JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2026, 27(11), 404–419
https://doi.org/10.12911/22998993/231138
ISSN 2299–8993, License CC-BY 4.0

Received: 2026.08.18
Accepted: 2026.09.15
Published: 2026.10.01

Physicochemical, structural and thermal characterization of *Chlorella vulgaris*; *Arthrospira platensis* and *Nannochloropsis oculata* biomass for sustainable soil amendment applications

H'anane Sahil[1*], Zaina Idardare[1,2], Hakima Assendal[3], Fatima El Aamri[4], Mohamed Bakki[5], Chafia Hajji[1], Lamia Yakkou[6], Laila Bouqbis[1]

[1] Ecosystems and Environmental Sciences, Faculty of Applied Sciences, Ibn Zohr University, National Road No. 10, P.O. Box 6146, Agadir, Morocco
[2] Higher Institute of Maritime Fisheries, National Road No. 10, P.O. Box 479, Agadir, Morocco
[3] Ecosystems and Environmental Sciences, Faculty of Sciences, Ibn Zohr University, P.O. Box 8106, Agadir, Morocco
[4] Regional Center of the National Institute of Fisheries Research (INRH), P.O. Box 5268, Tangier, Morocco
[5] Plant Biotechnology Laboratory, Faculty of Sciences, Ibn Zohr University, P.O. Box 8106, Agadir, Morocco
[6] Laboratory of Microbial Biotechnologies and Plant Protection (LBVRN), Faculty of Sciences, Agadir, University Ibn Zohr, P.O. Box 8106, Agadir, Morocco
* Corresponding author's e-mail: hanane.sahil.84@edu.uiz.ac.ma

ABSTRACT

The increasing use of chemical fertilizers has raised issues of land deterioration and agricultural system sustainability. The use of microalgae biomass could be beneficial in soil due to its organic matter, minerals, and bioactive compounds. This paper deals with comparing three different microalgae species, namely *Chlorella vulgaris*, *Arthrospira platensis*, and *Nannochloropsis oculata*, concerning their potential for agricultural applications based on their physicochemical, morphological, thermal, and structural characteristics. Microalgae biomass mostly consists of organic matter. The content rate varied from 80.4 ± 2.6% to 96.8 ± 2.65%, while the organic carbon content ranged from 46.63 ± 1.50 to 56.14 ± 1.53%. Microalgae composition varied, with N. oculata having the largest concentration of K, Na, Ca, P, Fe, Mn, and Zn, while A. platensis had the highest amounts of Mg and nitrate. Cation exchange capacity varied in the samples from 7.88 ± 0.24 to 12.64 ± 0.55 meq/100 g, and the water holding capacity was highest for A. platensis (2.5 ± 0.13 g H_2O/g dry weight). The pH of both A. platensis and *N. oculata* was neutral, while C. vulgaris had slightly alkaline properties. Electrical conductivity differed markedly, with *N. oculata* showing the higher value (19,270 ±4.58 µS/cm), indicating a higher soluble salt content, consistent with the detection of NaCl and KCl as major crystalline phases by XRD. SEM–EDX analysis revealed diverse morphological features of the surface and detected several major elements. No detectable heavy metal signals were observed in the EDX spectra analyzed, although this does not confirm their absence. TGA/DTG and DSC allowed the identification of patterns sample decomposition and its phase changes, as well as FTIR, which confirmed the presence of functional groups corresponding to proteins, carbohydrates, lipids, and hydroxyl groups in the analyzed samples. In general, the results demonstrate distinct physicochemical and structural properties of the three biomasses and confirm their potential as soil amendments. However, their actual agronomic effectiveness and safety require further validation through soil and plant-based experiments and quantitative contaminant analyses.

Keywords: microalgae, soil amendment, soil fertility, *Chlorella vulgaris*, *Arthrospira platensis*, *Nannochloropsis oculata*.

INTRODUCTION

Agriculture has been a cornerstone of economic development over recent decades and has made a significant contribution towards poverty reduction and food security (Boureima et al., 2021). The rapid growth of the global population has accelerated demand for food, leading to widespread adoption of chemical fertilizers to enhance crop yields and maintain farm profitability (Alvarez et al.,2021; Gonçalves et al., 2023). These fertilizers typically contain inorganic nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Meysami et al., 2025). However, prolonged and increased use of fertilizers, together with the continued physical and biological degradation of soil, ensured that nutrient depletion and accelerated soil erosion have emerged to jeopardize long-term sustainability in agricultural systems (Bouramdane et al., 2023; Renganathan et al., 2024).

Attention is increasingly being directed toward more sustainable and renewable biological sources to meet these challenges. Of these, microalgae have gained considerable attention lately owing to their high photosynthetic efficiency, fast growth, and rich composition of bioactive compounds such as proteins, lipids, carbohydrates, pigments, vitamins, and minerals (Bule et al., 2018). These microorganisms do not provide a singular benefit but rather multiple benefits relating to enhanced soil fertility and plant growth, and contribute to crop protection (El-Shazoly et al., 2025), improving tolerance against environmental stress, and even serving as a substitute for conventional chemical inputs in a more environmentally friendly manner. They are being applied to soil in both their whole form and as fresh or dried extracts (Chanthini et al., 2024).

