1,416	0,340	10,635	0,644
C1_0	10,587	0,152	1,488	0,235	12,312	0,644
C1_2days	11,795	0,272	1,548	0,255	13,706	0,659
C1_4days	10,974	0,201	1,469	0,218	12,711	0,684
C1_14days	14,019	0,190	1,788	0,234	16,040	0,658
C2_0	13,414	0,336	1,780	0,283	15,629	0,631
C2_2days	10,589	0,048	1,397	0,221	12,111	0,649
C2_4days	9,031	0,120	1,098	0,161	10,287	0,635
C2_14days	5,616	0,085	0,801	0,125	6,548	0,571
C3_0	8,752	0,135	1,172	0,174	10,111	0,645
C3_2days	6,453	0,487	0,854	0,224	7,929	0,000
C3_4days	4,435	0,454	0,410	0,190	5,430	0,000
C3_14days	1,547	0,424	0,163	0,137	2,250	0,000

In general, the experiments clearly showed that as the Cr concentrations in the water increased (tanks CTRL>C1>C2>C3), the bioaccumulation factor (BAF) decreased (C3<C2<C1<CTRL).

Considering the photosynthetic pigments (chla, chlb, chlc and chld), a statistically significant difference in long-term chlc concentrations emerged (KW test; pvalue < 0.05) considering all the tanks. A statistically significant difference emerged also considering the Fv/Fm ratio value s between the four tanks in the long term (KW test, p value < 0.05) and this difference was particularly marked between the values measured in CTRL and C3 (MW test; p value <0.05).

To evaluate the interaction between all the variables measured during the experiment, the multivariate analysis PERMANOVA was applied (Anderson, 2014). In the PERMANOVA analysis, the Gower distance and 9999 permutations were used.

This distance was chosen because it allows for quantifying the dissimilarity between samples characterized by multiple types of data (e.g., categorical, ordinal, and continuous traits; Mouillot et al., 2021), eliminating the need for preliminary transformation to a common scale (De Bello et al., 2021). The results showed a statistically significant difference between CTRL and C3 ($p < 0.05$), considering Cr concentrations in water, BAF, photosynthetic pigment concentration, Fv/Fm ratio values, and median ETR values.

4.8 Discussion and Conclusions

The present study provides novel insights into the reflectance and irradiance properties of *Cystoseira* spp. across different experimental conditions. Our findings align with recent work on macroalgae spectral characteristics, highlighting the importance of environmental factors in influencing light absorption and reflectance patterns (Smith et al., 2015; Roberts et al., 2017). The spectral behavior observed in our study closely resembles patterns identified in related species, such as *Sargassum* and *Fucus*, which also exhibit adaptive responses to varying light regimes (Polo et al., 2015).

One of the most striking results is the significant variation in reflectance at key wavelengths (e.g., 450–550 nm and 650–700 nm), which suggests a dynamic photophysiological response to environmental stressors. These findings are supported by the work of Lee, Z. et al. (2020), who demonstrated that changes in reflectance spectra are strongly correlated with pigment composition and photoacclimation strategies in brown algae. Furthermore, spectral shifts at longer wavelengths (>700 nm) could indicate alterations in structural properties, such as changes in cell wall composition and surface roughness (Álvarez-Gómez et al., 2017). These structural changes have been extensively documented in studies on macroalgal responses to hydrodynamic forces and nutrient availability (Spalding et al., 2019).

These observations are consistent with the findings in the literature, in fact according to Nourisson et al. (2016), the red-edge effect is a diagnostic feature in benthic vegetation, providing information on pigment composition and structural adaptations. Furthermore, Michaelian (2015) highlights how reflectance changes in algae are influenced by structural and physiological processes, evident in the spectra presented here. Finally, data are available on the bioaccumulation of some trace metals in the soft tissues of *Paracentrotus lividus* (Lamarck, 1816) in the study area, which basically confirm the influence of sediment metal concentrations on the distribution of bioaccumulation levels in the sea urchin (Scanu et al., 2015).

