CUKUROVA UNIVERSITY

INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Antioxidant and antimicrobial effects of *Spirulina platensis* Supplemented with Magnesium and Zinc

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Biotechnology Department

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This Doctorate Thesis was evaluated by the following Jury Members on 14/11/2023 and was approved by unanimity.

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ABSTRACT

This study was carried out to investigate the effect of different levels of magnesium (Mg) and zinc (Zn) on the growth, bioaccumulative capacity, protein and amino acid content, phytochemical content, antioxidant activity and antimicrobial activity of *S. platensis*, a cyanobacterium. The aim is to produce *Spirulina* by exposing it to mineral stress conditions to enrich its magnesium and zinc content while enhancing the production of organic and bioactive compounds, followed by evaluating its antioxidant and antimicrobial activity. These results show that magnesium and zinc mineral enrichment did not significantly affect *Spirulina* cell density throughout the experiment. Optic density values in the control group (0,230 ± 0,000) were generally similar or close to those of the mineral enrichment treatments, while the control group had the highest protein content (70,34%). The total phenol content of the magnesium-enriched groups was significantly higher than that of the zinc-enriched and control groups. *Spirulina* biomass enriched with Zn-5 mg/L, showed the highest degree of DPPH inhibition 101,346±9,274% for the methanol extract and 103,941±13,81% for the ethanol extract. All extracts showed antimicrobial activity against *E. coli*, *K. pneumonia*, *S. aureus*, *B. cereus*, *C. albicans* and *A. flavus* at a concentration of 100 mg/mL. Gram-positive *S. aureus* was the most sensitive bacterial species to Mg-200 mg/L methanolic extracts, followed by Zn-5 mg/L and Mg-100 mg/L with inhibition zones of 20 ± 1,31; 20 ± 0,00 and 19,5 ± 0,61 mm diameter, respectively.

Keywords: *Spirulina*, magnesium, zinc, antioxidant activity, antimicrobial activity

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

DOKTORA TEZİ

Magnezyum ve Çinko ile zenginleştirilmiş *Spirulina platensis*'in antioksidan ve antimikrobiyal etkileri

Mahamat Ahmat HAMID

Danışman: Prof. Dr. Oya IŞIK

Biyoteknoloji Anabilim Dalı

ÖZ

Bu çalışma, farklı seviyelerde magnezyum (Mg) ve çinkonun (Zn) bir siyanobakteri olan *S. platensis'in* büyümesi, biyoakümülatif kapasitesi, protein ve amino asit içeriği, fitokimyasal içeriği, antioksidan aktivitesi ve antimikrobiyal aktivitesi üzerindeki etkilerini araştırmak amacıyla gerçekleştirilmiştir. Amaç, *Spirulina* üretimini, mineral stres koşullarına maruz bırakarak magnezyum ve çinko içeriği artırırken organik ve biyoaktif bileşiklerin üretimini iyileştirmeyi hedeflemek ve ardından bu zenginleştirilmiş *Spirulina'nın* antioksidan ve antimikrobiyal aktivitesini değerlendirmektir. Bu sonuçlar, magnezyum ve çinko mineral zenginleştirmesinin deneme boyunca *Spirulina* hücre yoğunluğunu önemli ölçüde etkilemediğini göstermiştir. Kontrol grubundaki hücre yoğunluğu değerleri (0,230 ± 0,000) genel olarak mineral zenginleştirme uygulamalarınınkine benzer bulunurken, kontrol grubu en yüksek protein içeriğine (%70,34) sahip bulunmuştur. Magnezyumla zenginleştirilmiş gruplardan toplam fenol içeriği, çinko ile zenginleştirilmiş ve kontrol gruplarına göre önemli düzeyde yüksek olmuştur Zn-5 mg/L ile zenginleştirilmiş *Spirulina* biyoması, metanol ekstraktı için %101,346±9,274 ve etanol ekstraktı için %103,941±13,81 ile en yüksek DPPH inhibisyon derecesini göstermiştir. Tüm ekstraktlar 100 mg/mL konsantrasyonda *E. coli*, *K. pneumonia*, *S. aureus*, *B. cereus*, *C. albicans* ve *A. flavus* 'a karşı antimikrobiyal etki göstermiştir. Gram-pozitif *S. aureus* Mg-200 mg/L metanolik ekstraktlara en duyarlı bakteri türü olmuş, bunu sırasıyla 20 ± 1,31; 20 ± 0,00 ve 19,5 ± 0,61 mm çapındaki inhibisyon zonları ile Zn-5 mg/L ve Mg-100 mg/L izlemiştir.

Anahtar Kelimeler: *Spirulina*, magnezyum, çinko, antioksidan aktivite, antimikrobiyal aktivite

GENİŞLETİLMİŞ ÖZET

Mikroalgler, prokaryotik (*Cyanophyta*) veya ökaryotik (*Charophyta*, *Chlorarachniophyta*, *Chlorophytum*, *Cryptophyta*, *Dinophyta*, *Euglenophyta*, *Glaucophyta*, *Haptophyta*, *Heterokontophyta* ve *Rhodophyta*) fotosentetik mikroorganizmalardan oluşan canlı grubudur (Ibrahim ve ark, 2023; Mutanda ve ark, 2020) ve çeşitli sucul ve karasal ortamlarda yaşarlar. Tek hücreli veya çok hücreli olabilirler ve hızlı büyümeleri ve nispeten kolay yetiştirilmeleri, biyokütlelerinde değerli bileşikler üretmelerini sağlar. Bu nedenle mikroalgler, biyoaktif bileşikler üretmek için karasal ürünlere sürdürülebilir bir alternatif olarak giderek daha fazla görülmektedir. Mikroalg yetiştiriciliği, ekilebilir arazi gerektirmemesi ve tatlı su, deniz suyu, acı su ve hatta atık su kullanılarak gerçekleştirilebilmesi açısından bitkisel kaynaklara göre bir avantaja sahiptir. Biyoteknoloji sayesinde mikroalgler, hayvan ve insan beslenmesi, doğal renklendirici olarak gıda endüstrisi, ilaç ve farmasötikler, gıda üretimi, fonksiyonel farmasötikler ve nutrasötiklerin yanı sıra nemlendirici ve kırışıklık önleyici maddeler olarak kozmetik endüstrisi de dahil olmak üzere çok çeşitli uygulamalarda kullanılabilir. Ayrıca biyoyakıt (biyodizel, biyogaz ve biyoetanol) ve yenilenebilir enerji için umut verici bir kaynak olarak, tarımda doğal gübre ve bitki hastalıklarının biyolojik kontrolü, atık su arıtma sistemleri, karbondioksit yakalama, ağır metal giderimi ve biyopolimer üretimi olarak da birçok farklı alanlarda kullanılmaktadırlar.

Mikroalgler, antioksidan özelliklere sahip çok çeşitli biyolojik olarak aktif bileşikleri sentezleyebilen organizmalardır. Karotenoidler, tokoferoller, çoklu doymamış yağ asitleri, fikobiliproteinler, astaksantin ve fukoksantin gibi bu bileşikler antioksidan ve anti-enflamatuar özellikleri nedeniyle gıda, kozmetik ve ilaç endüstrilerinde kullanılabilmektedir (Aburai ve ark., 2015; Guerin ve ark., 2003; Yen ve ark., 2013). Mikroalgler ayrıca proteinler, yağ asitleri ve vitaminler dahil olmak üzere besin kaynağı olarak da kullanılabilir. Çevresel strese yanıt olarak biyoaktif bileşikler üretme yetenekleri nedeniyle, mikroalgler bu bileşiklerin üretimini en üst düzeye çıkarmak için kontrollü koşullar altında yetiştirilebilir. Bununla birlikte, mikroalglerde bu bileşiklerin üretiminin türlere, kültür koşullarına, hasat süreçlerine ve ekstraksiyon yöntemlerine bağlı olarak önemli ölçüde değişebileceği unutulmamalıdır. Sonuç olarak, bu doğal bileşiklerin üretimini ve kullanımını optimize etmek için bu alanda devam eden araştırmalara ihtiyaç vardır.

Mikroalgler, metalleri biyolojik olarak biriktirme yetenekleri ve düşük ekonomik maliyetleri nedeniyle çevre dostu bir alternatif sunmaktadır (González-Camejo ve ark., 2021; Priya ve ark., 2022). Bu nedenle atık su arıtımında yeni bir biyoremediasyon teknolojisi olarak giderek daha fazla kullanılmaktadırlar. Metal kirliliğini azaltmadaki etkinlikleri, onları çevre dostu arıtma için tercih edilen bir seçenek haline getirmektedir. Bu genellikle çok sayıda çalışma tarafından ekonomik, etkili ve daha az toksik atık üreten yeşil teknikler olarak kabul edilen iki süreçle elde edilir. Bunlar biyoakümülasyon ve biyosorpsiyondur.

Siyanobakteriler fotosentetik prokaryotlardır ve 3,5 milyar yıl öncesine dayanan fosil kayıtları ile gezegendeki en eski organizmalardan biridir (Petrescu ve Ungureanu, 2022; Mazard ve ark., 2016). Dünya'nın karbondioksit açısından zengin bir atmosferden günümüzün nispeten oksijen açısından zengin atmosferine dönüşümünden sorumludurlar (Mazard ve ark., 2016). Siyanobakteriler çok çeşitlidir ve ekstrem koşullar da dahil olmak üzere çeşitli karasal, sucul ve havasal habitatları işgal ederler. Genellikle "mavi-yeşil algler" olarak adlandırılırlar, ancak kesin tür sayıları hala tartışmalıdır ve yaklaşık 8.000 olduğu tahmin edilmektedir. Morfolojik kriterlere ve moleküler analizlere göre 5185 tür tanımlanmış ve yedi takım halinde sınıflandırılmıştır: *Chroococcales*, *Gloeobacterales*, *Nostocales*, *Oscillatoriales*, *Pleurocapsales*, *Spirulinales* ve *Synechococcales* (Mehdizadeh Allaf ve Peerhossaini, 2022).

Son yıllarda, farklı endüstriyel sektörlerde, çeşitli uygulamalar için mikroalg kullanımına artan bir ilgi görülmektedir. Alg türleri özel uygulamalarına göre seçilmektedir. *Chlorella*, *Dunaliella* ve bazı siyanobakteriler, özellikle *Spirulina* gibi bazı alg türleri, hücre işlevini değiştirme ve aşırı koşullara uyum sağlama yetenekleri nedeniyle birçok biyoproseste model olarak yaygın şekilde kullanılmaktadır. Örneğin, mikroalgler besinleri, ışığı ve CO_2'yi yararlı moleküllere dönüştürebilir (Khan ve ark., 2018)

Spirulina platensis, besinsel özellikleri ve biyoremediasyon, mikroelement biyoakümülasyonu ve sağlık alanındaki yararları potansiyel uygulamaları nedeniyle bu doktora tezinde çalışma modeli olarak seçilmiştir. *S. platensis*'in model olarak seçilmesi, magnezyum ve çinkonun büyüme mekanizmaları üzerindeki etkisini, bu minerallerin biyo-akümülasyonu yoluyla biyo-zenginleştirme kapasitesini, biyokimyasal bileşimini ve antioksidan ve antimikrobiyal özelliklerini anlamamızı sağlayacaktır.

Bu doktora tez çalışmasında, farklı seviyelerde magnezyum (Mg) ve çinkonun (Zn) bir siyanobakteri olan *S. platensis*'in büyümesi, biyoakümülasyon kapasitesi, protein ve amino asit içeriği, fitokimyasal içeriği, antioksidan aktivitesi ve antimikrobiyal aktivitesi üzerindeki etkisi araştırılmıştır. Amaç, atık su arıtımı için bir kaynak oluşturmak üzere metal stresi altındaki *Spirulina*'dan organik bileşiklerin ve biyoaktif bileşiklerin üretimini ve aynı zamanda toksik olmayan mineral bakımından zenginleştirilmiş *Spirulina* üretmek için minerallerin biyoakümülasyonunu araştırmaktır. Bu sonuçlar, magnezyum ve çinko mineral zenginleştirmesinin deney boyunca *Spirulina* hücre yoğunluğunu önemli ölçüde etkilemediğini göstermektedir. Kontrol grubundaki hücre yoğunluğu değerleri (0,23 ± 0,00) genel olarak mineral zenginleştirme uygulama gruplarına benzer bulunmuştur.

Denemenin son gününde, farklı gruplardaki magnezyum seviyeleri şu şekildedir: Mg-200 mg/L grubu, Mg-100 mg/L grubu ve Kontrol grubu sırasıyla 348,150±44,845 mg/100 g, 249,626±73,274 mg/100 g ve 210,250±5,254 mg/100 g olarak ölçülmüştür. Bu sonuçlara göre, Mg-200 mg/L grubu deneyin son gününde (13. gün) en yüksek magnezyum konsantrasyonuna sahiptir.

Kontrol grubu ile karşılaştırıldığında, 5 mg/L ve 3 mg/L çinko konsantrasyonlarına maruz kalan *S. platensis* zamanla çinko içeriğinde önemli bir artış göstermiştir.

Kontrol grubu en yüksek protein içeriğine (%70,34) sahip bulunmuştur. Bu çalışmada, 5 mg/L çinko konsantrasyonunun kontrol grubuna kıyasla protein içeriğinde azalmaya sebep olduğu ve çinko konsantrasyonu arttıkça protein içeriğinde kademli bir düşüş gözlendiği belirlenmiştir.

Aspartat, sistin, histidin, tirozin ve metiyonin gibi spesifik amino asitler dahil olmak üzere amino asit konsantrasyonları da mineral zenginleştirme koşulları altında değişiklik göstermiştir.

Bu sonuçlardan, flavonoid içeriğinin farklı deneysel koşullara ve çözücülere göre önemli ölçüde değiştiği görülmektedir. Tanen içeriğinin Mg-200 mg/L ile muamele edilen grupta önemli ölçüde arttığı görülmektedir. Bu konsantrasyon Mg-200 mg/L grubunda, kontrol grubu da dahil olmak üzere diğer gruplardan önemli ölçüde yüksek bulunmuştur.

Magnezyumla zenginleştirilmiş grupların toplam fenol içeriği, çinkoyla zenginleştirilmiş ve kontrol gruplarınınkinden önemli ölçüde yüksek bulunmutur. Çinko grubu, özellikle de Zn-5 mg/L grubu, metanol ekstresi için %101,346±9,274 ve etanol ekstresi için %103,941±13,81 ile en yüksek DPPH inhibisyon derecesini göstermiştir. Tüm ekstraktlar 100 mg/mL konsantrasyonda antimikrobiyal etki göstermiştir. Gram-pozitif *S. aureus* Mg-200 mg/L metanolik ekstraktlara en duyarlı bakteri türü olmuş, bunu sırasıyla 20±1,31, 20±0,00 ve 19,5 ± 0,61 mm çapındaki inhibisyon zonlarıyla Zn-5 mg/L ve Mg-100 mg/L izlemiştir.

Sonuçlar, magnezyum ve çinko maruziyetinin sadece bu siyanobakterinin büyümesi üzerindeki etkisine değil, aynı zamanda magnezyum ve çinko eksikliğiyle mücadele için potansiyel bir besin takviyesi ve atık su arıtımı için potansiyel bir alg olarak *Spirulina platensis*'in kalitesi ve besin değeri üzerindeki etkisine dair önemli sorular ortaya çıkarmaktadır. Böylece, bu doktora çalışmasında, *S. platensis* mineralleri biriktirebiliği ve biyoaktif bileşikler üretebildiği ortaya konmuştur. Magnezyum ve çinko ile zenginleştirilmiş *Spirulina* fenolik özütleri antimikrobiyal aktivite göstermiş, bu da onları terapötik amaçlar için ilginç ve gıda maddelerini ve kozmetikleri kirleten mikroorganizmalarla mücadele etmek için gıda maddelerine dahil edilmeye uygun hale getirmiştir. *Spirulina platensis* bileşikleri bu nedenle mikroorganizmaların gelişimini önlemede oldukça etkilidir.

Minerallerin, pigmentlerin ve sekonder metabolitlerin biyoerişilebilirliğini anlamak ve çeşitli biyolojik aktiviteler üzerindeki etkilerini değerlendirmek, sağlık yararlarını göz önünde bulundurmak çok önemli hususlardır. Bu araştırma hattında umut verici bir yaklaşım, *Spirulina'nın* mineral bakımından zenginleştirilmiş özütlerinin kapsüllenmesini ve gıdalara dahil edilmesini araştırmaktır. İn vivo ve in situ çalışmalar, bu bileşiklerin biyolojik aktivitelerinin derinlemesine analiz edilmesini sağlayacaktır. Bu bakış açısı, gelecek için önemli bir araştırma alanını temsil etmekte ve bu unsurların sağlığı geliştirmek için nasıl kullanılabileceğini daha iyi anlamak için fırsatlar sunmaktadır.

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LIST OF SYMBOLS AND ABBREVIATONS

ABTS : 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid

DPPH : 2,2-diphenyl-1-picrylhydrazyl

DV : Daily value

FRAP : Ferric reducing antioxidant power

GAE : Gallic acid equivalent

Mg : Magnesium

PPO : Polyphenol oxidase

QUE : Quercetin equivalent

TPC : Total phenol content

TPF : Total flavonoid content

Zn : Zinc

1. INTRODUCTION

Currently, heavy metal pollution is primarily a result of human activities, although natural sources such as volcanoes and soil erosion also contribute. This pollution is predominantly attributed to anthropogenic activities associated with industrialization and increasing urbanization. The metallurgy sector, especially the automotive industry; intensive agriculture, involving the use of fertilizers and pesticides; municipal wastewater; and the chemical and pharmaceutical industries, discharging medicines and pharmaceutical products, constitute the primary sources of heavy metal emissions into the environment, leading to changes in ecosystems (He et al., 2005).

However, the elimination of heavy metals from the environment presents a substantial challenge, as these elements exhibit high persistence (Yan et al., 2020). The pollution of water, soil and air by these heavy metals poses a great threat to the well-being of living organisms, including human beings. In addition to that, various trace ions like magnesium, cobalt, copper, iron and zinc are acknowledged contaminants in ground and surface water (both in marine and freshwater), making it crucial to reduce the discharges and emissions of these metals into the environment.

Moreover, treating wastewater from industrial landfills is one of the most critical environmental challenges for preserving water quality as a natural resource. Although certain metals are essential for the growth of microorganisms beyond the recommended threshold, they become toxic to living organisms, resulting in physiological or morphological abnormalities (Choudhary et al., 2007) or genetic mutations, such as slowed or arrested growth or the appearance of mutations (Mitra et al., 2022). Some remediation techniques and technologies have been developed, such as filtration, precipitation, oxidation/reduction, reverse osmosis, and electrochemical methods, but these are still very costly and less effective.

Furthermore, the preservation of the quality of water as a natural resource is one of the leading environmental concerns. The remediation of wastewater from industrial waste collection centers is a crucial issue. Traditional methods are often expensive, ineffective at metal concentrations below 100 mg/L, and complex, often resulting in inadequate treatment or secondary pollution (Zinicovscaia et al., 2021). Thus, to reduce metal pollution, environmentally friendly metal disposal methods are a primary environmental concern. This is why using microalgae as a new bioremediation technology to treat wastewater is becoming increasingly common due to their high capacity to bioaccumulate metals, efficiency, and low economic cost.

Microalgae are a diverse group of photosynthetic microorganisms, either prokaryotic (*Cyanophyta*) or eukaryotic (*Charophyta*, *Chlorarachniophyta*, *Chlorophytum*, *Cryptophyta*, *Dinophyta*, *Euglenophyta*, *Glaucophyta*, *Haptophyta*, *Heterokontophyta*, et *Rhodophyta*) (Ibrahim et al., 2023; Mutanda et al., 2020), living in a variety of aquatic and terrestrial environments. They can be unicellular or multicellular, and their rapid growth and relatively easy cultivation enable them to produce valuable compounds in their biomass. As a result, microalgae are increasingly seen

as a sustainable option for terrestrial plants to produce bioactive compounds. Microalgae cultivation has an advantage over plant sources in that it does not require arable land and can be carried out using fresh water, seawater, brackish water or even waste. Biotechnology has opened up a wealth of opportunities for the use of microalgae in various applications. These include animal and human nutrition, the use of natural colourants in the food industry, applications in medicine and pharmaceuticals, use in food production, the development of functional pharmaceuticals and nutraceuticals, and even their role in the cosmetics industry as moisturisers and anti-wrinkle agents. They are also used as a promising source of biofuels (biodiesel, biogas and bioethanol) and renewable energy, as well as in agriculture as a natural fertilizer and biological control of plant diseases, wastewater treatment systems, carbon dioxide capture, heavy metal removal and biopolymer production.

Microalgae are organisms capable of synthesizing a wide range of biologically active compounds, some of which have antioxidant properties. These compounds, including carotenoids, tocopherols, polyunsaturated fatty acids, phycobiliproteins, astaxanthin and fucoxanthin can be utilized in the food, cosmetics and pharmaceutical firms for their antioxidant and anti-inflammatory characteristics (Aburai et al., 2015; Guerin et al., 2003; Yen et al., 2013). Microalgae can also be used as a source of nutrients, including proteins, fatty acids and vitamins. Because of their ability to produce bioactive compounds in response to environmental stress, microalgae can be grown under controlled conditions to maximize the production of these compounds. However, it should be noted that the production of these compounds in microalgae can vary considerably depending on species, culture conditions, harvesting processes and extraction methods. Consequently, ongoing research in this field is needed to optimize the production and use of these natural compounds.

Microalgae, like higher plants, require light, carbon dioxide, water and the inorganic salts carbon (C), nitrogen (N), potassium (K), phosphorus (P) and sodium (Na) to promote growth and productivity. These elements are essential for photosynthesis and microalgae's production of organic matter. In addition to these elements, microalgae also need other microelements such as iron (Fe), magnesium (Mg), zinc (Zn), sulfur (S), calcium (Ca), manganese (Mn), chlorine (Cl), molybdenum (Mo), Boron (B), cobalt (Co) and copper (Cu) for proper growth. These microelements are important for ion and energy transport, pigment production, chlorophyll synthesis and enzyme activation. Nutrients, light, temperature, salinity and pH are among the most important environmental factors affecting the productivity and biochemical composition of microalgae (Dolganyuk et al., 2020; Villarruel- Lopez et al., 2017). The limitation or excess of these factors directly impacts the synthesis of biologically active substances, biomass growth and productivity, and photosynthetic processes in microalgae. Microalgae are at the top of the marine food chain. They are an essential food resource for many marine organisms, such as zooplankton, mollusks, fish, crustaceans and marine animals.

Microalgae offer an environmentally friendly alternative due to their ability to bioaccumulate metals and their low economic cost (González-Camejo et al., 2021; Priya et al., 2022) . This is why they are increasingly used as a new bioremediation technology in wastewater treatment. Their effectiveness in reducing metal pollution makes them a preferred option for environmentally friendly treatment. This is generally achieved by two processes recognized by numerous studies as green techniques that are economical, effective and produce less toxic waste. These are bioaccumulation and biosorption.

Bioaccumulation is a metabolically active process combining heavy metal adsorption and concentration in living cells. This process generally occurs during biomass cultivation in the presence of metal. Conversely, bioabsorption is a passive process involving metabolism-independent mechanisms such as physical adsorption, chelation, ion exchange and precipitation (Kougia et al., 2023; Bilal et al., 2018) . Thus, the bioaccumulation of metals under controlled conditions can produce biomass enriched with targeted minerals. For example, it can contribute to wastewater remediation by promoting the production of *Spirulina* biomass bioenriched with microelements, phytonutrients and antioxidants. This helps combat nutritional deficiencies, oxidative stress and food preservation.

Micronutrient bioenrichment or biofortification is one of the low-cost, sustainable strategies developed by the World Health Organization and the Food and Agriculture Organization of the United Nations to address trace element deficiencies. Biofortification is the laudable process of improving the level and bioavailability of nutrients in food crops using genetic engineering or conventional plant breeding. Biofortification, therefore, combines agricultural and nutritional sciences to reduce malnutrition by enhancing levels of nutrients such as iron, vitamin A, and zinc in staple foods at no increased cost to consumers (Yadav et al., 2022) . Today, nutrient deficiency is a global problem in many countries for both humans and animals. Over-reliance on refined, nutrient-deficient food crops and a growing population highlight the need for strategies to improve food and nutritional security. Biofortification is a promising natural approach to enriching foods with essential nutrients, helping to combat malnutrition (Dhaliwal et al., 2022; Ofori et al., 2022).

In addition, microalgae are often exposed to high levels of oxidative stress and toxicity due to various environmental factors. This can include heavy metals, including cadmium, mercury, chromium and lead, which may be present in their natural environment due to pollution. In addition, chemicals from pesticides, herbicides, or other industrial sources can also affect the growth and productivity of microalgae. These stresses can cause excessive accumulation of free radicals (Saha et al., 2013), damaging cells and important cellular constituents such as lipids, proteins and nucleic acids. Consequently, this can lead to a reduction in microalgae growth, productivity and viability. So, to cope with these conditions, microalgae have developed defense mechanisms involving the production of antioxidant molecules like superoxide dismutase, glutathione reductase, catalase, and protective molecules like phytochelatins, pigments

(carotenoids, chlorophyll), polysaccharides, polyphenols, and vitamins C and E. However, high levels of oxidative stress can be toxic beyond the defense capabilities of microalgae. It is, therefore, essential to monitor and minimize the existence of environmental stressors, like heavy metals and chemicals, to preserve the growth and productivity of microalgae, which play an important role in aquatic ecosystems and also have the potential for industrial applications such as the production of biofuels, food supplements and sustainable chemicals. These antioxidant defense systems help microalgae maintain redox balance and protect their cellular structures and vital functions from oxidative damage. However, the capacity of microalgae to cope with oxidative damage can vary according to species and specific environmental conditions. Prolonged or extreme stress conditions can overwhelm microalgae's defense capabilities, leading to irreversible cell damage or cell death (Zhao et al., 2020; Berges and Choi, 2014; Poljšak and Milisav, 2012; Affenzeller et al., 2009; Barbosa et al., 2003).

In recent years, various industrial sectors have shown a growing interest in using microalgae for various applications. Algae species are selected according to their specific applications. Certain algal species, such as *Chlorella*, *Dunaliella* and certain cyanobacteria, particularly *Spirulina*, are commonly used as models in many bioprocesses due to their ability to modify cell function and adapt to extreme conditions. For example, microalgae can transform nutrients, light and CO_2 into desired molecules (Khan et al., 2018).

Cyanobacteria are photosynthetic prokaryotes and one of the oldest organisms on the planet, with a fossil record dating back 3.5 billion years (Petrescu and Ungureanu, 2022; Mazard et al., 2016). They are responsible for the Earth's conversion from a carbon dioxide-rich atmosphere to the comparatively oxygen-rich atmosphere of today(Mazard et al., 2016). *Cyanobacteria* are diverse and occupy various terrestrial, aquatic and aerial habitats, including extreme conditions. They are commonly referred to as "blue-green algae", but their exact number of species is still debatable and is estimated at around 8,000. According to morphological criteria and molecular analysis, 5185 species have been identified and classified into *Chroococcales*, *Oscillatoriales* *Gloeobacterales*, *Nostocales*, *Pleurocapsales*, *Spirulinales* and *Synechococcales* orders (Mehdizadeh Allaf and Peerhossaini, 2022).

Cyanobacteria are distinguished by their ability to carry out oxygenic photosynthesis, in which water is oxidized to generate oxygen and transform light into chemical energy, similar to the process in plants. With a few exceptions, they synthesize chlorophyll *a*, the primary photosynthetic pigment, and phycobiliproteins, which are light-collecting pigments (Gómez et al., 2023; Garcia-Pichel, 2009; Beale, 2008). *Cyanobacteria*, a type of prokaryote, have a productivity advantage over land plants due to their high growth rate and superior CO_2 capacity (Demirbas and Fatih Demirbas, 2011). They constitute a vast reservoir of a wide variety of metabolites that can be low-molecular-weight peptides, alkaloids, terpenoids, polysaccharides and lipopolysaccharides (Trabelsi et al., 2010) , phenolic compounds, flavonoids, photoprotective compounds,

phytohormones, cyanotoxins, biocides (Singh et al., 2017) that have application potential in several fields such as food, agriculture, pharmaceuticals and cosmetics. The growing desire of researchers, industrialists and consumers to find natural compounds that can replace synthetic antioxidants and preservatives illustrates the growing importance of functional foods. Cyanobacteria are well suited to this role and can be considered excellent candidates to meet this demand.

Spirulina platensis was chosen as the model for study in this doctoral thesis because of its nutritional properties and potential applications in bioremediation, bioaccumulation of microelement and health benefits. The choice of *S. platensis* as a model will enable us to understand the effect of magnesium and zinc on its growth mechanisms, its capacity for bio-enrichment through bioaccumulation of these minerals, its biochemical composition and its antioxidant and antimicrobial properties.

Spirulina platensis or *Arthrospira platensis* (commercially known as *Spirulina*), a blue-green, gram-negative, prokaryotic, photosynthetic, filamentous cyanobacterium that has been consumed since ancient times by the Kanembou of Lake Chad dried on the shores of Lake Chad in the form of dried cake called "dihé" and the Aztecs of Mexico for its nutritional and therapeutic potential (Fleurence and Levine, 2018; Masojídek and Torzillo, 2014). It thrives naturally in alkaline lakes located in sunny areas with high temperatures. Since then, this photosynthetic cyanobacterium has been rediscovered by scientists on numerous occasions, both for its nutritional and medicinal value. Today, it is produced commercially in large outdoor ponds or greenhouses under controlled conditions in various parts of the world. *Spirulina* and its by-products are marketed as tablets, dry powder, drinkable ampoules and capsules. The worldwide *Spirulina* market was estimated at $393.6 million in 2019 and is expected to rise $897.61 million by 2027 (https://www.alliedmarketresearch.com/spirulina-market). It was approved as a generally regarded as safe food by the FDA in 2002 (Marles et al., 2011). This cyanobacterium stands out for its composition, which is rich in highly digestible proteins (50-70%), peptides and essential amino acids, polyunsaturated fatty acids, minerals, vitamins and bioactive compounds. These characteristics make it a high-quality source of nutrients for human and animal nutrition. The consumption of *Spirulina* as a dietary supplement is highly recommended for its nutritional value and it is consumed in prevention and treatment of certain diseases. Moreover, *Spirulina* contains phytopigments including chlorophylls, phycocyanin, carotenoids and polyphenols (flavonoids, anthocyanins, tannins), which can be used as natural colorants and antioxidants. These molecules are mainly responsible for *Spirulina*'s bioactivities, for example its antioxidant property (Ramon-Mascarell et al., 2022; Grover et al., 2021; Agustini et al., 2015), anti-inflammatory (Hoseini et al., 2013) , antimicrobial (Kaushik and Chauhan, 2008), antiviral (Ayehunie et al., 1998; Hayashi et al., 1993) anticancer (Śmieszek et al., 2017; Koníčková et al., 2014) neuroprotective (Pérez-Juárez et al., 2016) probiotic (Cai et al., 2022), antiallergenic (Cingi et al., 2008), and immune system stimulant (Park et al., 2008) . This rich composition gives it interesting potential for various

functional food applications and related new technologies. In addition, it can be used in the production of biofuels (Rahman et al., 2017) cosmetics (Rizzi et al., 2021), bioplastic materials (Zhang et al., 2020), water treatment (Zhou et al., 2017), carbon dioxide (CO_2) fixation (Singh et al., 2016) and biofertilizers (Shedeed et al., 2022).

As a photoautotrophic aerobic species, *Spirulina*'s growth depends on its ability to photosynthesize using its chlorophyll pigments and photosystems I and II. For this process, *Spirulina* needs water and nutrients such as nitrogen, usually atmospheric carbon and phosphorus, which play an essential role in cellular metabolism and biochemical composition. *Spirulina*'s physiological growth and biochemical composition depend on environmental and nutritional conditions. These include light, pH, temperature, nutrient composition and availability, agitation speed and salinity. A change in the concentration of these nutrients can stress these organisms. This stress accumulates lipids, sugars, proteins, bioactive substances and pigments (Richmond, 2007) and produces reactive oxygen species (Ismaiel et al., 2016).

Like other microalgae, *Spirulina* is an essential source of polyphenols. Phenolic compounds are among the most numerous and widespread groups of secondary plant metabolites. They are stress metabolites that plants produce to protect themselves against abiotic and biotic factors. These compounds possess an aromatic ring containing one or more hydroxyl groups and function as antioxidants (Saranraj et al., 2019). The beneficial properties of polyphenol are manifold. Indeed, they possess biological activities identified as anticarcinogenic, antimutagenic, anti-inflammatory (Roleira et al., 2018) and antimicrobial (Zhang et al., 2022). These different activities are directly associated to the antioxidant activity of these compounds, making them extremely useful in the prevention and treatment of certain chronic or degenerative diseases such as cancer, diabetes, Alzheimer, cardiovascular disease and dementia.

Moreover, dietary supplements increasingly use antioxidants as bioactive compounds to combat oxidative stress. They also have an essential role in food preservation, colouring, and preventing undesirable lipid peroxidation. For example, *Spirulina* biomass or its phenolic compounds can replace synthetic antioxidants suspected of being carcinogenic (Lourenço et al., 2019). Hence, over the last decade, the need to develop environmentally friendly natural functional and bioactive compounds from marine plants, animals and microorganisms has become a sustainable alternative that offers new compounds with high biological activity (Šimat et al., 2020). This trend has attracted the interest of the food, pharmaceutical and cosmetics industries and consumers, who increasingly avoid synthetic food additives. As a result, research increasingly focuses on using natural products, leading to a growing number of studies on secondary metabolites.

Molecules derived from *Spirulina platensis* are promising due to their biological activities, large-scale cultivation and low production costs, and long consumption history. Given all their advantages, they can serve as reliable alternative antioxidant and antimicrobial agents. They can be

used as reliable alternative antioxidant and antimicrobial agents, as *Spirulina* has been consumed since ancient times. The extracts could be effective candidates for safe and effective medical applications (Abdel-Moneim et al., 2022) and have the advantage of being a natural food preservative (Ovando et al., 2018; Sun et al., 2016). *Spirulina* can produce bioactive molecules via photosynthesis, representing an economic interest supported by numerous applications in pharmacology, agri-food or cosmetology.

Thanks to their various functional groups, such as carboxyls, hydroxyls, sulfates, phosphates and other groups that they contain, microalgae can absorb pollutants (Dotto et al., 2012). However, research has focused on the impact of certain nutrients, such as nitrogen and phosphorus, light, temperature and pH, on the growth of microalgae and their biochemical composition. However, other microelements, such as magnesium and zinc also have an important role in various metabolic processes in microalgae. Magnesium is essential for chlorophyll synthesis and photosynthesis and is involved in lipid bioaccumulation in microalgae such as *Chlorella* (Gorain et al., 2013). For example, high levels of Mg^{2+} in the environment can cause environmental stress, leading to excess reactive oxygen species (ROS). These ROS can harm algal cells by oxidizing essential cellular components such as proteins, rendering metabolic enzymes inactive and affecting DNA and lipids. As a result, a defense mechanism against ROS is activated, comprising a range of non-enzymatic antioxidants, including SOD, which works together to neutralize the damaging effects of ROS (Salman et al., 2023).

Zinc is an essential micronutrient for developing the immune system and the general well-being of all living organisms. Zinc is a cofactor for many enzymes. It is involved in the synthesis of nucleic acids and proteins. However, an excess or deficiency of zinc can lead to health problems. Although zinc is essential for growth, high levels can inhibit growth and destroy the ecosystem. For example, three stress indicators were expressed in *Spirulina* biomass grown in Zn-containing multimetallic systems: a decrease in protein amount, a diminution in phycobiliprotein content and an augmentation in malondialdehyde quantity (Zinicovscaia et al., 2021). It is, therefore, essential to remove zinc from the environment by using microalgae to convert inorganic zinc into organic zinc, thereby improving its physiological activity and absorption rate while reducing its toxicity. It is well known that it improves tolerance to abiotic stress in various plant species. Various studies have evaluated the protective role of zinc on numerous abiotic stresses including drought, salinity, metals, low temperatures and acidic stresses. Studies have shown that exposure to zinc increases lipid peroxidation in certain algae (Li et al., 2006). Heavy metals, including zinc, can be bioaccumulated by microalgae such as *Spirulina platensis*, used in wastewater treatment.

Spirulina is also one of the microalgae used to treat wastewater, thanks to its rapid growth and adaptability and the accumulation of precious metabolites (Zhou et al., 2022). *Spirulina* has a high capacity for metal ion bio-enrichment. Essential metal ions such as magnesium and zinc, which are of fundamental importance to human health, can also stimulate *Spirulina* growth and are

involved in protein synthesis, lipid and carbohydrate metabolism (Zhou et al., 2018) . Recently, *Spirulina platensis* has been actively used for metal accumulation in single or multi-component systems (Cepoi et al., 2020 a; Cepoi, et al., 2020 b ; Zhou et al., 2018; Nalimova et al., 2005). Several studies have shown that *Spirulina* has the capacity for high uptake and accumulation of metals in their cells (Ghanbarzadeh et al., 2022) , suitable for enrichment (Akbarnezhad et al., 2019); it can, therefore, make an important contribution to producing functional foods rich in certain trace elements, such as zinc (Zhou et al., 2018), produce metabolites such as carotenoids (Ozturk Urek and Kerimoglu, 2019) and maintain optimal protein and lipid levels in biomass to ensure its ability to accumulate metal ions (Cepoi et al., 2020b). Only a few studies examine the influence of magnesium and zinc on *Spirulina* growth and their impact on biochemical composition. They have all been carried out in laboratory environments to treat natural or synthetic wastewater. These advances could lead to the production of functional foods beneficial to human health.

In recent years, the growing demand for dietary supplements rich in micronutrients and natural antioxidants to combat microorganism resistance and the spread of infections has fuelled increased interest in discovering biologically active cyanobacterial metabolites with antioxidant and antimicrobial activity. Several studies have shown that various cyanobacteria release biologically active substances with antibacterial, cytotoxic, antifungal, antiviral and immunosuppressive properties.

The general objective of this study was to determine the growth process of *S. platensis* in a medium enriched with magnesium and zinc outdoors under greenhouse conditions in a 1 m^3 pound, the capacity of the biomass to accumulate these trace ions, the biochemical change of the *Spirulina platensis* biomass as well as the antioxidant and antimicrobial activity.

The specific objectives are:

1- Determine the effect of magnesium and zinc on *Spirulina platensis* growth parameters, such as optical density and dry matter;
2- Assess the bioaccumulative capacity of *Spirulina platensis* for magnesium and zinc;
3- Analyze the protein and amino acid content of biomass enriched with magnesium and zinc;
4- Measure the content of pigments such as chlorophyll *a*, carotenoids and phycocyanin in *Spirulina platensis* enriched with magnesium and zinc;
5- Quantify total phenols, phenolic compounds, flavonoids and tannins in methanolic and ethanolic extracts of *Spirulina* enriched with magnesium and zinc;
6- Assess the antioxidant and antimicrobial activities of methanolic and ethanolic extracts of *Spirulina platensis* enriched with magnesium and zinc.

2. LITERATURE SUMMARY

2.1. Studies assessing the impact of magnesium and zinc on *Spirulina*

Ghanbarzadeh et al (2022) evaluated the effects different concentrations of iron (0.05, 0.1, 0.2 and 0.3 g/L), zinc (2, 4, 6 and 8 mg/L) and manganese (6, 12, 25 and 40 mg /L) on *A. platensis* MGH-1 growth and ability to accumulate these micronutrients. *A. platensis* MGH-1 showed maximum growth parameters at 0.1 g/L iron, 2.0 mg/L zinc, and 6.0 mg/L manganese. Highest growth values were obtained at 2 mg/L $ZnSO_4$. Dry matter, specific growth rate, and biomass productivity at 2.0 mg/L $ZnSO_4$ increased by 14.02, 5.88 and 17.02%. The highest bioaccumulation was found in 0.31 g/L of iron, 8.0 mg/L of zinc, and 40 mg/L of manganese. Maximum level of antioxidant enzyme activity (SOD, POX, and CAT levels, respectively: 15.31±0.82, 40.53±0.9, and 638.02±24.09 U/mg protein) and palmitic acid content (28.04±1.0) was found in *A. platensis* MGH-1 enriched with 4.0 mg/L zinc. Chlorophyll *a* concentration showed a significant increase at 25 mg/L manganese.

Jatupornpongchai et al (2022)studied the effects of zinc sulfate, nitrate and zinc chloride at levels of 0.4, 0.8 and 1.6 µmol Zn for 7 days. *Spirulina* (*S. platensis*) grown in medium containing zinc sulfate (1.6 µmol Zn) showed the highest biomass, with a dry weight of 4.03±0.01 g/L, a Zn content of 69.55 µg/g dry weight and a protein content of 63.11% compared with Zarrouk's modified media containing zinc nitrate and zinc chloride.

The increase of up to 10 times in the concentration of Fe^{2+}, Cu^{2+} and Zn^{2+} individually in the Zarrouk medium. No significant difference in weight was observed. The highest optical density was observed at Cu^{+2} (10 times).13.54 was the Zn^{+2} maximum enrichment factor. On day 7, chlorophyll *a* production reached 1.213±0.514 mg/L at all doses. After 14 days, Cu^{+2}-treated *S. platensis* had the highest total carotenoid concentration (0.0042±0.0004 mg/L). All treatments reduced phycobiliproteins (Akbarnezhad et al., 2020).

Urek and Kerimoglu (2019) investigated the impact of Mg^{2+} (0-4 mM) and Cu^{2+} (0–5 M) on biomass, photosynthetic metabolites, and photosystem II activity in *A. platensis* Gomont 1892. Maximum biomass was 49.11.6 mg/mL in the control condition with Mg^{2+} (0.8 mM) and Cu^{2+} (0.5 mM). Because of the Mg^{2+} content, the main components were phycocyanin and allophycocyanin. Chlorophyll reached its maximum at 0.5 M Cu^{2+}. Without Cu^{2+}, main carotenoid, *β*-carotene, reached 2400±60 µg mL^{-1}. Mg^{2+} and Cu^{2+} excess and deficiency reduced *A. platensis* photosystem II activity.

Balaji et al (2013) tested the biosorption of zinc and nickel by *S. indica, S. maxima and S. platensis* in laboratory conditions. They were grown in Zarrouk medium with varying concentrations of zinc and nickel (0.01, 0.05, and 0.1 mM). The biosorption of selected cyanobacterial strains was evaluated using doubling time, chlorophyll concentration (Chll *a*, Chll *c*), and protein value. Certain metals in cultural media had a significant impact on growth. At 0.1

mM, heavy metal stress slowed strain formation. *S. maxima* thrived at 0.01 mM zinc (6.9 mg/L) and 17 mg/L nickel.