Species like *Arthrospira platensis* (S)*, Chlorella vulgaris* (C)*,* and *Nannochloropsis oculata* (N) present particular importance due to their high nutritional value (Khanra et al.,2018; Ladjal-Ettoumi et al., 2024; Paterson et al., 2023). *Arthrospira* sp. is well known for its ability to stimulate plant growth and enhance soil mineral content (Anitha et al., 2019). Several studies have demonstrated that *Arthrospira* sp., extracts function as effective biostimulants, promoting seed germination, seedling growth, and development across various crops (Akgül et al., 2019; Selem et al.,2019; Shedeed et al., 2022). At moderate concentrations, they stimulate root-to-shoot length and the number of lateral roots (Akgül et al., 2019), biomass accumulation, photosynthesis, pigment content, and nutrient uptake, particularly N, P, and K (Selem et al.,2019), as well as carbohydrate accumulation. Additionally, they modulate the activity of antioxidant enzymes and influence the protein profile of seeds (Shedeed et al., 2022). *Chlorella* sp. is versatile in agriculture by acting as a biofertilizer and microalgal biostimulant (Hernandez et al., 2009; Kholssi et al., 2019). Various studies prove that *Chlorella* sp. possesses the ability to promote plants' growth, while Wang et al., (2024) defined "dual action", implying the use of both direct and indirect effects. The organic substances produced by microalgae stimulate root formation and biomass generation directly, while also improving soil microbial communities. Furthermore, *Chlorella* sp. is involved in nitrogen and phosphorus recycling processes in nutrient-deficient conditions, which shows its suitability for use in wastewater treatment systems. It was reported that the *Chlorella* extract, which consists of amino acids, peptides, growth-stimulating substances, as well as vitamins and minerals, has a positive effect on plants' height, stem diameter, root system performance, leaf area, and antioxidant enzyme activity in crops such as pepper (Tian et al., 2022). Other research has confirmed that *Chlorella* sp. can enhance nutrient retention in soils and prevent nutrient leaching (Garbowski et al., 2024). Likewise, *Nannochloropsis* sp. has been investigated as a natural biostimulant and microalgal biofertilizer, showing its positive effects on plant growth, development, and productivity under different cultivation conditions (Al Dayel et al., 2021; Gharib et al., 2024; Trinh et al., 2017). Its use has been correlated with variations in cell density, chlorophyll a level, and lipid accumulation in accordance with the stage of cultivation and phytohormone concentration, such as IAA, GA_3, and zeatin (Trinh et al., 2017). In addition, it has been noted that *Nannochloropsis* sp. can stimulate agricultural and medicinal crops like *Moringa oleifera* and modify their phytochemical composition, increasing the level of beneficial compounds, including rutin and gallic acid, even under the conditions of moderate salinity (Al Dayel et al., 2021). These findings confirm the possible significance of *Nannochloropsis* sp. as a natural and sustainable input for agricultural applications, although its effectiveness relies on the crop used and the conditions of application and cultivation (Gharib et al., 2024).

However, the successful application of microalgae in agriculture should be based on a detailed understanding of their physical and chemical properties, mineral content, morphology, thermal properties, and structure. The present study involved a comparative evaluation of physicochemical characteristics of the biomasses of *Chlorella vulgaris*, *Arthrospira platensis*, and *Nannochloropsis oculata* using various techniques, including water holding capacity measurement and physicochemical analysis, SEM-EDX, TGA/DSC, XRD, and FTIR. The aim was not only to characterize the biomass composition, but also to identify species-specific properties and potential constraints that may influence their behavior following incorporation into agricultural soils. This integrated comparative approach provides complementary information relevant to the preliminary assessment and selection of microalgal biomasses as potential soil amendments, while providing a basis for subsequent soil and plant experiments.

MATERIALS AND METHODS

Microalgae source

The microalgal biomasses used in this study were commercially obtained as dried powders. *Chlorella vulgaris Beijerinck* (C) and *Arthrospira platensis* (commercially marketed as Spirulina) (S) were purchased from Purasana. According to the supplier's product information, both biomasses consist of 100% organic algal powder without additional additives and are certified organic, vegan, and kosher. According to the supplier, *Chlorella vulgaris* and *Arthrospira platensis* are cultivated in open ponds using purified water and natural sunlight. Following harvesting, the biomass undergoes a brief heating step, followed by controlled drying. The supplier reports that these processing conditions are designed to preserve the nutritional characteristics of the products. According to the supplier's quality-control information, individual batches are tested for heavy metals, pesticides, and harmful microorganisms, and the products are supplied in accordance with IFS standards.

Nannochloropsis oculata (N) was obtained from Feed Algae Maroc SA, located in Akhfenir, Morocco. According to the information provided by the supplier, *N. oculata* was cultivated at 22 ± 1 °C, under a light intensity of 4.000 lux, with a 12 h light/12 h dark photoperiod. The cultivation medium was maintained at a salinity of 35 PSU, with continuous aeration throughout the cultivation period. The biomass was harvested and supplied in dried form for the present study.

The commercial products were supplied as dried powders and were directly subjected to the physicochemical, morphological, thermal, and structural characterization analyses described in the following sections. The information regarding cultivation, processing, and quality control presented above corresponds to information provided by the respective suppliers and was not independently verified in the present study.

Determination of the physicochemical properties of microalgae

The main physicochemical properties investigated in microalgal biomasses were organic matter (OM), organic carbon (OC), cation exchange capacity (CEC), mineral nutrients, pH, electrical conductivity (EC), and water holding capacity (WHC).

Organic matter was determined using the Walkley and Black (1934) method, while organic carbon was calculated from the measured organic matter content. The concentrations of nitrate nitrogen ($N–NO_3^-$) and available phosphorus were determined using colorimetric methods. The major cations, including Ca, K, Mg, and Na, were quantified by flame emission spectrophotometry, whereas the trace elements Fe, Cu, Mn, and Zn were determined by atomic absorption spectrophotometry. Cation exchange capacity was determined according to the method of Metson (1957).

The pH and electrical conductivity (EC) of the microalgal biomasses were determined following the procedures described by Uroić Štefanko et al. (2020), with modifications as described below. For EC determination, 0.5 g of dried microalgal biomass was placed in a 50 mL test tube and mixed thoroughly with 10 mL of deionized water, corresponding to a biomass-to-water ratio of 1:20 (w/v). The suspension was agitated for 1 h and then allowed to stand for 30 min before measurement. Electrical conductivity was measured at 18.2 °C.

WHC was determined in triplicate using small tubes measuring 5.5 cm in height and 4 cm in diameter. The microalgal biomasses were placed in pots lined at the bottom with fabric and filter paper. The pots were then placed in a plastic container filled with water and covered with aluminum foil. After 24 h, the tubes were removed and allowed to drain for an additional 24 h before weighing. The water-holding capacity was calculated relative to the dry weight of the biomass according to the method described by El Moussaoui et al. (2024).

All quantitative physicochemical measurements were performed in three independent analytical replicates, and the results are expressed as mean ± standard deviation (SD).

Morphological characteristics (SEM)

The morphology and surface structure of the microalgae, as well as the distribution of chemical elements present on their surface, were examined using scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDX) (Da Silva Veiga et al., 2021; Varkolu et al., 2025). The instrument used was a JEOL JSM IT-100 microscope equipped with an energy-dispersive X-ray spectroscopy detector (EDXS). This system enables determination of the local quantitative elemental composition of a sample through the analysis of characteristic elemental X-ray emissions. The instrument provides a maximum resolution of 100 nm.