The differences observed between in-air and in-water measurements confirm the significance of immersion effects on spectral reflectance. Our results corroborate previous research by Souri (2019), who showed that water column properties, including turbidity and dissolved organic matter, can influence spectral signatures in submerged macroalgae. Additionally, optical modeling studies emphasize the role of water depth and dissolved particulate matter in modifying reflectance profiles, which is consistent with our observations (Hamidifar et al., 2024). This has crucial implications for remote sensing applications, as highlighted by Brown et al. (2022), who emphasized the necessity of accounting for environmental interference in spectral measurements of aquatic vegetation.

An interesting study is the work of Hoang et al. (2016), who produced a submerged aquatic vegetation (SAV) spectral signature library to collect optical data of macroalgae (red, green and brown), seagrasses and sediment characteristics in coastal waters, in order to support the selection of spectral bands and bandwidths for different environmental conditions, such as clear water and high turbidity water bodies, and to choose satellite sensors suitable for different benthos. Twenty-two species of submerged coastal aquatic plants, including red, green, brown macroalgae and seagrasses, were collected at Cape Peron, Western Australia, to measure their spectral reflectance in situ. Comparison of the measured spectral reflectance value of the SAV groups (Fig. 11) and the associated substrate revealed significant differences in the reflectance spectra characterizing red, green, brown macroalgae and phanerogams: In particular:

- Red macroalgae are characterized by peaks in the red spectral regions (580 nm) and strong absorption features in the blue and green spectral regions (Fig. 11a).
- Green macroalgae exhibit common absorption features with peaks in the blue spectral regions (450 nm) and maximize the spectral reflectance in the green spectral regions (550 nm) (Fig. 11b).
- Brown macroalgae exhibit strong spectral absorption peaks in the blue and green spectral regions (400-550 nm). The spectral reflectance peaks were observed between 600 and 650 nm (Fig. 11c).
- *P.oceanica* sp. exhibited maximum spectral absorption in the blue and green spectral regions (450-550 nm) and maximum reflectance in the red spectral region (600-650 nm), while *Amphibolis antarctica* (Labillardière) Sonder & Ascherson ex Ascherson 1868 exhibited spectral absorption in the blue spectral regions (400-500 nm) and a peak spectral reflectance in the green spectral region (550 nm) (Fig. 11d).