Li et al (2017) investigated the growth-enhancing effects of external zinc on *A. platensis* under low-temperature (LT) stress. After 24 h of treatment, *A. platensis* dry weights in the LT and LT-zinc groups significantly declined by 60.38% and 40.25%, respectively. The LT group showed less green than the control group, and chlorophyll a, carotenoid and phycocyanin contents declined by 27.4, 22.5 and 33.2%, respectively. After low-temperature stress, soluble sugar content showed a significant increase of 18.6%, and the LT group with additional zinc increased by 27.7%, compared with the low-temperature group.

Zhou et al (2018) studied the effect of different initial Zn^{2+} concentrations on *S. platensis* growth and biochemical composition. *Spirulina platensis* biomass at each concentration peaked at the end of culture, but high levels could severely inhibit microalgal growth. OD_{560} was just 0.584 at 8.0 mg/L, well below 0 mg/L by 70.33%. Fluorescence activity changed in response to heavy metal exposure, and biochemical composition altered with zinc stress, with the highest levels of PC, chlorophyll *a*, carotenoid and zinc accumulation. Chlorophyll *a* and carotenoid levels were highest at 4.0 mg/L and 1.0 mg/L Zn^{2+}, respectively. The saturated and polyunsaturated fatty acid ratio increased constantly due to zinc exposition.

Nalimova et al (2005) examined how Cu and Zn affect *S. platensis* (Nordst.) Geitl growth and heavy metal accumulation. Copper was lethal at 5 mg/l in the lag phase and 4 mg/l in the linear growth. HM-treated *S. platensis* cells accumulated ten times more copper and zinc than controls.

Zinicovscaia et al (2021) studied the zinc accumulation ability of *S. platensis* and its chemical content by modelling four Zn-containing systems with various combinations of metal ions (Zn; Zn/Cu/Sr; Zn/Cu/Ni; Zn/Cu/Sr/Ba). *Spirulina* biomass cultivated in a mono-metallic system showed two indicators of biochemical stress. In contrast, in the case of multi-metal systems containing Zn, three stress parameters were observed: decreased protein levels, reduced phycobiliprotein contents, and increased malondialdehyde content. A neutron activation essay determined metal absorption by biomass. It demonstrated a high accumulation ability for all metals in the system tested, even at the highest metal concentration.

Cepoi et al (2020b) evaluated metal accumulation by *S. platensis*, using synthetic effluents containing different metals concentrations. These effluents had the chemical compositions below: Cr/Fe, Cr/Fe/Ni, Cr/Fe/Ni/Zn, and Cr/Fe/Ni/Zn/Cu. The study was carried out over several culture cycles. The researchers introduced metal ions at various concentrations at two key points in biomass growth: the exponential and stationary phases. They then measured the absorption of these metallic ions by biomass through neutron activation ssay. In addition, they also assessed the impact of these metal ions on the biomass and the main biochemical components of Spirulina platensis, namely proteins, carbohydrates, lipids, phycobilins and beta-carotene. They found that when metal

ions were introduced during the stationary growth phase, *Spirulina platensis* exhibited a notable capacity for metal accumulation over a period ranging from 2 to 3 culture cycles.

2.2. Studies relating to antioxidant effect of *Spirulina*

Several *in vitro* studies have demonstrated the promising antioxidant activity of *S. platensis* or its derivatives.

The results of the study by Wu et al (2005) revealed that DPPH essays also showed a similar trend to ABTS essays (EC50: 19.39 ± 0.65 μmol ascorbic acid equivalent/g *Spirulina* extract versus. 14.04 ± 1.06 μmol ascorbic acid equivalent/g *Chlorella* extract). Furthermore, aqueous extracts of both algae inhibited HSC and HepG2 proliferation, although *Spirulina* was shown to be a more powerful inhibitor than *Chlorella*.

Abdel-Moneim et al (2022) studied the antioxidant capacity of *S. platensis* extracts. All *S. platensis* extracts demonstred a significant ability to scavenge ABTS and DPPH radicals. Methanol extract showed an inhibition rate of 93% for ABTS radicals and 90% for DPPH radicals. Acetone extract had inhibition rates of 88% for ABTS radicals and 82% for DPPH radicals, and hexane extract showed lower but still substantial inhibition rates of 60% for ABTS radicals and 55% for DPPH radicals, compared with the standard compound (tert-butyl hydroquinone) with inhibition rates of 96% for ABTS radicals and 94% for DPPH radicals.

In the study conducted by Zaid et al (2015), the marine extracts of *S. platensis* recorded a percentage of inhibition of DPPH up to 81.1% (1.5 g/100 mL) and 40.45 mg/g of total phenolic content.

Anbarasan et al (2011) used the scavenging activity of the DPPH and nitric oxide radicals to assess the antioxidant capacity of a *Spirulina platensis* ethanol extract *in vitro*. The extract was evaluated at four various concentrations: 25, 50, 75, and 100 g/mL. According to the study results, the extract showed antioxidant activity in a dose dependent. The percentage of DPPH radical scavenging activity increased from 10.30±0.80% to 27.88±1.21% as the dose increased from 25 g/mL to 100 g/mL. At the same doses, the extract's radical nitric oxide increased from 1.84±0.44% to 20.27±0.1%.

El-Tantawy (2015) conducted a study to assess the antioxidant power of a *Spirulina* supplement on lead-induced liver damage in rats. Five groups of rats were used: one control group, one treatment group with lead acetate (100 mg/kg), two treatment groups with lead acid (100 mg/kg) and different doses of *Spirulina* (0,5 g/kg and 1 g/kg) and one treatment with lead Acetate (100 mg/kg) and 25 mg / 100 g of vitamin C (reference medicine). The results demonstrated that lead poisoning had caused significant liver damage, but that doses of *Spirulina* and vitamin C had improved biochemical parameters and prevented lead-induced changes in liver antioxidant status.

Pereira et al (2019) evaluated mixotrophic cultivation conditions using the Zarrouk medium supplemented with whey (0,0; 2,5; 5,0 and 10%) from the production of buffalo mozzarella. The antioxidant activity declines with the increase in the concentration of serum,

measured by FRAP and ABTS methods. The highest FRAP values were observed at 0.0% whey, while ABTS was also the highest at this level. Whey concentrations of 2.5% and 5.0% showed a slight decrease in antioxidant activity, while at the highest concentration of 10,0%, FRAP and ABTS values decreased further.

Dartsch (2008) tested the anti-free radical and anti-inflammatory activity of four preparations of *Spirulina platensis*. These are Biospirulina, SpiruComplex [*Spirulina* with naturally linked selenium (0.006%), chromium (0.003%) and zinc (0.115%), Charge L150492], SpiruZink [*Spirulina* with naturally linked zinc (0.25%), Charge L 110892] and Zinkspirulina + Acerola [*Spirulina* with naturally linked zinc (0.125%) and acerola powder (contains 5.5% ascorbic acid), Charge L 250692]. The results, therefore, suggest that the preparation Zinkspiruline + Acérola is most effective for inactivating the free radicals of oxygen, with an observed effective concentration of 100 µg/mL, followed by Spirucomplex and SpiruZink with an adequate level of 250 µg/mL, and finally BioSpirulina with an efficient level from 500 µg/mL.

Kepekçi and Saygideger (2012) tested the antioxidant activities of *S. platensis* methanol extracts cultivated at three varying doses (40, 60 and 120 µmol photons m^{-2} s^{-1}) of light irradiation. The highest antioxidant activities and quantities of polyphenols were found in cultures incubated at 120 µmol m^{-2} s^{-1} photons.

Magwell et al (2023) evaluated the activities of the antioxidant enzymes of the *Spirulina platensis* grown in a modified Jourdan environment with the supplementation of different concentrations of sodium bicarbonate (4, 8, 12 and 16 g/L) and 0 g/L for control. They show that increasing the concentration of $NaHCO_3$ between 4 to 16 g/L increases peroxidase activity (POX) up to a highest value. A significant increase ($P > 0,05$) in polyphenol oxidase (PPO) activity was noted in mediums containing 16 and 8 g/L of $NaHCO_3$ ($0,32 \times 10^{-3} \pm 0,00006$ EU/µg and $0,24 \times 10^{-3} \pm 0.00005$ EU/µg, respectively) versus to control ($0,13 \times 10^{-3} \pm 0.000008$ EU/µg). The levels of PPO activity were highest in environments at 16 and 8 g /L of $NaHCO_3$ ($1,22 \times 10^{-3} \pm 0,0004$ EU/µg and $1,09 \times 10^{-3} \pm 0,0006$ UE/ µg, respectively) compared to control ($0,65 \times 10^{-3} \pm 0,004$ EU/µg).

Ismaiel et al (2016) investigated the effect of pH changes on antioxidant activity. The highest percentage of radical scavenging activity, reducing power and chelating activities were observed at pH 9.0, with an augmentation of 567%, 250% and 206%, respectively, in comparison control. They also observed that the activities of antioxidant enzymes were dependent on pH. They found that SOD activity was greatest at pH 10.5 (12.53±0.16 U/mg protein), while the activity level was lowest at pH 8.5 (10.16±0.60 U/mg protein). On the other hand, CAT activity was greatest at pH 7.5 (4.11±0.05 U/mg protein) and lowest at pH 11.0 (2.58±0.02 U/mg protein), while POD activity was highest at pH 7.5 (30.50 ± 0.80 U/mg protein) and lowest at pH 11.0 (17.50±0.25 U/mg protein).

The effect of CO_2 enrichment on the antioxidant activity of methanolic extracts of *A. platensis* was tested by Aydi et al (2020). TAC, DPPH free radical neutralizing activity and FRAP

were utilized to determine the antioxidant activity. TAC was between 25 to 35 mg GAE/g dry wet, with CO_2-free (-CO_2) and CO_2-supplied (+CO_2) media, respectively.CO_2 enrichment stimulated antioxidant power; the IC_{50} were enhanced by 25% and 65% on DPPH and FRAP, respectively.

Gheda et al (2023) investigated the antioxidant activity of *A. platensis* methanolic extract. The DPPH radical scavenging activity of *Arthrospira platensis* methanolic extract was 79.7%.

Chauhan et al (2023) evaluated the antioxidant potential of the extract's methanol, ethanol, acetone, ethyl acetate, hexane, and dichloromethane of *Arthrospira* sp. Each extract was prepared for antioxidant potential assessment by diluting it from an initial stock solution with a concentration of 2048 µg/mL to a final concentration of 1 µg/mL. Antioxidant potential in relation to % DPPH free radical scavenging activity ranged from 15.23±0.38 to 89.21±0.14. Methanol showed the highest inhibition value at 66 µg/mL.

The antioxidant and anticarcinogenic properties of *Nostoc linckia* extracts were studied by Ramadan et al (2021) under stress conditions induced by heavy metals (zinc and copper) at different concentrations. The results showed that the antioxidant activity of the crude extract (DPPH and ABTS essays) increased with zinc concentration, reaching maximum activity at 0.44 mg/L zinc (92.36±0.55 and 94.22±0.78% for DPPH with 30 and 60 min, respectively, and 81.22± 0.69% for ABTS). The lowest copper concentration (0.079 mg/L) also demonstrated significant antioxidant activity against DPPH, superior to standard synthetic BHT. Furthermore, the highest antioxidant activity by the ABTS essay was observed at the same copper concentration (60.09±1.00%).

2.3. Studies relating to antimicrobial activities of *Spirulina*

The methanolic extract of Moroccan *Spirulina* did not show antimicrobial activity against bacteria such as *B. subtilis*, *S. aureus*, *E. coli*, *M. luteus* and molds studied in the study by Seghiri et al (2019). Nevertheless, they found antifungal activity against *C. versicolor*, with a highest inhibitory concentration (MIC) between 1/250 and 1/100 v/v of 1 g/10 ml.

Aydi et al (2020) investigated the effect of CO_2 enrichment of *A. platensis* on antimicrobial activity in methanolic extracts of *A. platensis* against six Gram-positive bacteria (*Enterococcus faecalis; Listeria monocytogenes; Bacillus subtilis; Bacillus cereus; Bacillus thuringiensis; Micrococcus luteus)*) and three Gram-negative bacteria (*Salmonella enterica; Escherichia coli; Klebsiella pneumoniae*). The results showed that the Gram-positive bacteria with the maximum mean zone of inhibition were *L. monocytogenes* at 19.3±0.2 mm for extracts without CO_2 enrichment and 19.4±0.4 mm with CO_2 enrichment and *B. thuringiensis* at 19.6±0.1 mm for extracts without CO_2 enrichment and 18.3±0.2 mm with CO_2 enriched extracts. Among Gram-negative bacteria, however, the highest zone of inhibition was recorded against *K. pneumoniae* (16.2±0.3 mm for extracts without CO_2 enrichment and 16.3±0.2 mm for extracts with CO_2

enrichment). Of all the bacteria studied, *E. coli* had the lowest zone of inhibition (13.4±0.3 mm for extracts without CO_2 enrichment and 16.3±0.2 mm for extracts with CO_2 enrichment).

Gheda et al (2023) investigated the antifungal activities of *Arthrospira platensis* extracts against many pathogenic fungi, including *Candida albicans*, *Trichophyton rubrum* and *Malassezia furfur*, using various solvents including ethanol, methanol, ethyl acetate, and acetone. The methanolic extract of *A. platensis* inhibited *Candida albicans* the most, with a diameter of 19.2 mm, followed by *Malassezia furfur* (17.3 mm) and *Trichophyton rubrum* (11 mm). Ethyl acetate extract, on the contrary, had a considerable influence on *Candida albicans*, reaching a zone of inhibition of 16 mm, followed by *Malassezia furfur* at 12.6 mm, and did not affect *Trichophyton rubrum*. Acetone extract inhibited *Candida albicans* reaching a zone of inhibition of 16.3 mm, *Malassezia furfur* with a zone of inhibition of 10.7 mm, and *Trichophyton rubrum* with no effect. For the Minimum Inhibitory Concentration (MIC), the lowest concentration of the methanolic extract of *Arthrospira platensis* only inhibited the growth of *Candida albicans*. Methanolic extract of *Arthrospira platensis* was effective against *Candida albicans* and *Malassezia furfur* at 8.29 mg/mL. Growth inhibition of all fungi studied increased with concentration, reaching a maximum of 66 mg/mL, with the highest zone of inhibition observed in *Candida albicans*.

A study revealed that extracts of *Arthrospira* sp have variable antibacterial activity against bacteria resistant to several antibiotics. The MeOH extract showed the highest activity in inhibition zone terms (24.20±0.1 mm) against *E. coli*. In contrast, the EtOH extract recorded relatively lower activity (9.01 mm to 13.29±0.3 mm). The Ace, AcOEt and DCM extracts showed no significant activity, as a small zone of inhibition (8 mm) was recorded against *E. coli*, *P. aeruginosa* and *S.* Typhimurium. The hexanal extract showed no antibacterial activity. The estimated MIC for methanol extracts was 256 µg/mL against *E. coli*, then 512 µg/mL against *E. albertii* and *P. fluorescence*, but was greater than 2048 µg/mL against *P. aeruginosa*, *S.* Typhimurium and *S. dysenteriae* (Chauhan et al., 2023).

Singh et al (2021) evaluated the antibacterial activity of various crude *Spirulina* extracts against *S. aureus*, *E. coli* and *S.* Typhi, using various concentrations of methanol, ethyl acetate and acetone. The results showed variable antibacterial activity depending on the solvent and the bacterial strain targeted. Ethyl acetate (50%) showed development inhibition of *E. coli* and *S.* Typhi, whereas methanol nor acetone (50%) were ineffective. The crude extract obtained in acetone (100%) exhibited the highest inhibition zone of 12 mm against *S.* Typhi, while the crude extract obtained in ethyl acetate (100%) was efficient against *E. coli* and *S.* Typhi at 10 and 11 mm, respectively. Only the 100% methanol crude extract showed zones of inhibition against *Candida* (8 mm) and *Cryptococcus* (10 mm). However, the other concentrations of the crude methanol extracts did not produce any effect on the fungi.

According to Ozdemir et al (2004) methanol extracts of *Spirulina* exhibited higher antimicrobial effects compared to ethanol, hexane, acetone and chloroform extracts. This activity

was comparable to that of tobramycin (10 µg/disc), particularly against *Streptococcus*, *Staphylococcus* and *Candida*.

Spirulina butanol extract demonstrated higher antimicrobial activity compared to other solvents against *Staphylococcus epidermidis* (19 mm), *Staphylococcus aureus* and *Aeromonas liquefaciens* (18 mm), *Candida glabrata* (13 mm), *Enterococcus faecalis* (12 mm), *Campylobacter coil* and *Vibrio cholerae* (11 mm); and lower activity against *Salmonella* Typhi (5 mm) (Sivakumar and Santhanam, 2011).

2.4. Bioaccumulation of trace elements in microalgae

Bioaccumulation refers to the process by which living cells absorb and store pollutants or toxins, resulting from a balance between absorption and elimination. It is a term often used alongside biomagnification to explain the toxicity of metals. Biomagnification refers to the increase in pollutant concentration as it moves from the environment to living organisms. The extent of bioaccumulation determines the expression of toxic effects. This process describes the transfer of pollutants and metals into a food chain and the accumulation of contaminants in the biological tissues of aquatic organisms. These contaminants come from water, food and suspended sediment (Blowes et al., 2003; Wang and Fisher, 1999).

According to Blowes et al (2003)"absorption" designates the penetration of a chemical substance into an organism through respiration, ingestion or dermal absorption, without taking into account the storage, metabolism and elimination that occur. Bioconcentration is a type of bioaccumulation in which the level of a chemical substance in an organism exceeds that in the atmosphere or water surrounding the organism. Nevertheless, the process is identical for natural and artificial chemicals, "bioconcentration" is generally used to designate chemicals extraneous to the organism. In fish and other marine animals, bioconcentration following absorption via the gills or, in certain cases, via the skin is the principal bioaccumulation process. The term "biomagnification" designates the trend of contaminants to aggregate as they pass from one trophic level to the next. This process takes place when a chemical or metal increases in concentration as it passes up the food chain, that is, when feeding interactions among unicellular organisms and higher animal develop. The natural bioaccumulation process is important to organisms' growth and development.

Several studies have shown that the bioaccumulation of heavy metals by algae can effectively remediate and control pollution and treatment in aquatic environments. Absorption, storage in cells and excretion depend on environmental factors, the characteristics of the microorganism species and the nature of the pollutant.

Arunakumara and Zhang (2008) describe the mechanism of metal bioaccumulation. Heavy metals are introduced into algal cells by active transfer or endocytosis via chelating proteins, impacting a range of algal physiochemical processes. Toxicity results mainly through binding to protein sulphydryl groups, disruption of protein structure or displacement of crucial elements.

Metals may disrupt the oxidation balance of algae, triggering antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and ascorbate peroxidase. The quantity of proteins and lipids oxidized in algal cells indicates the severity of the damage. The defense reaction against probable oxidative damage largely influences the tolerance of algae to metals. The synthesis of binding agents and proteins and the selective ion transporters' role in keeping metals out of cells, along with processes like excretion or compartmentalization, may mitigate the harmful effects of heavy metal toxicity. However, a comprehensive understanding of the mechanisms responsible for metal toxicity in microalgae and the development of tolerance mechanisms remains to be fully established.

Microalgae have reactive groups with active binding sites that may interact with wastewater contaminants to generate chemicals. Total dissolved solids and total suspended solids are reduced throughout this flocculation process. Some cyanobacteria, like *Anabaena*, *Oscillatoria*, *Phormidium*, and *Spirogyra*, can naturally survive in heavy metal-contaminated water due to their resistance to heavy metal stress (Balaji et al., 2016; Leong and Chang, 2020).

Heavy metals, such as mercury, chromium, cadmium, lead, arsenic, nickel, zinc, and copper, are dangerous even at low concentrations. They exist in the environment due to numerous industrial, residential, agricultural, medical, and technological uses. Heavy metals can persist in aquatic environments and biomagnify throughout the food chain due to natural processes and human activities such as urbanization, industrialization and wastewater discharges. They can cause respiratory, kidney, mental and cardiovascular disorders and lead to cancer (Chugh et al., 2022). Trace elements, on the other hand, are found in trace amounts in both natural and disturbed ecosystems, and excessive bioavailability can be hazardous to living species. Essential heavy metals needed for organisms, such as manganese, iron, cobalt, copper, and zinc, may be detrimental when their amounts exceed safe limits (Ali et al., 2019). So-called non-essential heavy metals, including cadmium, lead and mercury, are highly toxic but have no biological role to play. Moreover, some heavy metals are also essential for plants. They are used as trace elements by plants, contributing to growth, stress resistance and synthesizing important metabolites. Understanding the distribution and impact of heavy metals and trace elements is therefore crucial for assessing the state of the environment and protecting flora, fauna and human well-being.

Chojnacka (2007) investigated *Chlorella vulgaris*'s capacity to bioaccumulate metals. Her results demonstrated that the *Chlorella vulgaris* cell surface had three functional groups involved in metal bioaccumulation. *C. vulgaris* has naturally high quantities of microelements, including 380 mg/kg copper, 690 mg/ kg manganese, 1.600 mg/kg zinc, and 240 mg/ kg chromium.

Chojnacka et al (2005) study focuses on the biological uptake of heavy metal ions (Cr^{+3}, Cd^{+2}, Cu^{+2}) by *Spirulina platensis*. They used potentiometric titration and adsorption methods to assess binding capacity on the cell surface. Results showed rapid uptake and equilibrium governed by the Langmuir equation. Kinetic experiments showed that the equilibrium point was reached rapidly in less than 5 to 10 minutes. Biological uptake was mainly chemical rather than physical.

Metal ions were bound to functional groups on the cell surface, and changes occurred as a function of pH.

Liz et al (2005) studied the Cr (III) uptake mechanism by *Spirulina platensis* and explained this mechanism using thermodynamic isotherms. When chromium metal is present in the culture medium, it is initially physically adsorbed onto the algal cell wall by electrostatic effect, and most of the absorbed Cr is firmly bound to proteins, lipids, and polysaccharides. They reported that, in addition to factors such as temperature, light and cell concentration, pH was an important factor in chromium uptake by *Spirulina platensis*.

Ayed et al (2017) evaluated the uptake of Mg^{2+} ions by *Chlorella vulgaris* under mixotrophic systems (10 g/L of glucose) in an agitated photobioreactor. The results showed that *Chlorella vulgaris* reached a higher biomass level of 1.2 g/L with glucose consumption of 8.5 g/L and nitrogen (as nitrate) consumption of 0.5 g/L. Mg^{2+} ion uptake was measured in a culture medium containing an initial magnesium concentration of 17.7 mg/L. An average clearance rate of 4.0 mg Mg^{2+}.g/dry biomass during the autotrophic growth phase and 2.3 mg Mg^{2+}.g/dry biomass during the heterotrophic growth phase was reported. At the experiment's end (330 h), around 90% of the starting amount of Mg^{2+} in the medium was related to the microalgal biomass, with 4% adsorbed and 86% taken up by the cells.

2.5. Generality

2.5.1 Zinc

Physical and chemical properties

Zinc is an indispensable chemical element for life and a metal with a low melting point in the periodic table's Group 12 (formerly Group IIb or the zinc family). It is necessary for vital activity and is one of the Earth's most common and abundant metals. Zinc is represented by the symbol 'Zn' and has an atomic number of 30 and a standard atomic weight of 65.38 amu. On average, every metric tonne of the Earth's crust accounts for about 65 grams. Zinc is found mainly in franklinite, gahnite, goslarite, hemimorphite (zinc silicate), willemite, wurtzite, and sphalerite. Sphalerite (zinc sulphide) is the most abundant source of zinc. It is also present in minerals like smithsonite (zinc carbonate) and zincite (zinc oxide) in the +2 oxidation state (Goodwin, 2000).

Zinc is one of the most common and essential metals, with a bluish-white appearance, found in the Earth's crust, air, water, and nearly all food items. It holds significant commercial importance. The primary zinc mineral is sphalerite (zinc blende), which, along with its oxidation products smithsonite and hemimorphite, accounts for most of the world's zinc ores. Zinc is a complex, brittle metal at most temperatures. However, it changes between 100 and 150°C and becomes soft and malleable. Various forms of zinc exist, such as zinc carbonate, chloride, zinc acetate, and zinc sulfide (Environment Health Criteria, 2001).

Zinc in the environment

Zinc is a natural element in the earth's crust and an essential component of our environment. It is present in the lithosphere (earth), hydrosphere (water), biosphere (plants, animals and humans) and atmosphere (air). According to the American Galvanizers Association, zinc makes up 0.004% of the earth's crust, ranking 24th in order of abundance (https://galvanizeit.org). It is introduced into the environment through natural processes and human activities such as erosion, industrialization, metallurgy and mining. Zinc concentrations can be high at industrial and mining sites, and in soils where zinc-based fertilizers are overused. It can contaminate water and soil through runoff and infiltration, damaging aquatic life, plants and animals. Although zinc is necessary for human health, excessive exposure can be dangerous; however, cases of acute poisoning are rare. To reduce the risks to the environment and human health, it is essential to effectively regulate zinc emissions and monitor contamination levels.

As a result, on the one hand, the pollution of all ecosystems, and on the other, the race to recover secondary raw materials, i.e. the recycling of zinc-containing industrial waste, has become a global challenge. Several sustainable techniques have been developed to protect the environment and benefit the economy.

Zinc in water

Zn concentrations in surface waters are typically less than 10.0 µg/L, but groundwater values range between 10 and 40 µg/L (Elinder,1986). The average Zn concentrations in Chinese drinking water were 0.29 mg/l, below the EPA's equivalent level and WHO guideline values. The average daily intake of Zn in drinking water was 0.65 mg/d, i.e., 1.1% of the provisional tolerable daily intake (PMDI) set by the Joint FAO/WHO Expert Committee (Xu et al., 2006).

Kaçar et al (2022) found a higher Zn concentration in IR in the summer with a level of 2.343 mg/L which is higher than the Turkish Standards Institute (TS 266; 2005) 2,000 mg/L and lower than the Word Health Organization (WHO, 2011) 3,000 mg/L and the USA Environmental Protection Agency (EPA, 2008) 5.000 mg/L. However, the average Zn value (0.414±0.644 mg/L) in Çanakkale tap water does not exceed the thresholds set by national and international standards.

It is therefore important to analyze metal levels systematically and periodically to assess the daily intake of metals in drinking water and to prevent contamination risks and dangers to human health. Heavy metal concentrations in drinking water are hazardous to human health. They can build up in the human body and create physiological and morphological problems like cancer.

Zinc in food

Zinc is present in almost all food matrices at varying levels. Foods of protein-rich animal origin, such as meat products, shellfish and fish, are essential sources of (Cosgrove, 1980) zinc. For example, oysters have the highest zinc concentration per serving (28.2-32 mg) of all foods

(https://ods.od.nih.gov/factsheets). Beef also contributes around 20% of the zinc intake in the American diet (Foster et al., 2013). Plant-based products such as whole grains, legumes and nuts are also significant sources of zinc. Fruits, vegetables, and tubers are moderately low in zinc (Table 2.1). The difference in zinc content between animal and vegetable products depends on the quantity of food and the bioavailability of zinc. The bioavailability of zinc in plant-based foods is lower than in animal-based foods due to the presence of phytates.

Phytate, or phytic acid (inositol hexaphosphate), is the main form of phosphorus (P) and inositol storage in plants (Cosgrove, 1980). It has anti-nutritional activities by binding minerals such as zinc, iron and calcium, forming complexes that make their intestinal absorption difficult in humans and monogastric animals such as pigs and horses (Raboy, 2001).

Table 2.1. Zinc content, zinc density, phytate content, and phytate-to-zinc molar ratios of commonly consumed foods; data derived from the International MiniList[a] (Brown et al, 2004)

Food groups	Zinc content		Phytate content	
	mg/100 g	mg/100 kcal	mg/100 g	Phytate: zinc molar ratio
Liver, kidney (beef, poultry)	4.2–6.1	2.7–3.8	0	0
Meat (beef, pork)	2.9–4.7	1.1–2.8	0	0
Poultry (chicken, duck, etc.)	1.8–3.0	0.6–1.4	0	0
Seafood (fish, etc.)	0.5–5.2	0.3–1.7	0	0
Eggs (chicken, duck)	1.1–1.4	0.7–0.8	0	0
Dairy (milk, cheese)	0.4–3.1	0.3–1.0	0	0
Seeds, nuts (sesame, pumpkin, almond, etc.)	2.9–7.8	0.5–1.4	1,760–4,710	22–88
Beans, lentils (soy, kidney bean, chickpea, etc.)	1.0–2.0	0.9–1.2	110–617	19–56
Whole-grain cereal (wheat, maize, brown rice, etc.)	0.5–3.2	0.4–0.9	211–618	22–53
Refined cereal grains (white flour, white rice, etc.)	0.4–0.8	0.2–0.4	30–439	16–54
Bread (white flour, yeast)	0.9	0.3	30	3
Fermented cassava root	0.7	0.2	70	10
Tubers	0.3–0.5	0.2–0.5	93–131	26–31
Vegetables	0.1–0.8	0.3–3.5	0–116	0–42
Fruits	0–0.2	0–0.6	0–63	0–31

a. WorldFood Dietary Assessment Program, 2.0, University of California, Berkeley

Vegetarians and vegans have lower dietary zinc intakes and serum zinc levels than non-vegetarians (Foster et al., 2013). It should be noted, however, that well-planned vegetarian diets can offer enough levels of zinc from plant sources. Fermented foods such as tofu and fortified morning cereals are examples of complete cereals (Saunders et al., 2013).

Zinc requirements

Zinc requirements in humans vary according to age, sex, physiological state (such as pregnancy or breastfeeding) and the amount of zinc absorbed and excreted by the body. Since the mid-1990s, several organizations, including the WHO, the FAO, the International Atomic Energy Association and the Food and Nutrition Board (FNB) of the Institute of Medicine (IOM), have used a factorial method to calculate the average physiological zinc requirement (Table 2.2).

Table 2.2. Estimated physiologic requirements for absorbed zinc during childhood by age group and sex, and pregnancy and lactation, as developed by expert committees of the WHO, the US FNB/IOM and as reviewed by IZiNCG

WHO			**FNB/IOM**			**Revisions suggested by IZiNCG**		
Age, sex	Reference weight (kg)	Physiologic requirement (mg/day)	Age, sex	Reference weight (kg)	Physiologic requirement (mg/day)	Age, sex	Reference weight (kg)	Physiologic requirement (mg/day)
6–12 mo	9	0.84	7–12 mo	9	0.84	6–11 mo	9	0.84
1–3 yrs	12	0.83	1–3 yrs	13	0.74	1–3 yrs	12	0.53
3–6 yrs	17	0.97	4–8 yrs	22	1.20	4–8 yrs	21	0.83
6–10 yrs	25	1.12						
10–12 yrs, M	35	1.40	9–13 yrs	40	2.12	9–13 yrs	38	1.53
10–12 yrs, F	37	1.26						
12–15 yrs, M	48	1.82						
12–15 yrs, F	48	1.55						
15–18 yrs, M	64	1.97	14–18 yrs, M	64	3.37	14–18 yrs, M	64	2.52
15–18 yrs, F	55	1.54	14–18 yrs, F	57	3.02	14–18 yrs, F	56	1.98
Pregnancy	—	2.27	Pregnancy (1st, 2nd, 3rd trimester)	—	4.12, 4.42, 5.02	Pregnancy	—	2.68
Lactation	—	2.89	Lactation (0–3, 3–6, 6–12 mo)	—	4.92, 3.82, 4.52	Lactation	—	2.98

International Zinc Nutrition Consultative Group (IZiNCG), Hotz C, Brown KH. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 2004;25(suppl 2): S99-203.

Zinc deficiency occurs when insufficient zinc intake meets the body's physiological needs. Inflammatory bowel disease, ulcerative colitis, Crohn's disease, malabsorption syndrome (Valberg et al., 1986), chronic liver or kidney disease, diabetes, malignant tumors and other chronic diseases (Prasad, 2013) chronic diarrhoea/tropical enteropathy (Akhtar, 2013) all lead to excessive zinc loss.

Importance of zinc

Zinc is an essential trace element that plays many vital roles in the body. It is involved in many biological functions and is considered a versatile trace element due to its ability to bind to over 300 enzymes and more than 2 000 transcription factors (Chasapis et al., 2020). Zinc is required for many processes, including gene expression, enzymatic reactions, immune function, protein synthesis, DNA synthesis, wound healing, growth and development. Zinc also plays a role in the proper functioning of lipid and glucose metabolism and the regulation of insulin expression (Hara et al., 2017). It also reduces oxidative stress by participating in the synthesis of antioxidant enzymes. Due to its unique chemical properties, zinc is crucial to many biological processes. It has a strong affinity for electrons and generally binds to amino acids, peptides and nucleotides. This ability gives biological systems essential catalytic and structural functions (Shrimpton, 2001). Long-term zinc intake can stimulate the production of antioxidant proteins such as metallothioneins. Metallothioneins are cysteine-rich proteins that protect against free radicals and toxic metals such as iron and copper (Mezzetti et al., 1998).

2.5.2. Magnesium

Physical and chemical properties

Magnesium has a silvery or white appearance and belongs to the alkaline-earth metal group. It is relatively reactive, but less so than alkali metals such as sodium or potassium. In aqueous media, it forms Mg^{2+} ions and has the particularity of forming certain highly soluble salts.

Magnesium has a relatively low density of around 1.74 g/cm^3 and a melting point of around 650°C (Campbell, 2012). With the chemical symbol Mg, atomic number 12 and atomic mass 24.305 u, magnesium is an essential catalyst for photosynthesis in plants. It is also widely used in industry, particularly in sectors requiring light metal alloys. Given this advantage, magnesium alloys are attracting interest from several industrial sectors. They are used in automotive, aerospace, and personal electronics due to their low weight and the ease with which they are malleable (Atrens et al., 2018).

Magnesium in the environment

Magnesium is the sixth most abundant metal and one of the most common elements on the planet. It generally occurs in salt form and has a 0.13% concentration in saltwater. In 1808, it was first found in metallic form (Barbare, 2005). Certain human activities, such as fuel combustion and producing magnesium-based products and materials, can release magnesium into the environment. It occurs as magnesite (magnesium carbonate, $MgCO_3$), dolomite (calcium magnesium carbonate Ca Mg $(CO_3)_2$) and brucite (magnesium hydroxide Mg $(OH)_2$). It is also found as carnallite (magnesium chloride, $KMgCl_3 \cdot 6H_2O$) and kieserite (magnesium sulphate $MgSO_4 \cdot H_2O$) and in many common silicates, including talc, olivine and majority types of asbestos (Chen et al., 2023; Loring et al., 2019). It is present in minerals such as serpentine, chrysolite and sea foam. Seawater contains around 0.13% magnesium, mainly dissolved chloride, which gives it its characteristic bitter taste (Chapman, 2002). Magnesium is an important nutrient for human, animal, and plant well-being.

Magnesium in water

Magnesium can be found in particularly all natural water sources, such as rivers, lakes, seas, and oceans. It is dissolved in water by erosion, soil leaching and anthropogenic activities. Magnesium concentrations in natural waters vary depending on the geological composition of the region. Seawater contains about 1.3 g/L of magnesium, mainly in the form of dissolved chloride, which gives it its characteristic bitter taste (Tran et al., 2013).

Although present at low levels, drinking water is a significant source of daily intake of trace elements that play a key role in many biochemical reactions in living organisms. Their content is subject to national and international standards. Regarding the magnesium content in drinking water, the Turkish Standards Institute (Türk Standartları Enstitüsü) and the European Union (EC) have set a limit of 50 mg/L (https://dobisu.marmara.edu.tr/orta-menu/yararli-bilgiler/icme-suyu-kabul-edilebilir-degerler).

Magnesium in food

Magnesium is widely found in foods of plant and animal origin, as well as in beverages. Green leafy vegetables are excellent sources of magnesium. Legumes, nuts, and whole grains are also rich in magnesium (Fox et al., 2001b). Some food sources of magnesium are shown in Table 2.3.

Table 2.3. Magnesium Content of Selected Foods (ods.od.nih.gov/factsheets/Magnesium-HealthProfessional)

Food	Milligrams (mg) per serving	Percent DV*
Pumpkin seeds, roasted, 1 ounce	156	37
Chia seeds, 1 ounce	111	26
Almonds, dry roasted, 1 ounce	80	19
Spinach, boiled, ½ cup	78	19
Cashews, dry roasted, 1 ounce	74	18
Peanuts, oil roasted, ¼ cup	63	15
Cereal, shredded wheat, 2 large biscuits	61	15
Soymilk, plain or vanilla, 1 cup	61	15
Black beans, cooked, ½ cup	60	14
Edamame, shelled, cooked, ½ cup	50	12
Peanut butter, smooth, 2 tablespoons	49	12
Potato, baked with skin, 3.5 ounces	43	10
Rice, brown, cooked, ½ cup	42	10
Yogurt, plain, low fat, 8 ounces	42	10
Breakfast cereals, fortified with 10% of the DV for magnesium, 1 serving	42	10
Oatmeal, instant, 1 packet	36	9
Kidney beans, canned, ½ cup	35	8
Banana, 1 medium	32	8
Salmon, Atlantic, farmed, cooked, 3 ounces	26	6
Milk, 1 cup	24–27	6
Halibut, cooked, 3 ounces	24	6
Raisins, ½ cup	23	5
Bread, whole wheat, 1 slice	23	5
Avocado, cubed, ½ cup	22	5
Chicken breast, roasted, 3 ounces	22	5
Beef, ground, 90% lean, pan-broiled, 3 ounces	20	5
Broccoli, chopped and cooked, ½ cup	12	3
Rice, white, cooked, ½ cup	10	2
Apple, 1 medium	9	2
Carrot, raw, 1 medium	7	2

*DV = Daily Value. The U.S. Food and Drug Administration (FDA) developed DVs to help consumers compare the nutrient contents of foods and dietary supplements within the context of a total diet. The DV for magnesium is 420 mg for adults and children aged 4 years and older (food-labeling-revision-of-the-nutrition-and-supplement-facts-labels). FDA does not require food labels to list magnesium content unless magnesium has been added to the food.

Foods providing 20% or more of the DV are considered to be high sources of a nutrient, but foods providing lower percentages of the DV also contribute to a healthful diet.

The U.S. Department of Agriculture's (USDA's) FoodData Central (https://fdc.nal.usda.gov/) lists the nutrient content of many foods and provides a comprehensive list of foods containing magnesium arranged by nutrient contain and by food name.

Recommended magnesium intakes

Recommended magnesium intakes vary according to age and sex. Table 2.4 shows recommended daily intake values for different population categories.

Table 2.4. Recommended Dietary Allowances (RDAs) for Magnesium (IOM,1997)

Age	**Male (mg)**	**Female (mg)**	**Pregnancy (mg)**	**Lactation(mg)**
Birth to 6 months	30	30		
7–12 months	75	75		
1–3 years	80	80		
4–8 years	130	130		
9–13 years	240	240		
14–18 years	410	360	400	360
19–30 years	400	310	350	310
31–50 years	420	320	360	320
51+ years	420	320		

Importance of magnesium

It participates in approximately 300 processes involving enzymes. It is necessary for muscular function, a regular heartbeat, a healthy immune system, and blood pressure control. It also involved energy metabolism, protein synthesis, and nerve signal transmission (Jahnen-Dechent and Ketteler, 2012). Magnesium, like other electrolytes such as sodium, potassium and calcium, plays a crucial role in many physiological processes and is precisely regulated to maintain a proper balance in the human body (Glasdam et al., 2016). Many foods contain magnesium, and vitamins are often supplemented with it. Magnesium sulfate can be used topically as a dip, internally as a laxative or intravenously during pregnancy to control eclampsia and uterine activity (Barbare, 2005).

2.5.3. *Spirulina*

Spirulina is a blue-green cyanobacterium due to the presence of the pigments phycocyanin (blue) and chlorophyll (green). It is a photoautotrophic prokaryote capable of photosynthesis. *Spirulina* microalgae photosynthesize from mineral elements, water in the culture medium and light, thanks to their chlorophyll in the presence of light. In this way, *Spirulina* absorbs carbon dioxide and releases oxygen. *Spirulina* grows naturally in specific warm (around 30 °C), basic, brackish lakes in the tropics of Africa and Central America. This cyanobacterium discovered around 3.5 billion years ago, is one of planet oldest life forms (Schoofs et al., 1997). It was in 1844 that Wittrock and Nordsted described *Spirulina* as *Spirulina jenneri plantensis* Nordsted (Vonshak, 1997).

This microalga was rediscovered until 450 years later by Belgian botanist J. Léonard during a Belgian-French trip to Chad (1964-1965). He observed that the Kanembou of southern Kanem harvested these abundant algae from the natural ponds around Lake Chad, which are rich in sodium carbonates. They made a blue-green purée from it to prepare sun-dried local cakes called "dihé". As early as 1940, French phycologist Dangeard examined these cakes and found them made from edible blue-green algae. Subsequently, by studying samples from Léonard's expedition, Belgian researcher Compère confirmed that these cakes were mainly composed of the *S. platensis*. The Belgian researchers demonstrated that they were extremely protein-rich (Léonard and Compère, 1967). *Spirulina platensis* was subsequently cultured, and its composition was established in the 70s by Delpeuch et al (1975) and Sautier et al (1975). Since the 1980s, these blue-green algae have attracted growing interest in scientific research. Researchers worldwide have conducted numerous studies to better understand the positive effects of daily *Spirulina* consumption on being well. These studies have examined various aspects, such as *Spirulina*'s nutritional composition, effects on the immune system, role in combating malnutrition, antioxidant properties, metabolism, etc.

However, despite the many studies carried out, it's important to note that *Spirulina* research is still ongoing, and new discoveries are regularly made to confirm existing results and explore new areas of application due to its richness in nutrients and bioactive compounds.

The two best-known *Arthrospira* species are *Arthrospira platensis* and *Arthrospira maxima*. Alternatively, *Spirulina platensis* refers to *Arthrospira platensis* (Gershwin and Belay, 2007). *Spirulina* measures around 250 µm and 10 to 12 µm in diameter. Size generally varies according to strain and culture conditions. *Spirulina platensis* from Chad can reach 350 µm and a diameter of between 6 and 12.45 µm. It is the best known and most widely cultivated in the world. *Spirulina maxima* is distinguished by trichomes 70 µm 80 µm long and 7 to 9 µm in diameter (Fox, 1999a). It is made up of trichomes coiled in the form of spires (spira from Latin), supposedly end to end, called filaments (figure 2.1). This filament, in the form of a tiny *Spirulina* spiral, is the origin of the name "*Spirulina*". Its helical shape enables it to move by motility (Charpy et al., 2008). It is classified in the plant kingdom on account of its high chlorophyll content, and in the bacterial kingdom on account of its biochemical characteristics.