For each microalgal biomass, several areas were examined by SEM–EDX, and representative micrographs and EDX spectra were selected for presentation. Images obtained at comparable magnification were selected for Figure 1 to facilitate morphological comparison among *Chlorella vulgaris*, *Arthrospira platensis*, and *Nannochloropsis oculata*. These analyses were carried out at the Faculty of Sciences, Agadir, Ibn Zohr University, Morocco.

Thermal properties (TGA, DTG, DSC)

The thermal behavior of the microalgal biomasses was analyzed using a Setaram Themys ONE thermal analyzer at the Analysis and Characterization Platform of the National School of Rabat, within the Laboratory of Physicochemistry of Inorganic and Organic Materials, Rabat, Morocco. Each sample was heated under an air atmosphere up to 800 °C at 20 °C/min, with a gas flow rate of 45 mL/min.

Structural and chemical characteristics (XRD, FTIR)

The crystallinity and structural properties of the microalgae were analyzed using an X-ray diffractometer (XRD, Bruker D8 Advance Twin) equipped with a copper anticathode and operating with Cu-Kα radiation (λ = 1.5418 Å). Data were collected over a 2θ range of 5–100° with a step size of 0.05°. Before analysis, the samples were finely ground using an agate mortar. The diffraction patterns obtained for each sample were interpreted using HighScore software (Singh et al., 2021).

In addition, Fourier-transform infrared spectroscopy (FTIR) was performed to identify the functional groups present in the samples. This technique is based on the absorption of infrared radiation by chemical bonds, the characteristic vibrational modes of which allow the identification of the material's functional groups (Arif et al., 2021; Kritikaki et al., 2025). FTIR-ATR analyses were carried out using an IRAffinity-1S spectrometer (Shimadzu) equipped with a Jasco ATR PRO ONE module. The samples were prepared as pellets containing 1% finely ground powder mixed with 99% KBr. Spectra were recorded in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. These analyses were carried out at the Faculty of Sciences, Agadir, Ibn Zohr University, Morocco.

RESULTS AND DISCUSSION

Physicochemical characterization

The physicochemical characterization revealed marked differences among the three microalgal biomasses investigated (Table 1). All samples exhibited high organic matter (OM) contents, ranging from 80.40 ± 2.6% to 96.80 ± 2.65%, indicating that the biomasses were predominantly composed of organic material. *C. vulgaris* (C) showed the highest OM content (96.80± 2.65%), followed by *A. platensis* (S) (93.71± 1.3%), whereas *N. oculata* (N) exhibited a comparatively lower value (80.40± 2.6%), which may be associated with a higher mineral fraction. The organic carbon (OC) contents differed among the three microalgal biomasses, with values of 54.35 ± 0.75% for *Chlorella* sp., 56.14 ± 1.53% for *Arthrospira platensis*, and 46.63 ±

1.50% for *N. oculata* (N). The maximum value of OC was obtained in the case of Arthrospira platensis, while the minimum value was determined in the case of N. oculata (N). The observed differences indicate that the samples have different carbon compositions, which may be indicative of various biochemical compositions and conditions of cultivation. The microalgal biomass is generally composed of proteins, carbohydrates, and lipids. However, the proportions of the components listed above may vary for different species and cultivation conditions (Wang and Yin, 2018; Sharma et al., 2025). Moreover, microalgae can accumulate extracellular polymeric substances (EPS), which are mainly composed of polysaccharides, proteins, lipids, and nucleic acids. This means that EPS can be considered as an important carbon and energy source (Xiao and Zheng, 2016). These characteristics contribute to the organic nature of microalgal biomass and may be relevant when considering its potential interactions with soil organic matter.

Differences were also observed in the CEC. *C. vulgaris* (C) exhibited the highest CEC value (12.66 ± 0.55 meq/100 g), followed by *A. platensis* (S) (10.50 ± 0.44 meq/100 g) and *N. oculata* (N) (7.88 ± 0.24 meq/100 g). Such differences may be related to variations in the composition of the cell surface and cell wall, particularly the presence of negatively charged functional groups that can contribute to cation binding or exchange (Monteiro et al., 2012).

The three biomasses contained measurable amounts of mineral elements, although their concentrations differed considerably. *N. oculata* (N) exhibited the highest concentrations of potassium (25.85 ± 0.22 meq/100 g), sodium (34.31 ± 1.20 meq/100 g), calcium (16.15 ± 0.33 meq/100 g), phosphorus (852.47 ± 2.56 ppm), iron (27.66 ± 0.44 ppm), manganese (12.38 ± 0.39 ppm), and zinc (3.90 ± 0.65 ppm). In contrast, *A. platensis* (S) showed the highest magnesium (11.96 ± 0.54 meq/100 g) and nitrate (466.46 ± 4.83 ppm) contents, whereas *C. vulgaris* (C) generally presented intermediate concentrations for most of the analyzed parameters.

The mineral profiles, therefore, differed substantially among the three biomasses, highlighting their distinct elemental compositions. In particular, the relatively high concentrations of P, K, Ca, Fe, Mn, and Zn accrued in *N. oculata* (N) provide a mineral nutrient supply when added to the soil as an amendment. However, its markedly higher sodium concentration should also be considered, as excessive Na inputs, it may contribute to an increase in soil salinity at a high rate of application.

Studies indicate that microalgal biomass contains various compounds of potential interest for agricultural applications, such as compounds with bioactive properties, antioxidants, biostimulants, and micronutrients (Parmar et al., 2023; Kapoore et al., 2021). These properties should provide microalgal biomass with promising potential as an additive to the soil. Nevertheless, the actual agronomic effects of these biomasses require further validation under soil and plant conditions.

The pH values of the three analyzed biomasses showed some variation (Table 1). *N. oculata* (N) (pH 7.055 ± 0.04) and *A. platensis* (S) (pH 7.094 ± 0.06) exhibited values close to neutrality, whereas *C. vulgaris* (C) presented a slightly alkaline pH (8.360 ± 0.16). The pH observed for *A. platensis* (S) is consistent with the value reported by Eissa et al., (2025), who measured a pH of approximately 7.2 for A. platensis (S) grown in freshwater. Previous studies have shown that the pH values associated with microalgal cultivation can vary according to species and cultivation conditions, with values generally ranging from 8 to 10 for *Chlorella vulgaris*, around 8 for *Nannochloropsis bacillaris*, and between 9 and 11 for *Arthrospira platensis* (Dineshkumar et al., 2017). In the present study, the pH of *C. vulgaris* (C) was 8.360 ± 0.16, slightly higher than the value of 8.05 ± 0.04 reported by Agüero-Quiñones et al., (2025) during the 8th to 16th days of cultivation. Such differences may be related to the differences in cultivation and biomass processing conditions. Since the present measurements were performed on dried biomass, variations in moisture content and sample preparation may also influence the measured pH.