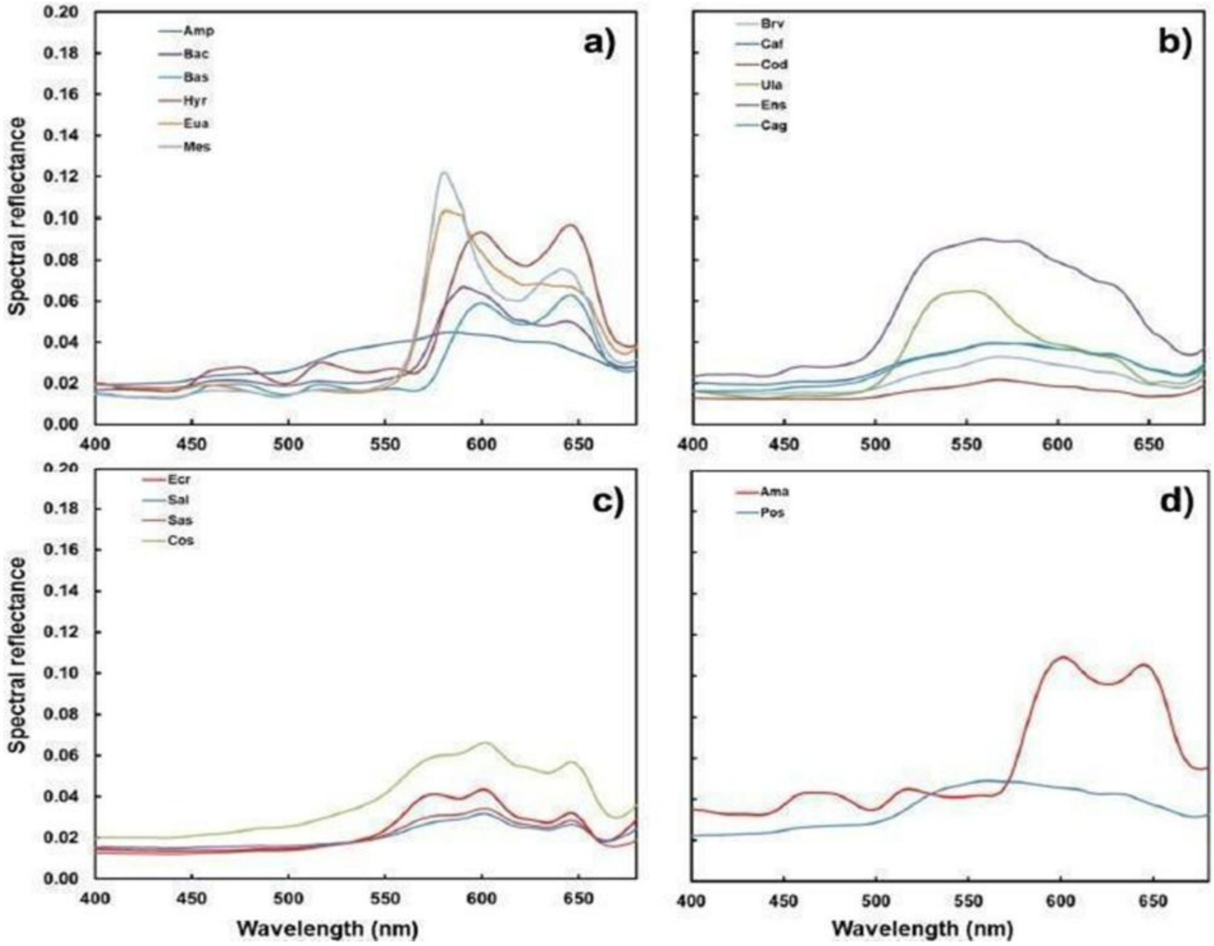

Fig.11. Spectral reflectance divided into groups: a) red algae; b) green algae; c) brown algae; d) seagrasses. (from Hoang et al., 2016).

To assess the effects of pollutant damage on the in vivo optical properties of macroalgae, applicable to laboratory and remote systems, May (2000) exposed the green macroalga E. intestinalis to selected trace metals (copper and zinc), triazine herbicides (lrgarol1051eatrazine) and a series of alcohols (n=1-9) in the laboratory. Each pollutant, except zinc, had a significant effect (P<0.05) on the in vivo spectral properties at the range of concentrations used. The results reported in Fig. 12 indicate that the technique has the potential to identify pollutant-affected algal systems, as the signatures obtained appear to indicate whether the change is due to structure or pigments.