(A) (B) (C)

Figure 2.1. (A) Trichome of *Arthrospira platensis*. Trichome diameter ~12µm (Borowitzka, 2018). (B) Trichome (Sili et al., 2012). (C) *Spirulina platensis* filament (objective x40) (original)

Natural habitat

The great lakes of Africa and Central America, such as Lake Chad and Lake Texcoco, as well as the lakes of the Great Rift Valley in East Africa, are regions where the *Spirulina* species can be found naturally when water conditions are suitable for its growth. This strict photoautotrophic cyanobacterium grows naturally in warm (30 to 40 degrees Celsius), alkaline (pH 8 - 11.5), soft waters, preferably highly mineralized (nitrate, phosphate, iron) waters rich in sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3). For several centuries, lakes Bodou and Rombou in Chad have been home to a stable monoculture of *Spirulina*. The alkaline environment in which *Spirulina* thrives makes it difficult, if not impossible, for other micro-organisms to survive (Kebede and Ahlgren, 1996) . Moreover, in its natural state, because of its thermophilic nature and its need for light for photosynthesis, this microalgae colonizes certain lakes in tropical Africa and Central America (Holman and Malau-Aduli, 2013) .

***Spirulina* cultivation**

Nowadays, growing *Spirulina* is relatively simple. It can be found just about anywhere in aquatic and terrestrial environments. This ease of cultivation has made it, along with other microalgae such as *Chlorella* and *Dunaliella*, the focus of research in many biotechnology laboratories.

Two microalgae production systems exist open ponds and closed systems, known as photobioreactors and bioreactors. Open systems can be either natural (Figure 2.2) or artificial (circular or raceway) (Figure 2.3).

(A)

(B)

Figure 2.2. (A) *Spirulina* in Lake Chad (https://www.unesco.org). (B) *Spirulina* harvesting by the Aztecs in the lakes of the Valley of Mexico. (Peter, 1978).

Today, open microalgae production systems are the most widespread worldwide, particularly in developed countries. This system is economical. Soni et al (2017)have shown that this system can have its drawbacks. The disadvantages of open ponds are poor light utilization, evaporation losses, carbon dioxide diffusion and land clutter. Inefficient aeration leads to low mass transfer rates, reduced biomass productivity and contamination by fast-growing organisms. Researchers are proposing closed systems combined with new technologies to solve these problems.

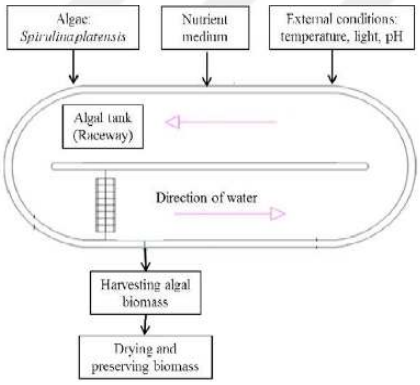


Figure 2.3. (A) Raceway tank for *Spirulina* cultivation. (B) Image Algal Biotechnology culture unit outdoor of Çukurova University in Adana, Turkey(original).

Photobioreactors are closed, lighted vessels used for the controlled cultivation of biomass. They are made of transparent materials to ensure sufficient light exposure for cultivation. Photobioreactors provide a controlled environment and high productivity in the cultivation of microalgae, allowing precise regulation of cultivation parameters such as CO_2 supply, water availability, optimum temperature, light intensity, levels pH, gas exchange, aeration and crop density. These cultivation systems can be illuminated using either artificial or natural light sources, or a combination of both. In the laboratory, fluorescent lamps or external light sources are commonly used to artificially illuminate photobioreactors. Temperature control is relatively

straightforward in some photobioreactors, although maintaining optimal conditions in large-scale outdoor systems requires significant technical expertise. Despite the associated costs, photobioreactors offer many advantages over open systems, including minimized contamination risks, improved environmental control, reduced carbon dioxide losses, prevention of water evaporation, potential higher cell concentrations, improved production of complex biopharmaceuticals, the ability to grow diverse species of microalgae, uniform light distribution, and minimal areas without light exposure. For the cultivation of microalgae and cyanobacteria, flat-plate photobioreactors (Figure 2.4 A) and tubular photobioreactors (Figure 2.4 B) are two commonly used types of systems (Soni et al., 2017). Many other types include coils, rigid panels, and ring reactors (Richmond, 2007).

(A) (B)

Figure 2.4. (A) Flat plate Photobioreactors, (B) Tubular Photobioreactors (Soni et al., 2017)

Growth

Algae have a wide variety of nutritional mechanisms. Most algae are photoautotrophic, meaning they use light, carbon dioxide and water to produce organic compounds through photosynthesis. As inorganic carbon sources for photosynthesis, they can absorb both dissolved CO_2 and bicarbonate (HCO_3^-). However, some algae have more specific nutritional needs and require organic molecules, such as vitamins, in addition to carbon dioxide, for their growth. These are known as auxotroph. On the other hand, some algae can adopt a heterotrophic, or chemoorganotrophic, mode of nutrition, feeding on simple organic compounds such as acetate or glucose in the absence of light. Thus, algae have developed a diversity of nutritional strategies to adapt to different environments and growth conditions (Borowitzka, 2018).

Spirulina is an aerobic photoautotrophic species, thanks to its chlorophyll pigments. Unlike anaerobic photoautotrophic bacteria, which possess photosystem I only, *Spirulina* possesses both photosystems I and II (Merceron, 2006).

Photosynthesis is a crucial physiological process, essential to life in all animal and human forms. It enables a photosynthetic organism to produce the organic matter necessary for its development by using light to transform mineral elements, carbon dioxide and water, and by producing oxygen.

To carry out photosynthesis, *Spirulina* requires water, carbon and nutrients, notably nitrogen. It takes in atmospheric carbon dioxide as a source of mineral carbon, which it transforms into biochemically available energy in the form of glucose. Unlike other cyanobacteria, *Spirulina* lacks the complete Krebs cycle (Fox, 1999a; Doumenge et al., 1993).

Chlorophyll pigments capture light energy. In *Spirulina* and other photosynthetic bacteria, chlorophyll is found in compartmentalized areas of the cell wall known as thylakoid phycobilisomes. Photosynthesis is composed into two phases: "light" reactions, which need the presence of light and take place in thylakoid membranes. The thylakoids comprise photosynthetic pigments arranged into two photosystems: PS I and PS II. These photosystems are located in membranes common to two thylakoids, and are generally near each other (Morot-Gaudry, 2006)

Photosystem I (PS I) has a higher diversity of pigments than Photosystem II (PS II) and is abundant in chlorophyll P700, which uptake at wavelengths of 430 nm in blue and 700 nm in red. Electrons come from minerals and organic molecules. Photosystem II is rich in P680-type chlorophyll *a*. This is where water photolysis occurs, where electrons released by water molecules are captured. Phycobiliprotein has a prolonged absorption capacity thanks to phycocyanobilin, a chromophore. In addition, it exhibits optimal energy overlap to transfer energy to photosystem II (Shively et al., 2009).

During the light phase, water photolysis occurs, separating oxygen and hydrogen molecules. The synthesis of ATP, a molecule that liberates large amounts of energy when consumed, and NADP is also observed (Morot-Gaudry, 2006)

Dark reactions occur in the thylakoid's stroma (matrix in which the energy generated in the light phase is accumulated in the form of ATP and reduced NADP. That energy is utilized to synthesize organic molecules by reducing carbon dioxide. The dark phase takes place in the absence of light, although this does not mean that it takes place exclusively at night. These reactions form the Calvin cycle, which leads to the synthesis of glucose (Morot-Gaudry, 2006).

The general formula for photosynthesis can thus be expressed as follows:

$$6\ CO_2 + 6\ H_2O + (\text{light energy}) \rightarrow C_6H_{12}O_6 + 6\ O_2$$

Although *Spirulina maxima* contains only chlorophyll *a*, other types of chlorophyll, such as chlorophylls *b*, *c* and *d*, are found in other types of algae.

All chlorophyll pigments are chromoproteins with a common molecular structure, featuring a hydrophilic porphyrin-based prosthetic group constituted of four tetrapyrrolic rings centered on a magnesium atom and esterified with a hydrophobic carbon alcohol called phytol. The differences between chlorophylls lie in their pyrrolic substituents, molecular structure, and light absorption

wavelength. In thylakoids, chlorophyll granules are associated with proteins, forming pigment-protein complexes.

Chlorophylls are photosynthetic pigments that are excitable by light. Their excitability is caused by the existence of conjugated bonds with delocalized electrons. When excited by a photon, electrons change from a ground state to an excited state.

Chlorophyll then returns to its ground state, which can happen in several ways: by emitting light (fluorescence), by transferring energy to a neighbouring molecule (resonance), or by losing electrons (photochemistry), enabling chlorophyll to trap electrons, reduce an electron acceptor and participate in the photosynthetic chain.

Before cyanobacteria appeared on Earth, photosynthesis was called "primitive bacterial photosynthesis". At that time, photosynthetic pigments formed a single photosystem, and the source of electrons was reduced sulphur mineral compounds. With evolution and the appearance of cyanobacteria, photosynthesis evolved:

- The electron source became water;
- Oxygen was produced as the final electron acceptor;

PS I and PS II photosystems were present.

There was a coupling between photochemical reactions and carbohydrate synthesis.

Spirulina produces energy by respiration during the night to ensure its survival and growth. Glucides generated during the day are oxidized to convert them into protides, while CO_2 dissolved in the culture medium and water is produced (Fox, 1999a). When daylight returns, the CO_2 takes part in a new photosynthesis cycle.

Biochemical compositions

The macronutrient and micronutrient composition of *Spirulina* varies according to several factors. *Spirulina* composition varies according to strain, growing conditions, production conditions, medium design, harvesting and post-harvest processes such as drying, grinding and storage. *Spirulina* generally contains 55-70% protein, 20% carbohydrates, 5% lipids and 7% minerals. It is also rich in vitamins and other substances, such as the pigment chlorophyll and phycobiliproteins.

Spirulina's proteins are qualitatively complete, containing all the essential amino acids (Table 2.5) that make up around 47% of the total protein content. Moreover, these proteins are of high biological value, as they are highly digestible due to the absence of cellulose walls. However, *Spirulina* contains reduced amounts of methionine, cysteine and lysine compared to commonly known protein sources such as meat, eggs, or milk (Falquet, 1996).

Table 2.5. Percentage of *Spirulina* amino acids

Amino acids	Jacquet 1974	Clement 1975	Fox 1999
Essential amino acids			
Isoleucine	5,60	6,40	5,98
Leucine	8,00	9,00	8,71
Lysine	4,20	4,80	5,28
Méthionine	2,25	2,60	2,85
Phénylalanine	4,40	4,60	5,09
Thréonine	4,70	5,50	5,58
Tryptophane	1,00	1,60	1,48
Valine	5,70	6,90	7,72
Non-essential amino acids			
Alanine	7,25	7,90	8,24
Arginine	6,60	6,70	7,92
Acide aspartique	9,30	9,20	9,50
Cystéine	0,95	0,90	0,93
Acide glutamique	NC	12,90	13,20
Glycine	4,80	5,00	5,07
Histidine	1,60	1,60	1,50
Proline	3,60	3,90	4,32
Sérine	5,00	5,60	5,46
Tyrosine	4,30	4,90	-

Lipids account for 6–8% of *Spirulina* sec and are divided into two fractions: a saponifiable fraction (83%) and a non-saponifiable fraction (17%). *Spirulina* biomass contains a high concentration of the essential fatty acid polyunsaturated fatty acids (PUFAs), i.e., 1.5 to 2% of the 5 to 6% of total lipids (Habib, 2008). It is also a significant source of gamma-linolenic fatty acid (GLA), a precursor of several hormones such as prostaglandins, leukotrienes and thromboxanes. These hormones are essential in metabolism and modulate the immunological response, reducing inflammation and blood pressure (Lordan et al., 2011; Ronda and Lele, 2008).

Carbohydrates make up 15–25% of *Spirulina'*s dry weight. They can generally be found in simple sugars, such as rhamnoses and glucosan amines; and polyols such as glycogen and starch. *Spirulina* also contains calcium-spirulan, a sulfated polysaccharide with biological properties(Hayashi et al., 1996; Mishima et al., 1998).

The vitamin content of *Spirulina* can vary widely based on the species and the environmental conditions in which it is cultivated. Virtually all vitamins are present in *Spirulina* in optimal quantities (Table 2.6). Most of these organic substances are bioavailable. This is the case of vitamin B12, which is especially important for vegetarians, who are often deficient in this nutrient, mainly due to limited consumption of foods of animal origin or limited assimilation of this cyanocobalamin.

Table 2.6. Vitamin content in µg/g *Spirulina* dry matter (Charpy et al., 2008)

Vitamin	**Content**
Hydrosoluble vitamins	
B1 (thiamin)	34 – 50
B2 (riboflavin)	30 – 46
B3 (niacin)	130
B5 (pantothenate)	4.6 – 25
B6 (pyridoxin)	5 – 8
B8 (biotin)	0.05
B9 (folate)	0.5
B12 (cobalamin)	0.10 - 0.34*
C (ascorbic acid)	-
Liposolubles vitamin	
Provitamin A (β-carotene)	700 – 1700
Cryptoxanthin	100
E (alpha-tocopherol)	50 – 190

Like other nutrients, *Spirulina's* mineral composition can vary according to various factors such as cultivation conditions, *Spirulina* strain and laboratory analysis methods. In general, *Spirulina* is a good source of minerals (Table 2.7). It contains all the essential minerals, representing around 7% of its dry weight.

Table 2.7. Mineral composition *Spirulina* in µg/g of dry matter from (Falquet and hurni, 2006)

Mineral	**Content (mg/kg)**	**Doses required (mg /day)**
Calcium	1300 – 14000	1200
Phosphorus	6700 – 9000	1000
Magnesium	2000 – 2900	250 – 350
Iron	580 – 1800	18
Zinc	21 – 40	15
Copper	8 – 10	1.5 – 3
Chromium	2.8	0.5 – 2
Manganese	25 – 37	5
Sodium	4500	500
Potassium	6400 – 15400	3500
Selenium	0.01 - 50	0.05

Pigments

Cyanobacteria are the only prokaryotes with the ability to perform oxygenic photosynthesis. Their main light-harvesting capacity is associated with large antenna complexes, phycobilisomes, located on photosynthetic membranes' surfaces (Morançais et al., 2018). Pigments also have antioxidant, anticancer, anti-inflammatory, anti-obesity and neuroprotective proprieties (Cuellar-Bermudez et al., 2015; Guedes et al., 2011). The three main types of pigment present in *Spirulina* are chlorophylls, carotenoids and phycobiliproteins(Bortolini et al., 2022). Phycobiliproteins and carotenoids are commercially important and are utilized in food ingredients, cosmetics, pharmaceuticals, food colorings and biomedical products. The lability of coordinated magnesium, which causes yellow to brownish colouring, limits the usage of chlorophyll and the water-soluble chlorophyllin (Mortensen, 2006).

Chlorophyll

Animals also do not synthesize chlorophyll. It must be provided by food. Marine organisms are also an essential source of chlorophyll. *Spirulina* is a good source of chlorophyll. It is attracting the attention of scientists and industrialists because of its ease of cultivation. This pigment is responsible for the green coloring of *Spirulina* and many other algae. Apart from its utilize as a natural colorant in the food industry, it can also be extracted and used as an antioxidant and anticancer agent (Asghari et al., 2016). The chlorophyll molecule contains a magnesium atom in its center (Figure 2.5)

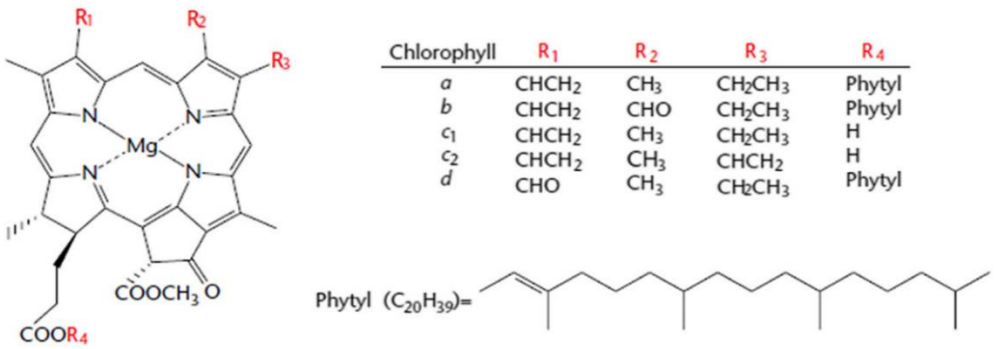


Figure 2.5. Structure of chlorophyll molecules (Fiedor et al., 2008).

Carotenoids

Carotenoids are a class of natural, liposoluble pigments responsible for the red, yellow and orange hues observed in various plant species and micro-organisms. They are divided into two groups: provitamin A, such as beta-carotene, and the others (lycopene, phycoxanthin, zeaxanthin, lutein, astaxanthin and cryptoxanthin) (Figure 2.6). They can be converted into vitamins in mammals. Carotenoids are used as food colourants in the food industry. They also protect against oxidative stress, ageing, cancer, degenerative diseases and eye diseases. Around 80% of the

carotenoids in *Spirulina* are beta-carotenes, or provitamin A, which can be converted into vitamin A by animals. It is one of the hydrocarbon carotenoids synthesized in plants, phytoplankton and certain photosynthetic microorganisms. It is involved, for example, in the biosynthesis of zeaxanthin from phytoene (Dey and Rathod, 2013) and plays an essential role in the fight against cancer and xerophthalmia (Seshadri et al., 1991). Carotenoids are also used as colorants in the food industry.

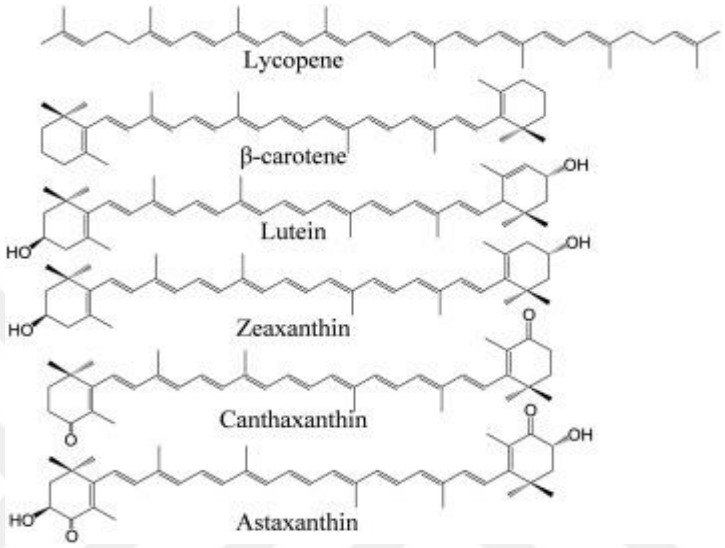


Figure 2.6. Structure of carotenoids found in microalgae (Gong and Bassi, 2016)

Phycocyanin

Phycobiliproteins are non-toxic, hydrophilic pigments that function as a highly fluorescent chromophore. They are classified into three classes based on the colour of the phycobilin moiety: phycocyanin (blue chromophore), phycoerythrin (red chromophore), and allophycocyanin (yellowish chromophore) (Sinha et al., 1995) and phycoerythrocyne (Stadnichuk et al., 2015) .

Phycocyanin is a water-soluble pigment protein accounting for more than 15% of its weight of *Spirulina* (Hayashi et al., 1996). Phycobilisomes (PBS) serve as antennas for the photosynthetic pigment machinery in cyanobacteria (Figure 2.6). Light-absorbing pigments may be classified into chlorophylls, which take up light at wavelengths > 640 nm (red) and < 440 nm (blue) of the luminous spectrum, and carotenoids, phycobiliproteins, phycoerythrin and phycocyanin (470-630 nm) (Chan, 2003). Phycocyanin is composed of two subunits and possesses a linear tetrapyrrole, phytochromobilin, covalently linked to its polypeptides by a thioether bond to a conserved Cys residue (Wu et al., 2016). Responsible for blue coloration, phycocyanin, depending on its properties, can be divided into c-phycocyanin and R-phycocyanin. The term phycocyanin refers to the phycocyanin in *Spirulina*. In addition to its photosynthetic properties, this pigment is used as a natural colorant in food, nutraceuticals, cosmetics, fluorescent dyes and pharmaceuticals.

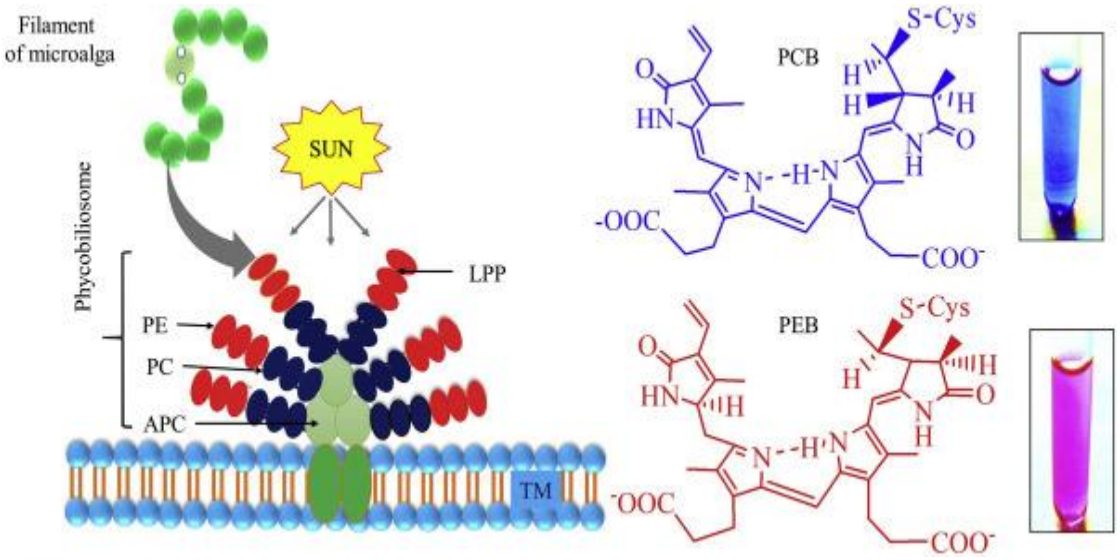


Figure 2.7. Schematic diagram of phycobilisomes on thylakoid membrane of cyanobacteria: PC (blue color), PE (pink-red color), APC (blue-green color), and linker polypeptides (LPP) with PCB and PEB chromophores. PC: phycocyanin; PE: phycoerythrin, APC: allophycocyanin; PCB: phycocyanobilin, PEB: phycoerythrobilin (Kannaujiya et al., 2021).

PC is present in cyanobacteria (blue-green), Rhodophyceae (red), and Cryptophyceae and is known as C-PC and R-PC depending on the species. R-Phycocyanin is a minor pigment found in red microalgae. An (αβ)3 trimer is the structure. Both α and β subunits have the PCB chromophore, however, the β subunit has a second chromophore: PEB. The most prevalent, C-PC, is made up of two α and β subunits that have a hexameric conformation (αβ)6 at pH 5-6 and a trimeric conformation (αβ)3 at pH 7. Only the PCB chromophore is found in both the α and β subunits.

Phycocyanin, an antioxidant molecule, has several beneficial therapeutic effects. It has been shown to neutralize alkoxy (RO) and hydroxyl (OH) radicals and lipid peroxidation, thereby reducing oxidative stress (Eriksen, 2008; Li et al., 2019; Riss et al., 2007; Udayan et al., 2017). Phycocyanin has immunomodulatory, anticancer, antiviral and cholesterol-reducing effects (Belay, 2002). In addition, phycocyanin has demonstrated antimalarial activities (Wulandari et al., 2017) and possesses chemopreventive properties against tumors (Li et al., 2005). Furthermore, it has been shown to inhibit histamine release from mast cells, thus playing an important role in allergic inflammation (Remirez et al., 2002) and antibacterial (Sarada et al., 2011).

Phenolic compounds

Phenolic compounds, or polyphenols, are plants' most common secondary metabolites. Phenolic compounds are categorized as secondary metabolites since they do not serve a direct role in the essential processes of plant organisms, such as growth or production. They are defense molecules that plants synthesize to cope with various biological and physical stresses. These compounds are among the main constituents of the human diet and are provided by fruit,

vegetables and legumes. Natural polyphenols can be simple molecules such as phenolic acids or highly polymerized complex substances such as tannins. They are characterized by benzene rings carrying one or more hydroxyl functions. The presence of these hydroxyl groups gives phenols their antioxidant properties. These substances are widely diversified. They are divided into phenolic acids (hydroxybenzoic and hydroxycinnamic acids), stilbenes, lignins (non-flavonoids) and flavonoids (Manach et al., 2004; Robards et al., 1999). Each subclass contains various compounds with a wide range of activities. The antioxidant power of a phenolic compound varies according to the number and position of the hydroxyl group (Figure 2.7). They are synthesized by the shikimate pathway from L-phenylalanine and L-tyrosine and contain one or more hydroxyl groups attached directly to the aromatic ring (Kumar and Goel, 2019) .

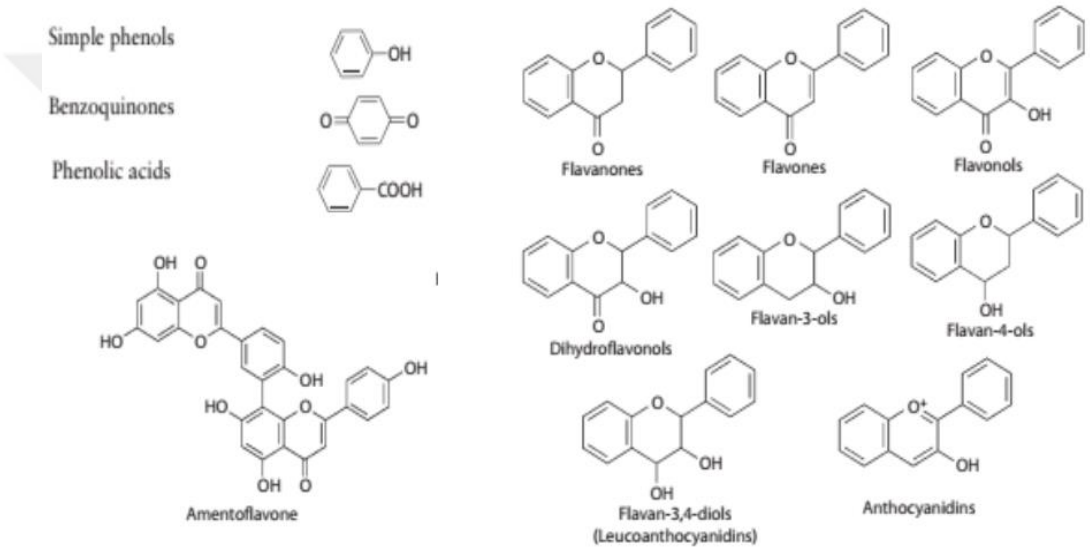


Figure 2.8. General structure of polyphenols (Nollet and Gutierrez-Uribe, 2018)

Phenolic acids

Phenolic acids (non-flavonoids) are simple forms of polyphenols, the best known of which are hydroxybenzoic and hydroxycinnamic acids. This class includes p-hydroxybenzoic, protocatechic, vanillic, gallic, syringic, p-coumaric, caffeic and ferulic acids (Figure 2.8). They can demonstrate antioxidant activity by scavenging the hydroxyl radical, the superoxide radical anion, several organic radicals, the peroxyl radical, peroxynitrite and singlet oxygen, among others. The HPLC-DAD profiles of the *Spirulina* phenolic extracts revealed the presence of a significant variety of phenolic acids and flavonoids at various amounts. Gallic, chlorogenic, cinnamic acids, pinostrobin, and p-OH-benzoic acids are the most prevalent elements in the extracts (Abd El Baky et al., 2009).

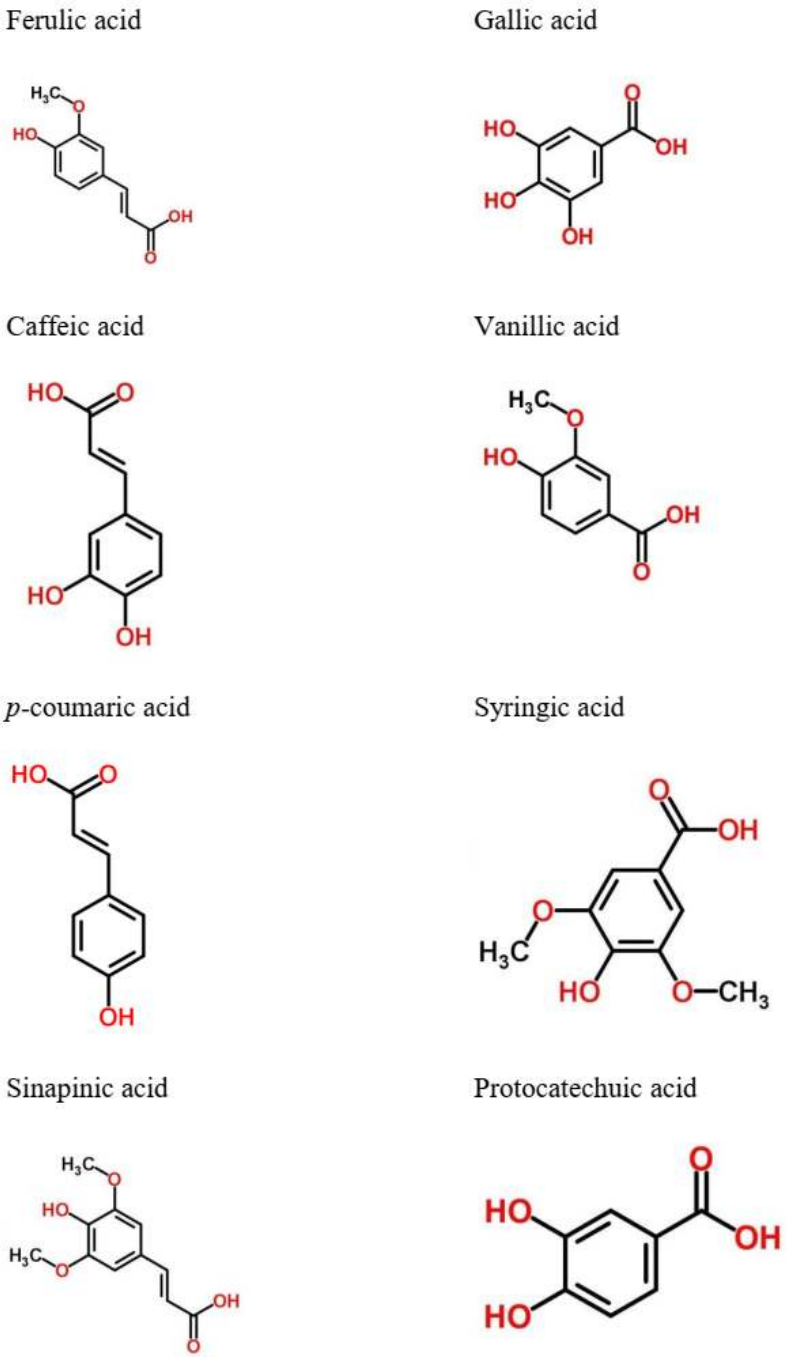


Figure 2.9. Chemical structure of phenol acids (Wang, 2016; Stalikas, 2007).

Flavonoids

Flavonoids are ubiquitous phenolic compounds in the plant kingdom. They are the main metabolites responsible for pigmentation, like chlorophyll and carotenoids in plant products. Some 4,000 flavonoids have been identified in plants. Flavonoids have a basic 15-carbon skeleton (C6-C3-C6). They are classified according to their degree of oxidation and saturation of the C ring: Flavanones, flavones, flavonols, dihydroflavonols, flavan-3-ols, flavan-4-ols, flavan-3,4-diols (leucoanthocyanidins), and anthocyanidins (Nollet and Gutierrez-Uribe, 2018).

Anthocyanins

Anthocyanins are the pigments responsible for the color (red, blue, and violet of flowers and fruit) most widespread in the different species of the plant. They play a role in pollination and the absorption of UV radiation. A skeleton of flavylium cations characterizes their basic structure, hydroxylated in different positions to give rise to anthocyanidins (Mattioli et al., 2020). Anthocyanins share a common structure of 15 carbon atoms, with aromatic rings linked by three carbon atoms to form an oxygenated heterocycle (Figure 2.9).

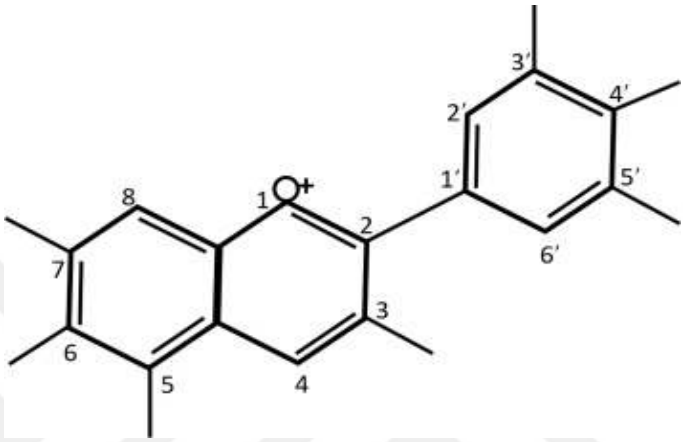
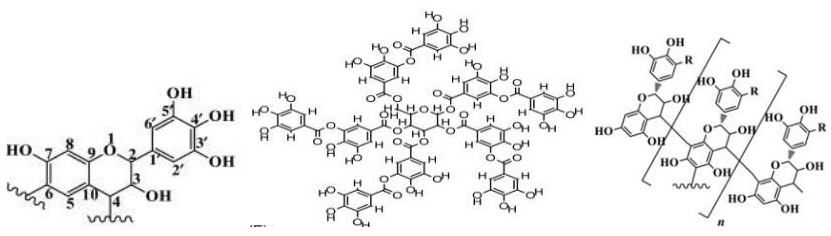


Figure 2.10. The General anthocyanin structure (Kent et al., 2018).

Tannins

Tannins are a structurally complex group of polyphenols, including hydrolyzable tannins, proanthocyanidins (also known as condensed tannins) and phlorotannins (Figure 2.10) (Bule et al., 2020; Suvanto et al., 2017). Since antiquity, tannins, widespread polyphenols found in plants, have been utilized in the tanning industry to render skin resistant to decay. From a food point of view, they are responsible for the astringency of many fruits, vegetables, and products derived from them. This characteristic is due to their property of complexation with salivary proteins. Tannins are also used in plant protection as anti-predation agents or pesticides (Patachia and Croitoru, 2016). Condensed tannins are the types most associated with the medicinal properties attributed to tannins(Bule et al., 2020).



(A) (B) (C)

Figure 2.11. Structure of tannins. (A) Tannin general structure, (B) tannic acid, (C) condensed tannin (Bule et al., 2020) .

Polyphenols and Spirulina

Microalgae are an important source of polyphenols. These phenols are a major antioxidant contributor (Maadane et al., 2015; Hajimahmoodi et al., 2010). Thanks to their varied structures, these substances enable microalgae to resist various stresses through their antioxidant, antimicrobial and photoprotective properties.

Several studies have shown the presence of many polyphenols and flavonoids at varying levels in *Spirulina*. These metabolites are generally produced to protect against environmental stresses. The content of these compounds varies according to the conditions under which *Spirulina* is grown and the method used to prepare the extracts. Nutrients such as sodium nitrate (Abd El-Baky et al., 2009) , temperature, (Colla et al., 2007) ,cultivation method (Kepekçi and Saygideger, 2012) , or growth mediums (Asnake Metekia et al., 2022) are the most important factors influencing the content of *Spirulina* phenolic compounds. In addition, the content of these secondary metabolites varies according to the solvents used and the extraction conditions (Table 2.8).

Table 2.8. Polyphenols contain in *Spirulina*

Strain	**Medium**	**Solvent**	**Treatment**	**Phenolic compounds**	**Flavonoids**	**Authors**
Spirulina sp. LEB-18	Logon water + Zarrouk medium 20% (v/v)	Methanol		700 µg /g		(Pagnussatt et al., 2016)
Spirulina platensis M2 strain	Schlösser's Medium (Schlösser 1982)	methanol+sonication	40 µmol photons m^{-2} s^{-1}	6.32±3.89 mg GAE /g		(Kepekçi and Saygideger, 2012)
			60 µmol photons m^{-2} s^{-1}	25.73±3.16 mg GAE/ g		
			120 µmol photons m^{-2} s^{-1}	49.83±5.56 mg GAE/ g		
		MeOH + gel silica gel column chromatography		44.48 ± 1.71 mg GAE/g		(Alshuniaber et al., 2021)
				7.80 ± 0.15 mg GAE/g		
				4.19 ± 0.21 mg GAE/g	15.60 ± 2.74 mg RE/g dw	(Seghiri et al., 2019)
Spirulina				2.28mg GAE/g		(Machado et al., 2019)
				26.64±0.16 mg GAE/g		(Hidayati et al., 2020)

Role of phenolic compounds

They form the basis of active plant ingredients with a wide range of biological activity, making them interesting to researchers and industrialists. As a result, these compounds' antioxidant, antiviral, antimicrobial, anti-inflammatory, antitumor, anti-platelet aggregation, antihypertensive, antiallergic and cholesterol-lowering activities are well known. On the other hand, secondary metabolites are associated with plant colors, tastes and odors (Ashraf et al., 2018). Examples include vanillin, used to flavor foods; anthocyanins, responsible for fruit coloring or bitterness; and salicylic acid, a precursor of aspirin.

Antioxidant activity

Cyanobacteria have considerable promise as a source of phenolic compounds with strong antioxidant properties, which might assist various sectors, including medicines, food, and cosmetics (Ijaz and Hasnain, 2016). The antioxidant potential of algae and *Spirulina* species is attracting increasing attention. Numerous in vitro and in vivo studies have shown that treating or supplementing *Spirulina* or its compounds significantly reduces oxidative stress. These antioxidant and protective effects are provided by phycocyanin, phenolic compounds, β-carotene and other vitamins and minerals in *Spirulina* (Abdel-Daim et al., 2013; Abd El-Baky et al., 2009; Upasani and Balaraman, 2003). The results of the study by Wu et al. (2005) revealed that DPPH- essays also showed a similar trend to ABTS-$^{+}$ essays (EC50: 19.39±0.65 µmol ascorbic acid equivalent/g *Spirulina* extract versus 14.04±1.06 µmol ascorbic acid equivalent/g *Chlorella*. Furthermore, aqueous extracts of both algae demonstrated antiproliferative effects on hepatic stellate cells (HSCs) and HepG2, but *Spirulina* proved to be a more potent inhibitor than *Chlorella*. *Spirulina* protects organs from damage caused by metal such as lead exposure (Upasani and Balaraman, 2003). Kalafati et al (2010) found that *Spirulina* supplementation significantly improved physical performance, glucide oxidation, and fat oxidation rates, while also increasing glutathione (GSH) levels. The levels of Thiobarbituric acid reactive substances (TBARS) and protein carbonyls, catalase, and TAC also increased immediately and 1 hour after exercise. This suggests that *Spirulina* supplementation may enhance lipid peroxidation and improve overall performance.

Antimicrobial activity

Many studies have shown that microalgae and their extracts are effective antimicrobial agents (antiviral, antibacterial, antifungal, antiprotozoal). Among microalgae, *Spirulina* possesses natural antimicrobial activity against various microorganisms. Several solvents, such as water, methanol, ethanol, dichloromethane, hexane, petroleum ether and ethyl acetate were used to extract *Spirulina platensis* bioactive molecules with antimicrobial activity against microorganisms in vitro. *Spirulina*'s antimicrobial activity depends also on the extraction method, the extract doses used and the bacterial strains targeted. *Spirulina platensis* can inhibit the growth of certain Gram-negative

and Gram-positive bacteria. It has shown inhibitory activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus pumulis*, with maximum activity against *Proteus vulgaris* (Bhowmik et al., 2009). For example ,*Spirulina* methanol extract showed higher antimicrobial activity than extracts of hexane (Kaushik and Chauhan, 2008), dichloromethane (Kaushik and Chauhan, 2008; Ozdemir et al., 2004), petroleum (Ozdemir et al., 2004), ethyl acetate (Kaushik and Chauhan, 2008; Ozdemir et al., 2004) and volatile components (heptadecane and tetradecane) (Ozdemir et al., 2004), in particular against *Streptococcus faecalis* (Ozdemir et al., 2004), *Staphylococcus epidermidis* (Ozdemir et al., 2004) and *Candida albicans* (Ozdemir et al., 2004), as well as Gram-positive *Staphylococcus aureus* (Kaushik and Chauhan, 2008) and Gram-negative *Escherichia coli* (Kaushik and Chauhan, 2008). Purified C-phycocyanin from *S. platensis* significantly suppressed the development of many drug-resistant bacteria, including *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Sarada et al., 2011). *Spirulina* peptides also have antimicrobial properties. In a study by Sun et al (2016), the SP-1 peptide was found to have a minimum inhibitory concentration of 8 mg/mL for *E. coli* and 16 mg/mL *S. aureus*. 0.1% *Spirulina* was found to improve the ability of 3-week-old chicks injected with *E. coli* or *S. aureus* suspensions to rid themselves of bacteria, as evidenced by the improved activities of various phagocytic cells in chickens, including heterophils, thrombocytes, macrophages, and monocytes (Qureshi et al., 1996).

Spirulina biomass also has a stimulating effect on growth during fermentation and/or improves survival during storage of lactic acid bacteria such as *Bifidobacterium* (Mocanu et al., 2013), *Lactobacillus casei* and *Lactobacillus acidophilus* (Bhowmik et al., 2009), *Lactobacillus bulgaricus* (Akalin et al., 2009) and *Streptococcus thermophilus* (Fadaei et al., 2019; Malik et al., 2013).

Spirulina extracts also have antifungal activity. Methanolic extracts containing 1.15 mg of phenolic compounds per gram of *Spirulina platensis* have demonstrated antifungal activity against *Aspergillus flavus*, inhibiting glucosamine production by up to 56% (Souza et al., 2011). In Balb/C mice infected with candidiasis, the immunostimulatory activity of *S. platensis* extract was tested. Mice pretreated with 800 mg/kg extract for 4 days before intravenous inoculation with *Candida albicans* produced more of the cytokines TNF- and interferon-gamma (IFN-), resulting in enhanced survival and clearance of fungal infection compared to control groups (Soltani et al., 2012).