Electrical conductivity (EC), which reflects the concentration of soluble ionic species, showed substantial differences among the three biomasses. The highest EC was recorded for *N. oculata* (N) (19270 ± 4.58 μS/cm), followed by *A. platensis* (S) (2482 ± 4.35 μS/cm) and *C. vulgaris* (C) (2115 ± 4.50 μS/cm). The markedly higher EC observed for *N. oculata* (N) is consistent with its marine origin and the presence of a higher concentration of soluble salts in the biomass. Similar observations were reported by Gharib et al., (2023), who associated the elevated EC of *N. oculata* (N) biomass with its saline cultivation environment. This result is also consistent with the X-ray diffraction (XRD) analysis, which identified NaCl and KCl as the major crystalline phases in *N. oculata* (N) biomass. The presence of these salts provides a plausible explanation for its markedly higher EC. Therefore, the high salt content of *N. oculata* (N) biomass should be considered when evaluating its potential agricultural use, particularly in relation to application rate and soil salinity.

Water holding capacity (WHC)

Water holding capacity is an important physicochemical property commonly considered when evaluating the potential suitability of biomass materials for soil amendment applications. In the present study, this parameter was measured to characterize the moisture-retention capacity of the analyzed microalgal biomasses.

Results from the current study are presented in Table 2. Among the evaluated microalgae, the obtained data suggest that (S) has the highest water-holding capacity, at 2.5 ± 0.13 g H_2O/g dry weight, whereas that of (C) was 1.87 ± 0.08 g H_2O/g DW, and 1.70 ± 0.18 g H_2O/g DW for (N). Such a difference could be due to the more fibrous cellular structure of (S), which makes it more capable of absorbing and holding water (Liu et al., 2023).

However, when compared with the values from the previous research, water holding capacity is lower than that described by Ma et al., 2018, who established a value of 340% for (S) and 240% for (C). Bleakley et al., (2021) reported a WHC for (S) at a value of about 2.25 g H_2O/g, while Hayes et al., 2025 measured a value of 1.83 g H_2O/g for (C).

Morphological and elemental characterization (SEM-EDX)

SEM observations showed clear differences in morphology for the three microalgae studied (Figure 1).

Cells of (C) were mostly oval to spherical, with an irregular cell surface and a high tendency to form aggregates. This can be ascribed to the presence of extracellular polysaccharides that induce cell aggregation and the formation of clusters. These aggregates have been reported to contribute to the interactions with soil microorganisms and may influence soil organic carbon dynamics as well as physicochemical properties under soil conditions (Redmile Gordon et al., 2020). However, these effects require further validation for the microalgal biomasses investigated in the present study.

The microstructure of (N) showed a typical spherical cell morphology, tightly packed in compact clusters with a rough and uneven surface. This is characteristic of the reported multi-layered, thick cell wall for these microalgae (Khamila et al.,2025). Filaments for (S) were folded and in spiral morphology, as is typical for this cyanobacterium. Liu et al., (2023) reported that the ribbon-like

Table 1. The physicochemical properties of the microalgae (C), (S), and (N)

Parameter	(C)	(S)	(N)
OM (%)	93.71±1.30	96.80±2.65	80.40±2.60
OC (%)	54.35±0.75	56.14±1.53	46.63±1.50
CEC (meq/100g)	12.64±0.55	10.50±0.44	7.88±0.24
K (meq/100g)	18.52±0.54	3.52±0.57	25.85±0.22
Na (meq/100g)	20.68±0.48	13.40±0.70	34.31±1.20
Ca (meq/100g)	10.99±0.47	12.50±0.52	16.15±0.33
Mg(meq/100g)	6.64±0.43	11.96±0.54	10.33±0.47
P(ppm)	783.04±4.5	742.53±3.35	852.47±2.56
Fe (ppm)	10.73±0.32	13.88±0.65	27.66±0.44
Mn (ppm)	6.35±0.61	5.91±0.27	12.38±0.39
Zn (ppm)	1.99±0.33	0.25±0.13	3.90±0.65
Cu (ppm)	0.61±0.24	0.37±0.06	0.40±0.08
N-NO_3 (ppm)	266.80±5.35	466.46±4.83	81.11±3.90
pH	8.36±0.16	7.094±0.06	7.055±0.04
EC (µS/cm)	2115±4.50	2482±4.35	19,270±4.58

Note: meq/100 g = milliequivalents per 100 g of dry material.

Table 2. Water holding capacity (WHC) of microalgae (C), (S), and (N)

Microalgae	WHC (g H_2O /g dry weight)
(C)	1.87±0.08 (g H_2O /g DW)
(S)	2.50±0.13 (g H_2O /g DW)
(N)	1.70 ±0.18 (g H_2O /g DW)

Table 3. SEM–EDX elemental composition of the investigated microalgal biomasses: *Chlorella vulgaris*, *Arthrospira platensis*, and *Nannochloropsis oculata*

A)

Element	Mass (%)	Atomic (%)	Sigma
C	47.07	54.35	0.02
N	6.14	6.08	0.02
O	44.17	38.29	0.07
Na	0.25	0.15	0.02
Mg	0.68	0.39	0.02
P	1.16	0.52	0.02
S	0.45	0.2	0.02
Cl	0.07	0.03	0.02

B)

Element	Mass (%)	Atomic (%)	Sigma
C	47.07	54.27	0.02
N	8.28	8.19	0.01
O	41.91	36.28	0.04
Na	0.13	0.08	0.01
Mg	0.55	0.31	0.01
P	1.05	0.47	0.01
S	0.56	0.24	0.01
K	0.45	0.16	0.02
Cl	nd	nd	-

C)

Element	Mass (%)	Atomic (%)	Sigma
C	45.16	59.48	0.03
N	2.46	2.78	0.02
O	20.71	20.48	0.04
Na	13.09	9.01	0.04
S	0.59	0.29	0.02
Cl	16.47	7.35	0.05
K	1.51	0.61	0.03

morphology of *Spirulina* may enhance cohesiveness and facilitate interactions with soil matrices, like organic fibers. However, this behavior was not evaluated in the present study. In contrast, various studies have observed other SEM morphologies indicating that structural changes may take place upon drying, which could confirm that different drying methods influence the quality of the microalgal biomass (Aljabri et al., 2023).