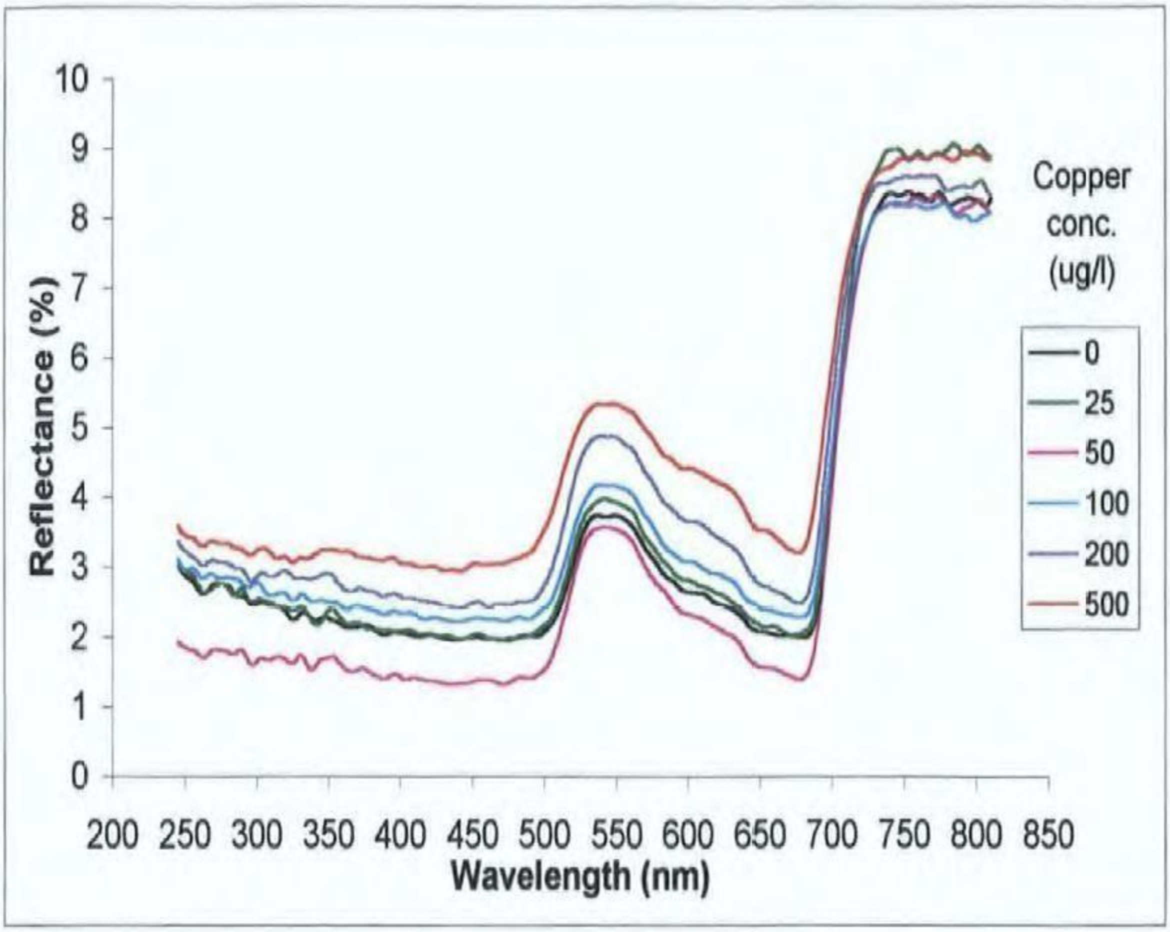

Fig.12. Effect of copper (ug/1) on in vivo reflectance (%) of E. intestinalis after 7 days (from May, 2000)

Fig. 14 also shows the normalized reflectance spectra of sixteen species of green, brown and red macroalgae measured by Douay et al. (2022). Green algae are characterized by a single large reflectance peak at 545 nm, while brown algae showed three reflectance peaks, including a brown algae-specific peak located at 570 nm. The other two reflectance peaks were also detected in red algae and are located at 600 nm and 645 nm, respectively. Red algae are characterized by three reflectance peaks, the two common to brown algae and one specific to this group detected at 515 nm.