Spirulina is rich in bioactive compounds such as phenols, biopeptides, β-carotene, polyunsaturated fatty acids (PUFAs), calcium-spirulan, B-complex vitamins, particularly vitamin B12, and antioxidant minerals such as selenium and zinc. These elements stimulate the immune system and strengthen the body's defense mechanisms against infection. Recently, the use of *Arthrospira maxima* powder inhibited SARS-CoV-2 infection using a concentration of 50 TCID50/ml for both experimental regimens, but the percentage of protection was lower when the viral inoculum was increased to 100 TCID50/ml; viral RNA decreased 48 hours after infection,

reaching a 250-fold difference at 72 hours. The study carried out by Daoud and Soliman (2015) confirmed the biological activity of the ethanolic extract of *S. platensis* against types O, A and SAT2 of the foot-and-mouth disease virus in old baby Swiss mice. Calcium-spirulan (Ca-SP), a sulfated polysaccharide extracted from *Spirulina platensis*, has demonstrated antiviral properties against various enveloped viruses such as herpes simplex type I, measles, HIV-I and influenza. This powerful antiviral activity has been attributed to the chelating effect of calcium ions on the sulfate groups, which affects the molecular conformation of the polysaccharide (T. Hayashi et al., 1996).

2.5.4 Oxidants and Antioxidants

Oxidants

Free radicals are chemical compounds with unpaired electrons, making them highly reactive. To achieve stability, they seek to fill this free electron by accepting it from or transferring it to another molecule (Afonso et al., 2007). The two most important types of free radicals in the body are oxygen and nitrogen free radicals (Table 2.5). They are a radical species or non-radical species. RNS (Reactive Nitrogen Species) are a group of p-oxidizing molecules involved in various biological processes, primarily derived from the nitric radical (NO•). They include nitric oxide (NO^-), nitrite peroxide (ONOOH) and nitrite (NO_2^-). Free radicals are generated by normal physiological processes in organisms, including aerobic metabolism and inflammatory responses, to eliminate invading pathogenic microorganisms (Govindarajan et al., 2005). However, external factors such as exposure to pollution, smoking, UV radiation, heavy metal ions, ozone, allergens, drugs, toxins, pollutants, pesticides, or insecticides can also contribute to an increase in free radical production in cells (Oke et al., 2019; Mahajan et al., 2018; Antunes dos Santos et al., 2018). The endogenous production of free radicals can occur in the body during various metabolic reactions. For example, during the transport of electrons in the respiratory chain, the superoxide radical ($O_2^{\bullet -}$) is formed in the mitochondria generally by dismutation. Phagocytic cells, polymorphs, and macrophages contain a membrane enzyme known as NADPH oxidase. This specialized enzyme generates superoxide radicals (Bedard and Krause, 2007). Superoxide can also be formed during the catabolism of adenosine triphosphate by xanthine oxidase. Certain metal ions, such as iron and copper, generate ROS by converting hydrogen peroxide (H_2O_2) into a more dangerous hydroxyl radical ($OH^{\bullet}$). Free radicals can also be formed during the production of humoral mediators such as prostaglandins and leukotrienes, as well as during the catabolism of certain xenobiotics. Moreover, the RNS, particularly nitric oxide ($NO^{\bullet}$), can be synthesized in certain cells through NO-synthase from amino acids such as arginine and oxygen. When its concentration increases, nitric oxide can combine with the superoxide radical ($O_2^{\bullet -}$) to form peroxynitrite ($ONOO^-$), a powerful oxidant (Oldham and Bowen, 1998).

The production of reactive oxygen species (ROS) in the chloroplasts of green plants and algae, especially under stressful conditions such as intense light or limitation of CO_2 fixation. During photosynthesis, the oxygen produced accepts electrons, thereby generating ROS such as $O_2^{\cdot -}$ and H_2O_2 (Telfer et al., 1994; Asada et al., 1974). In addition, energy transfer between photosystems can produce $^1O_2$. These ROS can damage proteins and cellular structures. There are protective mechanisms in place, such as the reduction of oxygen by superoxide dismutase (SOD) and the presence of antioxidants such as ascorbate and tocopherol. The balance between ROS production and neutralization is essential to preserve chloroplast integrity and photosynthetic function.

In normal quantities, free radicals are necessary for life (Valko et al., 2006), but if their overproduction, they can cause cellular damage and alter components such as proteins, lipids and DNA (Pizzino et al., 2017). This contributes to ageing and the development of chronic and degenerative diseases. Therefore, the balance between free radicals and antioxidants is important to maintain cell protection and prevent free radical damage. This phenomenon is known as oxidative stress. It is characterized by an imbalance between the production of reactive oxygen species by cellular metabolism and the body's ability to neutralize and repair oxidative damage (Sies, 2019). Under normal conditions, the body has a complex system of antioxidant defense mechanisms to maintain a balance between the production and elimination of ROS, known as redox homeostasis. Enzyme systems such as superoxide dismutase, catalase and glutathione peroxidase play an important role in neutralizing and detoxifying ROS.

Although oxidative stress is a normal phenomenon, it is important to maintain a balance in redox homeostasis to prevent excessive damage and promote overall good health. This can be achieved through a healthy lifestyle, including varied and balanced diet rich in fruits, vegetables and marine products, rich in antioxidants (beta-carotene, flavonoids, vitamins C and E), regular physical activity, stress management and avoiding or reducing exposure to environmental toxins.

The superoxide radical or superoxide ion ($O_2^{\bullet -}$) is one of the most important reactive oxygen species (ROS), resulting from the reaction of oxygen with an electron in the electron transport chain of the mitochondrial membrane. They are produced by NADPH oxidases, cyclooxygenase, lipoxygenase, xanthine oxidoreductase and cytochrome p450 (Finkel, 2003). It is neutralized by SOD (superoxide dismutase), an enzyme dependent on dietary microelements (Mn-SOD, Cu-SOD) (Evans and Halliwell, 2001).

The hydroxyl radical ($OH^{\bullet}$) is one of the most reactive oxygen species and the most damaging to cells and biological molecules. It is mainly formed in vivo during reactions of metal ions such as iron II or copper I with hydrogen peroxide, described as Fenton reactions (Fenton, 1894).

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-}$ (Fenton reactions)

Under stressful conditions, biological systems can generate hydroxyl radicals (OH^{-}) from superoxide anion and hydrogen peroxide in the presence of metal ions such as iron and copper, according to the Haber-Weiss reaction (Haber and Weiss, 1934):

$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^{\bullet} + OH^{-}$ (Haber-Wei reaction)

These reactions can lead to disorders such as atherosclerosis, cancer and neurological pathologies (Lipinski, 2011).

Hydrogen peroxide (H_2O_2): one of the most stable oxidants. It is formed by the reaction of two superoxide ions ($O_2^{\bullet-}$) thanks to an enzyme called superoxide dismutase (SOD).

Singlet oxygen ($^1O_2$) is an extremely reactive and unstable form of oxygen. It gets generated when two oxygen molecules (O_2) absorb energy, often from UV light. This absorption of energy excites the oxygen molecules, causing them to enter an excited electronic state. This excited state is referred to as singlet oxygen.

In addition to ROS and RNS, there is also a Species of Reactive Sulfur (RSS) (Table 2.9). RSS is a broad group of reactive species that includes radical and non-radical sulfur-based fractions. This category includes radicals such as the RS radical (where R can represent different alkyl or aryl groups) and the glutathionyl radical ($GSSG^{\bullet}$) generated from glutathione. It also includes non-radical reactive sulfane species, such as polysulfides, persulfides and thiosulfates (Ali et al., 2020).

Table 2.9. Reactive oxygen, nitrogen and sulfur species (Ali et al., 2020; Tvrdá and Benko, 2020)

Reactive Oxygen Species (ROS)			
Radical species		**Nonradical species**	
Name	Formula	Name	Formula
Superoxide	O_2 •$^-$	Hydrogen peroxide	H_2O_2
Hydroxyl radical	OH•	Hypochlorous acid	HOCL
Peroxyl radical	ROO•	Hypobromous acid	HOBr
Alkoxyl radical	RO•	Ozone	O_3
Hydroperoxyl radical	HO_2 •	Singlet oxygen	$^1O_2$
Lipid peroxyl radical	LOO•	Lipid peroxide	LOOH
Reactive Nitrogen Species (RNS)			
Name	Formula	Name	Formula
Nitric oxide	NO•	Nitrous acid	HNO_2
Nitrogen dioxide	$NO_2^•$	Nitrosyl cation	NO+
		Nitroxyl anion	NO-
		Dinitrogen tetroxide	N_2O_4
		Dinitrogen trioxide	N_2O_3
		Peroxynitrite	ONOO-
		Peroxynitrous acid	ONOOH
		Nitronium (nitryl) cation	NO_2^+
		Nitryl chloride	NO_2Cl
		Alkyl peroxynitrite	ROONO
Reactive Sulfur species			
Name	Formula	Name	Formula
Thiyl radical	RS•	Reactive sulfane species	RSR
Glutathionyl radical	GSSG•	Reducing sulfur species	H2S, GSH
Sulfur atom	RSR•	Reactive sulfur substances	SO_2, SO_3
		Sulfur secondary metabolites	Allicin $C_6H_{10}OS_2$

When prolonged stress conditions occur, cells can significantly increase oxidative damage caused by reactive oxygen species (ROS) or free radicals. The damaging effects of ROS on cells generally manifest themselves in the 3 main macromolecules (Juan et al., 2021).

- **Oxidative stress and DNA**

  They can damage DNA and RNA, leading to alterations in genetic structure and RNA sequence, disrupting normal cellular functions and thus leading to mutagenesis, mutagenesis, carcinogenesis and ageing (Juan et al., 2021; Valko et al., 2006). The DNA damage caused by ROS includes various modifications such as breaks in the DNA strands, alterations in the bases or deoxyribose sugar, telomeres, and DNA cross-link formation

(Figure 2.11). These DNA damages can have significant effects, including the interruption or activation of transcription, the activation of signalling pathways, errors during DNA replication, and the promotion of genomic instability. These consequences are closely linked to the development of cancer (Cooke et al., 2003; Juan et al., 2021; Marnett, 2000; Valko et al., 2006).

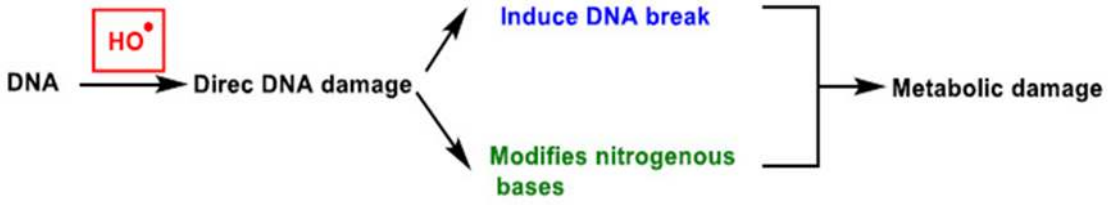


Figure 2.12. ROS-induced DNA oxidative stress (Juan et al., 2021)

- **Oxidative stress and protein**

  When proteins undergo oxidation, they are either broken down through hydrolysis or processed by the proteasome to prevent them from spreading in the body or interacting with other proteins. The impact of reactive oxygen species (ROS) on proteins is diverse and includes the oxidation of amino acid residues, the cleavage of peptide bonds, and the aggregation of proteins (Juan et al., 2021). Sulfur-containing amino acids such as cysteine and methionine are easily oxidized (Mythri et al., 2013). Several metabolic diseases, such as Alzheimer's disease, rheumatoid arthritis, mitochondrial damage and neurodegeneration, have been linked to protein oxidation (Milkovic et al., 2019; Mythri et al., 2013).

- **Oxidative stress and lipids**

  Lipid peroxidation (LPO) is a chain reaction generally induced by free radicals, where one radical can oxidize many substrate molecules, mainly polyunsaturated fatty acids (PUFAs). The initiation of this reaction occurs through the abstraction of a hydrogen atom from a reactive methylene group of a PUFA. Monounsaturated and saturated fatty acids are less reactive and generally do not participate in LPO. These reactions play an important role in oxidative stress and associated cellular damage (Abuja and Albertini, 2001). The process is generally initiated by a sufficiently reactive radical (R·):

  $$LH + R^{\bullet} \rightarrow L^{\bullet} + RH$$

Molecular oxygen is rapidly added to the carbon-centered radical (L·) formed during this process, resulting in the lipid peroxyl radical ($LOO^{\bullet}$):

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

In turn, the lipid peroxyl radical (LOO·) can also extract a hydrogen atom from another polyunsaturated fatty acid (PUFA), similar to what is described in equation (1).

$$LH + LOO^{\bullet} \rightarrow L^{\bullet} + LOOH$$

This reaction step is known as propagation, implying that a single initiator radical can catalyze the conversion of many polyunsaturated fatty acids (PUFAs) into lipid hydroperoxides.

Lipid hydroperoxide (LOOH) represents the initial, comparatively stable product formed during lipid peroxidation. When lipid peroxidation is initiated continuously, a termination reaction occurs to limit the extent of the reaction, producing non-radical products (NRPs) and simultaneously removing two radicals:

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow NRP$$

Antioxidant

Antioxidants are any substances present at low concentrations relative to oxidizable substrates that retard, prevent, or inhibit oxidation or damage to these substrates (Halliwell and Gutteridge, 1995). This defense mechanism is achieved by the release of one or more electrons. In the body, antioxidants are synthesized, forming the primary defense system comprising enzymes such as superoxide dismutase, catalase and glutathione peroxidase. In addition to this system, the diet provides a secondary defense system comprising minerals such as selenium and zinc, vitamins C and E, polyphenols and carotenoids.

Both mechanisms, enzymatic and non-enzymatic antioxidants, are essential to sustaining the life of organisms by maintaining an intracellular redox balance and minimizing unwanted cellular damage caused by free radicals.

Natural antioxidants have a wide range of biological activities, generating considerable interest in industry. They are widely distributed in plants, algae and various food groups. Tocopherols, flavonoids, and phenolic acids are the most significant classes of natural antioxidants. As a result, several efficient techniques for extracting these bioactive compounds have been developed and evaluated for various applications, such as promoting functional foods, nutraceuticals, pharmaceuticals and food additives.

Natural antioxidants include enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione reductase (GRD), glutathione transferase (GRT), quinone reductase, as well as vitamins such as vitamin C, tocopherols, vitamin Q (Ubiquinone),

vitamin A; minerals such as selenium, zinc, copper, magnesium and manganese; and phytochemicals such as polyphenols, carotenoids and terpenes.

Synthetic antioxidants include, for example, BHT (3,5-ditert-butyl-4-hydroxytoluene), BHA (3-tert-butyl-4-hydroxyanisole), TBHQ (tert-butylhydroxyquinone), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), propyl, octyl and dodecyl gallate; citric and tartaric acid, ethylenediaminetetraacetic acid (EDTA), sodium and calcium salts (Pokorny et al., 2001).

Antioxidants are commonly used in the food industry as additives in fats, oils and processed foods to prevent or retard oxidative deterioration. The oxidation of dietary fats can lead to the formation of free radicals and lipid peroxidation, which affect the quality of foods, both from the point of view of flavor and nutritional security (Taghvaei and Jafari, 2015).

Both natural and synthetic antioxidants have advantages and disadvantages, as shown in (Table 2.9)

Table 2.10. Advantages and disadvantages of natural antioxidants, as compared with synthetic antioxidants (Valenzuela and Nieto, 1996)

Synthetic antioxidants Inexpensive	**Natural antioxidants Expensive**
Wikely applied	Use restricted to some products
Medium to high antioxidant activity	Wide-ranging antioxidant activity
Increasing safety concern	Perceived as innocuous substances
Use banned for some of them	Increasing use and expanding applications
Low water-solubility Decreasing interest	A broad range of solubilities Increasing interest

3. MATERIAL AND METHOD

3.1. Material

3.1.1. *Spirulina platensis*

Spirulina platensis M2 strain was used in this study.

Spirulina platensis in the systematics is given.

Section: Cyanophyta

Class: Cyanophyceae

Order: Nostocales

Family: Oscillatoriacea

Genus: *Spirulina* Turpin (1827)

Species: *Spirulina platensis*

3.1.2. *Spirulina platensis* Stock Cultures

Spirulina platensis produced as inoculum in the experiment is maintained under laboratory conditions with a constant illumination of 80 µmol m^{-2} s^{-1} and an ambient temperature of 30±1 °C, regulated by an air conditioning device. Initially, the stocks were produced in 250 and 500 mL Erlen Mayer under laboratory conditions (Figure 3.1 A). Then, they were used to inoculate 2, 4 and 8-liter containers (Figure 3.1 B and C). These inoculums are acclimatized outdoors and multiplied to 200-250 liters (Figure 3.1 D). Before being used as inoculums in the experimental ponds (Figure 3.1 E).

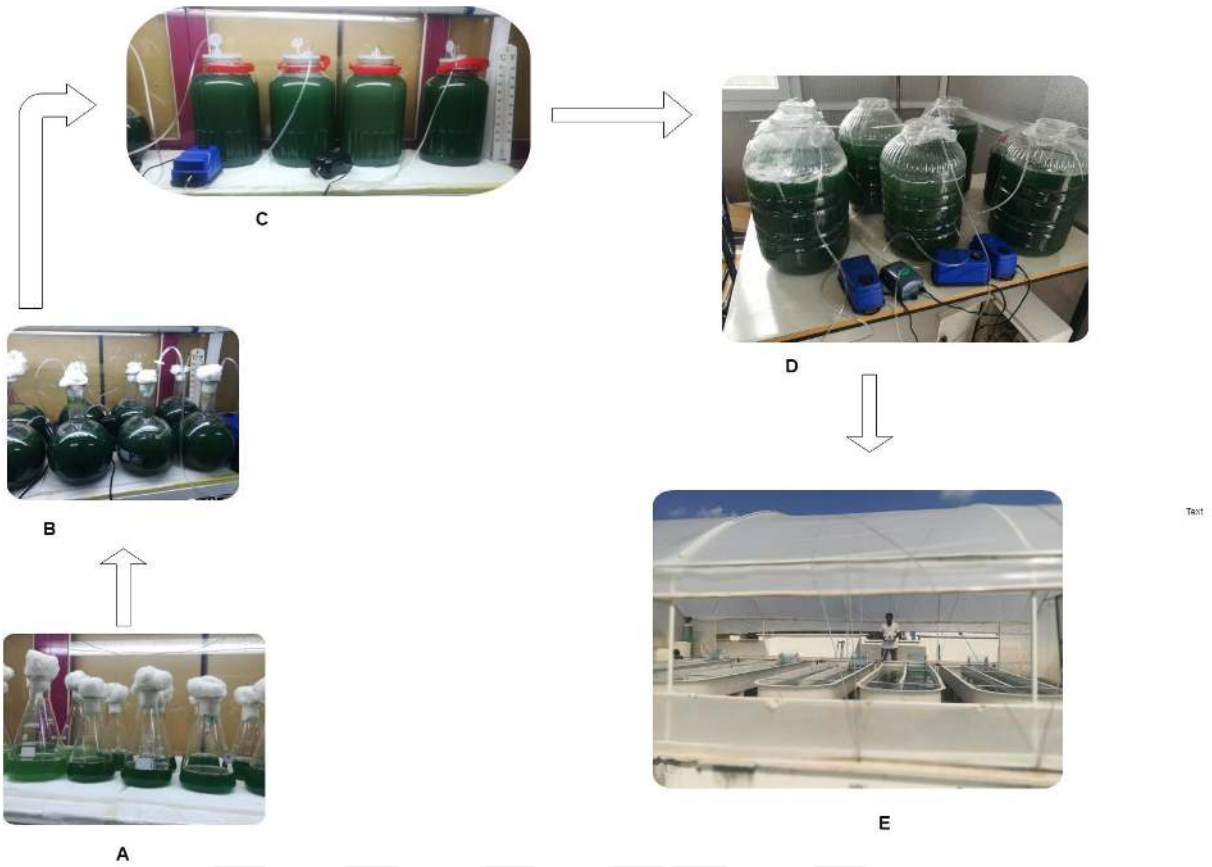


Figure 3.1. Multiplication of inoculum (original)

3.1.3. Culture medium

In this experiment, *Spirulina* medium was used to produce *S. platensis*. This medium consists of a mixture of three different solutions (Part 1, part 2 and Microelement solution), the composition of which is shown in Table 3.1.

Table 3.1. *Spirulina* medium

The medium solution	**Matter**	**Quantity**
Part 1	$NaHCO_3$	18.6g
	$Na2CO_3$	8.06g
	$K2HPO_4$	1.00g
	distilled water	500 mL
Part 2	$NaNO_3$	5.00g
	K_2SO_4	2.00g
	NaCl	2.00g
	$MgSO_4$. $7H_2O$	0.40g
	$CaCl_2.2H_2O$	0.02g
	$FeSO_4.7H_2O$	0.02
	$EDTANa_2$	0.16g
	Micro element solution	10.0 mL
	Distilled water	500 mL
Microelement solution	$ZnSO_4.7H_2O$	0.001g
	$MnSO_4.7H2O$	0.002g
	H_3BO_3	0.01g
	$Na2MO_4.2H_2O$	0.001g
	Co $(NO_3)2.6H_2O$	0.001g
	$CuSO_4.5H2O$	0.00005g
	$FeSO_4.7H_2O$	0.7g
	$EDTANa_2$	0.8g
	Distilled water	1L

3.1.4. Culture of *Spirulina*

The experiment used ponds of 1x5x0.2 m, 1 m³ capacity, fiberglass (Figure 3.2). A pedal system provides the mixture in ponds. The mixing speed was adjusted to 20 cm/s. The ponds are in the greenhouse on the building's roof of the Faculty of Fisheries Faculty of Cukurova University.

Figure 3.2. Ponds used in the experiment (original)

3.2. Methods

In this thesis, the culture of *Spirulina platensis* was carried out using the *Spirulina* culture medium (Table 3.1). As part of the experiment, this medium was enriched with two doses of magnesium (100 g/L and 200 g/L) for the experimental magnesium group and two different doses of zinc (3 mg/L and 5 mg/L) for the experimental zinc group. No enrichment was performed for the control group.

At the beginning of the experiment, several parameters were measured daily to monitor the growth of the *Spirulina*, including temperature, pH, light intensity (Licor, model LI-250) and optical density. Dry matter, phycocyanin, chlorophyll and carotenoids were determined every two days to assess biomass composition during growth. Magnesium and zinc bioaccumulation in biomass was assessed at 1, 3, and 5 hours after start-up, as well as on day 5, day 9, and at the end of the experiment.

At the end of the experimentation, harvesting of *S. platensis* was carried out by natural gravity filtration using a fine mesh of 200 µm, 100 µm, and 45 µm. The filtrate was washed, scraped, and pressed to obtain a *Spirulina* paste. That paste was lyophilized. Finally, the resulting biomass was reduced to powder before being stored at -24 °C to determine the biochemical composition, antioxidant and antimicrobial activity. Figure 3.3 shows the experimental design.

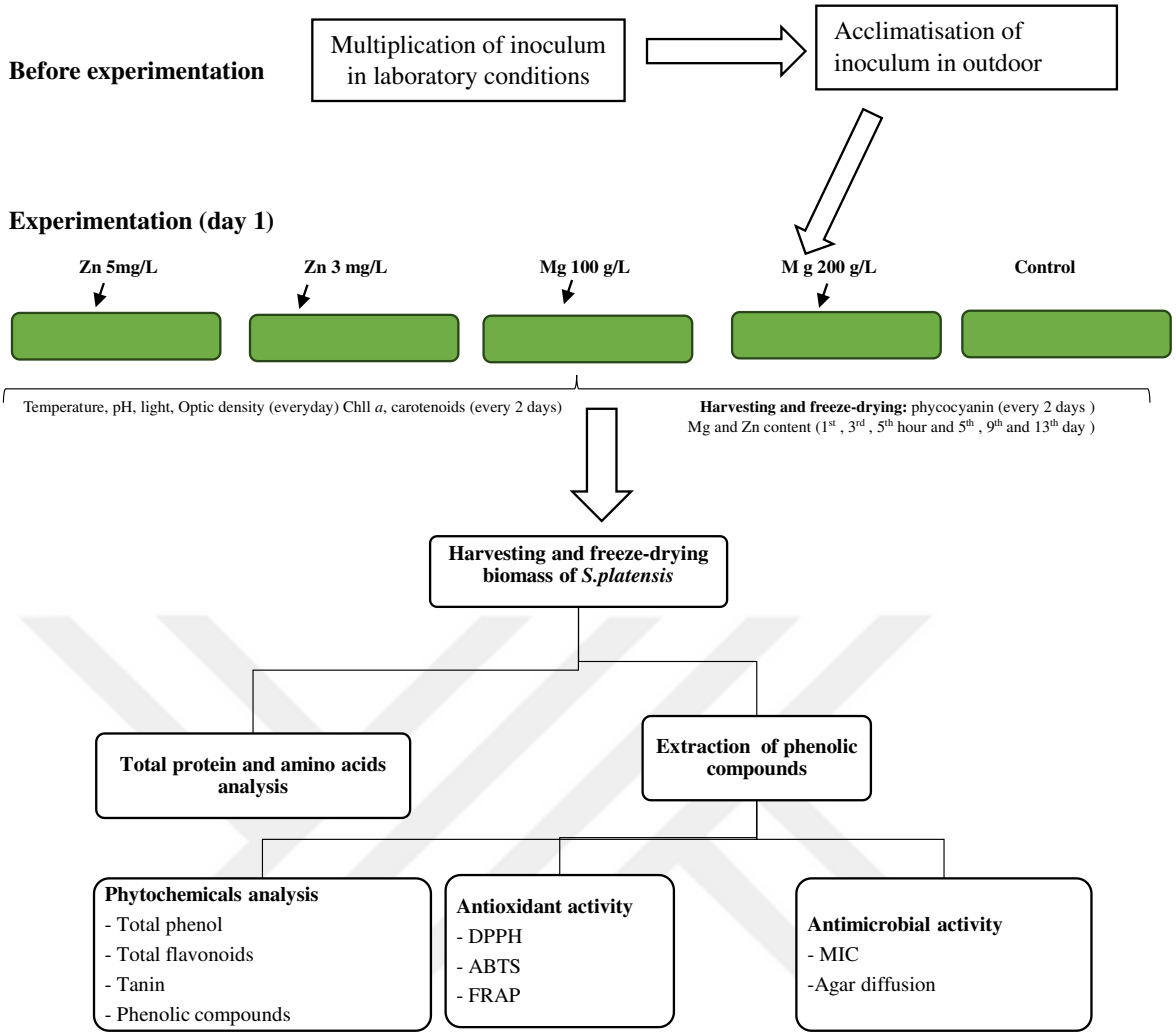


Figure 3.3. Experimental design

3.2.1. *Spirulina platensis* growth condition

pH, Temperature and light intensity

The pH and the temperature of the culture medium were evaluated using a pH meter, while the light intensity was measured using the Licor device, model LI-250.

Cell Density

The optical density is measured daily at the wavelength OD_{680} nm using a spectrophotometer (Spectrophotometer UV-Vis SP-3000 nano).

Dry matter

10 mL of *Spirulina* culture was taken daily from each flask. The *Spirulina* cells were filtered using a Whatman GF/C filter paper with a mesh size of 0.45 µm, which were previously kept in an oven at 105°C for 1 hour, cooled in a desiccator and tared. The filter papers were then dried in an oven at 105° C. for 2 hours. After cooling in a desiccator, the samples were weighed on a precision balance and the dry matter content was calculated (Vonshak, 1997).

Chlorophyll and carotenoids

Five mL of each culture of *Spirulina* (enriched with magnesium, zinc and control) was filtered through a filter (Whatman GF/CTM, 1.2 µm, UK). The filters were placed in glass tubes, and 10 mL of a 90% ethanol/water mixture was added for chlorophyll and carotenoid extraction. The tubes were placed in the refrigerator for 24 hours and analyzed the following day. Tubes were centrifuged (Hettich EBA-20) at 6000 rpm for 15 minutes at room temperature. Extracts were collected using Pasteur pipettes and chlorophyll was determined spectrophotometrically using 3 wavelengths: 470, 649 and 665 nm. Chlorophyll *a*nd carotenoids were calculated using the equations of (Strickland & Parsons, 1965).

$$\text{Chl } a = 13.36\,\text{Absorbance } 665 - 5.19\,\text{Absorbance } 649 \quad (3.1)$$

$$\text{Chl } b = 27.43\,\text{Absorbance } 649 - 8.12\,\text{Absorbance } 665 \quad (3.2)$$

$$\text{Carotenoids} = \frac{(1000\,\text{Absorbance } 470 - 2.13\,\text{Chl } a - 97.64\,\text{Chl } b)}{209} \quad (3.3)$$

Harvesting and drying

harvesting of *S. platensis* was carried out by natural gravity filtration using a fine mesh of 200 µm, 100 µm, and 45 µm. the filtrate was washed, scraped, and pressed to obtain a *Spirulina* paste that was lyophilized in the Department of Animal Science of Cukurova University (Figure 3.3). The lyophilized *Spirulina* was ground into powder and stored at -24 °C for to prepare future different analysis. All subsequent analyzes were performed using the freeze-dried sample.

Figure 3.4. Lyophilization of the biomass of *Spirulina* (original)

Phycocyanin

The extraction was done by mixing 100 mg of freeze-dried *Spirulina* with 10 mL of 0.1 M phosphate buffer pH 7.0. After homogenization and ultrasonication for 10 minutes at room temperature, the samples were subjected to freezing (- 24 °C) and thawing (room temperature). This procedure was repeated twice. The extracts were then centrifuged at 6000 rpm for 20 min. After centrifugation (Figure 3.4), the optical density of the supernatant is measured at 615 and 652 nm. Phycocyanin (PC) concentration, according to Bennett and Bogorad (1973), was defined as follows:

$$\mathrm{PC} = \frac{(\mathrm{OD615} - 0{,}474(\mathrm{OD652}))}{5{,}34} \tag{3.4}$$

Where PC is the phycocyanin concentration (mg/mL), OD615 is the optical density of the sample at 615 nm, OD652 is the optical density of the sample at 652 nm.

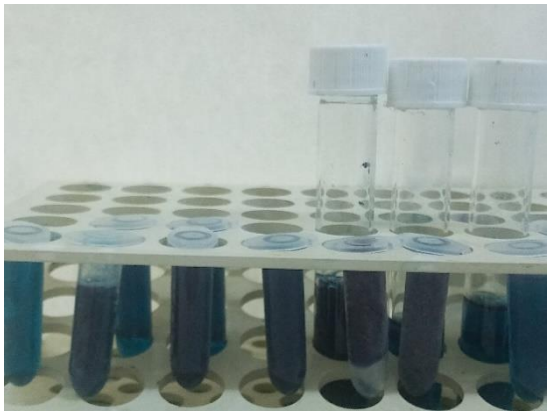


Figure 3.5. Phycocyanin extraction

Microelements analysis

For magnesium and zinc analysis, 200 mg of freeze-dried and ground *Spirulina* samples were weighed and incinerated in a muffle furnace at 550°C for 8 hours. After incineration, the ash was dissolved in 1/3 HCl and filtered through a blue-band filter (Figure 3.5 A) and the concentrations of Magnesium and Zinc were determined by an atomic absorption spectrometer (Agilent) (Figure 3.5 C) (H. D. Chapman & Pratt, 1961).

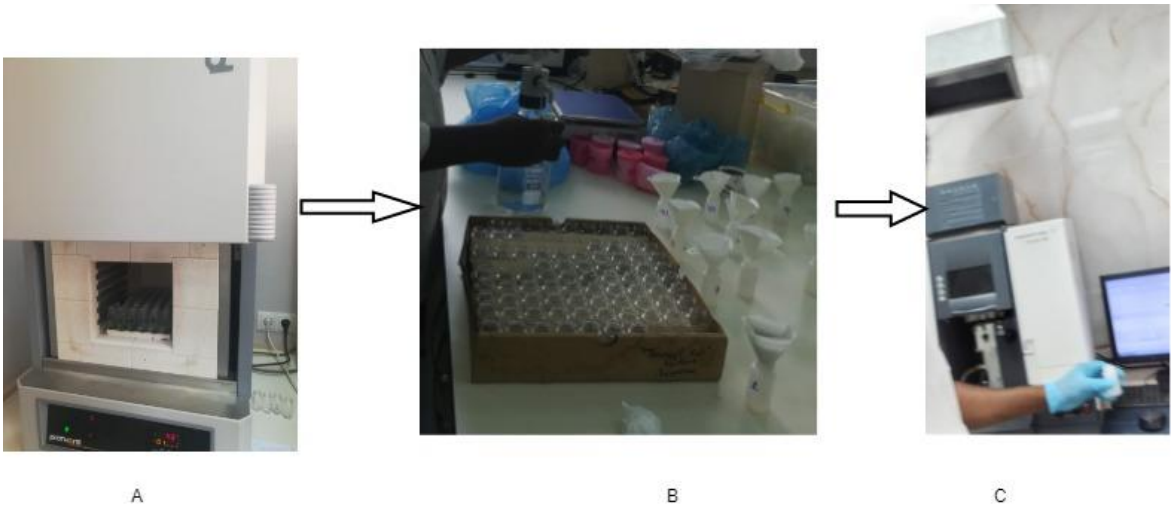


Figure 3.6. Microelements analysis

3.2.2. Protein analysis

Total crude protein and dry matter were determined using the AOAC (Association of Official Agricultural Chemists) method of 1995 (AOAC, 1995).

Amino acid analysis

The amino acid profile was determined following the method described by (Zeng et al., 2015) with minor modifications. Dried samples of 0.5 g were hydrolyzed using 10 mL of 0.1 N HCl at 110 °C for 24 hours under reflux. Then, 2 mL of the liquid was withdrawn and filtered through a 0.45 size filter. The analysis was conducted using a Shimadzu HPLC device (LC-20A) at the Horticulture Department, Cukurova University. Samples derivatized with 3-MPA, OPA, and FMOAC were injected into the Agilent Eclipse Plus C18 column (3.5 µm, 4.6×100 mm). The mobile phase, 20 mM potassium phosphate buffer (45/40/15: acetonitrile/methanol/water) (pH 6.9), was used. Amino acids were detected based on the retention times of the standards, and calculations were performed using peak areas at 262 nm for proline and at 338 nm for other amino acids.

3.2.3. Extractions of polyphenols

The extractions of polyphenols was determined using the method of Papalia et al (2019) with slight modifications. 1 g of freeze-dried *Spirulina* biomass was mixed with 5 mL of 80% methanol-water and 80% ethanol-water (v/v) (milli Q-water). The whole is vortexed for 3 minutes at room temperature. The tubes were placed in a sonication bath (Bandelin Sonorex RK 510 H, 35 kHz, Germany) at room temperature in dark for 45 minutes. The extracts were centrifuged at 5500 g at 4°C for 10 minutes, and the supernatants were separated. Then, the collected supernatant was filtered through a 0.45 µm membrane and then concentrated to dryness using a freezer dryer (FD8518, ll Shin Lab Co. Ltd., Korea) at -72°C.

3.2.4. Determination of phenolic compounds

Determination of total phenol content

The total phenol content was determined by the folin-Ciocalteu method (Singleton and Rossi, 1965). 125 µL of the *Spirulina* extracts at optimum conditions was added to 12 µL folin Ciocalteu and incubated for 6 min in the dark at room temperature. Then, 1,250 µL of 7% Na_2CO_3 was added to the mixture, and the solution was maintained at a volume of 3 mL with the addition of water. The mixture was incubated for 2 h in the dark at room temperature and the absorbance was read at 760 nm. Each essay was performed in triplicate. The result was expressed as mg gallic acid GAE/g dry weight.

Determination of total flavonoid content

The total flavonoid content was determined using the method of Dewanto et al (2002) with slight modifications. Briefly, 250 µL of diluted *Spirulina* extracts was added to 75 µL of a 15 % $NaNO_2$ solution. After incubating for 6 minutes at room temperature, 150 µL of a 10% $AlCl_3$ solution was added to the mixture. Following a 6 minute resting period at room temperature, 500 µL of 1M NaOH solution was added to the mixture. The final volume was adjusted to 2500 µL with distilled water. The absorbance of the preparation was measured at 510 nm using a spectrophotometer. The flavonoid content was expressed as milligrams of catechin equivalent per gram of dry biomass of *Spirulina* (mg CE/g DW).

Determination of condensed tannins

The condensed tannin content was determined according to the Broadhurst and Jones (1978) method using catechin as a standard. In brief, 1,500 µL vanillin solution (4%) was added to 25 µL *Spirulina* extract. Afterward, 750 µL of concentrated H_2SO_4 was added to the mixture. The latest was incubated for 15 min in the dark at room temperature and then the absorbance was read at 500 nm using a spectrophotometer. The total condensed tannins were expressed as mg equivalents of catechin (CTE) per gram dry weight.

Determination Phenolic compounds by HPLC

Phenolic compounds analysis s *Spirulina* extracts were analyzed by HPLC (Agilent Technologies, 1290 infinity model), and analysis was performed on an LC-18 İNTERSİL ODS-3 column (4,6x250mm, 5um particle size) (GL Sciences, TOKYO). The mobile phase consisted of acetic acid (3%, solvent A) and methanol (solvent B) gradient methods, with gradient elution of 0.8 ml/min. An Agilent Technologies, 1260 infinity model detector was used to detect phenolic compounds from λ 254 to 330 nm at a colon temperature of 25°C and an injection volume of 20 µl. Quantification of phenolic compounds was determined with standard curves of 3-hydroxy benzoic acid, 4-hydroxy benzoic acid, benzoic acid, chlorogenic acid, caffeic acid, gallic acid, p-coumaric acid, rosmarinic acid, synaptic acid, syringic acid, t-cinnamic acid, t-ferulic acid, catechin, epicatechin, hesperidin and quercetin. This analysis was conducted at the Scientific and Technological Application and Research Center (ASÜBTAM) of Aksaray University.

3.2.5. Antioxidant activities

Three different methods (DPPH, ABTS and FRAP) were used to assess antioxidant capacity.

DPPH (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity

The DPPH essay was done according to the method of Brand-Williams et al (1995) with some modifications. 50 µL of the *Spirulina* extracts was taken from Falcon tubes and placed in 2 mL Eppendorf tubes in a dark place; 1950 µL of a 0.06 millimolar DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was added. 250 µL of each sample was loaded onto the plate. 50 µL of pure water and 1950 µL of DPPH solution were used as controls. Blank: 50 µL of 80% methanol or ethanol and 1950 µL of DPPH solution were used. Readings were taken at a wavelength of 515 nm in the spectrophotometer. The percentage of DPPH radical scavenging was calculated using equation 3.7.

$$\text{DPPH scavenging activity (\%)} = \left(\frac{\text{Control Abs} - \text{Sample Abs}}{\text{control Abs}}\right) \text{x } 100 \qquad (3.7)$$

Where Abs represents absorbance.

ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid) essay

The ABTS scavenging activity of the extract was assessed following the method of Ozgen et al (2006) with slight modifications. In brief, a freshly prepared methanolic solution of ABTS (7 mM) was mixed with 2.45 mM of potassium persulfate and incubated for 12 hours in the dark at room temperature, with the absorbance set at 0.700±0.01. Subsequently, 100 µL of the *Spirulina*

extracts was added to 900 µL of the mixture, and the absorbance was measured at 734 nm using a spectrophotometer. As a blank control, 80% methanol and ethanol were used. The ABTS radical scavenging activity was calculated using the following equation 3.8.

$$\text{ABTS scavenging activity (\%)} = ((\text{Control abs} - \text{Sample abs})/(\text{control abs})) \times 100 \quad (3.8)$$

Where Abs represents absorbance.

FRAP (Ferric reducing antioxidant power) essay

FRAP essay was performed according to the method of Benzie and Strain (1996) . Briefly, the FRAP mix (300 mM acetate buffer, pH 3.6, 10 mM TPTZ, 40 mM HCl, and 20 Mm $FeCl_3$) was freshly prepared. At optimum extraction conditions, 2,950 µL of FRAP mix was added to a 50 µL *Spirulina* extracts. The mixture was incubated in the dark at room temperature for 4 min, and then the absorbance was read at 593 nm using a spectrophotometer against a blank. The results were expressed as µmol Trolox/g dry weight.

3.2.6. Antimicrobial activity

Growing media

For the disc diffusion method, Muller Hinton Agar (MHA), Sabouraud Dextrose Agar (SBA), and Malt Extract Agar (MEA) were used, respectively, for bacteria, yeast, and mold. For the microdilution technique, the broths used are Sabouraud Dextrose Broth (SBB) for yeast and Muller Hinton Broth (MHB) for bacteria, and Malt Extract Broth (MEB) for mold. All media were autoclaved at 121°C for 15 minutes. All media were obtained from Biokar, France. The studies were conducted in the Food Microbiology Laboratory of the Food engineering department of Cukurova University.

Microbial strains

In this study, the microbial activity of plant extracts was tested against four bacterial strains: two Gram-negative strains: *Escherichia coli,* and *Klebsiella pneumonia*; Gram-positive strains: *Staphylococcus aureus*, and *Bacillus cereus;* one yeast, *Candida albicans*; one mold: *Aspergillus flavus*. These strains were conserved in glycerol at -20°C. All the strains were obtained from the Food Engineering Department, Cukurova University, Adana, Turkey.

Inoculum standardization

The bacterial inoculum was obtained by taking 2-3 colonies from 24-hour cultures which were aseptically collected and suspended in 0.9% sterile saline solution and shacked for 15 seconds, the turbidity was adjusted to 0.5 McFarland. The bacterial suspensions contain approximately 1-2 x 10^8 CFU/ mL, while the yeast suspension contains approximately 1-5 x 10^6 CFU/ mL. Regarding the fungal inoculum, sporulation was obtained by culturing *A. flavus* on MEA medium at 27°C for 7 days. Briefly, the spores were collected by flooding the plate with 5 mL of 0.05 % (v/v) tween 20, using a sterile spread rod. The number of spores in the mother suspension was counted using a hemocytometer calculator (area: 0.0025 mm^2; depth: 0.2 mm) with light microscopy before being diluted to 10^6 spores/mL in 0.9% NaCl solution.

Well agar diffusion method

The plates containing the agar mediums MHA, SDA, and MEA were inoculated with impregnated swabs with bacterial and fungal standardized suspensions; then, the plates were dried for 10 min. Afterwards, 6-mm wells were performed on the surface of pre-inoculated agar. Then, 100 µL of the *Spirulina* extracts (100 mg/mL of 5% Dimethyl sulfoxide (DMSO) were added to the wells, 1 to 10. Ampicillin 10 µg/well (AMP antibiotic) and kanamycin 10 µg/well (KAN antibiotic) were used as positive controls for bacteria and fluconazole X µg/well (FLU: antifungal) for fungal species. DMSO (Dimethyl sulfoxide) 5% was used as a negative control. Finally, the plates were incubated for 24 hours at 37°C for pathogens, and 30°C for Bacillus spp. Yeast and mold plates were incubated at 25-27°C for 3 days and 5 days, respectively. After incubation, the diameters of the inhibition zones were measured in millimeters using a transparent ruler.