Complementary EDX spectra (Table 3) showed that these biomasses are rich in carbon, oxygen, and nitrogen, and contain essential nutrients like P, S, Mg, Na, K, and Cl, all of which are vital for plant development. These results support the findings of Coppens et al. (2016). These results are consistent with previous studies reporting that microalgal biomass contains macro- and micronutrients, including N, P, and K (Coppens

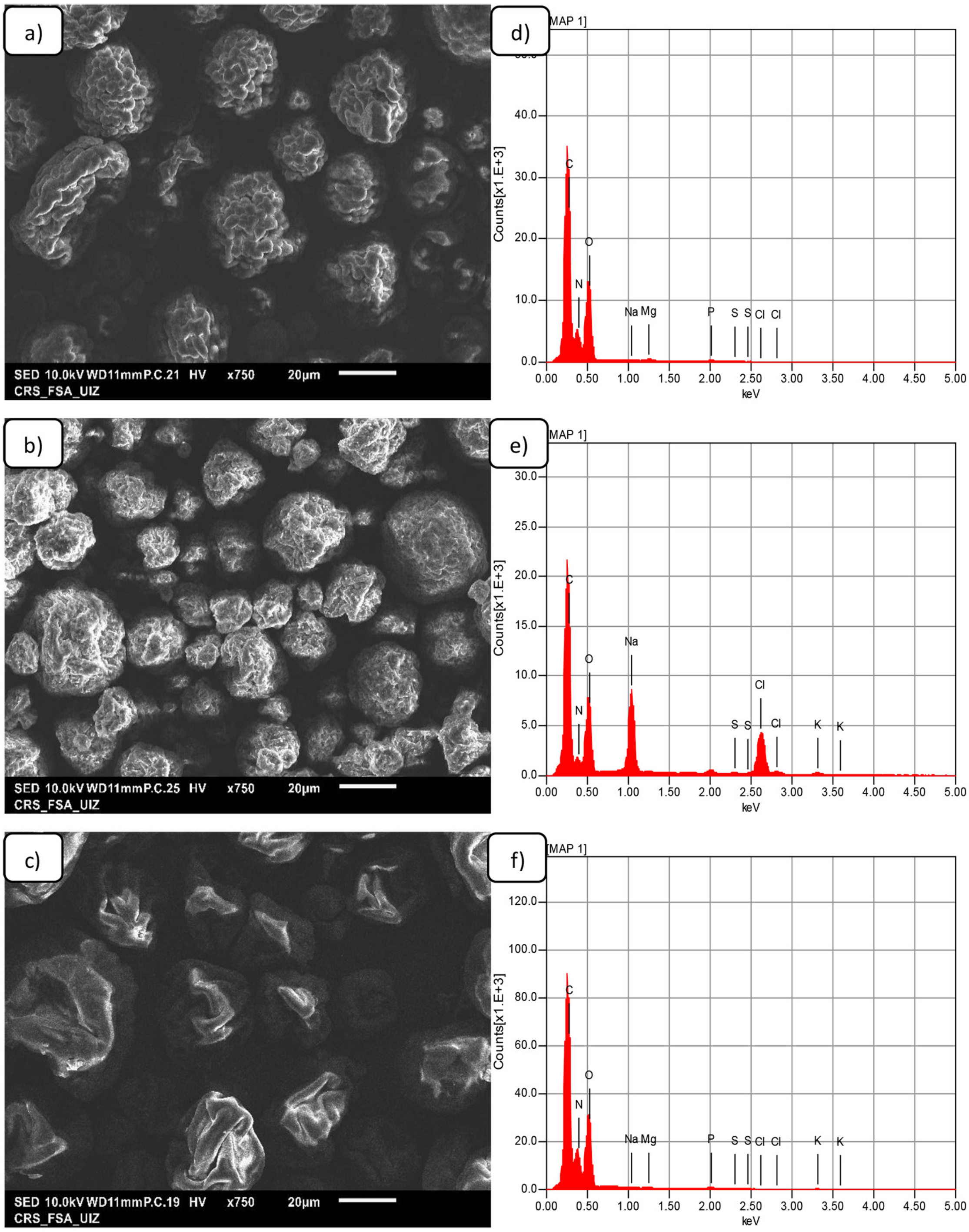

Figure 1. SEM imaging with EDX elemental analysis of microalgal cells: (a) *C. vulgaris* (C) cells, (b) *N. oculata* (N) cells, (c) *A. platensis* (S) cells, (d) EDX analysis of elemental composition of *C. vulgaris* (C) cells, (e) EDX analysis of elemental composition of *N. oculata* (N) cells, (f) EDX analysis of elemental composition of *A. platensis* (S) cells

et al., 2016). In the present study, quantitative physicochemical analyses (Table 1) confirmed the presence of organic matter, organic carbon, mineral nutrients (P, K, Ca, Mg), and trace elements (Fe, Mn, Zn, and Cu) in the investigated biomasses. These chemical characteristics highlight the potential interest of microalgal biomass as a soil amendment material; however, nutrient availability, release behavior, and agronomic effectiveness require further validation through soil incubation and plant-growth experiments. In addition, these elements have important functions related to photosynthesis, enzymatic activation, and plant cell wall structure (Díaz et al., 2024).

The conducted study showed that no detectable lead signal was observed in the SEM–EDX spectra of the analyzed microalgal biomasses. This observation is consistent with the findings of Aly et al., (2008), who reported no detectable lead concentration in *Spirulina* sp. biomass and suggested its potential use as a plant growth stimulator for agricultural purposes. However, SEM–EDX provides localized elemental information and cannot confirm the complete absence of heavy metals.