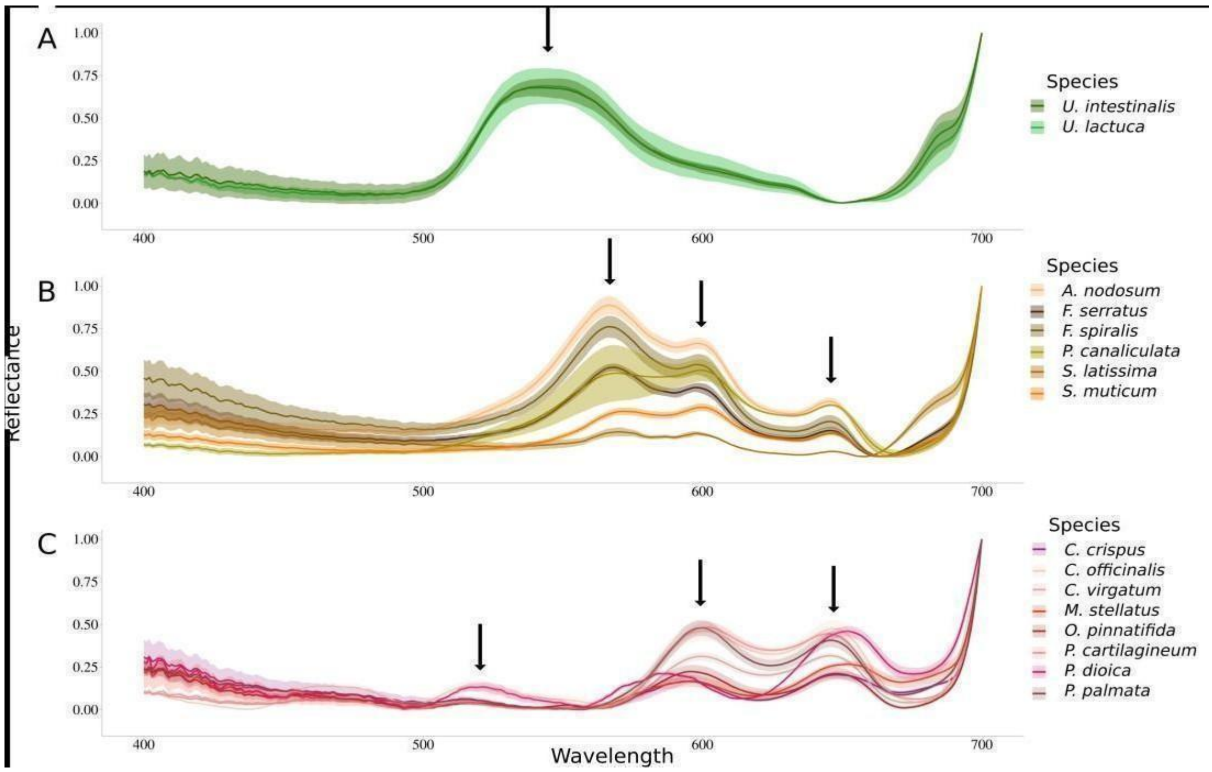

Fig.14. Normalized reflectance spectra measured for sixteen species of green, brown and red macroalgae (from Douay et al., 2022).

Moreover, our findings provide additional evidence of the role of photoprotection mechanisms in brown algae. The observed increase in reflectance within the 500–600 nm range under stress conditions aligns with the work of Seys et al. (2022), who found that xanthophyll cycle activation and increased carotenoid content contribute to the modulation of reflectance under high irradiance levels. Such mechanisms are vital for mitigating oxidative stress and ensuring algal survival in fluctuating light environments. Additionally, studies by Melo-Santos et al. (2018) have shown that brown algae exposed to environmental stressors such as salinity changes and UV radiation develop photoprotective adaptations that are reflected in their spectral properties.

Our study also highlights the potential use of spectral signatures for detecting physiological stress in *Cystoseira* spp. Under conditions of prolonged stress, the decline in near-infrared reflectance could be indicative of cellular damage and chlorophyll degradation, a phenomenon previously reported by Zahir et al., (2022) in seagrasses and macroalgae. This suggests that hyperspectral reflectance analysis could serve as an early-warning system for assessing macroalgal health in response to environmental disturbances, a crucial tool for conservation strategies Wahl et al., 2014).

In conclusion, our study demonstrates the intricate relationship between spectral reflectance properties and environmental conditions in *Cystoseira* spp. The observed spectral variations, both in-air and in-water, highlight the photophysiological adaptability of these macroalgae, in agreement with recent literature (Polo et al., 2015; Lee et al., 2020; Seys et al., 2022). These findings have significant implications for the development of remote sensing methodologies aimed at monitoring macroalgal health and productivity.

Future research should focus on refining spectral models to improve the accuracy of remote sensing applications, particularly in distinguishing stress-induced spectral signatures from natural variability (Álvarez-Gómez et al., 2017). Additionally, integrating physiological and biochemical analyses with spectral data could provide deeper insights into the adaptive strategies of *Cystoseira* spp. under different environmental stressors (Brown et al., 2022). The inclusion of long-term monitoring approaches, such as those proposed by Carvalho (2019), could further enhance our understanding of macroalgal responses to climate-driven environmental changes.

Moreover, advances in hyperspectral imaging and machine learning algorithms offer new opportunities for the automated classification of spectral data, improving the detection and quantification of algal biomass in coastal ecosystems (Zhang et al., 2022). Such advancements could revolutionize ecological assessments, aiding in habitat conservation and marine resource management.