Determination of the minimum inhibitory concentration MIC

Microdilution essays determined the MICs according to the standards of the NCCLS (Patel and Clinical and Laboratory Standards Institute, 2017) . 50 µL of the growth medium was deposited in each well of the microplate; MHB for bacterial strains and SBB for the yeast strain. Then, one hundred microliters of each *Spirulina* extract (1/10 diluted in growth medium) were poured into the first well. After that, a micro-dilution was done by diluting the sample by a factor of 2 in each well, except for the last well, which acted as the positive control for growth. Finally, the inoculation was carried out by depositing 50 µL of the microbial suspension (1/150 diluted in growth medium) whose turbidity had been verified previously. After that, the plates were incubated under agitation for 24 h at 37°C. 20 µL of the 0.2% 2,3,5-triphenyltetrazolium chloride (TTC) revealer (Biokar, France) was added to each well, giving a pinkish coloration where there is growth due to the activity of the dehydrogenases after incubation for 2 hours. After 2 h of incubation, the MIC corresponds to the lowest concentration that does not produce a red color.

3.2.7. Statistical Analysis

The experiment was performed in triplicate, and the data were expressed as the mean ± standard deviation (SD). Statistical analysis was conducted using the SPSS statistical program (Version 25.0). One-way analysis of variance (ANOVA) was applied, followed by Duncan's comparison test. The χ_2 test was used to compare means for two groups.

4. RESULTS AND DISCUSSION

4.1. Growth of *Spirulina platensis*

4.1.1. Temperature

During this study, the temperature in *S. platensis* cultures was measured daily. Temperature changes (°C) are shown in Figure 4.1. There was no significant difference between mean temperatures ($p > 0,05$) among the groups, statistically. The mean culture temperature values for each treatment were 33,21±2,82 °C for Mg-100 mg/L, 34,06±3,39 °C for Mg-200 mg/L, 32,59±2,38 °C for Zn-3 mg/L, 32,75±2,37 °C for Zn-5 mg/L and 32,876±2,673 °C for the control, during the experiment. The overall mean culture temperature was 33,10±2,71 °C.

Spirulina is a thermophilic cyanobacterium. According to Richmond (2018), the optimal temperature for *Spirulina* growth is between 35°C and 37°C. Temperature is the fundamental factor for all living organisms, particularly microalgae, given its impact on metabolic processes and the cellular biochemical composition. The ideal growth temperature and resistance to extreme temperatures typically differ from one strain to another. At a temperature of 45°C, no growth has been observed, accompanied by the disappearance of pigmentation in *Spirulina* (Kumar et al., 2011). Kumar et al (2011) reported that the growth rate of *S. platensis* increases with increasing temperature, reaching a maximum of 35°C. Above this temperature, a reduction in pigments was observed.

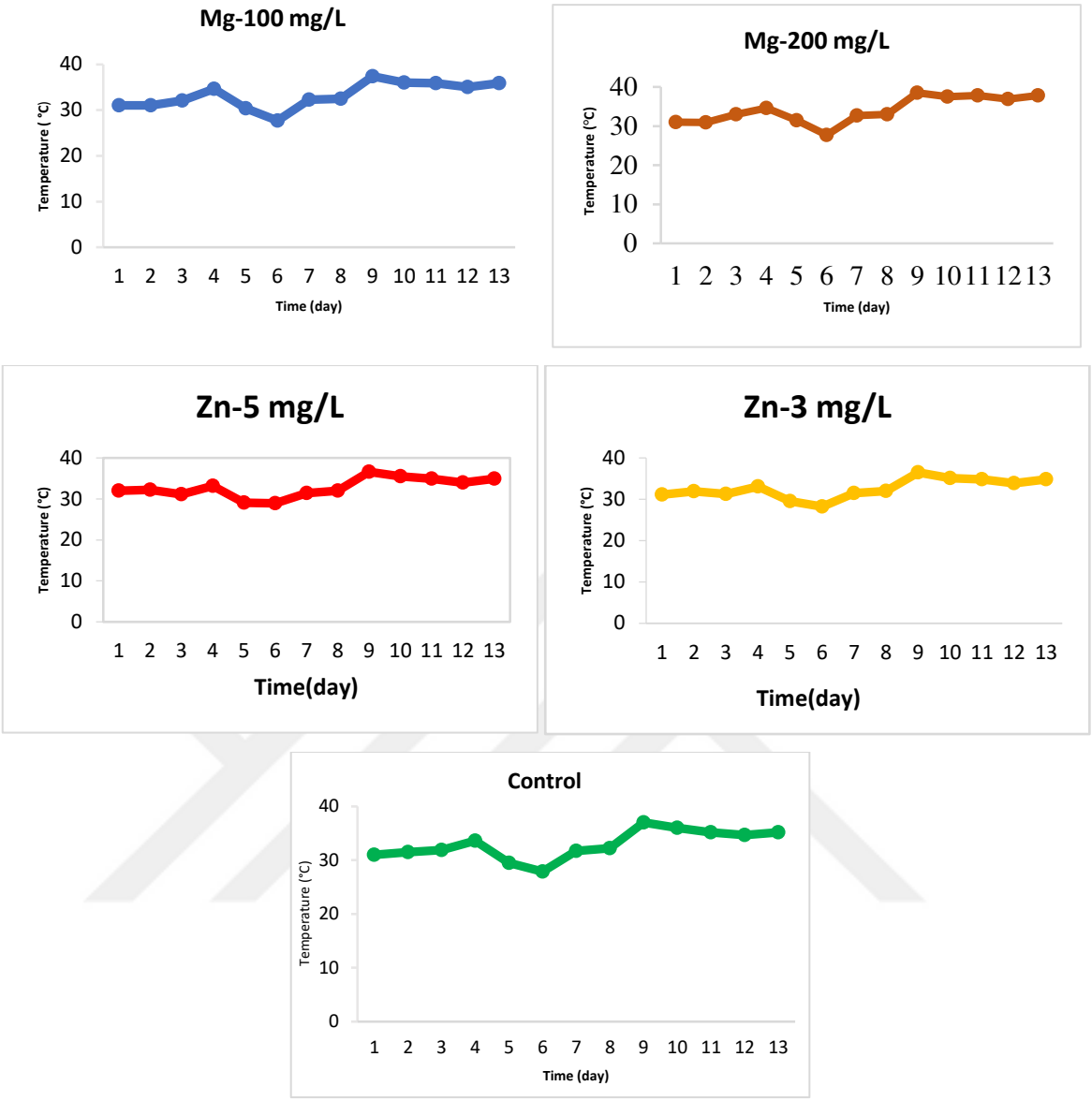


Figure 4.1. Changes in temperature of *S. platensis* cultures grown with different concentrations of Magnesium (100 and 200 mg/L) and Zinc (3 and 5 mg/L) during the experiment

4.1.2. pH

During this study, the daily pH in *S. platensis* cultures was measured daily. pH changes are shown in Figure 4.2. There was no significant difference between mean pH ($p > 0,05$), statistically. The mean pH values for each treatment were 10,10±0,14 for Mg-100 mg/L, 10,10±0,13 for Mg-200 mg/L, 10,12±0,13 for Zn-3 mg/L, 10,12±0,11 for Zn-5 mg/L and 10,16±0,160 for the control, during the study. The overall mean culture pH was 10,1215±,02516.

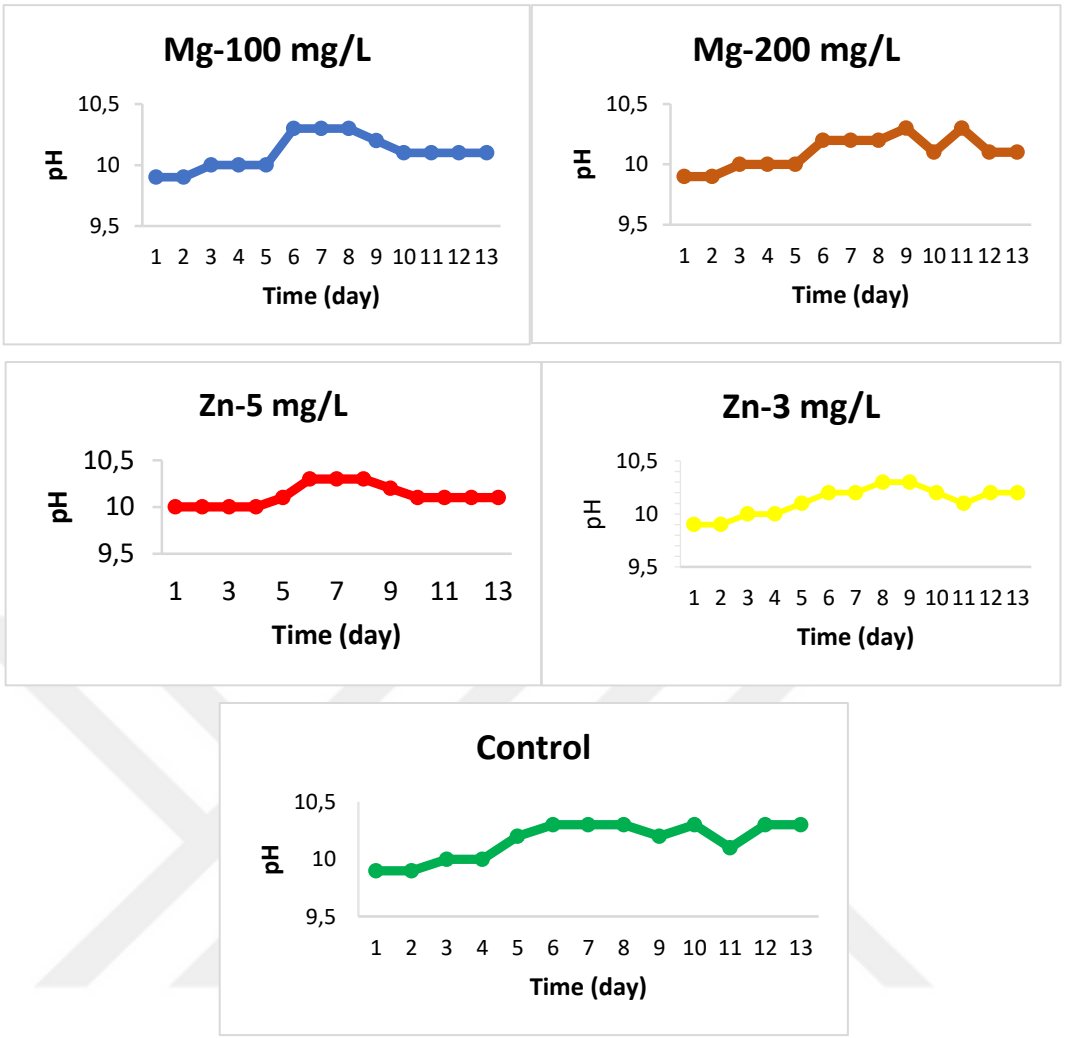


Figure 4.2. Changes in pH of *S. platensis* cultures grown with different concentrations of Magnesium (100 and 200 mg/L) and Zinc (3 and 5 mg/L) during the experiment.

4.1.3. Light intensity

During this study, the light intensity in *S. platensis* cultures was measured daily. pH changes are shown in Figure 4.3. There was no significant difference between mean temperatures ($p > 0,05$), statistically. The mean the light intensity values for each treatment were 1108,33±180,05 µmol $m^{-2}s^{-1}$for Mg-100 mg/L, 1006,99±232,42 µmol $m^{-2}s^{-1}$for Mg-200 mg/L, 1141,27±212,02 µmol $m^{-2}s^{-1}$ for Zn-3 mg/L, 1035,923±176,48 µmol $m^{-2}s^{-1}$ for Zn-5 mg/L and 1130,54±175,57 µmol $m^{-2}s^{-1}$ for the control, during the study. The overall mean culture pH was 1084,61±197,88 µmol $m^{-2}s^{-1}$.

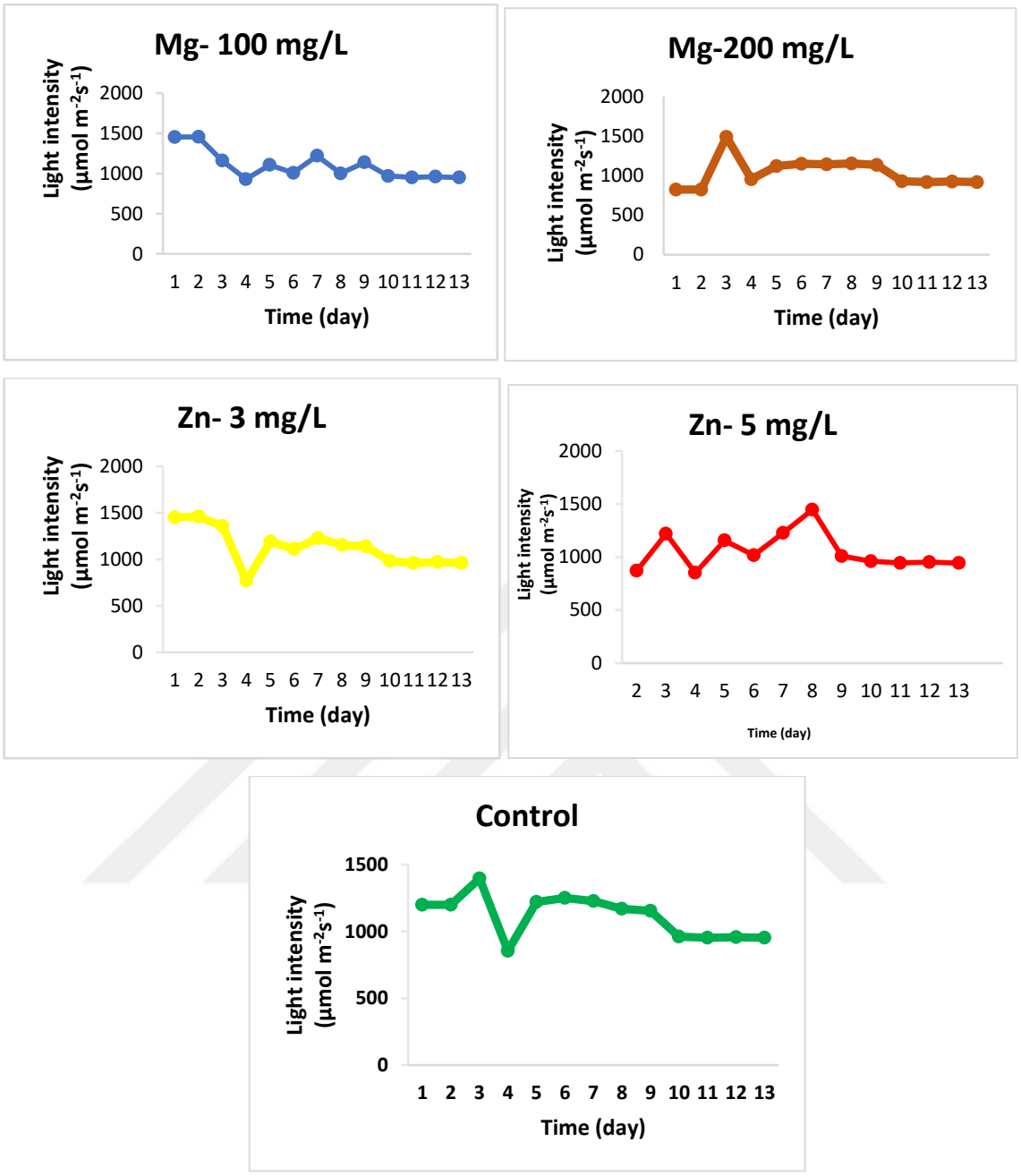


Figure 4.3. Changes in light intensity of *S. platensis* cultures grown with different concentrations of magnesium (100 and 200 mg/L) and zinc (3 and 5 mg/L) during the experiment

4.1.4. Optical density

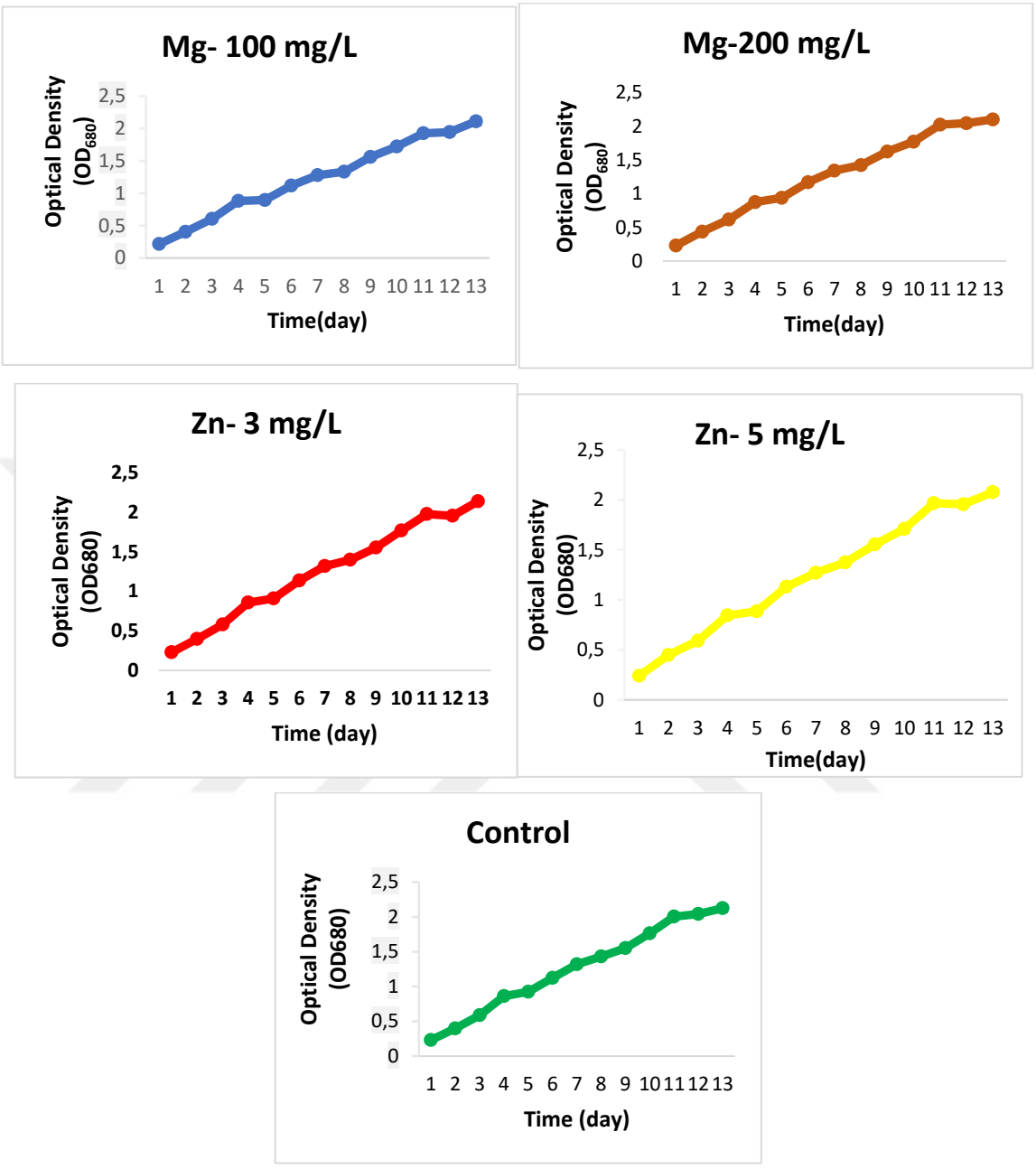


Figure 4.4. Changes in OD_{680} of *S. platensis* cultures grown with different concentrations of magnesium (100 and 200 mg/L) and zinc (3 and 5 mg/L) during the experiment

Two different magnesium concentrations (100 mg/L and 200 mg/L) were used for evaluating the magnesium effect on *S. platensis* growth. The experiment was continued for 13 days, and the optical absorbance values (OD_{680}) indicating the initial cell density were determined at 0,21±0,005, 0,23±0,00 and 0,23±0,00 for the Mg-100 mg/L groups, Mg-200 mg/L and control, respectively. During the experiment, the cell density increased steadily and no decrease was observed until the end of the experiment, which ended on the 13th day when the culture was

harvested. The maximum cell densities observed on the last day were 2,11±0,26, 2.10±0.06 and 2.12±0.056 for the Mg-100 mg/L, Mg-200 mg/L and control groups, respectively.

Also, two different zinc concentrations were used in the zinc group: 3 mg/L and 5 mg/L for the experiment. The experiment was conducted for 13 days, and the optical density values (OD_{680}), which indicate the initial cell density, were measured on the first day. Initial cell density values were 0,23±0,01, 0.24± 0,01 and 0,23±0,00 for the Zn-3 mg/L, Zn-5 mg/L and control groups, respectively. During the experiment, the cell density increased steadily and no decrease was observed until the end of the 13th day when the culture was harvested. On the last day of the experiment, the maximum cell densities were recorded at 2,14±0,01, 2,08±0,05 and 2,12±0,05 for the Zn-3 mg/L, Zn-5 mg/L and control groups, respectively.

These results show that the mineral enrichments of magnesium and zinc had no significant effect on the cell density of the *Spirulina* during the experiment. The cell density values in the control group are generally similar or close to those of the mineral enrichment treatments. This suggests that mineral enrichments did not have a significant impact on *S. platensis* growth over the duration of the experiment.

Thus, the outdoor cultivation of *S. platensis* in different concentrations of magnesium (100 and 200 mg/L) and zinc (3 and 5 mg/L) led to rapid adaptation of the microalgae and discrete lag phase. These observations indicate an excellent performance of *S. platensis* against abiotic stress. Furthermore, the results show that *S. platensis* can photosynthesize efficiently, allowing it to accumulate biomass in the presence of light. The optical density of each mineral concentration reached its maximum value at 13 days of culture.

During the incubation period, Ozturk Urek and Kerimoglu (2019) observed that the presence of Mg^{2+} increased the OD values of *Arthrospira platensis*. Ben Amor-Ben Ayed et al (2015) reported similar results. They kept the same level of growth of *C. vulgaris* from 8.9 to 456 mg/L of Mg^{2+} and concluded that magnesium is not toxic for *C. vulgaris*, even at high concentrations.

Magnesium plays a crucial role in the chlorophyll molecule by acting as a constituent element of the chlorophyll skeleton. It is essential for the formation of chlorophyll and directly impacts the biogenesis of this molecule for photosynthesis (Sarma et al., 2014; Carvalho et al., 2011). It also acts as a transporter and contributes as a cofactor for many enzymes. It is one of the main constituents of the microalgae culture medium. When microalgae are subjected to magnesium deprivation during their growth, it significantly reduces chlorophyll production, negatively affecting their ability to carry out photosynthesis efficiently, while starch accumulation increases dramatically (Volgusheva et al., 2014).

Similar results showed no significant differences in OD values between the control group and the other treatments after the 14th day of culture (Akbarnezhad et al., 2019). Balaji et al (2014) report that *S. indica*, *S. maxima*, and *S. platensis* could tolerate low concentrations of zinc and

nickel (0.01 mM) for 13 days. According to Zhou (2018), *Spirulina* can grow in a medium containing minerals, particularly zinc. However, high zinc concentrations can decrease the growth curve from the 14th day of incubation in a medium containing a tenfold higher zinc concentration (Akbarnezhad et al., 2019). The previous study showed that growth parameters increased with varying concentrations of $ZnSO_4$ except at 8.0 mg L^{-1} zinc (Ghanbarzadeh et al., 2022). Thus, exposure to a high zinc level could be toxic, reducing biological activity and potentially lethal to *Spirulina platensis* (Zhou et al., 2018). Therefore, *S. platensis* has a high growth potential and is a potential candidate for treating wastewater containing low concentrations of heavy metals.

4.1.5. Dry matter

Biomass measurements were checked on the first, third, sixth, and ninth day, as well as the last day of the experiment, as shown in Figure 4.5. A general trend of gradual increase in the biomass of *S. platensis* over time is observed for all conditions tested.

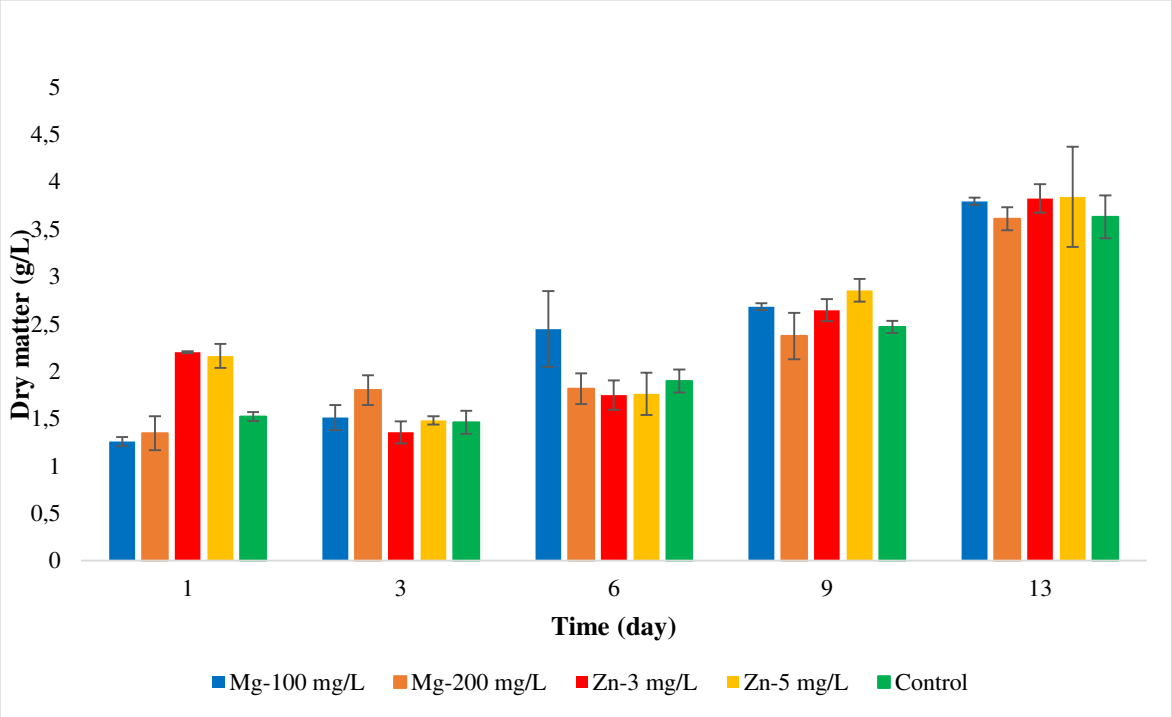


Figure 4.5. Changes in dry matter (g/L) of *S. platensis* cultures grown with different concentrations of magnesium (100 and 200 mg/L) and zinc (3 and 5 mg/L) during the experiment.

The maximum biomasses were observed on the 13th day of the experiment for all the groups. The final biomass values were for the groups exposed to a zinc concentration of 5 mg/L (3,84±0,52 g/L), followed by the group exposed to 3 mg/L zinc (3,82±0,15 g/L), the group exposed to 100 mg/L magnesium (3,79±0,03 g/L), the unenriched control group (3,63±0,22 g/L), and finally the group exposed to 200 mg/L magnesium (3,61±0,12 g/L). No significant difference was observed in the biomass on the last day of the experiment.

The effects of zinc on the biomass of *S. platensis* may not be as pronounced as those of magnesium. Magnesium enrichment also benefits *Spirulina* growth but to a lesser extent than zinc enrichment. These results suggest that mineral enrichment, particularly zinc, and magnesium, could effectively improve *Spirulina* biomass production, which is essential in algae production for food, nutrition, medical, or environmental purposes.

Similar results were observed in a previous study by Zinicovscaia et al (2021). In this study, they also found that when they exposed *Spirulina* to a medium containing zinc ions at different concentrations (2.5, 5.0 and 10.0 mg/L), biomass production was similar to that of the control group. On the other hand, according to the study carried out by Pane et al (2008), the addition of zinc ions at concentrations ranging from 0.5 to 2 mg/L caused a reduction of around 50% in the biomass of *S. platensis*. Omar (2002) observed that very low zinc concentrations stimulated the growth of *Scenedesmus obliquus* and *S. quadricauda*. In contrast, when zinc concentrations were lower (i.e. 1.5, 4.5 and 8.0 µg L^{-1}), the growth of *S. quadricauda* was inhibited. This suggests that the effect of zinc on algal growth depends on the concentration used. According to the study conducted by Baścik-Remisiewicz et al (2009), zinc promotes growth rate because it is an essential metabolic requirement for microalgae and acts as an important enzyme cofactor.

4.1.6. Chlorophyll *a*

The quantities of Chlorophyll *a* (µg/L) are shown in Figure 4.6. In the Mg-100 mg/L essay group, the maximum chlorophyll *a* concentration was reached on day 6, with a value of 2,493±0,03 µg/L, while the minimum chlorophyll *a* concentration was 0,62±0,07 µg/L on day 13. As for the in Mg-200 mg/L group the experiment, the maximum chlorophyll *a* concentration observed was 2,70±0,89 µg/L on day 9, while the minimum value recorded was 0,46±0,13 µg/L on day 13.

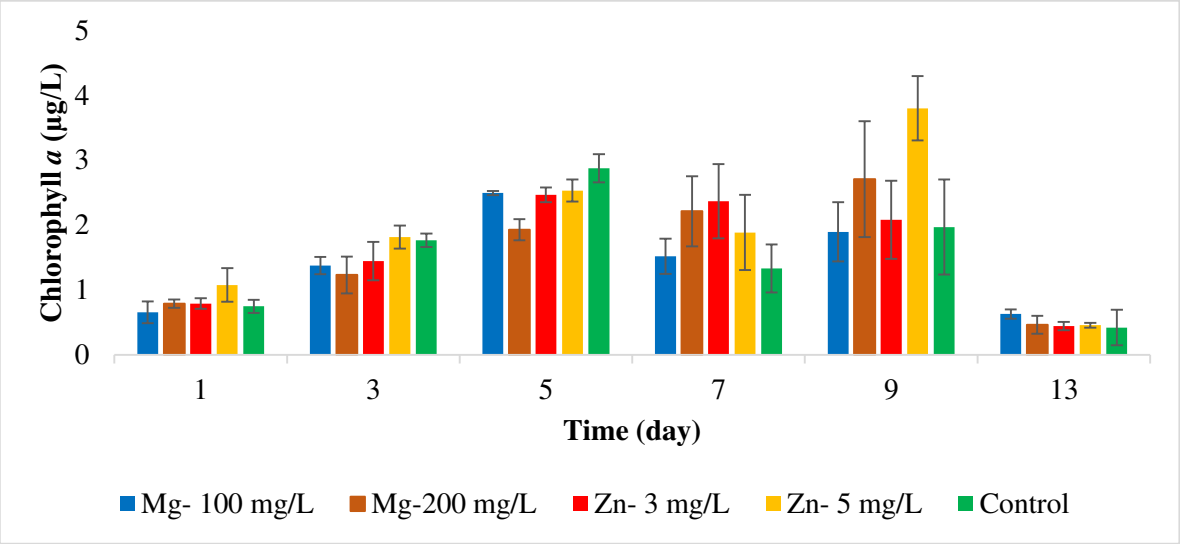


Figure 4.6. Changes in Chlorophyll *a* of *S. platensis* cultures grown with different concentrations of magnesium (100 and 200 mg/L) and zinc (3 and 5 mg/L) during the experiment.

In the Zn-3 mg/L experiment group, the maximum chlorophyll *a* value was observed on day 3, with a value of 2,46±0,11 µg/L, while the minimum chlorophyll value was 0,44±0,06 µg/L on day 13. In the Zn-5 mg/L experiment group, the maximum chlorophyll *a* value was observed on day 9, with a value of 3,79±0,49 µg/L, while the minimum value was 0,45±0,03 µg/L on day 13.

In the Zn-5 mg/L experiment group, the maximum chlorophyll *a* value was observed on day 9, with a value of 3,79±0,49 µg/L, while the minimum value was 0,45±0,03 µg/L on day 13. In the control group, the maximum chlorophyll *a* value was observed on day 6, reaching 2,87±0,21 µg/L, and the minimum value was 0,42±0,27 µg/L on day 13. No statistically significant difference ($P < 0,05$) was observed in chlorophyll *a* level between the different groups on the last day of experimentation.

4.1.7. Total carotenoids

The quantities of total carotenoids (µg/L) are shown in Figure 4.7. The maximum concentration of total carotenoids in the Mg-100 mg/L experiment group was 1,01± 0,28 µg/L on the 9th day, and the minimum concentration was 0,21± 0,01 µg/L on the 1st day of the experiment. In the Mg-200 mg/L experiment group, the maximum concentration of total carotenoids was 0,87± 0,09 µg/L on the 5th day, and the minimum concentration was 0,23± 0,05 µg/L on the 1st day of the experiment.

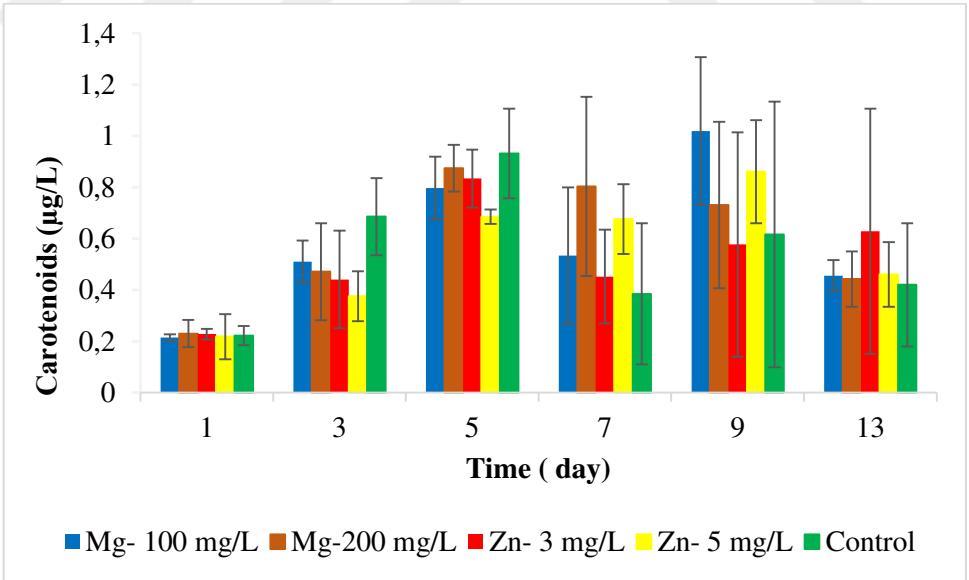


Figure 4.7. Changes in carotenoids of *S. platensis* cultures grown with different concentrations of magnesium (100 and 200 mg/L) and zinc (3 and 5 mg/L) during the experiment.

The maximum value of total carotenoids in the Zn-3 mg/L group was 0,83± 0,11 µg/L on the 5th day, and the minimum value was 0,22±0,02 µg/L on the 1st day of the experiment. The maximum value of total carotenoids in the Zn-5 mg/L group was 0,86± 0,20 µg/mL on the 9th day, and the minimum value was 0,218± 0,08 µg/L on the 1st day of the experiment. In the Control

group, a maximum value of total carotenoids of 0,93± 0,17 µg/L was observed on the 5th day and a minimum value of 0,222± 0,037 µg/L on the 1st day of the experiment. chlorophyll *a* and Carotenoid levels throughout the culture showed a consistent accumulation, as depicted in Figure 4.6. and 4.7. The results show that mineral enrichment, particularly magnesium and zinc, can affect carotenoid concentrations in *S. platensis*. However, no obvious pattern emerges since concentrations and mineral enrichment concentrations change with time. The interactions between minerals and *Spirulina* may be complex, depending on nutrient availability, the growth of cells, and other factors.

Chlorophyll and Carotenoids are vital natural pigments found in plants and algae, playing a crucial role in photosynthesis. They are important indicators of the growth and health of plants and microalgae. These pigments are essential for capturing light energy and facilitating the process of photosynthesis, which is vital for producing energy and organic compounds in plants and algae.

The reduction in the amount of chlorophyll between days 9 and 13 could be explained by several factors, such as nutrient depletion, the accumulation of metabolic waste produced during growth, environmental conditions, the natural growth cycle of *Spirulina*, which naturally reaches a stage of reduced growth, as well as the biological responses of *Spirulina*.

The previous study showed that the chlorophyll *a* levels declined in media with 0.2 mM and without Mg^{2+} after the 6th day, as the dry biomass was lower at these concentrations. This decrease might be attributed to the essential nutrient Mg^{2+} since its deficiency did not promote dry biomass growth. A positive correlation was observed between maximum dry biomass and Chl *a* values, indicating that chlorophyll *a* indicates microalgae growth. After 6 days, chlorophyll *a* levels decreased in Mg^{2+}-deficient and Mg^{2+}-absent conditions, suggesting that chlorophyll *a* molecules might lose Mg ions in the active center (Ozturk Urek and Kerimoglu, 2019). on the other hand, Finkle and Appleman (1953) reports that excessive amounts of Mg in culture media cause an increase in the chlorophyll content of cells. this could reduce light diffusion in the culture and thus lead to a decrease in culture productivity (Mitra and Melis, 2008). Magnesium plays a crucial role in the photosynthesis process as the central element of chlorophyll. It is essential for microalgae growth because it ensures ribosome stability, helps chlorophyll to function properly and activates the enzymes essential for photosynthesis and respiration. Ermis et al (2020) reported no significant differences in biomass quantity and chlorophyll content between the different samples studied. However, they did observe variation in carotenoid concentrations. The 0.4 mM $MgSO_4$ group (equivalent to 7.4 mg L^{-1}) showed the highest concentration of carotenoids among all samples and enrichment concentrations. In the control group, the carotenoid concentration was 3.3 mg L^{-1}, while it reached 5.8 mg L^{-1} in the presence of 5.1 mM $MgSO_4$.

Ghanbarzadeh et al (2022) reported a significant increase in chlorophyll *a* levels when zinc concentrations were 4.0 mg L^{-1}, with an increase of only 2.70% compared to the control group. In contrast, carotenoid content at 4.0 mg L^{-1} zinc increased significantly by 14.73% compared with the

control sample. Zhou et al (2018) also reported that under zinc stress, chlorophyll-a concentration increased rapidly to 4.0 mg/L zinc ions between 12 and 15 days, reaching its maximum level compared to other stressed groups. In multi-component systems, β-carotene content varied between 0.23% and 0.29% of dry biomass, remaining very close to the value of the control group (0.26%) (Zinicovscaia et al., 2021). In the mono-metal system, the chlorophyll content in the biomass was closely linked to the zinc concentration in the solution. At a zinc concentration of 2.5 mg/L, chlorophyll *a* was comparable to that in the control group but gradually decreased with increasing zinc concentration in the medium. When the zinc concentration reached 10 mg/L, chlorophyll *a* in the biomass decrease by 31% compared with the control group (Zinicovscaia et al., 2021).

Carotenoids are accumulated by microalgae as a function of metal concentrations and types when growth and production are affected by stress. They are synthesized in the chloroplast by the action of a series of nuclear-encoded membrane proteins.

4.1.8. Phycocyanin

The maximum and minimum phycocyanin concentrations of each group, with their respective times as a function of the different experimental conditions, are shown in Figure 4.8. In the experimental group with a zinc concentration of 5 mg/L, phycocyanin levels reached a maximum concentration of 0,29±0,003 mg/L on day 3, while they reached a minimum concentration of 0,25±0,003 mg/L on day 11. In the experimental group with a zinc concentration of 3 mg/L, phycocyanin levels reached a maximum concentration of 0,26±0,008 mg/L on day 7, while they reached a minimum concentration of 0,24±0,008 mg/L on day 11.

In the experimental group with a magnesium concentration of 100 mg/L, phycocyanin levels reached a maximum concentration of 0,26±0,002 mg/L on day 1 and a minimum of 0,24±0,008 mg/L on day 5. In the experimental group with a magnesium concentration of 200 mg/L, phycocyanin levels reached a maximum concentration of 0,29±0,000 mg/L on day 7, while they reached a minimum concentration of 0,24±0,004 mg/L on day 13.

In the experimental control group, phycocyanin levels reached a maximum concentration of 0,29±0,004 mg/L on day 9, while they reached a minimum concentration of 0,25±0,004 mg/L on day 11.

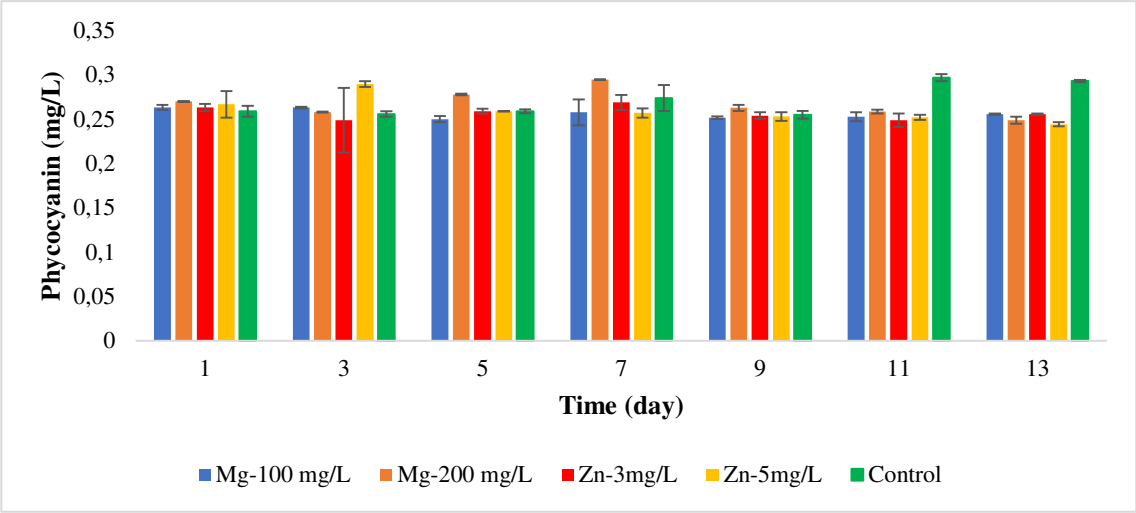


Figure 4.8. Content of phycocyanin in *S. platensis* biomass cultivated in different metal enrichment

These results show variations in phycocyanin values on day 13 between the different experimental groups. The control group had the highest phycocyanin value (0,29±0,001 mg/L) compared with the other groups.