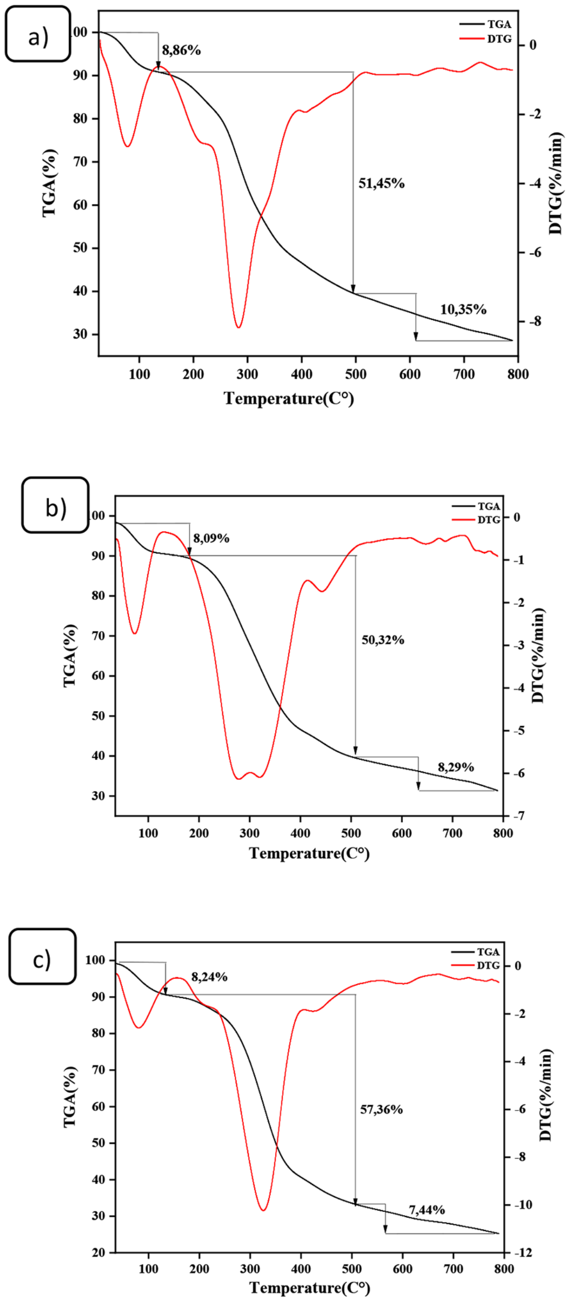

Figure 2. TGA/DTG analysis of (C) (a), (N) (b), and (A) (c) at a heating rate of 20 °C/min

Thermal gravimetric analysis (TGA/DTG)

The TGA analysis gives an indication of thermal stability and phase composition, enabling the identification of the temperature at which various components either degrade or volatilize (Gholami et al., 2025; Lothenbach et al.,2016). The data obtained from TGA are presented as curves displaying a relationship between temperature and the percentage of mass loss; these are used to obtain the onset and completion temperatures of decomposition. The DTG curve, which is the derivative of the TGA curve, is also utilized in determining the maximum decomposition temperatures (Ladjal-Ettoumi et al., 2024). Figure 2 presents the results of TGA and DTG, which describe the thermal behavior of the samples. The experiments were carried out up to 800 °C with a heating rate of 20 °C/min.

Thermal decomposition of the three biomasses is distinguished by three different stages, namely, between 30–150 °C, corresponding mainly to water dehydration (Díaz et al., 2024; Ladjal-Ettoumi et al., 2024) and evaporation of light volatile compounds (Díaz et al., 2024) with a mass loss of about 8% for all three biomasses.

The second stage, between 150–500 °C, shows high mass loss of about 57.36% for (S) this is due to devolatilization reactions, whereby the sample loses a significant part of its mass as volatile compounds that come from the thermal degradation of carbohydrates, polysaccharides, proteins, and lipids (Dessì et al., 2021; Jacinto et al., 2023). Similarly, previous studies (Chen et al., 2018; Ladjal-Ettoumi et al., 2024) indicated that the protein, carbohydrate, and lipid fractions in microalgae mainly undergo pyrolysis between 150–500 °C.

Specific polysaccharides like pectin, agar, or alginates are commonly present in the external cell wall of microalgae, whereas the internal wall is mainly made up of cellulose, which is a hydrophilic molecule that degrades only at high temperatures around 300–330 °C (Cebrián-Lloret et al., 2022). Within the third thermal stage, in the temperature range of 500–700 °C, mass losses of about 7.44%, 8.29%, and 10.35% was recorded for (S), (N), and (C), respectively. Beyond 700 °C, the mass loss became very minimal, and the remaining residue stabilized, which accounts for the formation of thermally stable char. The overall yields of the final pyrolysis residues were 27%, 29%, and 34% for (S), (C), and (N), respectively, values close to those reported for

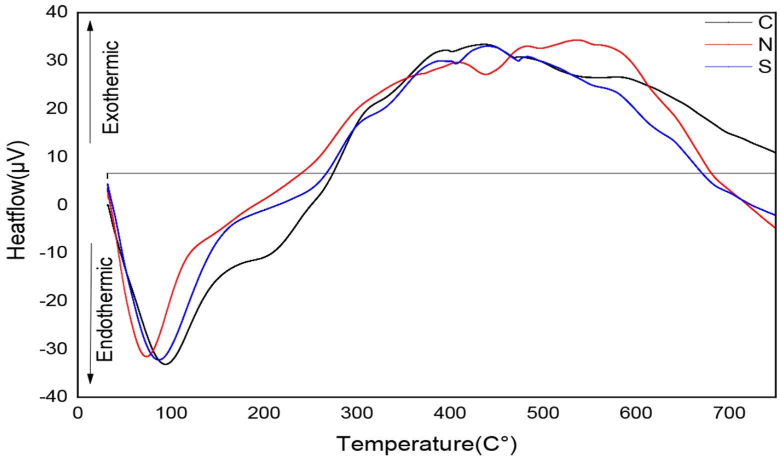

Figure 3. DSC thermograms of microalgae (C), (N), and (S). The downward peaks reflect endothermic events, and the upward peaks reflect exothermic events, obtained at a heating rate of 20°C/min

Spirulina sp., biomass, which is in the range of 28.47% (Ziyue et al., 2023). Other studies have documented the final residue yields of 30% and 23% for *A. platensis* (S) and *C. vulgaris* (C), respectively (Chen et al., 2018; Ladjal-Ettoumi et al., 2024; Marcilla et al., 2009).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) allows for the identification of phase transitions (such as melting or crystallization) that are essential in understanding material thermal and physical behavior. The obtained thermogram provides not only quantitative data on specific heat capacity but also detailed information on different phase transitions (Gholami et al., 2025).