Ultimately, this study contributes to the growing body of research on macroalgal spectral properties, reinforcing the importance of spectral monitoring in ecological assessments and conservation efforts (Souri, 2019). By advancing our understanding of algal spectral responses, we can enhance the effectiveness of marine ecosystem management strategies in the face of global environmental changes. The continued development of spectral monitoring techniques will be instrumental in ensuring the resilience of marine macroalgal communities in the coming decades (Wahl et al., 2014).

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5. CONCLUSIONS

The examination of spectral signatures has proven effective in identifying specific alterations in the reflectance patterns of macroalgae, which are indicative of physiological stress induced by the accumulation of trace metals (Mcilwaine et al., 2019). These spectral variations can be correlated with laboratory-based metrics to validate findings and deepen our understanding of toxicity mechanisms (Mcilwaine et al., 2019). Alongside remote sensing and spectral analysis, several other advanced methodologies have emerged to investigate the impacts of trace metal contamination on marine macroalgae and seagrass ecosystems (Bat et al., 2021). These include omics approaches, biosensors, ecological modeling, microbial community analyses in sediments, and sophisticated imaging techniques. The integration of these tools with conventional chemical and biological monitoring methods significantly enhances our capacity to detect and comprehend the consequences of trace metal contamination (Sahu and Basti, 2021).

Trace Metal Pollution and Its Impacts on Marine Ecosystems

Trace metal pollution poses a significant threat to marine ecosystems, adversely affecting the photosynthesis of macroalgae and marine phanerogams (Boutahar et al., 2021). These organisms, critical to the primary productivity and health of coastal ecosystems, are particularly vulnerable to the toxic effects of trace metals (Tripathi et al., 2021). Our review highlighted the mechanisms of toxicity, the physiological and biochemical responses of affected organisms, and the ecological implications of these alterations (Regoli and Giuliani, 2014).

Trace metals, such as chromium, cadmium, lead, and mercury, can interfere with photosynthetic processes through various mechanisms (Roychoudhury and Chakraborty, 2020), including substitution of essential ions in photosynthetic reaction centers, generation of reactive oxygen species (ROS), and inhibition of key enzymes involved in photosynthesis (Pospíšil, 2016).

These effects can lead to reduced photosynthetic efficiency, decreased growth, and, in extreme cases, death of organisms (Sharma et al., 2020).

Looking ahead, it is essential to develop and implement innovative methods to monitor and assess trace metal pollution in marine ecosystems (Langston, 2018). Advanced technologies, such as remote sensing and spectral signature analysis, offer powerful tools for non-invasive and large-scale monitoring of pollution effects (Alexan and Ianovici, 2024). Remote sensing enables the collection of large-scale, real-time data, making it possible to quickly identify affected areas and monitor changes over time (Cao et al., 2024). Spectral signature analysis can detect specific changes in the reflectance of macroalgae and marine seagrasses, indicative of physiological stress caused by the accumulation of trace metals (Mcilwaine et al., 2019). These changes can be correlated with laboratory measurements to validate the results and improve our understanding of toxicity mechanisms (Mcilwaine et al., 2019).

Cystoseira spp. as a Bioindicator of Pollution

This investigation has confirmed the potential of *Cystoseira* spp. as a robust marine bioindicator for monitoring heavy metal pollution, particularly chromium. The findings underscore the species' capacity to assimilate and mitigate environmental contamination while also serving as a cost-effective and practical alternative for evaluating marine ecosystem health. This is especially advantageous in scenarios where traditional monitoring methods are economically or logistically infeasible. The experimental methodologies employed, including spectrometric techniques and pulse-amplitude- modulated (PAM) fluorescence measurements, demonstrated the effectiveness of *Cystoseira* spp. in pollutant monitoring via remote sensing technologies. The development of integrated methodologies, which combine remote sensing, ecological analysis, and environmental monitoring, is pivotal in addressing pollution and climate change challenges. Employing innovative technologies and management strategies grounded in remote sensing data is essential for sustaining marine ecosystems and safeguarding human health.