Zinc enriched groups showed slightly lower values than the control group at concentrations of 5 mg/L (0,24±0,002 mg/L) and 3 mg/L (0,25±0,001 mg/L). Similarly, the magnesium enriched groups, with concentrations of 100 mg/L (0,25±0,001 mg/L) and 200 mg/L (0,24±0,004 mg/L), also showed slightly lower values, and this difference was significant ($P < 0,05$) when compared to the control group (0,29±0,001 mg/L).The reduced phycocyanin concentrations in the mineral-enriched groups could be explained by a possible inhibition of phycocyanin production due to the effects of these metal ions.

Akbarnezhad et al (2019) also observed phycobiliproteins decrease in the *Spirulina* treatment by Fe^{2+}, Cu^{2+} and Zn^{2+}. Phycobiliproteins, including phycocyanin and allophycocyanin, exhibit distinct preferences in binding to heavy metal ions, with phycocyanin displaying a higher affinity for such ions (Bellamy-Carter et al., 2022). These proteins, found in microalgae, are distinguished by their brilliant colors and fluorescence, making them one of the most colorful and luminescent proteins in nature. Phycobiliproteins, found in microalgae, are distinguished by their bright colors and intense fluorescence, making them among the most colorful and luminescent proteins in nature. These characteristics enable them to capture light energy in auxiliary photosynthetic complexes called phycobilisomes (Dagnino-Leone et al., 2022). These natural pigments are of great importance to sectors such as food, cosmetics, nutraceuticals and pharmaceuticals (Pagels et al., 2019). Phycocyanin, the most abundant phycobiliprotein, is an intense blue water-soluble protein produced by the phycobilisome in cyanobacteria and red algae. It can bind to heavy metal ions, inhibiting their fluorescence. This property has led to phycocyanin being considered a potential biosensor for heavy metals. Bellamy-Carter et al (2022) observed that phycocyanin and allophycocyanin had minimal binding to metal cations such as zinc and iron.

Other authors have reported that phycocyanin is a potential biosensor for heavy metals, particularly mercury (Hg^{2+})(Chi et al., 2020; Bhayani et al., 2016).

4.1.9. Metal accumulation

Magnesium accumulation

Table 4.1. Variation in magnesium accumulation in *Spirulina platensis* harvested at different times and with varying concentrations of mg

	***S. platensis* Magnesium content (mg/100 g DW)**		
	Magnesium enrichment		
Time	**Mg-100 mg/L**	**Mg-200 mg/L**	**Control**
1st hour	3065,87±24,30[a]	2254,16±98,64[b]	1637,63±,17,72[c]
3rd hour	2874,67±45,14[b]	3133,76±61,47[a]	1034,65±24,57[c]
5th hour	5663,50±46,47[c]	7189,42±107,97[a]	6471,97±204,23[b]
Day 5	119,79±8,54[a]	126,35±23,57[a]	127,97±17,52[a]
Day 9	136,26±40,28[a]	125,33±35,87[a]	140,43±33,32[a]
Day 13	249,62±73,27[ab]	348,150±44,84[a]	210,25±5,25[b]

Different letters in the same row indicate statistically significant differences ($p<0.05$).

The table 4.1. demonstrates the levels of variation in the magnesium content of *Spirulina platensis* at different times and under different experimental conditions.

In the Mg-100 mg/L group of the experiment, the maximal value of magnesium concentration was 5663,50±46,47 mg/100 g at the 5th hour and the minimal was 119,79±8,54 mg/100 g at the 5th day. In the Mg-200 mg/L group of the experiment, the maximal value of magnesium concentration was 7189,42±107,97 mg/100 g at the 5th hour and the minimal was 125,33±35,87 mg/100 g at the 9th day. In the experiment's control group, the maximal value of magnesium content was 6471,97±204,23 mg/100 g at the 5th hour and the minimal was127,97±17,52 mg/100 g at the 5th day.

On the last day of the experiment, the magnesium levels in the different groups were respectively 348,150±44.845 mg/100 g, 249,626±73,274 mg/100 g, 210,250±5,254 mg/100 g in the Mg-200 mg/L group, Mg-100 mg/L group and Control group. According to these results, the Mg-200 mg/L group had the highest magnesium concentration on the experiment's last day (13th day). It was followed by the Mg-100 mg/L group with a lower concentration and the Control group with the lowest magnesium concentration.

These results show that magnesium enrichment at a concentration of 200 mg/L led to a more significant accumulation of magnesium in *Spirulina platensis* compared with the other groups. This confirms that the higher the magnesium concentration, the higher the magnesium

content. Magnesium enrichment had a significant effect on magnesium bioaccumulation by *Spirulina platensis*.

Zinc accumulation

Table 4.2. shows variations in zinc accumulation in *S. platensis* biomass collected at different times (1h, 3h, 5h, day 5, day 9 and day 13) during the experiment. In the Zn-3 mg/L experiment group, the maximum zinc content value was observed on the 5th hour of the experiment, with a value of 117,98±27,91 mg/100g, while the minimum value was 1,77±1,40 mg/100 g on the 9th day. In the Zn-5 mg/L experiment group, the maximum zinc content value was observed on the 5th hour of the experiment, with a value of 178,54±5,61 mg/100g, while the minimum value was 1,65±0,52 mg/100 g on the 1st hour.

In the control experiment group, the maximum zinc content value was observed on the 9th day of the experiment, with a value of 2,70±0,78 mg/100g, while the minimum value was 1,76±0,48 mg/100 g on the 3rd hour.

Table 4.2. Variation in zinc accumulation in *S. platensis* harvested at different times and with different Zn concentrations

	***Spirulina platensis* Zinc content (mg/100 g DW)**		
	Zinc enrichment		
Time	**Zn-3mg/L**	**Zn-5mg/L**	**Control**
1st hour	2,18±1,31[a]	1,65±0,52[a]	1,87±0,11[a]
3rd hour	16,05±0,77[b]	9,47±1,65[a]	1,76±0,48[c]
5th hour	117,98±27,9[b]	178,54±5,61[a]	1,77±0,11[c]
Day 5	2,48±0,27[a]	2,49±0,07[a]	2,21±0,36[a]
Day 9	1,77±1,40[a]	1,80±0,14[a]	2,70±0,78[a]
Day 13	3,57±0,14[a]	2,84±0,36[a]	2,54±0,97[a]

Different letters in the same row indicate statistically significant differences ($p<0.05$).

S. platensis exposed to zinc concentrations of 5 mg/L and 3 mg/L showed a significant increase in zinc content over time compared with the control group. A progressive increase in zinc content was observed for both zinc concentrations (Zn 5mg/L and Zn-3 mg/L) up to day 5, followed by a decrease on day 9. This could be explained by a certain saturation or equilibrium between *Spirulina platensis* and zinc after a specific exposure. Long-term exposure to zinc in *S. platensis* produces different effects from those observed in the short term, suggesting an adaptation of this microalga to prolonged exposure. The results raise pertinent questions about the impact of zinc exposure, not only on the quality and nutritional value of *Spirulina platensis* as a potential dietary supplement to combat zinc deficiency but also as a potential alga for wastewater treatment.

Zhou et al (2018) also observed a significant increase in zinc accumulation resulting from increased zinc concentrations before 4.0 mg/L. A 27.2-fold increase in total zinc content at 4.0 mg/L Zn^{2+} compared with the control group was found. Zinicovscaia et al (2021) evaluated the capacity of *Spirulina platensis* to accumulate Zn, Ni and Cu ions in the context of environmental bioremediation. They found that *Spirulina platensis* had a better zinc bioaccumulation capacity than nickel and copper ions. This alga could be an exciting option for removing heavy metals from the environment due to its strong capability adsorb and accumulate these toxic heavy metal ions (Zinicovscaia et al, 2021).

4.2. Protein

Figure 4.9. shows the results of protein levels in *Spirulina platensis* as a function of different zinc and magnesium concentrations. The control group presented the highest protein content (70,34%), followed by the magnesium 200 mg/L group (63,73%) and the Mg-100mg/L group (61,89%). The zinc group had a lower protein content than the other groups. The protein content was 55,59% for the Zn-3 mg/L group and 51.43% for the Zn-5 mg/L group.

This study shows that the higher concentrations of zinc harm protein content compared with the control group, with a progressive decrease in protein content observed as the zinc concentration increases. In contrast, higher magnesium concentrations have a slightly positive effect on the protein content of *Spirulina*. Protein content values increased somewhat with increasing magnesium concentration.

The control group received no additional treatment and had the highest protein content (70,34%).

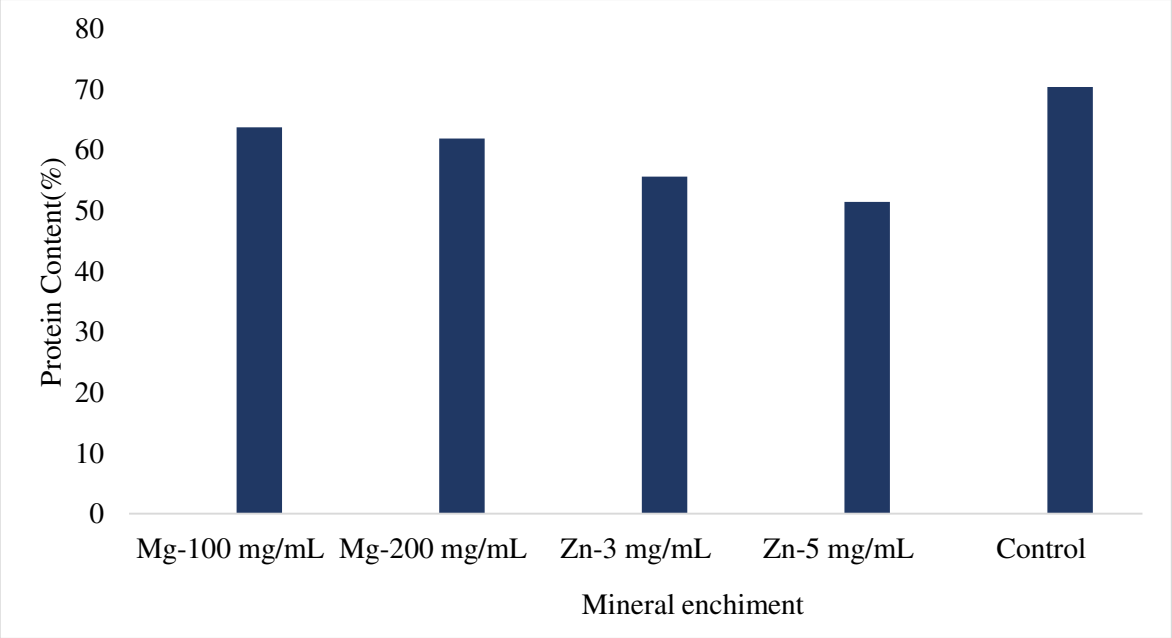


Figure 4.9. Protein content of *Spirulina platensis* enriched with magnesium and zinc

4.3. Amino acid

In the Mg-100 mg/L group, histidine has the highest amino acid concentration, reaching 2,58±0,010 g/100g DW, while glycine reaches its minimum level at 0,09±0,005 g/100g DW. In the Mg-200 mg/L group, histidine has the highest amino acid content, reaching 2,30±0,08 g/100g DW, while glycine reaches its minimum level at 0,06±0,004 g/100g DW. In the Zinc-3 mg/L group, histidine has the highest amino acid concentration, reaching 2,24±0,05 g/100g DW, while glycine comes to its minimum level at 0,07±0,005 g/100g DW. In the Zinc-5 mg/L group, glutamate has the highest amino acid content, reaching 2,37±0,07 g/100g DW, while glycine comes to its minimum level at 0,07±0,004 g/100g DW. In the control group, histidine maintained a high 2,55±0,02 g/100g DW as the most predominant amino acid. In comparison, glycine presented the weakest amino acid, represented by 0,09±0,001 g/100g DW. The amino acid values (g/100 g) for *Spirulina platensis* in the different experimentation groups are given in Table 4.3.

Uslu et al (2009) reported that climate change significantly influences protein content and that amino acid concentration is higher during the summer growth period than during the winter. Amino acids such as proline, cystine and arginine were only observed in winter.

Table 4.1. Changes in the amino acid content of *S. platensis* cultures grown with different

	***S. platensis* amino acid content (g/100 g DW)**				
	Mineral enrichment				
Amino acid	**Mg-100 mg/L**	**Mg-200 mg/L**	**Zn-3 mg/L**	**Zn-5 mg/L**	**Control**
Aspartate ASP	1,58±0,01^{a}	1,27±0,04^{c}	1,40±0,03^{b}	1,37±0,03^{b}	1,56±0,05^{a}
Glutamate GLU	2,43±0,003^{a}	2,30±0,08^{b}	2,18±0,04^{c}	2,37±0,07^{ab}	1,29±0,03^{d}
Serine SER	1,009±0,003^{a}	0,732±0,036^{c}	0,87±0,02^{b}	0,83±0,02^{b}	1,007±0,006^{a}
Glycine GLY	0,09±0,005^{a}	0,06±0,004^{c}	0,07±0,005^{b}	0,07±0,004^{b}	0,09±0,001^{a}
Histidine HIS	2,58±0,01^{a}	1,92±0,09^{c}	2,245±0,05^{b}	2,14±0,06^{b}	2,55±0,02^{a}
Threonine THR	0,68±0,003^{a}	0,62±0,02^{b}	0,60±0,007^{b}	0,67±0,01^{a}	0,68±0,004^{a}
Arginine ARG	1,01±0,004^{a}	0,75±0,03^{c}	0,87±0,02^{b}	0,83±0,02^{b}	1,01±0,008^{a}
Alanine ALA	1,40±0,001^{a}	1,03±0,04^{d}	1,22±0,03^{b}	1,15±0,01^{c}	1,40±0,007^{a}
Tyrosine TYR	0,81±0,004^{a}	0,59±0,05^{c}	0,70±0,01^{b}	0,65±0,01^{b}	0,81±0,02^{a}
Cystine CY2	0,297±0,019^{a}	0,259±0,016^{a}	0,26±0,02^{a}	0,265±0,023^{a}	0,29±0,026^{a}
Valine VAL	0,43±0,01^{a}	0,32±0,01^{d}	0,38±0,01^{b}	0,36±0,01^{c}	0,42±0,002^{a}
Methionine MET	0,35±0,001^{a}	0,12±0,01^{d}	0,30±0,009^{b}	0,19±0,01^{c}	0,36±0,002^{a}
Phenylalanine PHE	0,61±0,001^{a}	0,46±0,02^{c}	0,54±0,012^{b}	0,52±0,006^{b}	0,61±0,001^{a}
Isoleucine ILE	0,41±0,004^{a}	0,31±0,01^{d}	0,37±0,01^{b}	0,34±0,01^{c}	0,40±0,001^{a}
Leucine LEU	1,27±0,001^{a}	0,94±0,05^{c}	1,12±0,03^{b}	1,07±0,04^{b}	1,26±0,001^{a}
Lysine LYS	0,78±0,005^{a}	0,56±0,02^{c}	0,69±0,02^{b}	0,56±0,03^{c}	0,79±0,02^{a}
Proline PRO	0,54±0,03^{a}	0,36±0,01^{c}	0,48±0,01^{b}	0,35±0,01^{c}	0,52±0,04^{ab}

Different letters in the same row indicate statistically significant differences ($p<0.05$)

Zinc and magnesium concentrations can decrease or increase the amino acid content of *S. platensis*, but variations depend on the specific amino acid. These results show the variability of amino acid concentrations under different processing conditions, including specific amino acids such as aspartate, cystine, histidine, tyrosine and methionine. These variations could majorly impact the nutritional value and potential applications of *Spirulina platensis*.

4.4. Polyphenols

4.4.1. Total phenolic content

The total phenol content (mg GAE/ g DW) of *S. platensis* in the different experimental groups is shown in Table 4.4. In the Mg-100 mg/L group, total phenol content was 10,56±1,50 mg GAE/g DW for methanol and 14,25±0,50 mg GAE/g DW for ethanol extract. In the Mg-200 mg/L group, total phenol content was 10,85±0,38 mg GAE/g DW for methanol and 17,52±1,29 mg GAE/g DW for ethanol extract. In the zinc 3 mg/L group, total phenol content was 11,25±0,78 mg GAE/ g DW for methanol and 11,98±0,59 mg GAE/ g DW for ethanol extract. In the 5 mg/L zinc group, total phenol content was 10,41±1,43 mg GAE/ g DW for methanol and 10,85±0,54 mg GAE/ g DW for ethanol extract. In the control group, the total phenol content was 9,85±0,94 mg GAE/g DW for methanol and 14,03±0,66 mg GAE/g DW for ethanol extract.

Table 4.2. Total phenol content (mg GAE/g DW) of *Spirulina platensis* in the different experimental groups

		***S. platensis* total phenol content (mg GAE/g DW)**				
		Mineral enrichment				
		Mg-100 mg/L	**Mg-200 mg/L**	**Zn-3 mg/L**	**Zn-5 mg/L**	**Control**
Solvent	Methanol	$10,56±1,50^{aA}$	$10,85±0,38^{aA}$	$11,25±0,78^{aA}$	$10,41±1,43^{a\ A}$	$9,85±0,94^{aA}$
	Ethanol	$14,25±0,50^{bA}$	$17,52±1,29^{aB}$	$11,98±0,59^{cA}$	$10,85±0,54^{cA}$	$14,03±0,66^{bB}$

Different letters (a-c) in the same row indicate statistically significant differences ($p<0.05$). Different letters (A-B) in the same column indicate statistically significant differences ($p<0.05$).

The effect of zinc on total phenol content in the zinc-enriched groups shows no clear trend at different concentrations. No statically significant differences were observed between zinc-enriched groups using the two solvents. However, the ethanol extract appears slightly higher than the methanol extract.

In contrast to the zinc group, a progressive increase in total phenol content with increasing magnesium concentration was observed, independent of the solvent used.

Total phenol content values in the magnesium-enriched groups were significantly higher than in the zinc and control groups, independent of the solvent used. Phenol content was also found to vary according to the solvent used. Thus, ethanol extracts generally showed higher total phenol contents than those extracted with methanol.

4.4.2. Total flavonoid content

The total flavonoid content (mg catechin equivalent/g DW) of *S. platensis* in the different experimental groups is shown in Table 4.5.

In the Mg-100 mg/L group, total flavonoid content was 3,42±0,18 mg CE/g DW for methanol and 36,15±0,83 mg CE/g DW for ethanol extract. In the Mg-200 mg/L group, the total flavonoid content was 5,33±0,31 mg CE/g DW for the methanol and 27,29±3,62 mg CE/g DW for the ethanol extract. In the Zn-3 mg/L group, total flavonoid content was 2,63±0,18 mg CE/g DW for methanol and 26,030±3,709 mg CE/g DW for ethanol extract. In the 5 mg/L zinc group, total flavonoid content was 6,22±0,34 mg CE/g DW for methanol and 33,86±3,02 mg CE/g DW for ethanol extract. In the control group, total flavonoid content was 6,51±0,64 mg CE/g DW for methanol and 25,14±4,56 mg CE/g DW for ethanol extract.

From these results, it appears that flavonoid content varied significantly according to the different experimental conditions and solvents. For example, in the zinc treatment group, flavonoid concentrations appeared to increase with increasing zinc concentrations. Consequently, the Zn-5 mg/L group had the highest flavonoid content among extracts obtained with both solvents. No significant difference ($P<0,05$) in total flavonoid content was observed between the methanol extract of the Zn-5 mg/L group and that of the control group.

For the magnesium group, the methanol and ethanol extracts from the magnesium group had the highest total flavonoid content compared to the other groups. The use of ethanol as an extraction solvent produced higher values for flavonoid content than extracts obtained with methanol.

Table 4.3. Total flavonoid content (mg CE/g DW) of *Spirulina platensis* in the various experimental groups

		***S. platensis* total flavonoid content (mg CE/g DW)**				
		Mineral enrichment				
		Mg-100mg/L	**Mg-200 mg/L**	**Zn-3 mg/L**	**Zn-5 mg/L**	**Control**
Solvent	Methanol	$3,42±0,18^{cA}$	$5,33±0,31^{bA}$	$2,63±0,18^{dA}$	$6,22±0,34^{aA}$	$6,51±0,64^{aA}$
	Ethanol	$36,15±0,83^{aB}$	$27,29±3,76^{bB}$	$26,03±3,70^{bB}$	$33,86±3,02^{aB}$	$25,14±4,56^{bB}$

Different letters (a-c) in the same row indicate statistically significant differences ($p<0.05$). Different letters (A-B) in the same column indicate statistically significant differences ($p<0.05$).

4.4.3. Tannin Content

Table 4.6. shows the tannin content (mg catechin equivalent /g DW) of *Spirulina platensis* in the various experimental groups.

In the Mg-100 mg/L group, tannin content was 18,34±0,44 mg CE/g DW for methanol and 24,16±1,12 mg CE/g DW for ethanol extract. In the Mg-200 mg/L group, the tannin content was 32,0±3,76 mg CE/g DW for the methanol and 21,35±1,73 mg CE/g DW for the ethanol extract.

In the zinc 3 mg/L group, tannin content was 18,23±0,63 mg CE/g DW for methanol and 30,20±4,30 mg CE/g DW for ethanol extract. In the 5 mg/L zinc group, tannin content was 21,02±2,79 mg CE/g DW for methanol and 23,41±0,50 mg CE/g DW for ethanol extract.

In the control group, tannin content was 24,37±0,69 mg CE/g DW for methanol and 18,05±0,98 mg CE/g DW for ethanol extract.

Tannin content appears to vary with different zinc concentrations. The results show that tannin content in the Zn-5 mg/L group was significantly higher than in the zinc group and even in the control group.

Tannin content seems to increase significantly in the group treated with Mg-200 mg/L. This concentration was significantly higher in the Mg-200 mg/L group than in the other groups, including the control group.

A variation in *Spirulina platensis* tannin content as a function of solvent was noted. Ethanol extracts generally had a higher tannin content than methanol extracts.

Table 4.4. Total tannin content (mg CE/g DW) of *Spirulina platensis* in the different experimental groups

		***Spirulina platensis* tannin content (mg catechin equivalent/g DW)**				
		Mineral enrichment				
		Mg-100 mg/L	**Mg-200 mg/L**	**Zn-3 mg/L**	**Zn-5 mg/L**	**Control**
Solvent	Methanol	$18,34±0,440^{cA}$	$32,00±3,76^{aA}$	$18,23±0,63^{cA}$	$21,02±2,79^{bcA}$	$24,37±0,69^{bA}$
	Ethanol	$24,16±1,12^{bB}$	$21,35±1,73^{bBc}$	$30,20±4,30^{aBB}$	$23,41±0,50^{bA}$	$18,05±0,98^{cB}$

Different letters (a-c) in the same row indicate statistically significant differences ($p<0,05$). Different letters (A-B) in the same column indicate statistically significant differences ($p<0,05$).

4.4.4. HPLC analysis of phenolic compounds

Figure 4.10. shows chromatograms of the mixtures of standards used, analyzed at four different wavelengths: 254, 278, 287 and 330 nm. The identification and quantification of phenolic compounds contained in *Spirulina* methanolic extracts is carried out by comparing the retention times and surface areas of the standards used with those obtained by analyzing the extracts. A total of 16 phenolic compounds including 3-hydroxy benzoic acid, 4- hydroxy benzoic acid, benzoic acid, catechin, hydrate, chlorogenic acid, caffeic acid, epicatechin, gallic acid, hesperidin, p-coumaric acid, quercetin, rosmarinic acid, synaptic acid, syringic acid t-cinnamic acid and t-ferrulic acid was used to determine the quantities of these compounds in the *Spirulina* extract.

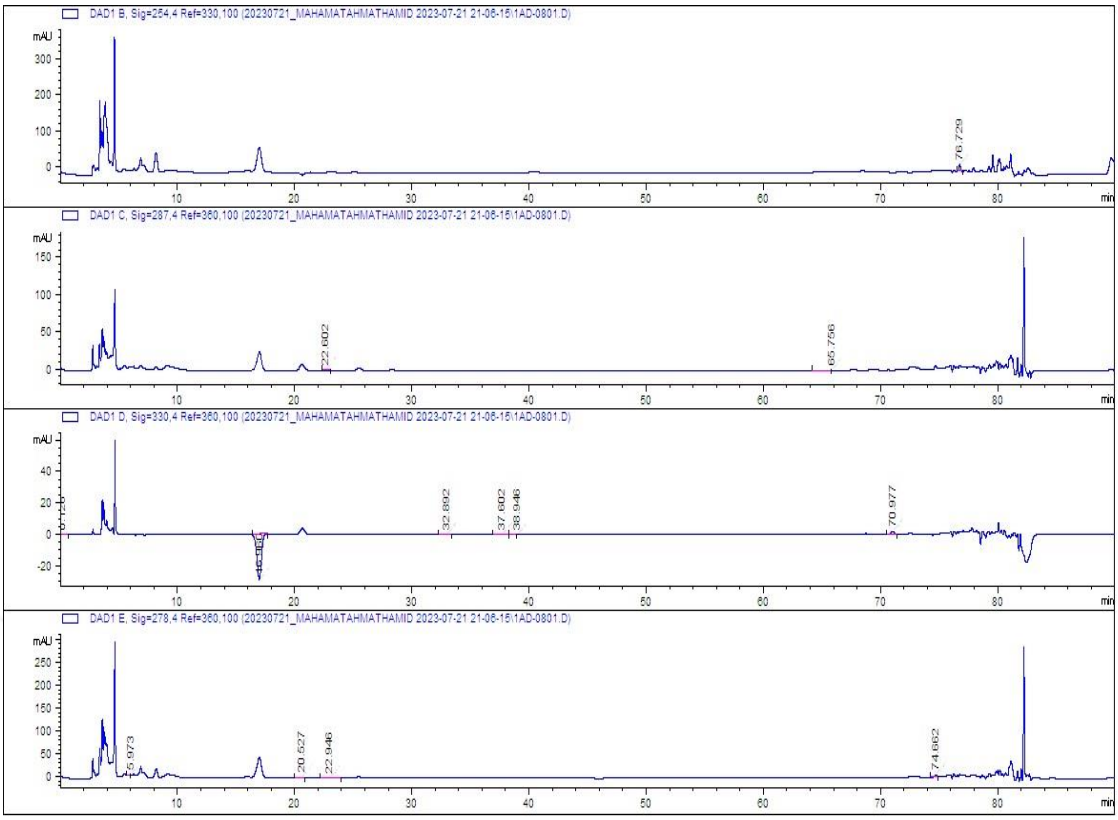


Figure 4.10. Chromatograms of phenolic compounds

The HPLC profile of *Spirulina* phenolic extracts showed the presence of phenolic acids (3-hydroxy benzoic acid, 4-hydroxy benzoic acid benzoic acid, chlorogenic acid, caffeic acid, gallic acid, p-coumaric acid, rosmarinic acid, synaptic acid, syringic acid, t-cinnamic acid, t-ferulic acid) and flavonoids (catechin, epicatechin, hesperidin, quercetin).

These results (Table 4.7.) showed a significant change in the quantity of phenolic compounds as a function of magnesium and zinc concentrations. Quercetin and rosmarinic acid are the majority in the zinc group, and their quantities increased with zinc concentration. In the magnesium group, compounds such as 4-hydroxybenzoic acid, gallic acid, catechin hydrate, quercetin and epicatechin are in the majority. The amount of chlorogenic acid is much lower in the mineral-rich group than in the control group (21,83±1,96 mg/kg DW). Catechin hydrate (6,39±1,07 mg/kg DW), a catechin derivative, was only detected in the Mg-100 mg/L group. 3-Hydroxy benzoic acid (1,11±0,01 mg/kg) was only detected in control group and Benzoic Acid only in Zn-5mg/L group (0,13±0,10 mg/kg).

It is important to note that variations in the amount of this compound did not follow a clear trend with zinc and magnesium doses. However, the enrichment of *Spirulina* cultures medium with zinc and magnesium affected the phenolic compounds in *S. platensis*. Changes in the concentrations of these compounds may be linked with the various metabolic mechanisms involved in their biosynthesis under normal conditions, or with *Spirulina's* response to stress, notably involving changes in enzymes.

Table 4.5. Phenolic compounds and their amounts in *S. platensis* (mg/Kg DW) as a function of different zinc and magnesium concentrations

	Phenolic Compounds	Correlation (r2)	λ (nm)	RT (min.)	Mg-100 mg/L	Mg-200 mg/L	Zn-3 mg/L	Zn-5 mg/L	Control
1	**3-Hydroxy Benzoic Acid**	0,99928	287	22.545	N.D	N.D	N.D	N.D	1,11±0,01
2	**4-Hydroxy Benzoic Acid**	0,99994	278	17.647	2,48±0,72^{b}	5,24±0,184^{a}	4,78±0,05^{a}	1,94±0,16^{b}	N.D
3	**Benzoic Acid**	0,99986	278	47.629	N.D	N.D	N.D	0,135±0,101	N.D
4	**Catechin hydrate**	0,99906	278	11.499	6,399±1,077	N.D	N.D	N.D	N.D
5	**Chlorogenic acid**	0,9997	330	16.239	N.D	2,05±0,02^{b}	1,77±0,25b	N.D	21,83±1,96^{a}
6	**Caffeic Acid**	0,99892	330	21.476	N.D	N.D	N.D	N.D	N.D
7	**Epicatechin**	0,99879	278	20.169	N.D	6,03±0,81^{a}	4,94±0,065^{a}	N.D	5,14±0,03^{a}
8	**Gallic Acid**	0,99966	278	5.912	2,48±0,51^{a}	3,21±0,23^{a}	2,96±0,28^{a}	0,82±0,09^{b}	0,54±0,13^{b}
9	**Hesperidin**	0,99705	287	65.989	3,83±0,17^{a}	N.D	3,63±0,07^{a}	3,43±0,01^{a}	3,54±0,14^{a}
10	**P-Coumaric acid**	0,99982	330	33.597	0,37±0,01^{a}	N.D	N.D	0,44±0,05^{a}	0,36±0,01^{a}
11	**Quercetin**	0,99962	254	76.313	4,60±0,02^{c}	4,61±0,06^{c}	4,71±0,02^{c}	5,26±0,29^{b}	6,05±0,02^{a}
12	**Rosmarinic Acid**	0,99907	330	70.655	2,39±0,07^{c}	3,02±0,05^{b}	3,21±0,13^{b}	4,83±0,25^{a}	2,90±0,09^{b}
13	**Sinnapic Acid**	0,99925	330	37.264	N.D	N.D	N.D	N.D	0,24±0,26
14	**Syringic Acid**	0,99839	278	22.628	N.D	N.D	N.D	N.D	0,99±0,02
15	**t-Cinnamic Acid**	0,99998	278	75.207	0,63±0,00^{a}	0,55±0,08^{ab}	0,45±0,00^{b}	0,62±0,05^{a}	0,47±0,01^{b}
16	**t-Ferrulic Acid**	0,99993	330	37.202	N.D	N.D	N.D	N.D	0,02±0,007

Different letters in the same row indicate statistically significant differences ($p<0.05$). N.D : Not Detected

4.5. Antioxidant activities

DPPH, ABTS and FRAP essays were used to assess the antioxidant capacity of *S. platensis*.

4.5.1. DPPH

Table 4.8 shows the percentages of inhibition of DPPH by *Spirulina platensis* for the different experimental conditions and extraction solvents, namely methanol and ethanol. DPPH is a stable free radical soluble in alcohols (methanol and ethanol) and is widely used to assess the antioxidant properties of chemical compounds by spectrophotometry. It is generally expressed as a percentage or Trolox equivalent. The highest percent inhibition exhibits the strongest antioxidant or inhibition capacity.

In the Mg-100 mg/L group, the percentage inhibition was 78,83±13,36% for the methanol extract and 62,76±23,31% for the ethanol extract. In the Mg-200 mg/L group, the inhibition percentage was 71,91±9,96% for the methanolic extract and 64,47±9,84% for the ethanolic extract.

In the Zn-3 mg/L group, the inhibition percentage was 72,36±7,27% for the methanol extract and 70,09±4,33% for the ethanol extract. In the Zn-5 mg/L group, the inhibition percentage was 101,34±9,27% for the methanolic extract and 103,94±13,81% for the ethanolic extract. In the control group, the inhibition percentage was 92,93±6,73% for the methanol extract and 78,73±11,47% for the ethanol extract.

These results show that the zinc group, particularly the Zn-5 mg/L group showed the highest degree of DPPH inhibition 101,34±9,27% for methanol extract and 103,94±13,81% ethanol extract, compared with the magnesium and the control groups with extracts from both solvents (Table 4.8). It should also be noted that the rate of DPPH inhibition of *Spirulina* in these studies also varied according to the solvent used. In all groups, the rate of DPPH inhibition was higher for the methanol extract than for ethanol. This may indicate that the active compounds in *S. platensis* are better extracted with methanol, resulting in this higher antioxidant capacity.

Table 4.6. The percentages of inhibition of DPPH by *S. platensis* for the different experimental conditions and extraction solvents

		***Spirulina platensis* DPPH (% inhibition)**				
		Mineral enrichment				
		Mg-100 mg/L	**Mg-200 mg/L**	**Zn-3 mg/L**	**Zn-5 mg/L**	**Control**
Solvent	Methanol	78,83±13,36^{bc}	71,91±9,96^{c}	72,36±7,27^{c}	101,34±9,27^{a}	92,93±6,73^{ab}
	Ethanol	62,76±23,31^{b}	64,47±9,84^{b}	70,09±4,33^{b}	103,94±13,81^{a}	78,73±11,47^{ab}

Different letters in the same row indicate statistically significant differences ($p<0,05$).

4.5.2. ABTS

The results of the percentages of inhibition of the ABTS radical by *S. platensis* for the different experimental conditions and extraction solvents, namely methanol and ethanol, are showed in Table 4.9. In the Mg-100 mg/L group, the percentage inhibition was 82,49±0,06 % for the methanol extract and 46,95±14,71 % for the ethanol extract. for the Mg-200 mg/L group, the inhibition percentage was 77,65±1,58 % for the methanolic extract and 74,18±1,60 % for the ethanolic extract.

For the zinc 3 mg/L group, the inhibition percentage was 20,44±0,83 % for the methanol extract and 46,50±5,84 % for the ethanol extract. In the 5 mg/L zinc group, the inhibition percentage was 76,19±6,04% for the methanolic extract and 52,68±15,90 % for the ethanolic extract.

In the control group, the inhibition percentage was 79,98±1,90 % for the methanol extract and 53,07±1,03 % for the ethanol extract.

These results show that the ABTS radical's inhibition percentage varies significantly as a function of the extraction solvent and different mineral enrichment concentrations. For example, the Mg-100 mg/L group and the control group showed a higher percentage of inhibition of the ABTS radical than the other groups with methanol extracts. The Zn-3mg/L group, on the other hand, showed a lower percentage of inhibition with the extracts of two solvents than the other groups. Unlike DPPH, where the zinc group showed strong antioxidant activity, with ABTS, the magnesium group showed strong antioxidant activity.

Table 4.9. The percentages of inhibition of ABTS by *Spirulina platensis* for the different experimental conditions and extraction solvents

		***Spirulina platensis* ABTS (% inhibition)**				
		Mineral enrichment				
		Mg-100 mg/L	**Mg-200 mg/L**	**Zn-3 mg/L**	**Zn-5 mg/L**	**Control**
Solvent	Methanol	82,49±0,06^{a}	77,65±1,58^{ab}	20,44±0,83^{c}	76,19±6,04^{b}	79,98±1,90^{ab}
	Ethanol	46,95±14,71^{b}	74,18±1,60^{a}	46,50±5,84^{b}	52,68±15,90^{b}	53,07±1,03^{b}

Different letters in the same row indicate statistically significant differences ($p<0.05$).

4.5.3. FRAP

The results of FRAP essay of *S. platensis* for the different experimental conditions and extraction solvents, namely methanol and ethanol, are showed in Table 4.10. The ferric-reducing antioxidant power (FRAP) values of extracts were expressed in μmol Trolox equivalent/g dry weight (μmol TE/g DW).

In the Mg-100 mg/L group, the FRAP was 37,54±8,80 μmol TE/g DW for the methanol extract and 130,29±17,32 μmol TE/g DW for the ethanol extract. for the Mg-200 mg/L group, the

FRAP was 67,10±5,26 µmol Trolox/g dry weight for the methanolic extract and 128,88±36,80 µmol TE/g DW weight for the ethanolic extract.

For the zinc 3 mg/L group, the FRAP was 59,19±9,52 µmol TE/g DW for the methanol extract and 123,59±26,98 µmol TE/g DW for the ethanol extract. In the 5 mg/L zinc group, the FRAP was 120,80±7,83 µmol µmol TE/g DW for the methanolic extract and 183,89±15,58 µmol TE/g DW weight for the ethanolic extract.

In the control group, the FRAP was 68,81±16,25 µmol TE/g DW for the methanol extract and 152,36±33,76 µmol TE/g DW for the ethanol extract.

These results show significant variation in the antioxidant activity of FRAP with different concentrations of minerals and solvents. Unlike other methods (DPPH and ABTS), the FRAP value is generally related to mineral enrichment in zinc and magnesium, suggesting that the enrichment of *Spirulina platensis* with these minerals affects *Spirulina*'s antioxidant activity. The variations in *Spirulina* antioxidant activity results using the DPPH, ABTS and FRAP methods may be explained by differences in the radicals or ions they use, and also by specific metal reduction or chelation mechanisms. Each of these methods has its advantages and limitations, and they are often used in complementary ways to obtain a more complete assessment of antioxidant activity.

However, the FRAP activity value obtained with the ethanol extract was significantly higher than that of the methanol extract. No significant difference was observed between the groups in the FRAP values of the ethanolic extract. The highest FRAP value was observed in the Zn-5mg/L group and the lowest in the Mg-100 mg/L group with the methanol extracts.

The results of this study show that the mineral enrichment of *Spirulina*, particularly with high levels of zinc and magnesium, and the choice of extraction solvent significantly impact the antioxidant activity of *Spirulina platensis*.

Table 4.10. FRAP essay by *Spirulina platensis* for the different experimental conditions and extraction solvents

		***Spirulina platensis* FRAP (µmol TE/g DW)**				
		Mineral enrichment				
		Mg-100 mg/L	**Mg-200 mg/L**	**Zn-3 mg/L**	**Zn-5 mg/L**	**Control**
Solvent	Methanol	37,54±8,80^{c}	67,10±5,26^{b}	59,19±9,52^{b}	120,80±7,83^{a}	68,81±16,25^{b}
	Ethanol	130,29±17,32^{a}	128,88±36,80^{a}	123,59±26,98^{a}	183,89±15,58^{a}	152,36±33,76^{a}

Different letters in the same row indicate statistically significant differences ($p<0.05$).

In the present study, the phytochemical constituents such TPC, TFC and tannin of *S. platensis* enriched by different concentrations of zinc and magnesium methanol and ethanol extract were determined (Table 4.4 et 4.5 and 4.6).

Microalgae are a rich source of phytochemicals containing high levels of compounds such as phenolic acids, flavonoids and tannins, which contribute to the antioxidant activity of their

extracts. Phenolic compounds are essential antioxidants because they can scavenge radicals by donating a hydrogen atom or an electron, which gives them numerous biological activities (Machu et al., 2015) . In general, these compounds play a vital role in protecting plants against ultraviolet rays and pathogens (Manach et al., 2004). They also play an essential role in pigmentation, reproduction and plant growth (Zern and Fernandez, 2005). As a result, several methods, including so-called unconventional methods such as pulsed electric fields (PEF), high-pressure homogenization, the use of artificial intelligence, ultrasound, microwaves, subcritical and supercritical fluid extraction, have been suggested as suitable techniques for maximizing the extraction of these compounds. The phenolic content of *Spirulina* varies according to drying technique, choice of solvent and extract concentration. For the quantitative phytochemical screening of *S. platensis* in this study, the ethanol extract presented a maximum content of phenolic compounds, flavonoids and tannin compared to those obtained with methanol. Although methanolic extracts had lower levels of total polyphenols, flavonoids and tannins than ethanolic extracts, they showed higher antioxidant and antimicrobial activity than ethanolic extracts in all *Spirulina* groups. This may be due to the high polarity of the solvents and the solubility of the active compounds present in *S. platensis*.

This study confirms the results of some researchers on the appropriate choice of solvent for the extraction of phytochemicals in general and those of *S. platensis* in particular. Kavisri et al (2023) recorded 96.7±0.008 µg/mg phenolics, 56.3±0.004 flavonoids and 18.1±0.92 µg/mg tannins in the methanolic extract of *S. platensis*. Silva et al (2023) reported that although continuous drying at 65°C gave the best results for TPC, reaching 319.22 mg gallic acid/100 g DW of sample, compared with fresh *Spirulina* (462, 12 mg gallic acid/100 g DW), the intermittent combination alternating between 65°C and 50°C produced a similarly higher TPC of 423.67 mg gallic acid/100 g DW, approaching the values observed in fresh material. Golmakani et al (2018) reported that the amounts of TPC and TFC in *Spirulina* methanolic extract were 38.04±3.07 mg GAE/g and 1.86±0.33 mg QUE/g, respectively. Boyanova et al (2022) found that *Spirulina* extracts obtained with 80% methanol had a TPC quantity of 140.2±3.0 mg GAE/100g DW. Machu et al (2015) reported that the total phenolic content of *Spirulina platensis* (mg g^{-1} GAE) varied according to the different extraction methods: 43.2 ± 1.0 for distilled water extraction, 17.0 ± 0.5 for methanol-water-acetic acid extraction (30: 69:1, v/v/v), 23.9±0.1 for 80% methanol extraction, 18.4±0.1 for 70% acetone extraction and 24.4 ± 0.2 for 100% methanol extraction. Maadane et al (2015) reported that the DPPH IC_{50} effect of the ethanolic extract of Moroccan marine microalgae *Tetraselmis* sp., *Dunaliella* sp. and *Nannochloropsis gaditana* was higher, at 247±0.01, 283±0.09 and 365±0.05 µg/ml, respectively.

4.6. Antimicrobial activity

4.6.1. Minimal Inhibitory Concentration (MIC) determination

Table 4.11 shows the results of the minimum inhibitory concentration (MIC) test for different mineral-enriched extracts of *S. platensis* (Mg-100 mg/L, Mg-200 mg/L, Zn-3 mg/L, Zn-5 mg/L, Control) on different strains of Gram-negative bacteria (*Escherichia coli*, *Klebsiella Pneumonia*), Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*), yeasts (*Candida albicans*) and molds (*Aspergillus flavus*). These values indicate the lowest concentration of methanolic and ethanolic extracts of *S. platensis* required to inhibit the growth of each microorganism. In this study, the MICs were determined by microdilution essays according to the (Patel and Clinical and Laboratory Standards Institute, 2017) NCCLS standards (Figure 4.11).