The DSC curves (Figure 3) of the samples under study show, in the temperature range of 50 and 120 °C, an endothermic peak with maxima at 83 °C (C), 77 °C (N), and 95 °C (S). This phenomenon is associated with dehydration or the loss of free and bound water (Lima et al.,2024) and may also correspond to the onset of protein denaturation (Díaz et al., 2024). In detail, the DSC analysis of (S) biomass exhibits a characteristic endothermic transition with a maximum around 95 °C, which may be associated with protein denaturation and/or residual water loss. This value is slightly lower than that reported by Zeller et al. (2013), who observed a maximum near 100 °C, and lower than that reported by other studies where denaturation peaks appeared at 146 °C for unwashed *Spirulina sp*., and at 158 °C for the washed one, at the same heating rate of 10 °C/min (Díaz et al., 2024). These differences can be due to the influence of the operating conditions, especially the heating rate (Díaz et al., 2024; Nyoni et al., 2020). Variations in peak intensities among the three curves may reflect differences in water content or soluble protein composition (Molino et al., 2018).

All the curves then present a wide exothermic region between 200 and 550 °C, with maximum activities between 400–450 °C for samples (C) and (S) and between 500 and 550 °C for (N). This region corresponds to the exothermic decomposition of organic compounds such as proteins, polysaccharides, and lipids (Zeller et al., 2013). Noticeably, *N.oculata* (N)exhibited a more intense exothermic peak, which may be related to differences in biomass composition, including lipid content, as reported for this genus (Navalho et al., 2025).

X-ray diffraction

The X-ray diffraction patterns obtained for microalgal biomasses are presented in Figure 4. Samples (C) and (S) showed an amorphous microstructure with a strong diffraction peak at around 2θ≈19.8° and a weaker secondary signal at about 8.9°. These signals are generally associated with the amorphous contribution of organic components, including polysaccharides and nucleic acids, within the microalgal matrix (Patil et al.,2025). These observations agree with the findings of Ladjal Ettoumi et al., 2024, as well as other studies, which reported that the carbon structure in *C. vulgaris* (C) and *A. platensis* (S)

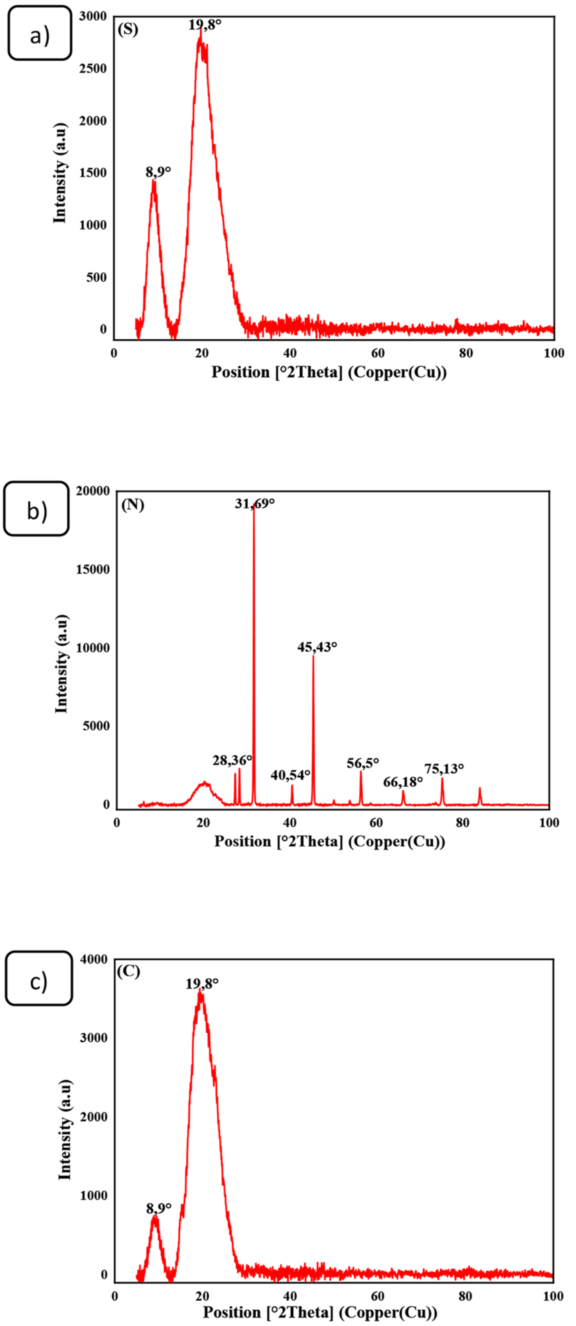

Figure 4. X-ray diffraction pattern of (a) (C), (b) (N), and (c) (S)