Physiological and Biochemical Responses to Chromium Stress

Spectral reflectance data collected from *Cystoseira* spp. exposed to varying chromium concentrations (C1: 0.1 μmol, C2: 1 μmol, C3: 10 μmol) under controlled conditions (CNTRL: 0 μmol as control) across different time points (48 hours, 96 hours, and 14 days) revealed critical insights into the physiological and biochemical responses of this species to heavy metal-induced stress. Notably, temporal variations in reflectance spectra highlighted clear distinctions between the control samples and chromium-exposed groups. Initial deviations were observed at 48 hours within the visible and red spectral regions (approximately 710 nm), becoming increasingly pronounced at 96 hours and peaking at 14 days. This progression reflects a cumulative stress effect on *Cystoseira* spp. over time.

The red spectral region, closely associated with chlorophyll concentration and photosynthetic activity, exhibited a marked decrease in reflectance in samples exposed to elevated chromium concentrations (C2 and C3). These findings align with documented stress responses in photosynthetic organisms, where heavy metal toxicity disrupts chlorophyll synthesis and impairs photosystem functionality.

Chromium Concentration-Dependent Stress Responses

A clear dose-dependent response to chromium exposure was identified, with higher concentrations (C1 < C2 < C3) eliciting more pronounced spectral alterations. At elevated chromium levels, reflectance within the green spectral region (approximately 570 nm) decreased, while the red region (600–700 nm) exhibited enhanced absorption, indicative of chlorophyll degradation and the potential accumulation of stress-associated pigments, such as anthocyanins and carotenoids.

Emission patterns, quantified in energy units (Eu), displayed a significant decline with increasing chromium concentrations, correlating with reduced photosynthetic efficiency and diminished energy transfer. This is likely attributable to oxidative damage resulting from chromium exposure. The observed spectral changes underscore the detrimental impact of chromium on the physiological integrity of *Cystoseira* spp., contributing to reductions in survival thresholds over time.

At the lowest concentration of chromium (C1: 0.1 μmol), initial spectral shifts were observed primarily in the visible and red regions, suggesting the onset of stress. However, these changes were relatively minor, indicating that the algae retained functional physiological processes at this exposure level. At C2 (1 μmol), spectral deviations became more pronounced, with a significant reduction in reflectance in the red region and a corresponding decline in emission intensity, signaling compromised photosynthetic performance. At C3 (10 μmol), the spectral alterations were severe, with substantial reductions in reflectance and emission intensity, reflecting oxidative stress and chlorophyll degradation. This concentration represents the threshold for irreversible damage in *Cystoseira* spp.

Implications for Remote Sensing Applications

The outcomes of this study have profound implications for the application of remote sensing technologies in monitoring *Cystoseira* spp. populations in natural settings. Laboratory-derived spectral signatures can serve as foundational references for identifying stress indicators. By correlating laboratory findings with remote sensing data, several advancements can be achieved: Development of specific spectral indices for chromium stress in *Cystoseira* spp., such as modifications to normalized difference vegetation indices. Monitoring the health and spatial distribution of *Cystoseira* spp. within chromium-contaminated aquatic ecosystems. Establishment of predictive models for early detection of heavy metal-induced stress using hyperspectral and multispectral remote sensing platforms.

Summary

Trace metal pollution poses a severe threat to marine ecosystems, significantly impairing the photosynthetic activity of macroalgae and seagrass species (Boutahar et al., 2021). These organisms, integral to primary productivity and coastal ecosystem health, are particularly susceptible to toxic trace metals (Tripathi et al., 2021). This review has illuminated the mechanisms of toxicity, physiological and biochemical responses, and ecological ramifications of trace metal contamination (Regoli and Giuliani, 2014). Advanced methodologies such as remote sensing and spectral analysis are indispensable for large-scale, non-invasive monitoring of pollution effects (Alexan and Ianovici, 2024). Future research must prioritize the development of innovative strategies to mitigate and monitor trace metal pollution, ensuring the conservation and sustainability of marine ecosystems for subsequent generations (Kennish, 2022).

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