Table 4.11. MIC results of *S. platensis* mineral enriched extracts

		Minimum inhibitory concentration (mg/ml)					
		Gram-negative bacteria		**Gram-positive bacteria**		**Yeast**	**Mold**
		E. coli	***K. pneumonia***	***S. aureus***	***B. cereus***	***C. albicans***	***A flavus***
***Spirulina* (Mg, Zn)**	**Solvent**						
Mg-100 mg/L	Methanol	10	10	5	10	5	20
	Ethanol	0,625	2,5	1,25	5	5	20
Mg-200 mg/L	Methanol	5	10	2,5	1,25	5	20
	Ethanol	0,625	0,625	0,625	0,625	5	5
Zn-3 mg/L	Methanol	2,5	10	1,25	1,25	10	10
	Ethanol	0,625	0,625	0,625	0,625	5	5
Zn-5 mg/L	Methanol	5	5	2,5	2,5	2,5	20
	Ethanol	1,25	1,25	1,25	2,5	5	20
Control	Methanol	2,5	1,25	0,312	1,25	5	20
	Ethanol	2,5	5	2,5	5	5	5

Overall, the inhibitory effect of the extracts varied according to solvent, microbial strain and mineral concentration. In Gram-negative bacteria, the inhibitory activity of the ethanolic extract was stronger than that of the methanolic extract. *E. coli* is the strain most sensitive to strains other than ethanolic extracts. For example, for *E. coli*, the MIC was lowest with the ethanolic extract of *S.platensis* enriched with Zn-3 mg/L, Mg-100 mg/L and Mg-200 mg/L with a MIC of 0,625 mg/ml. Whereas, for *K. pneumonia*, the lowest MIC (0,625 mg/ml) was obtained with the ethanolic extract of *S.platensis* enriched with Zn-3 mg/L and Mg-100 mg/L. In Gram-positive bacteria, MIC varied according to *S.platensis* extract, microorganism and solvent. For example, *S. Aureus*, a lower MIC value (0,312 mg/mL) was observed with the methanolic extract of the control group. However, for *S. aureus* and *B. cereus*, the lowest MIC (0,625 mg/mL) was obtained with the ethanolic extract of *S. platensis* enriched with Zn-3 mg/L and Mg-100 mg/L.

Concerning yeast (*C. albicans*) and mold (*A. flavus*), the MIC values are higher than for bacteria, thus suggesting a relative resistance to extracts of *S.platensis*. It varies between 2,5 to 10 mg/mL in *C. albicans* and 5-20 mg/mL in *A. flavus*. However, *C. albicans* seems less resistant than *A. flavus*. Zn-5 mg/L group.

The MIC results obtained in this study ranged from 0,312-20 mg/mL. They differ from those obtained by Abdel-Moneim et al (2022) who obtained a lower MIC (1-2 mg/mL) with methanolic *S. platensis* compared to other extracts. Moreover, the methanolic extract of *Spirulina* also showed higher antifungal activity than the other extracts, with MICs of between 15 and 21 mm compared to diniconazole.

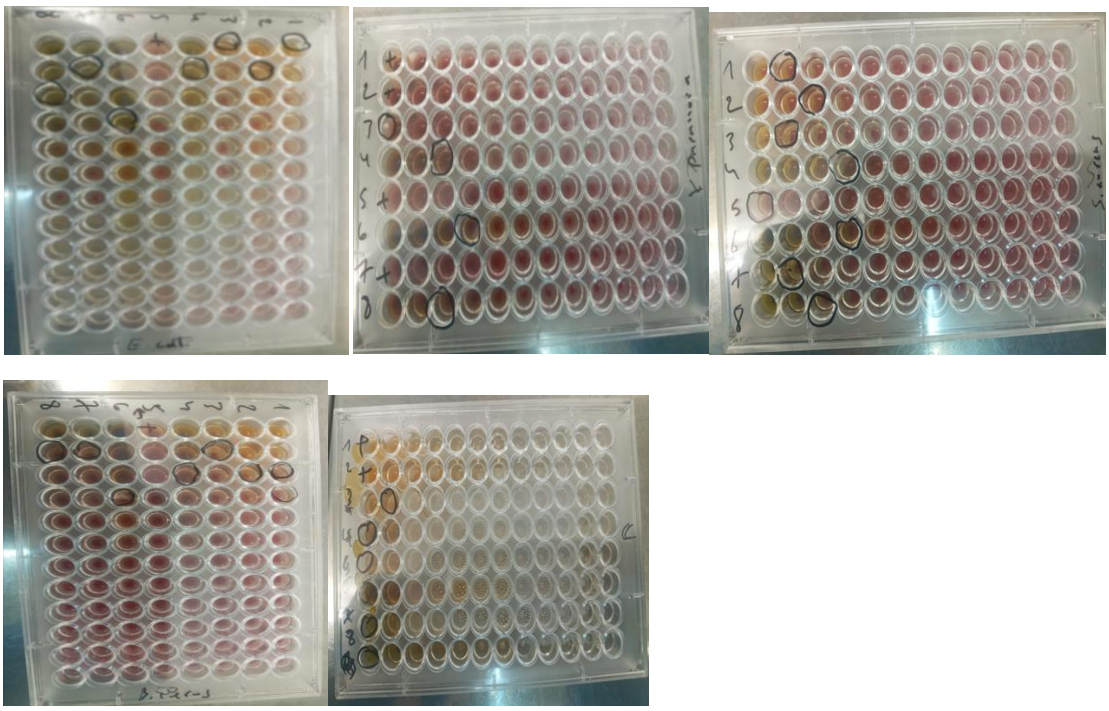


Figure 4.11. Plates for determining the minimum inhibitory concentration (MIC) of *Spirulina* extracts

4.6.2. Agar diffusion essay

Table 4.12 shows the inhibition test results for various *S.platensis* extracts on the growth of different bacteria, yeasts and molds. The values in the table are the mean diameters of the inhibition zones in millimeters. These inhibition zones indicate the extent to which the various *S.platensis* extracts tested were able to inhibit the growth of micro-organisms, namely Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*), Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*), a yeast (*Candida albicans*) and a mold (*Aspergillus flavus*). The larger the diameter of the zone of inhibition, the greater the inhibitory effect of the extracts on the growth of the microorganism. Concerning the antimicrobial activity, the first test with a concentration of 10 mg/ml showed no antimicrobial activity. It was decided to increase the concentration to look for an activity.

All extracts showed antimicrobial effects with a 100 mg/mL. Gram-positive bacteria, particularly *Staphylococcus aureus*, appeared highly sensitive to methanolic extracts of *S.platensis* enriched with Mg-200 mg/L and Zn-5 mg/L. Variations in the antimicrobial activity of extracts indicate variations in their chemical compositions. The antimicrobial activity of the various *S. platensis* extracts against the four bacterial strains, one yeast strain and one mold strain tested in this study and their potency are qualitatively and quantitatively assessed by the presence or absence of a zone of inhibition and the diameter of this zone (Figure 4.12).

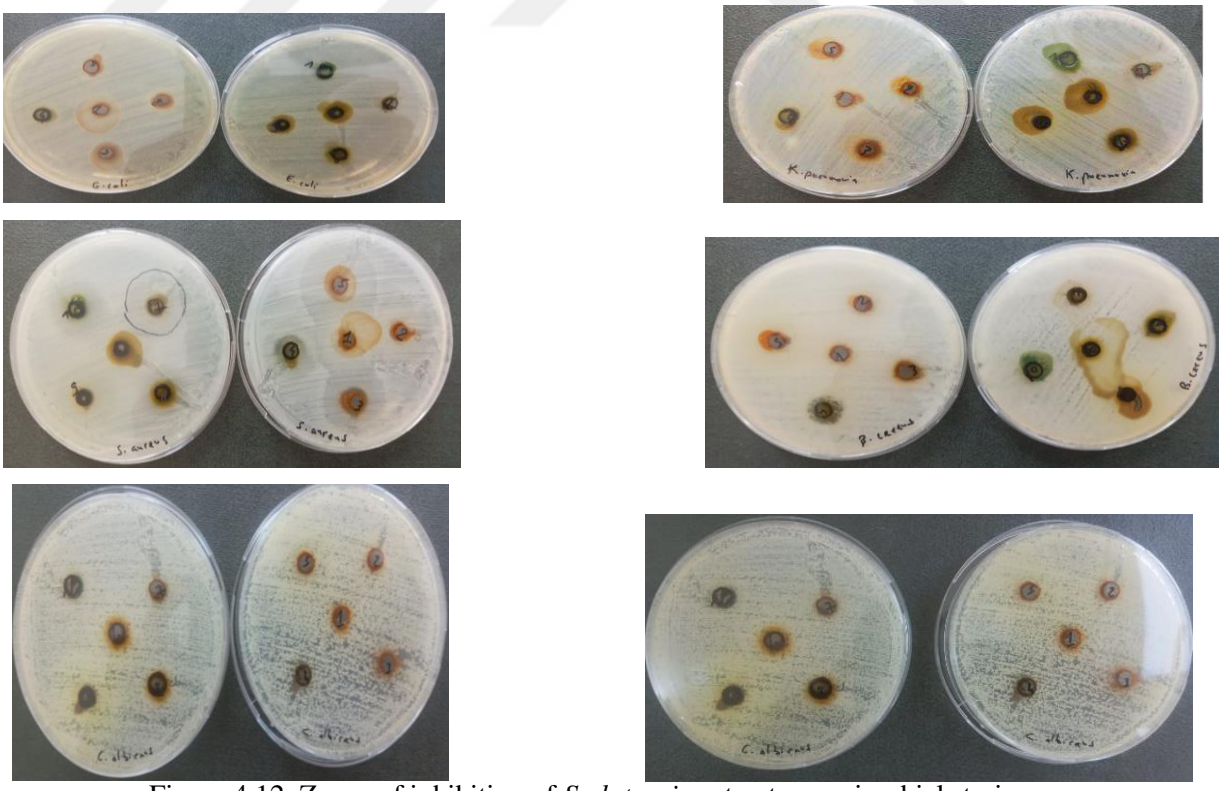


Figure 4.12. Zones of inhibition of *S.platensis* extracts on microbial strains

Table 4.7. Agar diffusion essay

			Diameter of inhibition zone (mm)					
			Gram-negative bacteria		**Gram-positive bacteria**		**Yeast**	**Mold**
			E.coli	***K. pneumonia***	***S. aureus***	***B. cereus***	***C. albicans***	***A. flavus***
***Spirulina* (Mg,Zn)**	**Solvent**	**Concentration (mg/ml)**						
Mg-100 mg/L	Methanol	10	-	-	-	-	-	-
		100	7,5±2,12^{b}	6,5±0,71^{a}	19,5±0,61^{a}	15,5±4,95^{a}	6,00±0,00^{a}	6,00±0,00^{a}
	Ethanol	10	-	-	-	-	-	-
		100	6±0,00^{b}	7,5±2,12^{a}	17±4,24^{b}	10,5±3,54^{a}	6,00±0,00^{a}	6,00±0,00^{a}
Mg-200 mg/L	Methanol	10	-	-	-	-	-	-
		100	9±1,41^{a}	6,5±0,71^{a}	20±1,31^{a}	15±4,24^{a}	6,00±0,00^{a}	6,00±0,00^{a}
	Ethanol	10	-	-	-	-	-	-
		100	9±4,24^{a}	6±0,00^{a}	16±5,66^{b}	14±2,83^{a}	6,00±0.00^{a}	6,00±0,00^{a}
Zn-3 mg/L	Methanol	10	-	-	-	-	-	-
		100	6±0,00^{b}	6,5±0,71^{a}	16±5,66^{b}	9,5±4,95^{a}	6,00±0,00^{a}	6,00±0,00^{a}
	Ethanol	10	-	-	-	-	-	-
		100	6±0,00^{b}	6,5±0,71^{a}	17,5±1,78^{b}	15±4,24^{a}	6,00±0,00^{a}	6,00±0,00^{a}
Zn-5 mg/L	Methanol	10	-	-	-	-	-	-
		100	9,5±0,71^{a}	8±2,83^{a}	20±0,00^{a}	14±4,24^{a}	6,00±0,00^{a}	6,00±0,00^{a}
	Ethanol	10	-	-	-	-	-	-
		100	6±0,00^{b}	6±0,00^{a}	17,5±3,54^{b}	7,5±0,71^{b}	6,00±0,00^{a}	6,00±0,00^{a}
Control	Methanol	10	-	-		-	-	-
		100	8±2,83^{b}	6,5±0,71^{a}	15±4,24^{b}	7,5±2,12^{b}	6,00±0,00^{a}	6,00±0,00^{a}
	Ethanol	10	-	-	-	-	-	-
		100	6±0,00^{b}	6±0,00^{a}	17,5±3,54^{b}	7,5±0,71^{b}	6,00±0,00^{a}	6.00±0.00^{a}
DMSO			0.00±0.00^{b}	0.00±0.00^{ab}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
AMP			33.00±4.24^{c}	0±0.00^{ab}	48±0.00^{c}	13±0.00^{a}	NA	NA
KAN			28±1.41^{c}	20±0.00^{ac}	29±0.00^{a}	29±0.00^{c}	NA	NA
FLU			NA	NA	NA	NA	9±0.00^{b}	11±0.00^{b}

Different letters in the same column indicate statistically significant differences ($p<0.05$)

The Gram-positive bacteria *S. aureus* was the most sensitive bacterial species to methanolic extracts of Mg-200 mg/L followed by Zn-5 mg/L and Mg-100 mg/L with respective inhibition zones of 20±1.31, 20±0.00 and 19.5±0.61 mm of diameter. No significant statistical difference was observed between these different groups, but they were above the zone of inhibition of the control group, which was 15.15±4.24 mm of diameter. These values differ from those obtained with antibiotics such as ampicillin and kanamycin, which presented a zone of inhibition of 48±0. 00 and 29±0.00 mm of diameter, respectively. While Gram-negative *K. pneumonia* was the least sensitive to methanol and ethanol extracts in all groups. The inhibition zones varied from 6-8±2.83 mm in diameter. No antibiotic activity against this bacteria was observed with Ampicillin with a 0±0.00 mm diameter, while a 20±0.00 mm diameter zone of inhibition was observed with Kanamycin. The results of the present study revealed that *S. platensis* extracts have a higher bactericidal potential against gram-positive bacteria than gram-negative bacteria. This effect can be attributed to gram-positive bacteria's complex bacterial wall structure.

These results could be linked to the high phytochemical compounds in these groups compared to the control group of *Spirulina*. It could also be linked to the magnesium and zinc content high in the Mg-200 mg/L group and Mg-100mg/L, Zn-5mg/L. These minerals are known to exhibit antimicrobial activities. Thus, Imani and Safaei, (2019) reported that magnesium oxide nanoparticles could significantly reduce the number of Gram-positive and Gram-negative bacteria and be a suitable alternative to commonly used antibacterial agents to combat drug resistance in pathogens.

Kaushik and Chauhan (2008) reported that polyphenol extracts from *S. platensis* inhibited the growth of *S. aureus*, *E. coli*, *P. aeruginosa*, *S. Typhi* and *K. pneumoniae*. The most effective minimum inhibitory concentrations were obtained with methanolic extracts compared with the other extracts (hexane, ethyl acetate, dichloromethane). They were 128 µg/mL and 256 µg/mL in *S. aureus* and *E. coli*, respectively.

The antimicrobial activity of the methanolic and ethanolic extracts of *Spirulina* studied was attributed to the presence of functional compounds that are antibiotically active, such as phenolic acids, flavonoids and tannins. These compounds act as antimicrobial agents, in particular as a bacteriostat, bacteriocides, fungistatics or fungicides, inhibiting the growth and killing bacteria, fungi and yeasts by reaching the bacterial cell walls and causing their disintegration and reducing the viability of the microorganisms (Bush, 2012)

So, according to this study, the phenolic extracts of *Spirulina* enriched with magnesium and zinc have shown antimicrobial activity, making them not only interesting for therapeutic purposes but also suitable for incorporation into foodstuffs to combat the microorganisms that contaminate foodstuffs and cosmetology. The compounds in *Spirulina platensis* are, therefore, highly effective in preventing the development of microorganisms.

5. CONCLUSION

The thesis aims to investigate the impact of varied concentrations of magnesium (Mg) and zinc (Zn) on the growth rate of *S. platensis*, its bioaccumulative potential for both Mg and Zinc, its biochemical and phytochemical composition, and its antimicrobial and antioxidant activities. This study shows that magnesium (100 and 200 mg/L) and zinc (3 and 5 mg/L) enrichment produced results similar to those of the control group concerning *S. platensis* growth. At the end of the experiment, it was observed that *Spirulina* had accumulated higher levels of magnesium and zinc. Specifically, magnesium levels were high in the group exposed to 200 mg/L magnesium, reaching a concentration of 348,15±44,84 mg/100 g DW. Compared with the control group, a significant increase in zinc content was also observed in *S. platensis* exposed to zinc concentrations of 5 mg/L and 3 mg/L.

The highest protein content (70,34%) was observed in the control group, and as zinc content increased, protein content decreased polyphenols' concentration exhibits variations depending on the solvents employed; for example, ethanol extracts displayed elevated levels of secondary metabolites, including tannins and flavonoids. Significantly, the magnesium-enriched groups exhibited a markedly higher content of these compounds than the zinc-enriched and control groups. In contrast, methanol extracts showed high levels of biological activity. The zinc group, particularly the Zn-5 mg/L group, showed the highest degree of DPPH inhibition for the methanol extract. All extracts showed an antimicrobial effect at 100 mg/mL.

The results raise significant questions about the impact of exposure to magnesium and zinc on both the growth of *Spirulina* and the quality and nutritional value of *S. platensis* as a possible dietary supplement for addressing deficiencies in these minerals. Furthermore, *S. platensis* has demonstrated an ability to accumulate minerals and generate bioactive compounds when subjected to stress. *Spirulina* phenolic extracts enriched with magnesium, zinc and the control group exhibited antimicrobial activity. This renders them intriguing for therapeutic usage and inclusion in food products to combat microorganisms that contaminate foodstuffs and cosmetics.

This study focused on the effects of minerals such as magnesium and zinc on the growth of *Spirulina* algae. It can be extended to study the growth of *Spirulina* and other microalgae with a multimetal or multimineral system. These aspects need to be addressed if the benefits of wastewater treatment are to be real. Understanding the bioaccessibility of minerals, pigments and secondary metabolites, as well as assessing their effects on various biological activities, are crucial aspects in considering health benefits. A promising approach in this line of research is to explore the encapsulation and incorporation of *Spirulina's* mineral-enriched extracts into foods. In vivo and situ studies would enable in-depth analysis of the biological activities of these compounds. This perspective represents a significant avenue of research for the future, offering opportunities to better understand how these elements can be harnessed to promote health.

REFERENCES

Abd El Baky, H., El-Baz, F., and El baroty, G., 2009. Production of phenolic compounds from *Spirulina maxima* microalgae and its protective effects. African Journal Of Biotechnology, 8.

Abd El-Baky, H. H., El Baz, F. K., and El-Baroty, G. S., 2009. Enhancement of antioxidant production in *Spirulina platensis* under oxidative stress. Acta Physiologiae Plantarum, 31(3), 623–631.

Abdel-Daim, M. M., Abuzead, S. M. M., and Halawa, S. M., 2013. Protective Role of *Spirulina platensis* against Acute Deltamethrin-Induced Toxicity in Rats. PLoS ONE, 8(9), e72991.

Abdel-Moneim, A.-M. E., El-Saadony, M. T., Shehata, A. M., Saad, A. M., Aldhumri, S. A., Ouda, S. M., and Mesalam, N. M., 2022. Antioxidant and antimicrobial activities of *Spirulina platensis* extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi Journal of Biological Sciences, 29(2), 1197–1209.

Abdel-Moneim, A.-M. E., El-Saadony, M. T., Shehata, A. M., Saad, A. M., Aldhumri, S. A., Ouda, S. M., and Mesalam, N. M., 2022. Antioxidant and antimicrobial activities of *Spirulina platensis* extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi Journal of Biological Sciences, 29(2), 1197–1209.

Abuja, P. M., and Albertini, R., 2001. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clinica Chimica Acta, 306(1), 1–17.

Aburai, N., Sumida, D., and Abe, K., 2015. Effect of light level and salinity on the composition and accumulation of free and ester-type carotenoids in the aerial microalga Scenedesmus sp. (Chlorophyceae). Algal Research, 8, 30–36. https://doi.org/10.1016/j.algal.2015.01.005

Affenzeller, M. J., Darehshouri, A., Andosch, A., Lütz, C., and Lütz-Meindl, U., 2009. Salt stress-induced cell death in the unicellular green alga Micrasterias denticulata. Journal of Experimental Botany, 60(3), 939–954. https://doi.org/10.1093/jxb/ern348

Afonso, V., Champy, R., Mitrovic, D., Collin, P., and Lomri, A., 2007. Radicaux libres dérivés de l'oxygène et superoxydes dismutases: Rôle dans les maladies rhumatismales. Revue du

Agustini, T. W., Suzery, M., Sutrisnanto, D., Ma'ruf, W. F., and Hadiyanto., 2015. Comparative Study of Bioactive Substances Extracted from Fresh and Dried *Spirulina* sp. Procedia Environmental Sciences, 23, 282–289. https://doi.org/10.1016/j.proenv.2015.01.042

Ahsan, M., Habib, B., Parvin, M., Huntington, T. C., and Hasan, M. R., 2008. A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals.

Akalin, A. S., Ünal, G., and Dalay, M. C., 2009. İnfluence of *Spirulina* platensis biomass on microbiological viability in traditional and probiotic yogurts during refrigerated storage. Italian Journal of Food Science, 21(3), 357–364.

Akbarnezhad, M., Shamsaie Mehrgan, M., kamali, A., and Javaheri Baboli, M., 2019. Effects of microelements (Fe, Cu, Zn) on growth and pigment contents of Arthrospira (*Spirulina*) *platensis*. Iranian Journal of Fisheries Sciences, Online First. https://doi.org/10.22092/ijfs.2019.120614

Akbarnezhad, M., Shamsaie Mehrgan, M., kamali, A., and Javaheri Baboli, M., 2019. Effects of microelements (Fe, Cu, Zn) on growth and pigment contents of Arthrospira (*Spirulina*) *platensis*. Iranian Journal of Fisheries Sciences, Online First.

Akhtar, S., 2013. Zinc Status in South Asian Populations-An Update. Journal of Health, Population and Nutrition, 31(2), Article 2. https://doi.org/10.3329/jhpn.v31i2.16378

Ali, H., Khan, E., and Ilahi, I., 2019. Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. Journal of Chemistry, 2019, e6730305. https://doi.org/10.1155/2019/6730305

Ali, S. S., Ahsan, H., Zia, M. K., Siddiqui, T., and Khan, F. H., 2020. Understanding oxidants and antioxidants: Classical team with new players. Journal of Food Biochemistry, 44(3).

Alshuniaber, M. A., Krishnamoorthy, R., and AlQhtani, W. H., 2021. Antimicrobial activity of polyphenolic compounds from *Spirulina* against food-borne bacterial pathogens. Saudi Journal of Biological Sciences, 28(1), 459–464. https://doi.org/10.1016/j.sjbs.2020.10.029

Anbarasan, V., Kumar, V. K., Kumar, P. S., and Venkatachalam, T., 2011. In vitro evaluation of antioxidant activity of blue green algae *Spirulina platensis*. International journal of pharmaceutical sciences and research, 2(10), 2616.

Antunes dos Santos, A., Ferrer, B., Marques Gonçalves, F., Tsatsakis, A. M., Renieri, E. A., Skalny, A. V., Farina, M., Rocha, J. B. T., and Aschner, M., 2018. Oxidative Stress in Methylmercury-Induced Cell Toxicity. Toxics, 6(3), Article 3.

AOAC, A., 1995. Official Method 990.12 for Aerobic Plate Count in Foods, Official Methods of Analysis. Washington, DC: Association of Official Analytical Chemists.

Arunakumara, K. K. I. U., and Zhang, X., 2008. Heavy metal bioaccumulation and toxicity with special reference to microalgae. Journal of Ocean University of China, 7(1), 60–64.

Asada, K., Kiso, K., and Yoshikawa, K., 1974. Univalent Reduction of Molecular Oxygen by Spinach Chloroplasts on Illumination. Journal of Biological Chemistry, 249(7), 2175–2181. https://doi.org/10.1016/S0021-9258(19)42815-9

Asnake Metekia, W., Garba Usman, A., Hatice Ulusoy, B., Isah Abba, S., and Chirkena Bali, K., 2022. Artificial intelligence-based approaches for modeling the effects of *Spirulina* growth mediums on total phenolic compounds. Saudi Journal of Biological Sciences, 29(2), 1111–1117.

Atrens, A., Song, G.-L., Shi, Z., Soltan, A., Johnston, S., and Dargusch, M. S., 2018. Understanding the Corrosion of Mg and Mg Alloys. In K. Wandelt (Ed.), Encyclopedia of Interfacial Chemistry (pp. 515–534).

Aydi, S. sassi, Aydi, S., Ben Abdallah Kolsi, R., Haddeji, N., Rahmani, R., Ktari, N., and Bouajila, J., 2020. CO2 enrichment: Enhancing antioxidant, antibacterial and anticancer activities in *Arthrospira platensis*. Food Bioscience, 35, 100575. https://doi.org/10.1016/j.fbio.2020.100575

Ayed, H. B. A. B., Taidi, B., Ayadi, H., Pareau, D., and Stambouli, M., 2017. The Use of *Chlorella Vulgaris* to Accumulate Magnesium under Different Culture Conditions. Journal of Applied Biotechnology and Bioengineering, 2(5), 43. https://doi.org/10.15406/jabb.2017.02.00043

Ayehunie, S., Belay, A., Baba, T. W., and Ruprecht, R. M.,1998. Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, 18(1), 7–12.

Balaji, S., Kalaivani, T., and Rajasekaran, C., 2014. Biosorption of Zinc and Nickel and Its Effect on Growth of Different *Spirulina* Strains: Biosorption Potentials of *Spirulina* Strains. CLEAN - Soil, Air, Water, 42(4), 507–512.

Balaji, S., Kalaivani, T., Shalini, M., Gopalakrishnan, M., Rashith Muhammad, M. A., and Rajasekaran, C., 2016. Sorption sites of microalgae possess metal binding ability towards Cr(VI) from tannery effluents—A kinetic and characterization study. Desalination and Water Treatment, 57(31), 14518–14529. https://doi.org/10.1080/19443994.2015.1064032

Barbare, R., 2005. Magnesium. In P. Wexler (Ed.), Encyclopedia of Toxicology (Second Edition) (pp. 1–3).

Barbosa, M. J., Albrecht, M., and Wijffels, R. H., 2003. Hydrodynamic stress and lethal events in sparged microalgae cultures. Biotechnology and Bioengineering, 83(1), 112–120.

Baścik-Remisiewicz, E. Tomaszewska, K. Labuda, and Z. Tukaj., 2009. The Effect of Zn and Mn on the Toxicity of Cd to the Green Microalga *Desmodesmus armatus* Cultured at Ambient and Elevated (2%) CO_2 Concentrations. Polish Journal of Environmental Studies, 18(5), 775–780.

Bush, K., 2012. Antimicrobial agents targeting bacterial cell walls and cell membranes. Scientific qnd Technical Review. 31 (1): 43-56. doi: http://dx.doi.org/10.20506/rst.31.1.2096.

Beale, S. I., 2008. Photosynthetic Pigments: Perplexing Persistent Prevalence of 'Superfluous' Pigment Production. Current Biology, 18(8), R342–R343. https://doi.org/10.1016/j.cub.2008.02.064

Bedard, K., and Krause, K.-H., 2007. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. Physiological Reviews, 87(1), 245–313. https://doi.org/10.1152/physrev.00044.2005

Belay, A., 2002. The Potential Application of *Spirulina* (*Arthrospira*) as a Nutritional and Therapeutic Supplement in Health Management.

Bellamy-Carter, J., Sound, J. K., and Leney, A. C., 2022. Probing heavy metal binding to phycobiliproteins. The FEBS Journal, 289(15), 4646–4656.

Ben Amor-Ben Ayed, H., Taidi, B., Ayadi, H., Pareau, D., and Stambouli, M., 2015. Effect of magnesium ion concentration in autotrophic cultures of *Chlorella vulgaris*. Algal Research, 9.

Benzie, I. F., and Strain, J. J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP essay. Analytical Biochemistry, 239(1), 70–76.

Berges, J. A., and Choi, C. J., 2014. Cell death in algae: Physiological processes and relationships with stress. Perspectives in Phycology, 103–112. https://doi.org/10.1127/pip/2014/0013

Bhayani, K., Mitra, M., Ghosh, T., and Mishra, S., 2016. C-Phycocyanin as a potential biosensor for heavy metals like Hg2+ in aquatic systems. RSC Advances, 6(112), 111599–111605.

Bhowmik, D., Dubey, J., and Mehra, S., 2009. Probiotic Efficiency of *Spirulina platensis*—Stimulating Growth of Lactic Acid Bacteria.

Bilal, M., Rasheed, T., Sosa-Hernández, J. E., Raza, A., Nabeel, F., and Iqbal, H. M. N., 2018. Biosorption: An Interplay between Marine Algae and Potentially Toxic Elements—A Review. Marine Drugs, 16(2), 65.

Blowes, D. W., Ptacek, C. J., Jambor, J. L., and Weisener, C. G., 2003. 9.05—The Geochemistry of Acid Mine Drainage. In H. D. Holland and K. K. Turekian (Eds.), Treatise on Geochemistry (pp. 149–204). Pergamon. https://doi.org/10.1016/B0-08-043751-6/09137-4

Borowitzka, M. A., 2018. Chapter 3—Biology of Microalgae. In I. A. Levine and J. Fleurence (Eds.), Microalgae in Health and Disease Prevention (pp. 23–72). Academic Press.

Bortolini, D. G., Maciel, G. M., Fernandes, I. de A. A., Pedro, A. C., Rubio, F. T. V., Branco, I. G., and Haminiuk, C. W. I., 2022. Functional properties of bioactive compounds from *Spirulina* spp.: Current status and future trends. Food Chemistry: Molecular Sciences, 5, 100134.

Boveris, A., Oshino, N., and Chance, B., 1972. The cellular production of hydrogen peroxide. The Biochemical Journal, 128(3), 617–630. Scopus. https://doi.org/10.1042/bj1280617

Boyanova, P., Gradinarska, D., Dobreva, V., Panayotov, P., Momchilova, M., and Zsivanovits, G., 2022. Effect of *Spirulina platensis* on the quality and antioxidants characteristics of ice cream. BIO Web of Conferences, 45, 01009.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25–30.

Broadhurst, R. B., and Jones, W. T., 1978. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture, 29(9), 788–794. https://doi.org/10.1002/jsfa.2740290908

Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lönnerdal B, Ruel MT, Sandtröm B, Wasantwisut E, Hotz C.,2004. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull, 25(1 Suppl 2):S99-203. PMID: 18046856.

Bryant, D. A.,1982. Phycoerythrocyanin and Phycoerythrin: Properties and Occurrence in Cyanobacteria. Microbiology, 128(4), 835–844. https://doi.org/10.1099/00221287-128-4-835

Bule, M., Khan, F., Nisar, M., andNiaz, K., 2020. Tannins (hydrolysable tannins, condensed tannins, phlorotannins, flavono-ellagitannins) (pp. 132–146).

Cai, B., Yi, X., Han, Q., Pan, J., Chen, H., Sun, H., and Wan, P., 2022. Structural characterization of oligosaccharide from *Spirulina platensis* and its effect on the faecal microbiota in vitro. Food Science and Human Wellness, 11(1), 109–118. https://doi.org/10.1016/j.fshw.2021.07.012

Campbell, F., 2012. Lightweight Materials: Understanding the Basics. ASM International. http://gen.lib.rus.ec/book/index.php?md5=abb6665bc33b51b998f241f3054adcba

Carvalho, A. P., Silva, S. O., Baptista, J. M., and Malcata, F. X., 2011. Light requirements in microalgal photobioreactors: An overview of biophotonic aspects. Applied Microbiology and Biotechnology, 89(5), 1275–1288.

Cepoi, L., Zinicovscaia, I., Rudi, L., Chiriac, T., Miscu, V., Djur, S., Strelkova, L., and Grozdov, D., 2020. *Spirulina platensis* as renewable accumulator for heavy metals accumulation from multi-element synthetic effluents. Environmental Science and Pollution Research, 27(25), 31793–

Cepoi, L., Zinicovscaia, I., Rudi, L., Chiriac, T., Miscu, V., Djur, S., Strelkova, L., Vergel, K., andNekhoroshkov, P., 2020b. Growth and heavy metals accumulation by *Spirulina platensis* biomass from multicomponent copper containing synthetic effluents during repeated cultivation cycles. Ecological Engineering, 142, 105637. https://doi.org/10.1016/j.ecoleng.2019.105637

Chan, E. C. S., 2003. 1—Microbial nutrition and basic metabolism. In D. Mara and N. Horan (Eds.), Handbook of Water and Wastewater Microbiology (pp. 3–33). Academic Press.

Chapman, G. R., 2002. Magnesium—Making light of resources. Mineral Processing and Extractive Metallurgy, 111(2), 49–52. https://doi.org/10.1179/mpm.2002.111.2.49

Chapman, H. D., and Pratt, P. F., 1961. Methods of analysis for soils, plants, and waters. University of California, Division of Agricultural Sciences.

Charpy, L., Langlade, M. J., and Alliod, R., 2008. « La Spiruline peut-elle être un atout pour la santé et le développement en Afrique? ».

Chasapis, C. T., Ntoupa, P.-S. A., Spiliopoulou, C. A., and Stefanidou, M. E., 2020. Recent aspects of the effects of zinc on human health. Archives of Toxicology, 94(5), 1443–1460.

Chauhan, R., Tripathi, A., Chauhan, A., Basniwal, R. K., Ranjan, A., Kumari, A., Rajput, V. D., Prazdnova, E. V., Minkina, T., Chauhan, S. C., Jindal, T., and Prasad, R., 2023. Bioactive compounds from high altitude lake *Arthrospira platensis* HANL01: Antioxidant property,

thermal stability and antibacterial assessment against multiple antibiotics resistant bacteria. Bioresource Technology Reports, 22, 101398.

Chen, Y., Yang, X., Wu, L., Tong, L., and Zhu, J., 2023. Recovery of Mg from $H2SO_4$ Leaching Solution of Serpentine to Precipitation of High-Purity Mg $(OH)_2$ and $4MgCO_3{\cdot}Mg(OH)_2{\cdot}4H_2O$. Minerals, 13(3), Article 3. https://doi.org/10.3390/min13030318

Chi, Z., Hong, B., Tan, S., Wu, Y., Li, H., Lu, C.-H., and Li, W., 2020. Impact Assessment of heavy metal cations to the characteristics of photosynthetic phycocyanin. Journal of Hazardous Materials, 391, 122225.

Chojnacka, K., 2007. Using biosorption to enrich the biomass of *Chlorella vulgaris* with microelements to be used as mineral feed supplement. World Journal of Microbiology and Biotechnology, 23(8), 1139–1147. https://doi.org/10.1007/s11274-006-9344-9

Chojnacka, K., Chojnacki, A., and Górecka, H., 2005. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae *Spirulina* sp.: Kinetics, equilibrium and the mechanism of the process. Chemosphere, 59(1), 75–84. https://doi.org/10.1016/j.chemosphere.2004.10.005

Choudhary, M., Jetley, U. K., Abash Khan, M., Zutshi, S., and Fatma, T., 2007. Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. Ecotoxicology and Environmental Safety, 66(2), 204–209.

Chugh, M., Kumar, L., Bhardwaj, D., and Bharadvaja, N., 2022. Chapter 11 - Bioaccumulation and detoxification of heavy metals: An insight into the mechanism. In M. P. Shah, S. Rodriguez-Couto, and R. T. Kapoor (Eds.), Development in Wastewater Treatment Research and Processes (pp. 243–264).

Cingi, C., Conk-Dalay, M., Cakli, H., and Bal, C., 2008. The effects of *Spirulina* on allergic rhinitis. European Archives of Oto-Rhino-Laryngology: Official Journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS): Affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery, 265(10), 1219–1223. https://doi.org/10.1007/s00405-008-0642-8

Colla, L. M., Oliveira Reinehr, C., Reichert, C., and Costa, J. A. V., 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresource Technology, 98(7), 1489–1493. https://doi.org/10.1016/j.biortech.2005.09.030

Cooke, M. S., Evans, M. D., Dizdaroglu, M., and Lunec, J., 2003. Oxidative DNA damage: Mechanisms, mutation, and disease. The FASEB Journal, 17(10), 1195–1214. https://doi.org/10.1096/fj.02-0752rev

Cosgrove, D. J., 1980. The determination of myo-inositol hexakisphosphate (phytate). Journal of the Science of Food and Agriculture, 31(12), 1253–1256. https://doi.org/10.1002/jsfa.2740311206

Dagnino-Leone, J., Figueroa, C. P., Castañeda, M. L., Youlton, A. D., Vallejos-Almirall, A., Agurto-Muñoz, A., Pavón Pérez, J., and Agurto-Muñoz, C., 2022. Phycobiliproteins: Structural aspects, functional characteristics, and biotechnological perspectives. Computational and Structural Biotechnology Journal, 20, 1506–1527.

Dartsch, P. C., 2008. Antioxidant potential of selected *Spirulina platensis* preparations. Phytotherapy Research, 22(5), 627–633. https://doi.org/10.1002/ptr.2310

Delpeuch, F., Joseph, A., and Cavelier, C., 1975. [Consumption and nutritional contribution of the blue algae (*Oscillatoria platensis*) among some populations of Kanem (Tchad)]. Annales de la nutrition et de l'alimentation, 29(6), 497–516.

Demirbas, A., and Fatih Demirbas, M., 2011. Importance of algae oil as a source of biodiesel. Energy Conversion and Management, 52(1), 163–170. https://doi.org/10.1016/j.enconman.2010.06.055

Dewanto, V., Wu, X., Adom, K. K., and Liu, R. H., 2002. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. Journal of Agricultural and Food Chemistry, 50(10), 3010–3014.

Dey, S., and Rathod, V. K., 2013. Ultrasound assisted extraction of β-carotene from *Spirulina platensis*. Ultrasonics Sonochemistry, 20(1), 271–276.

Dhaliwal, S. S., Sharma, V., Shukla, A. K., Verma, V., Kaur, M., Shivay, Y. S., Nisar, S., Gaber, A., Brestic, M., Barek, V., Skalicky, M., Ondrisik, P., and Hossain, A., 2022. Biofortification—A Frontier Novel Approach to Enrich Micronutrients in Field Crops to Encounter the Nutritional Security. Molecules, 27(4), Article 4.

Dolganyuk, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S., Katserov, D., Patyukov, N., and Sukhikh, S., 2020. Microalgae: A Promising Source of Valuable Bioproducts. Biomolecules, 10(8), 1153.

Dotto, G. L., Cadaval, T. R. S., and Pinto, L. A. A., 2012. Use of *Spirulina platensis* micro and nanoparticles for the removal synthetic dyes from aqueous solutions by biosorption. Process Biochemistry, 47(9), 1335–1343. https://doi.org/10.1016/j.procbio.2012.04.029

Doumenge, F., Durand-Chastel, H., and Toulemont, A., 1993. Spiruline, algue de vie.

El-Tantawy, W. H., 2015. Antioxidant effects of *Spirulina* supplement against lead acetate-induced hepatic injury in rats. Journal of Traditional and Complementary Medicine, 6(4), 327–331.-

Eriksen, N. T., 2008. Production of phycocyanin—A pigment with applications in biology, biotechnology, foods and medicine. Applied Microbiology and Biotechnology, 80(1), 1–14.

Ermis, H., Guven-Gulhan, U., Cakir, T., and Altinbas, M., 2020. Effect of iron and magnesium addition on population dynamics and high value product of microalgae grown in anaerobic liquid digestate. Scientific Reports, 10(1), Article 1.

Evans, P., and Halliwell, B., 2001. Micronutrients: Oxidant/antioxidant status. British Journal of Nutrition, 85(S2), S67–S74. https://doi.org/10.1049/BJN2000296

Fadaei, V., Mohamadi-Alasti, F., and Khosravi-Darani, K., 2019. Influence of *Spirulina platensis* powder on the starter culture viability in probiotic yoghurt containing spinach during cold storage.

Falquet, J., 1996. Un module d'apprentissage pour la production de la spiruline. Antenna Technology, Genève.

Falquet, J., and hurni J-P., 2006. Spiruline Aspects Nutritionnels.

Fenton, Henry John Horstman. "LXXIII.—Oxidation of tartaric acid in presence of iron." Journal of the Chemical Society, Transactions 65 (1894): 899-910.

Fiedor, L., Kania, A., Myśliwa-Kurdziel, B., Orzeł, Ł., and Stochel, G., 2008. Understanding chlorophylls: Central magnesium ion and phytyl as structural determinants. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1777(12), 1491–1500. https://doi.org/10.1016/j.bbabio.2008.09.005

Finkel, T., 2003. Oxidant signals and oxidative stress. Current Opinion in Cell Biology, 15(2), 247–254. https://doi.org/10.1016/S0955-0674(03)00002-4

Finkle, B. J., and Appleman, D., 1953. The Effect of Magnesium Concentration on Chlorophyll and Catalase Development in *Chlorella*. Plant Physiology, 28(4), 652–663.

Fleurence, J., and Levine, I. A., 2018. Chapter 14—Antiallergic and Allergic Properties. In I. A. Levine andJ. Fleurence (Eds.), Microalgae in Health and Disease Prevention (pp. 307–315). Academic Press.

Foster, M., Chu, A., Petocz, P., and Samman, S., 2013. Effect of vegetarian diets on zinc status: A systematic review and meta-analysis of studies in humans. Journal of the Science of Food and Agriculture, 93(10), 2362–2371. https://doi.org/10.1002/jsfa.6179

Fox, C., Ramsoomair, D., and Carter, C., 2001b. Magnesium: Its proven and potential clinical significance. Southern Medical Journal, 94(12), 1195–1202.

Fox, R. D., 1999a. La spiruline: Technique, pratique et promesse. Edisud.

Garcia-Pichel, F., 2009. Cyanobacteria. In Encyclopedia of Microbiology, Third Edition (pp. 107–124).

Gershwin, M. E., and Belay, A., 2007. *Spirulina* in Human Nutrition and Health. CRC Press.

Ghanbarzadeh, M., Moazami, N., Shahavi, M. H., and Mirdamadi, S., 2022. Study of bioactive compounds in *Arthrospira platensis* MGH-1 fortified with micronutrients of iron, zinc, and manganese. Journal of Applied Phycology, 34(5), 2449–2462.

Gheda, S., Abd El-Zaher, E. H. F., Abou-Zeid, A. M., Bedair, N. A., and Pereira, L., 2023. Potential Activity of *Arthrospira platensis* as Antioxidant, Cytotoxic and Antifungal against Some Skin Diseases: Topical Cream Application. Marine Drugs, 21(3), Article 3.

Giusti, M. M., and Wrolstad, R. E., 2001. Anthocyanins. Characterization and measurement with UV-visible spectroscopy. Current Protocols in Food Analytical Chemistry, 1, 1–13.

Glasdam, S.-M., Glasdam, S.,and Peters, G. H., 2016. Chapter Six - The Importance of Magnesium in the Human Body: A Systematic Literature Review. In G. S. Makowski (Ed.), Advances in Clinical Chemistry (Vol. 73, pp. 169–193). Elsevier. https://doi.org/10.1016/bs.acc.2015.10.002

Gómez, P. I., Mayorga, J., Flaig, D., Castro-Varela, P., Jaupi, A., Ulloa, P. A., Soto-Bartierra, J., Henríquez, V., and Rojas, V., 2023. Looking beyond *Arthrospira*: Comparison of antioxidant and anti-inflammatory properties of ten cyanobacteria strains. Algal Research, 74, 103182.

Gong, M., and Bassi, A., 2016. Carotenoids from microalgae: A review of recent developments. Biotechnology Advances, 34(8), 1396–1412. https://doi.org/10.1016/j.biotechadv.2016.10.005

González-Camejo, J., Ferrer, J., Seco, A., and Barat, R., 2021. Outdoor microalgae-based urban wastewater treatment: Recent advances, applications, and future perspectives. WIREs Water, 8(3), e1518. https://doi.org/10.1002/wat2.1518

Goodwin, F. E., 2000. Zinc Compounds. In Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Sons, Ltd. https://doi.org/10.1002/0471238961.2609140307151504.a02

Gorain, P. C., Bagchi, S. K., and Mallick, N., 2013. Effects of calcium, magnesium and sodium chloride in enhancing lipid accumulation in two green microalgae. Environmental Technology, 34(13–14), 1887–1894. https://doi.org/10.1080/09593330.2013.812668

Grover, P., Bhatnagar, A., Kumari, N., Narayan Bhatt, A., Kumar Nishad, D., and Purkayastha, J., 2021. C-Phycocyanin-a novel protein from *Spirulina platensis*- In vivo toxicity, antioxidant and immunomodulatory studies. Saudi Journal of Biological Sciences, 28(3), 1853–1859. https://doi.org/10.1016/j.sjbs.2020.12.037

Guerin, M., Huntley, M. E., and Olaizola, M., 2003. Haematococcus astaxanthin: Applications for human health and nutrition. Trends in Biotechnology, 21(5), 210–216. https://doi.org/10.1016/S0167-7799(03)00078-7

Haber, F., & Weiss, J. (1934). The catalytic decomposition of hydrogen peroxide by iron salts. Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences, 147(861), 332-351

Hajimahmoodi, M., Faramarzi, M. A., Mohammadi, N., Soltani, N., Oveisi, M. R., and Nafissi-Varcheh, N., 2010. Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. Journal of Applied Phycology, 22(1), 43–50. https://doi.org/10.1007/s10811-009-9424-y

Hara, T., Takeda, T., Takagishi, T., Fukue, K., Kambe, T., and Fukada, T., 2017. Physiological roles of zinc transporters: Molecular and genetic importance in zinc homeostasis. The Journal of Physiological Sciences, 67(2), 283–301. https://doi.org/10.1007/s12576-017-0521-4

Hayashi, K., Hayashi, T., Morita, N., and Kojima, I., 1993. An extract from *Spirulina platensis* is a selective inhibitor of herpes simplex virus type 1 penetration into HeLa cells. Phytotherapy Research, 7(1), 76–80. https://doi.org/10.1002/ptr.2650070118

Hayashi, T., Hayashi, K., Maeda, M., and Kojima, I., 1996. Calcium Spirulan, an Inhibitor of Enveloped Virus Replication, from Blue-Green Alga *Spirulina platensis*. Journal of Natural Products, 59(1), 83–87. https://doi.org/10.1021/np960017o

He, Z. L., Yang, X. E., and Stoffella, P. J., 2005. Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology, 19(2), 125–140. https://doi.org/10.1016/j.jtemb.2005.02.010

Hidayati, J. R., Yudiati, E., Pringgenies, D., Oktaviyanti, D. T., and Kusuma, A. P., 2020. Comparative Study on Antioxidant Activities, Total Phenolic Compound and Pigment Contents of Tropical *Spirulina platensis*, Gracilaria arcuata and Ulva lactuca Extracted in Different Solvents Polarity. E3S Web of Conferences, 147, 03012.

Holman, B. W. B., and Malau-Aduli, A. E. O., 2013. *Spirulina* as a livestock supplement and animal feed. Journal of Animal Physiology and Animal Nutrition, 97(4), 615–623.

Hoseini, S. M., Khosravi-Darani, K., and Mozafari, M. R., 2013. Nutritional and Medical Applications of *Spirulina* Microalgae. Mini Reviews in Medicinal Chemistry, 13(8), 1231–1237.

Ibrahim, T. N. B. T., Feisal, N. A. S., Kamaludin, N. H., Cheah, W. Y., How, V., Bhatnagar, A., Ma, Z., and Show, P. L., 2023. Biological active metabolites from microalgae for healthcare and pharmaceutical industries: A comprehensive review. Bioresource Technology, 372, 128661.

İçme Suyu Kabul Edilebilir Değerler | Doğa Bitkileri ve Su Ürünleri Araştırma ve Uygulama Merkezi. Marmara Üniversitesi. Retrieved July 6, 2023, from https://dobisu.marmara.edu.tr/orta-menu/yararli-bilgiler/icme-suyu-kabul-edilebilir-degerler

Ijaz, S., and Hasnain, S., 2016. Antioxidant potential of indigenous cyanobacterial strains in relation with their phenolic and flavonoid contents. Natural Product Research, 30(11), 1297–1300.

Imani, M. M., and Safaei, M., 2019. Optimized Synthesis of Magnesium Oxide Nanoparticles as Bactericidal Agents. Journal of Nanotechnology, 2019, e6063832.

Ismaiel, M. M. S., El-Ayouty, Y. M., and Piercey-Normore, M., 2016. Role of pH on antioxidants production by *Spirulina* (*Arthrospira*) *platensis*. Brazilian Journal of Microbiology, 47(2), 298–304. https://doi.org/10.1016/j.bjm.2016.01.003

Jahnen-Dechent, W., and Ketteler, M., 2012. Magnesium basics. Clinical Kidney Journal, 5(Suppl 1), i3–i14. https://doi.org/10.1093/ndtplus/sfr163

Jatupornpongchai, A., Phoopat, N., Limpirat, W., and Ningsanond, S., 2022. Biosorbtion, localization and bioavailability of zinc in *Spirulina platensis* culture. Food Research, 6(4), 228–235. https://doi.org/10.26656/fr.2017.6(4).471

Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., and Pérez-Lebeña, E., 2021. The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. International Journal of Molecular Sciences, 22(9), 4642. https://doi.org/10.3390/ijms22094642

Kaçar, H., Yilmaz, S., Türkoğlu, M., and Sadikoglu, M., 2022. Seasonal variations in tap water quality parameters in Çanakkale, Türkiye. Turkish Journal of Analytical Chemistry, 4(1), 6–18. https://doi.org/10.51435/turkjac.1111456

Kalafati, M., Jamurtas, A. Z., Nikolaidis, M. G., Paschalis, V., Theodorou, A. A., Sakellariou, G. K., Koutedakis, Y., and Kouretas, D., 2010. Ergogenic and antioxidant effects of *Spirulina* supplementation in humans. Med Sci Sports Exerc, 42(1), 142–151.

Kannaujiya, V. K., Kumar, D., Singh, V., and Sinha, R. P., 2021. Chapter 4 - Advances in phycobiliproteins research: Innovations and commercialization. In R. p. Sinhaand D.-P. Häder (Eds.), Natural Bioactive Compounds (pp. 57–81). Academic Press. https://doi.org/10.1016/B978-0-12-820655-3.00004-5

Kaushik, P., and Chauhan, A., 2008. In vitro antibacterial activity of laboratory grown culture of *Spirulina platensis*. Indian Journal of Microbiology, 48(3), 348–352. https://doi.org/10.1007/s12088-008-0043-0

Kavisri, M., Marykutty A., Gopal P., M., and Meivelu M., 2023. Phytochemistry, bioactive potential and chemical characterization of metabolites from marine microalgae (*Spirulina platensis*) biomass. Biomass Conversion and Biorefinery, 13(11), 10147–10154.

Kebede, E., and Ahlgren, G., 1996. Optimum growth conditions and light utilization efficiency of *Spirulina platensis* (*Arthrospira fusiformis*) (Cyanophyta) from Lake Chitu, Ethiopia. Hydrobiologia, 332(2), 99–109. https://doi.org/10.1007/BF00016689

Kent, K., Hölzel, N., and Swarts, N., 2018. Chapter 10 - Polyphenolic Compounds in Sweet Cherries: A Focus on Anthocyanins. In R. R. Watson, V. R. Preedy, and S. Zibadi (Eds.), Polyphenols: Mechanisms of Action in Human Health and Disease (Second Edition) (pp. 103–118). Academic Press. https://doi.org/10.1016/B978-0-12-813006-3.00010-6

Kepekçi, R. A., and Saygideger, S. D., 2012. Enhancement of phenolic compound production in *Spirulina platensis* by two-step batch mode cultivation. Journal of Applied Phycology, 24(4), 897–905.

Khan, M. I., Shin, J. H., and Kim, J. D., 2018. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories, 17(1), 36. https://doi.org/10.1186/s12934-018-0879-x

Koníčková, R., Vaňková, K., Vaníková, J., Vánová, K., Muchová, L., Subhanová, I., Zadinová, M., Zelenka, J., Dvořák, A., Kolář, M., Strnad, H., Rimpelová, S., Ruml, T., Wong, R. J., and Vítek, L., 2014. Anti-cancer effects of blue-green alga *Spirulina platensis*, a natural source of bilirubin-like tetrapyrrolic compounds. Annals of Hepatology, 13(2), 273–283.

Kougia, E., Ioannou, E., Roussis, V., Tzovenis, I., Chentir, I., and Markou, G., 2023. Iron (Fe) biofortification of *Arthrospira platensis*: Effects on growth, biochemical composition and in vitro iron bioaccessibility. Algal Research, 70, 103016. https://doi.org/10.1016/j.algal.2023.103016

Kumar, M., Kulshreshtha, J., and Singh, G. P., 2011. Growth and biopigment accumulation of cyanobacterium *Spirulina platensis* at different light intensities and temperature. Brazilian Journal of Microbiology, 42(3), 1128–1135.

Kumar, N., and Goel, N., 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports, 24, e00370. https://doi.org/10.1016/j.btre.2019.e00370

Léonard, J., and Compère, P., 1967. *Spirulina platensis* (Gom.) Geitl., algue bleue de grande valeur alimentaire par sa richesse en proteines. Ministère de l'Agriculture.

Leong, Y. K., and Chang, J.-S., 2020. Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. Bioresource Technology, 303, 122886.

Li, B., Zhang, X., Gao, M., and Chu, X., 2005. Effects of CD59 on antitumoral activities of phycocyanin from *Spirulina platensis*. Biomedicine and Pharmacotherapy, 59(10), 551–560.

Li, M., Hu, C., Zhu, Q., Chen, L., Kong, Z., and Liu, Z., 2006. Copper and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in the microalga Pavlova viridis (Prymnesiophyceae). Chemosphere, 62(4), 565–572.

Li, W., Su, H.-N., Pu, Y., Chen, J., Liu, L.-N., Liu, Q., and Qin, S., 2019. Phycobiliproteins: Molecular structure, production, applications, and prospects. Biotechnology Advances, 37(2), 340–353.

Lipinski, B., 2011. Hydroxyl Radical and Its Scavengers in Health and Disease. Oxidative Medicine and Cellular Longevity, 2011, e809696. https://doi.org/10.1155/2011/809696

Lordan, S., Ross, R. P., and Stanton, C., 2011. Marine Bioactives as Functional Food Ingredients: Potential to Reduce the Incidence of Chronic Diseases. Marine Drugs, 9(6), 1056–1100.

Loring, J. S., Miller, Q. R. S., Thompson, C. J., and Schaef, H. T. (2019). Chapter 4—Experimental Studies of Reactivity and Transformations of Rocks and Minerals in Water-Bearing Supercritical CO2. In P. Newell and A. G. Ilgen (Eds.), Science of Carbon Storage in Deep Saline Formations (pp. 63–88). Elsevier. https://doi.org/10.1016/B978-0-12-812752-0.00004-6

Lourenço, S. C., Moldão-Martins, M., and Alves, V. D., 2019. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. Molecules, 24(22), 4132.

Maadane, A., Merghoub, N., Ainane, T., El Arroussi, H., Benhima, R., Amzazi, S., Bakri, Y., and Wahby, I., 2015. Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. Journal of Biotechnology, 215, 13–19.

Machado, A. R., Pinheiro, A. C., Vicente, A. A., Souza-Soares, L. A., and Cerqueira, M. A., 2019. Liposomes loaded with phenolic extracts of *Spirulina* LEB-18: Physicochemical characterization and behavior under simulated gastrointestinal conditions. Food Research International (Ottawa, Ont.), 120, 656–667. https://doi.org/10.1016/j.foodres.2018.11.023

Machu, L., Misurcova, L., Vavra Ambrozova, J., Orsavova, J., Mlcek, J., Sochor, J., and Jurikova, T., 2015. Phenolic Content and Antioxidant Capacity in Algal Food Products. Molecules, 20(1), 1118–1133.

Magwell, P. F. R., Djoudjeu, K. T., Minyaka, E., Tavea, M.-F., Fotsop, O. W., Tagnikeu, R. F., Fofou, A. M., Darelle, C. K. V., Dzoyem, C. U. D., and Lehman, L. G., 2023. Sodium Bicarbonate (NaHCO3) Increases Growth, Protein and Photosynthetic Pigments Production and Alters Carbohydrate Production of *Spirulina platensis*. Current Microbiology, 80(2), 63.

Mahajan, L., Verma, P. K., Raina, R., Pankaj, N. K., Sood, S., and Singh, M., 2018. Alteration in thiols homeostasis, protein and lipid peroxidation in renal tissue following subacute oral exposure of imidacloprid and arsenic in Wistar rats. Toxicology Reports, 5, 1114–1119.

Malik, P., Kempanna, C., and Paul, A., 2013. Quality characteristics of ice cream enriched with *Spirulina* powder. International journal of food and nutritional sciences, Volume 2, 44–50.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L., 2004. Polyphenols: Food sources and bioavailability. The American Journal of Clinical Nutrition, 79(5), 727–747.

Marles, R. J., Barrett, M. L., Barnes, J., Chavez, M. L., Gardiner, P., Ko, R., Mahady, G. B., Dog, T. L., Sarma, N. D., Giancaspro, G. I., Sharaf, M., and Griffiths, J., 2011. United States Pharmacopeia Safety Evaluation of *Spirulina*. Critical Reviews in Food Science and Nutrition, 51(7), 593–604.

Marnett, L. J., 2000. Oxyradicals and DNA damage. Carcinogenesis, 21(3), 361–370.

Masojídek, J., and Torzillo, G. (2014). Mass Cultivation of Freshwater Microalgae☆. In Reference Module in Earth Systems and Environmental Sciences. Elsevier. https://doi.org/10.1016/B978-0-12-409548-9.09373-8

Mattioli, R., Francioso, A., Mosca, L., and Silva, P., 2020. Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. Molecules, 25(17), 3809. https://doi.org/10.3390/molecules25173809

Mazard, S., Penesyan, A., Ostrowski, M., Paulsen, I., and Egan, S., 2016. Tiny Microbes with a Big Impact: The Role of Cyanobacteria and Their Metabolites in Shaping Our Future. Marine Drugs, 14(5), 97. https://doi.org/10.3390/md14050097

Mehdizadeh Allaf, M., and Peerhossaini, H., 2022. Cyanobacteria: Model Microorganisms and Beyond. Microorganisms, 10(4), 696. https://doi.org/10.3390/microorganisms10040696

Mezzetti, A., Pierdomenico, S. D., Costantini, F., Romano, F., De Cesare, D., Cuccurullo, F., Imbastaro, T., Riario-Sforza, G., Di Giacomo, F., Zuliani, G., and Fellin, R., 1998. Copper/zinc ratio and systemic oxidant load: Effect of aging and aging-related degenerative diseases. Free Radical Biology and Medicine, 25(6), 676–681. https://doi.org/10.1016/S0891-5849(98)00109-9

Milkovic, L., Cipak Gasparovic, A., Cindric, M., Mouthuy, P.-A., and Zarkovic, N., 2019. Short Overview of ROS as Cell Function Regulators and Their Implications in Therapy Concepts. Cells, 8(8), Article 8. https://doi.org/10.3390/cells8080793.

Mishima, T., Murata, J., Toyoshima, M., Fujii, H., Nakajima, M., Hayashi, T., and Saiki, I., 1998. Inhibition of tumor invasion and metastasis by calcium-spirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, *Spirulina platensis*. Clinical & experimental metastasis, 16, 541-550.

Mitra, M., and Melis, A., 2008. Optical properties of microalgae for enhanced biofuels production. Optics Express, 16(26), 21807–21820. https://doi.org/10.1364/OE.16.021807

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., Idris, A. M., Khandaker, M. U., Osman, H., Alhumaydhi, F. A., and Simal-Gandara, J., 2022. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science, 34(3), 101865.

Mocanu, G.-D., Botez, E., Nistor, O. V., Georgeta, D., and Vlăsceanu, G., 2013. Influence of *Spirulina platensis* biomass over some starter culture of lactic bacteria. Journal of Agroalimentary Processes and Technologies.

Morançais, M., Mouget, J.-L., and Dumay, J., 2018. Proteins and Pigments. In Microalgae in Health and Disease Prevention (pp. 145–175). Elsevier. https://doi.org/10.1016/B978-0-12-811405-6.00007-4.

Morot-Gaudry, J.-F., 2006. La photosynthèse: Processus physiques, moléculaires et physiologiques. 1–460.

Mortensen, A., 2006. Carotenoids and other pigments as natural colorants. Pure and Applied Chemistry, 78(8), 1477–1491. https://doi.org/10.1351/pac200678081477

Mutanda, T., Naidoo, D., Bwapwa, J. K., and Anandraj, A., 2020. Biotechnological Applications of Microalgal Oleaginous Compounds: Current Trends on Microalgal Bioprocessing of Products. Frontiers in Energy Research, 8. https://www.frontiersin.org/articles/10.3389/fenrg.2020.598803

Mythri, R. B., Vali, S., and Bharath, M. M. S., 2013. Oxidative Stress, Protein Damage. In W. Dubitzky, O. Wolkenhauer, K.-H. Cho, and H. Yokota (Eds.), Encyclopedia of Systems Biology (pp. 1619–1620). Springer. https://doi.org/10.1007/978-1-4419-9863-7_650

Na wang., 2016. Biological activities of tropical green algae from Australia. https://unsworks.unsw.edu.au/entities/publication/7c999fa5-dd3d-4c1c-a551-9ff63c17ed09/full

Nalimova, A. A., Popova, V. V., Tsoglin, L. N., and Pronina, N. A., 2005. The effects of copper and zinc on *Spirulina platensis* growth and heavy metal accumulation in its cells. Russian Journal of Plant Physiology, 52(2), 229–234. https://doi.org/10.1007/s11183-005-0035-4

Nollet, L. M. L., and Gutierrez-Uribe, J., 2018. Phenolic Compounds in Food: Characterization and Analysis (1st ed.). CRC Press. https://doi.org/10.1201/9781315120157

Ofori, K. F., Antoniello, S., English, M. M., and Aryee, A. N. A., 2022. Improving nutrition through biofortification–A systematic review. Frontiers in Nutrition, 9, 1043655.

Oke, G. O., Abiodun, A. A., Imafidon, C. E., and Monsi, B. F., 2019. Zingiber officinale (Roscoe) mitigates CCl4-induced liver histopathology and biochemical derangements through antioxidant, membrane-stabilizing and tissue-regenerating potentials. Toxicology Reports, 6, 416–425.

Oldham, K. M., and Bowen, P. E., 1998. Oxidative Stress in Critical Care: Is Antioxidant Supplementation Beneficial? Journal of the American Dietetic Association, 98(9), 1001–1008.

Omar, H. H., 2002. Bioremoval of zinc ions by Scenedesmus obliquus and Scenedesmus quadricauda and its effect on growth and metabolism. International Biodeterioration and Biodegradation, 50(2), 95–100.

Ovando, C. A., Carvalho, J. C. de, Vinícius de Melo Pereira, G., Jacques, P., Soccol, V. T., and Soccol, C. R., 2018. Functional properties and health benefits of bioactive peptides derived from *Spirulina*: A review. Food Reviews International, 34(1), 34–51.

Ozdemir, G., Ulku Karabay, N., Dalay, M. C., and Pazarbasi, B., 2004. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytotherapy Research, 18(9), 754–757.

Ozgen, M., Reese, R. N., Tulio, A. Z., Scheerens, J. C., and Miller, A. R., 2006. Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method to Measure Antioxidant Capacity of Selected Small Fruits and Comparison to Ferric Reducing Antioxidant Power (FRAP) and 2,2‘-Diphenyl-1-picrylhydrazyl (DPPH) Methods. Journal of Agricultural and Food Chemistry, 54(4),

Pagels, F., Guedes, A. C., Amaro, H. M., Kijjoa, A., and Vasconcelos, V., 2019. Phycobiliproteins from cyanobacteria: Chemistry and biotechnological applications. Biotechnology Advances, 37(3), 422–443.

Pagnussatt, F. A., de Lima, V. R., Dora, C. L., Costa, J. A. V., Putaux, J.-L., and Badiale-Furlong, E., 2016. Assessment of the encapsulation effect of phenolic compounds from *Spirulina* sp. LEB-18 on their antifusarium activities. Food Chemistry, 211, 616–623.

Pane, L., Solisio, C., Lodi, A., Luigi Mariottini, G., and Converti, A., 2008. Effect of extracts from *Spirulina platensis* bioaccumulating cadmium and zinc on L929 cells. Ecotoxicology and Environmental Safety, 70(1), 121–126.

Park, H. J., Lee, Y. J., Ryu, H. K., Kim, M. H., Chung, H. W., and Kim, W. Y., 2008. A Randomized Double-Blind, Placebo-Controlled Study to Establish the Effects of *Spirulina* in Elderly Koreans. Annals of Nutrition and Metabolism, 52(4), 322–328. https://doi.org/10.1159/000151486

Papalia, T., Sidari, R., and Panuccio, M. R. (2019). Impact of different storage methods on bioactive compounds in *Arthrospira platensis* biomass. *Molecules*, *24*(15), 2810.

Patachia, S., and Croitoru, C., 2016. 14—Biopolymers for wood preservation. In F. Pacheco-Torgal, V. Ivanov, N. Karak, and H. Jonkers (Eds.), Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials (pp. 305–332). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100214-8.00014-2.

Patel, J. B. and Clinical and Laboratory Standards Institute., 2017. Performance standards for antimicrobial susceptibility testing.

Pereira, M. I. B., Chagas, B. M. E., Sassi, R., Medeiros, G. F., Aguiar, E. M., Borba, L. H. F., Silva, E. P. E., Neto, J. C. A., and Rangel, A. H. N., 2019. Mixotrophic cultivation of *Spirulina platensis* in dairy wastewater: Effects on the production of biomass, biochemical composition and antioxidant capacity. PLOS ONE, 14(10), e0224294. https://doi.org/10.1371/journal.pone.0224294

Pérez-Juárez, A., Chamorro, G., Alva-Sánchez, C., Paniagua-Castro, N., and Pacheco-Rosado, J., 2016. Neuroprotective effect of *Arthrospira* (*Spirulina*) *platensis* against kainic acid-neuronal death. Pharmaceutical Biology, 54(8), 1408–1412.

Petrescu, F. I. T., and Ungureanu, L. M., 2022. About the Cyanobacteria and Stromatolites. OnLine Journal of Biological Sciences, 22(1), 87–111.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A., 2017. Oxidative Stress: Harms and Benefits for Human Health. Oxidative Medicine and Cellular Longevity, 2017, 8416763. https://doi.org/10.1155/2017/8416763

Pokorny, J., Yanishlieva, N., and Gordon, M. H., 2001. Antioxidants in food: Practical applications. CRC press.

Poljšak, B., and Milisav, I., 2012. Clinical implications of cellular stress responses. Bosnian Journal of Basic Medical Sciences, 12(2), 122–126.

Prasad, A. S., 2013. Discovery of Human Zinc Deficiency: Its Impact on Human Health and Disease. Advances in Nutrition, 4(2), 176–190.

Priya, A. K., Jalil, A. A., Vadivel, S., Dutta, K., Rajendran, S., Fujii, M., and Soto-Moscoso, M., 2022. Heavy metal remediation from wastewater using microalgae: Recent advances and future trends. Chemosphere, 305, 135375.

Qureshi, M. A., Garlich, J. D., and Kidd, M. T., 1996. Dietary *Spirulina platensis* enhances humoral and cell-mediated immune functions in chickens. Immunopharmacology and Immunotoxicology, 18(3), 465–476.

Rahman, M. A., Aziz, M. A., Al-khulaidi, R. A., Sakib, N., and Islam, M., 2017. Biodiesel production from microalgae *Spirulina maxima* by two step process: Optimization of process variable. Journal of Radiation Research and Applied Sciences, 10(2), 140–147.

Ramadan, K. M. A., El-Beltagi, H. S., Shanab, S. M. M., El-fayoumy, E. A., Shalaby, E. A., and Bendary, E. S. A., 2021. Potential Antioxidant and Anticancer Activities of Secondary Metabolites of Nostoc linckia Cultivated under Zn and Cu Stress Conditions. Processes, 9(11), Article 11.

Ramon-Mascarell, F., Martí-Quijal, F. J., Castagnini, J. M., Phimolsiripol, Y., Ruksiriwanich, W., Rajoka, M. S. R., Mehwish, H. M., and Barba, F. J., 2022. Influence of Geographical Location of *Spirulina* (*Arthrospira platensis*) on the Recovery of Bioactive Compounds Assisted by Pulsed Electric Fields. Separations, 9(9), Article 9.

Remirez, D., Ledón, N., and González, R., 2002. Role of histamine in the inhibitory effects of phycocyanin in experimental models of allergic inflammatory response. Mediators of Inflammation, 11(2), 81–85.

Riss, J., Décordé, K., Sutra, T., Delage, M., Baccou, J.-C., Jouy, N., Brune, J.-P., Oréal, H., Cristol, J.-P., and Rouanet, J.-M., 2007. Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. Journal of Agricultural and Food Chemistry, 55(19), 7962–7967.

Rizzi, V., Gubitosa, J., Fini, P., Fraix, A., Sortino, S., Agostiano, A., and Cosma, P., 2021. Development of *Spirulina* sea-weed raw extract/polyamidoamine hydrogel system as novel platform in photodynamic therapy: Photostability and photoactivity of chlorophyll a. Materials Science and Engineering: C, 119, 111593.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., and Glover, W., 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry, 66(4), 401–436.

Roleira, F. M. F., Varela, C. L., Costa, S. C., and Tavares-da-Silva, E. J., 2018. Phenolic Derivatives From Medicinal Herbs and Plant Extracts: Anticancer Effects and Synthetic Approaches to Modulate Biological Activity. In Studies in Natural Products Chemistry (Vol. 57, pp. 115–156).

Ronda, S. R., and Lele, S. S., 2008. Culture Conditions stimulating high γ-Linolenic Acid accumulation by *Spirulina platensis*. Brazilian Journal of Microbiology, 39(4), 693–697.

Saha, S. K., Moane, S., and Murray, P., 2013. Effect of macro- and micro-nutrient limitation on superoxide dismutase activities and carotenoid levels in microalga Dunaliella salina CCAP 19/18. Bioresource Technology, 147, 23–28.

Salman, J. M., Grmasha, R. A., Stenger-Kovács, C., Lengyel, E., Al-sareji, O. J., AL-Cheban, A. M. A. AR., and Meiczinger, M., 2023. Influence of magnesium concentrations on the biomass and biochemical variations in the freshwater algae, *Chlorella vulgaris*. Heliyon, 9(1), e13072.

Sarada, D. V. L., Sreenath Kumar, C., and Rengasamy, R., 2011. Purified C-phycocyanin from *Spirulina platen*sis (Nordstedt) Geitler: A novel and potent agent against drug resistant bacteria. World Journal of Microbiology and Biotechnology, 27(4), 779–783.

Saranraj, P., Behera, S. S., and Ray, R. C., 2019. Traditional foods from tropical root and tuber crops. In Innovations in Traditional Foods (pp. 159–191).

Sarma, S. J., Das, R. K., Brar, S. K., Le Bihan, Y., Buelna, G., Verma, M., and Soccol, C. R., 2014. Application of magnesium sulfate and its nanoparticles for enhanced lipid production by mixotrophic cultivation of algae using biodiesel waste. Energy, 78, 16–22.

Saunders, A. V., Craig, W. J., and Baines, S. K., 2013. Zinc and vegetarian diets. Medical Journal of Australia, 199(S4), S17–S21.

Sautier, C., Trémolières, J., Billion, J., Flament, C., and Poivre, R., 1975. Valeur Alimentaire Des Algues Spirulines Chez L'homme. Annales de La Nutrition et de l'alimentation, 29(6), 517–534.

Schoofs, L., Veelaert, D., Vanden Broeck, J., and De Loof, A., 1997. Peptides in the Locusts, Locusta migratoria and Schistocerca gregaria. Peptides, 18(1), 145–156.

Seghiri, R., Kharbach, M., and Essamri, A., 2019. Functional Composition, Nutritional Properties, and Biological Activities of Moroccan *Spirulina* Microalga. Journal of Food Quality, 2019, e3707219.

Seshadri, C. V., Umesh, B. V., and Manoharan, R., 1991. Beta-carotene studies in *Spirulina*. Bioresource Technology, 38(2–3), 111–113.

Shedeed, Z. A., Gheda, S., Elsanadily, S., Alharbi, K.,and Osman, M. E. H., 2022. *Spirulina platensis* Biofertilization for Enhancing Growth, Photosynthetic Capacity and Yield of Lupinus luteus. Agriculture, 12(6), Article 6.

Shively, J. M., Cannon, G. C., Heinhorst, S., Fuerst, J. A., Bryant, D. A., Gantt, E., Maupin-Furlow, J. A., Schüler, D., Pfeifer, F., Docampo, R., Dahl, C., Preiss, J., Steinbüchel, A., and Federici, B. A., 2009. Intracellular Structures of Prokaryotes: Inclusions, Compartments and Assemblages. In M. Schaechter (Ed.), Encyclopedia of Microbiology (Third Edition) (pp. 404–424). Academic Press.

Shrimpton, R., 2001. Zinc Deficiency. In R. D. Semba and M. W. Bloem (Eds.), Nutrition and Health in Developing Countries (pp. 307–326). Humana Press.

Sies, H., 2019. Chapter 13 - Oxidative Stress: Eustress and Distress in Redox Homeostasis. In G. Fink (Ed.), Stress: Physiology, Biochemistry, and Pathology (pp. 153–163). Academic Press.

Sili, C., Torzillo, G., and Vonshak, A., 2012. *Arthrospira* (*Spirulina*). In B. A. Whitton (Ed.), Ecology of Cyanobacteria II (pp. 677–705).

Silva, N. C., Graton, I. S., Duarte, C. R., and Barrozo, M. A. S., 2023. Effects of Infrared and Microwave Radiation on the Bioactive Compounds of Microalga *Spirulina platensis* after Continuous and Intermittent Drying. Molecules, 28(16), Article 16.

Šimat, V., Elabed, N., Kulawik, P., Ceylan, Z., Jamroz, E., Yazgan, H., Čagalj, M., Regenstein, J. M., and Özogul, F., 2020. Recent Advances in Marine-Based Nutraceuticals and Their Health Benefits. Marine Drugs, 18(12), 627.

Singh, R., Parihar, P., Singh, M., Bajguz, A., Kumar, J., Singh, S., Singh, V. P., and Prasad, S. M., 2017. Uncovering Potential Applications of Cyanobacteria and Algal Metabolites in Biology, Agriculture and Medicine: Current Status and Future Prospects. Frontiers in Microbiology, 8, 515.

Singh, S. K., Rahman, A., Dixit, K., Nath, A., and Sundaram, S., 2016. Evaluation of promising algal strains for sustainable exploitation coupled with CO2 fixation. Environmental Technology, 37(5), 613–622.

Singh, U., Singh, P., Singh, A. K., Laxmi, Kumar, D., Tilak, R., Shrivastava, S. K., and Asthana, R. K., 2021. Identification of antifungal and antibacterial biomolecules from a cyanobacterium, *Arthrospira platensis*. Algal Research, 54, 102215.

Singleton, V. L., and Rossi, J. A., 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, 16(3), 144–158.

Sinha, R. P., Lebert, M., Kumar, A., Kumar, H. D., and Häder, D.-P., 1995. Spectroscopic and Biochemical Analyses of UV Effects on Phycobiliproteins of Anabaena sp. And Nostoc carmium. Botanica Acta, 108(2), 87–92.

Sivakumar, J., and Santhanam, P., 2011. Antipathogenic activity of *Spirulina* powder. Recent Research in Science and Technology, 3, 158–161.

Śmieszek, A., Giezek, E., Chrapiec, M., Murat, M., Mucha, A., Michalak, I., and Marycz, K., 2017. The Influence of *Spirulina platensis* Filtrates on Caco-2 Proliferative Activity and Expression of Apoptosis-Related microRNAs and mRNA. Marine Drugs, 15(3), 65.

Soltani, M., Khosravi, A.-R., Asadi, F., and Shokri, H., 2012. Evaluation of protective efficacy of *Spirulina platensis* in Balb/C mice with candidiasis. Journal de Mycologie Médicale, 22(4), 329–334.

Soni, R. A., Sudhakar, K., and Rana, R. S., 2017. *Spirulina* – From growth to nutritional product: A review. Trends in Food Science and Technology, 69, 157–171.

Souza, M. M. de, Prietto, L., Ribeiro, A. C., Souza, T. D. de, and Badiale-Furlong, E., 2011. Assessment of the antifungal activity of *Spirulina platensis* phenolic extract against Aspergillus flavus. Ciência e Agrotecnologia, 35, 1050–1058.

Stadnichuk, I. N., Krasilnikov, P. M., and Zlenko, D. V., 2015. Cyanobacterial phycobilisomes and phycobiliproteins. Microbiology, 84(2), 101–111.

Stalikas, C. D., 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of Separation Science, 30(18), 3268–3295.

Strickland, J. D. H., and Parsons, T. R.,1965. A manual of sea water analysis,(Bulletin 125). Fisheries Research Board of Canada.

Sun, Y., Chang, R., Li, Q., and Li, B., 2016. Isolation and characterization of an antibacterial peptide from protein hydrolysates of *Spirulina platensis*. European Food Research and Technology, 242(5), 685–692.

Suvanto, J., Nohynek, L., Seppänen-Laakso, T., Rischer, H., Salminen, J.-P., and Puupponen-Pimiä, R., 2017. Variability in the production of tannins and other polyphenols in cell cultures of 12 Nordic plant species. Planta, 246(2), 227–241.

Taghvaei, M., and Jafari, S. M., 2015. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. Journal of Food Science and Technology, 52(3), 1272–1282.

Telfer, A., Bishop, S. M., Phillips, D., and Barber, J., 1994. Isolated photosynthetic reaction center of photosystem II as a sensitizer for the formation of singlet oxygen. Detection and quantum yield determination using a chemical trapping technique. Journal of Biological Chemistry, 269(18), 13244–13253.

Trabelsi, L., Ben Ouada, H., and Bassa, H., 2010. Activités biologiques des métabolites excrétés par la cyanobactérie filamenteuse *Arthrospira platensis*. Phytothérapie, 8(5), 282–289.

Tran, K. T., Van Luong, T., An, J.-W., Kang, D.-J., Kim, M.-J., and Tran, T., 2013. Recovery of magnesium from Uyuni salar brine as high purity magnesium oxalate. Hydrometallurgy, 138, 93–99.

Tvrdá, E., and Benko, F., 2020. Free radicals: What they are and what they do. In Pathology (pp. 3–13).

Udayan, A., Arumugam, M., and Pandey, A., 2017. Nutraceuticals From Algae and Cyanobacteria. In Algal Green Chemistry (pp. 65–89).

Upasani, C. D., and Balaraman, R., 2003. Protective effect of *Spirulina* on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. Phytotherapy Research, 17(4), 330–334.

Urek, R. O., and Kerimoglu, Y., 2019. Evaluation of effects of Mg 2+ and Cu 2+ on pigment-metabolite production and photosystem II activity of *Arthrospira platensis* Gomont 1892. Turkish Journal of Fisheries and Aquatic Sciences, 19(10), 873-883.

Uslu, L. H., Oya, I., Sayin, S., Durmaz, Y., Göksan, T., and GÖKPINAR, Ş., 2009. The Effect of Temperature on Protein and Amino Acid Composition of Spirulina platensis. *Ege Journal of Fisheries and Aquatic Sciences*, *26*(2), 139-142.

Valberg, L. S., Flanagan, P. R., Kertesz, A., and Bondy, D. C., 1986. Zinc absorption in inflammatory bowel disease. Digestive Diseases and Sciences, 31(7), 724–731.

Valenzuela, A., and Nieto, S., 1996. Synthetic and natural antioxidants: Food quality protectors. Grasas y Aceites, 47(3), Article 3.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., and Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions, 160(1), 1–40.

Villarruel- Lopez, A., Ascencio, F., and Nuno, K., 2017. Microalgae, a potential natural functional food source – a review. Polish Journal of Food and Nutrition Sciences, 67(4).

Volgusheva, A., Kukarskikh, G., Krendeleva, T., Rubin, A., and Mamedov, F., 2014. Hydrogen photoproduction in green algae *Chlamydomonas reinhardtii* under magnesium deprivation. RSC Advances, 5(8), 5633–5637.

Vonshak, A., 1997. *Spirulina platensis* (*Arthrospira*): Physiology, cell-biology, and biotechnology. Taylor and Francis.

Wu, H.-L., Wang, G.-H., Xiang, W.-Z., Li, T., and He, H., 2016. Stability and Antioxidant Activity of Food-Grade Phycocyanin Isolated from *Spirulina platensis*. International Journal of Food Properties, 19(10), 2349–2362.

Wu, L., Ho, J. A., Shieh, M.-C., and Lu, I.-W., 2005. Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts. Journal of Agricultural and Food Chemistry, 53(10), 4207–4212.

Wulandari, D., Sidhartha, E., Setyaningsih, I., Marbun, J., Syafruddin, D., and Asih, P., 2017. Evaluation of antiplasmodial properties of a cyanobacterium, *Spirulina platensis* and its mechanism of action. Natural Product Research, 32, 1–4.

Yadav, L., Maurya, N. K., Yadav, L., and Maurya, N. K., 2022. Fight Hidden Hunger through National Programs and Food Based Approaches. In Combating Malnutrition through Sustainable Approaches. IntechOpen.

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., and Chen, Z., 2020. Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. Frontiers in Plant Science, 11.

Yen, H.-W., Hu, I.-C., Chen, C.-Y., Ho, S.-H., Lee, D.-J., and Chang, J.-S., 2013. Microalgae-based biorefinery – From biofuels to natural products. Bioresource Technology, 135, 166–174.

Zaid, A. A. A., Hammad, D. M., and Sharaf, E. M., 2015. Antioxidant and Anticancer Activity of *Spirulina platensis* Water Extracts. International Journal of Pharmacology, 11(7), 846–851.

Zeng, F., Ou, J., Huang, Y., Li, Q., Xu, G., Liu, Z., and Yang, S., 2015. Determination of 21 Free Amino Acids in Fruit Juices by HPLC Using a Modification of the 6-Aminoquinolyl-N-hydroxysuccinimidyl Carbamate (AQC) Method. Food Analytical Methods, 8(2), 428–437.

Zern, T. L., and Fernandez, M. L., 2005. Cardioprotective Effects of Dietary Polyphenols1. The Journal of Nutrition, 135(10), 2291–2294.

Zhang, C., Wang, C., Cao, G., Wang, D., and Ho, S.-H., 2020. A sustainable solution to plastics pollution: An eco-friendly bioplastic film production from high-salt contained *Spirulina* sp. residues. Journal of Hazardous Materials, 388, 121773.

Zhang, Y., Cai, P., Cheng, G., and Zhang, Y., 2022. A Brief Review of Phenolic Compounds Identified from Plants: Their Extraction, Analysis, and Biological Activity. Natural Product Communications, 17(1), 1934578X2110697.

Zhao, Y., Tang, X., Qu, F., Lv, M., Liu, Q., Li, J., Li, L., Zhang, B., and Zhao, Y., 2020. ROS-mediated programmed cell death (PCD) of *Thalassiosira pseudonana* under the stress of BDE-47. Environmental Pollution, 262, 114342.

Zhou, T., Li, X., Zhang, Q., Dong, S., Liu, H., Liu, Y., Chaves, A. V., Ralph, P. J., Ruan, R., and Wang, Q., 2022. Ecotoxicological response of *Spirulina platensis* to coexisted copper and zinc in anaerobic digestion effluent. Science of The Total Environment, 837, 155874.

Zhou, T., Wang, J., Zheng, H., Wu, X., Wang, Y., Liu, M., Xiang, S., Cao, L., Ruan, R., and Liu, Y., 2018. Characterization of additional zinc ions on the growth, biochemical composition and photosynthetic performance from *Spirulina platensis*. Bioresource Technology, 269, 285–291.

Zhou, W., Li, Y., Gao, Y., and Zhao, H., 2017. Nutrients removal and recovery from saline wastewater by *Spirulina platensis*. Bioresource Technology, 245, 10–17.

Zinicovscaia, I., Cepoi, L., Rudi, L., Chiriac, T., Grozdov, D., and Vergel, K., 2021. Effect of zinc-containing systems on *Spirulina platensis* bioaccumulation capacity and biochemical composition. Environmental Science and Pollution Research, 28(37), 52216–52224.

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