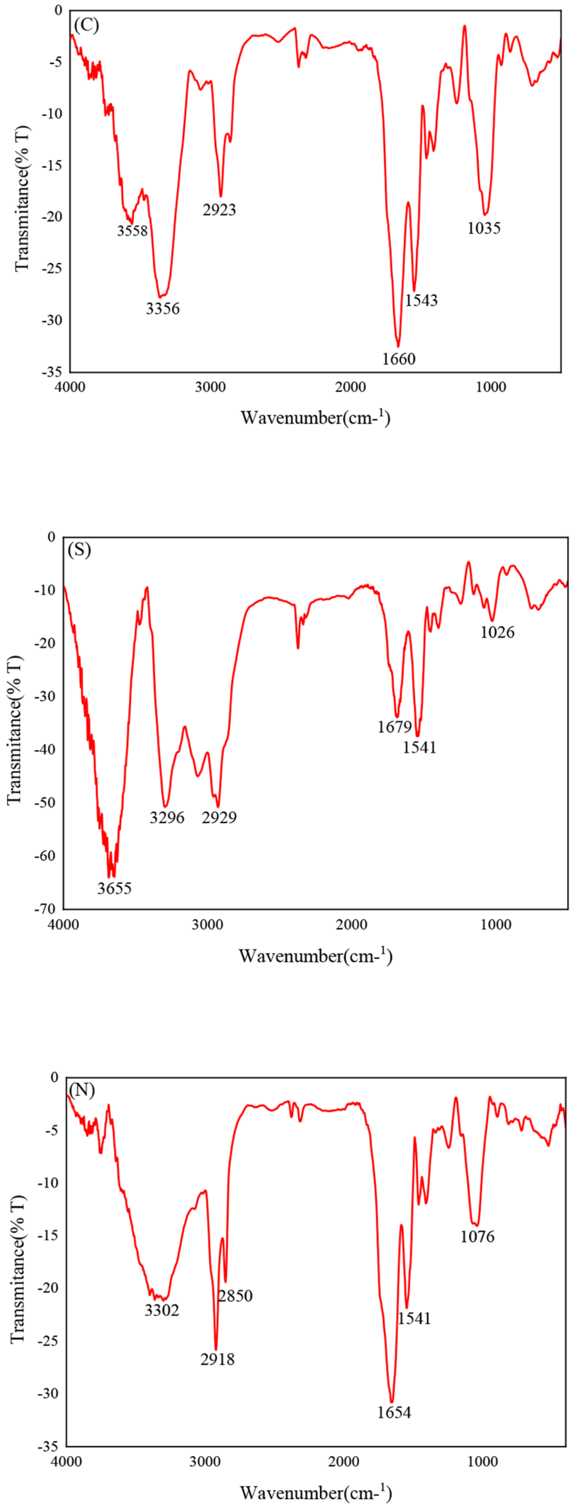

Figure 5. FTIR spectra of microalgae (C), (S), and (N)

is amorphous in nature (Mashhadi et al., 2024; Rohman et al., 2024; Wang et al., 2022).

In contrast, the diffraction pattern of (N) presents a mainly crystalline structure, characterized by distinct and well-defined peaks at about 28.5° and 40.7°. The peaks at 2θ =31.69°; 45.43°; 56.5°; 66.18° and 75.13° correspond to NaCl, while those at 2θ =28.36° and 40.54° correspond to KCl. According to the mineralogical analysis, there are two prevailing phases: the main phase was halite (NaCl), which accounted for about 91%, while the secondary one was sylvite (KCl), representing about 9%. Such a composition indicates a high salt content, which may be attributed to the cultivation of *N.oculata* (N) in a saline medium. This observation is consistent with the high electrical conductivity measured for the *N.oculata* (N) biomass.

Fourier transform infrared (FTIR)

Figure 5 presents the FTIR spectra for the various microalgae analyzed. Such analysis enables the identification of the chemical bonds and functional groups present in the samples. The three spectra are highly similar, with variations mainly in peak intensity, which suggests a comparable chemical composition among the microalgae, particularly with respect to their major constituents: proteins, lipids, and carbohydrates.

The absorption profile in the region between 4000–400 cm^{-1} can be differentiated by four characteristic regions. Bands in the range of 1600–1700 cm^{-1}, identified in all the spectra, point out that the three microalgae contain proteins. This is related to C=O stretching vibrations corresponding to the Amide I band (Ladjal-Ettoumi et al., 2024; Sočo et al., 2025). The band between 1500 and 1580 cm^{-1} is due to N-H bending and C-N stretching vibrations, from the Amide II band (Díaz et al., 2024; Ladjal-Ettoumi et al., 2024; Sočo et al., 2025). These observations are consistent with the results obtained from thermogravimetric analysis (TGA/DTG), which show significant mass losses corresponding to the decomposition of protein-related functional groups. Diaz et al. (2024) presented a peak at 1542 cm^{-1} for *Spirulina* sp., quite close to the values observed in this work.

A broad absorption within the region of 1200–900 cm^{-1} is considered representative of linkages of C-O-C and C-OH of carbohydrates (Marcilla et al., 2009), reflecting the presence of complex sugars or polysaccharides forming the microalgal cell wall (Martín-Juárez et al., 2018). A sharp peak at 2929 cm^{-1} (C), 2923 cm^{-1} (S), and 2918 cm^{-1} (N) represents stretching C-H in aliphatic chains, which is representative of methylene groups present in the fatty acid chains and amino acid side chains (Hu et al., 2025; Marcilla et al., 2009). In the spectrum of (N), a higher intensity was observed in the aliphatic C–H stretching region (2918–2850 cm^{-1}). This feature may be associated with differences in lipid-related functional groups.

This result is consistent with the previous findings reporting this strain as rich in lipids (Cebrián-Lloret et al., 2022; Hamed et al., 2016). Moreover, the cellulose-based cell wall of *Nannochloropsis* sp., is reinforced by the protective lipidic structure of algaenan (Hamed et al., 2016).

Finally, the band in the 3600–3100 cm^{-1} range, often extending to 4000–3700 cm^{-1}, is due to hydroxyl groups (-OH) (Bouaik et al., 2021; Cebrián-Lloret et al., 2022; Hamed et al., 2016; Wang et al., 2022).

It is a broadband attributed to the presence of alcohols, phenols, or carboxylic acids, and to adsorbed water (Bouaik et al., 2021; Wang et al., 2022).

Overall, the FTIR spectra confirmed the presence of characteristic functional groups associated with proteins, carbohydrates, lipids, and hydroxyl-containing compounds in the analyzed microalgal biomasses.

CONCLUSIONS

The comprehensive physicochemical, morphological, thermal, and structural characterization of *Chlorella vulgaris*, *Arthrospira platensis*, and *Nannochloropsis oculata* revealed distinct and complementary characteristics among the three microalgal biomasses. All biomasses showed high organic matter contents and measurable amounts of mineral nutrients, while *A. platensis* showed the highest water-holding capacity (2.5 g H_2O g^{-1} DW) and higher Mg and nitrate contents. *C. vulgaris* had the highest cation exchange capacity, whereas *N. oculata* demonstrated enhanced concentrations of several mineral elements, including P, K, Ca, Fe, Mn, and Zn. Nonetheless, the increased conductivity and sodium content in *N. oculata* make its utilization a complex process that requires special attention to soil salinity and application level.

The SEM–EDX, TGA/DTG, DSC, XRD, and FTIR analyses provided additional information about the morphology, elemental composition, thermal properties, crystalline phases, and functional groups of the biomasses. Notably, the occurrence of NaCl and KCl in *N.oculata* was in line with their high conductivity and mineral content. None of the heavy metals were detected in the EDX spectra analyzed. Nonetheless, this local measurement cannot reliably indicate the absence of heavy metals in the biomasses. For a final assessment of the environmental safety of the examined samples, methods such as ICP–OES and ICP–MS need to be utilized to determine concentration values of the heavy metals.

Overall, the findings suggest that the three types of microalgal biomass have complementary characteristics which may be important for their application in soil amendments. Rather than treating these types of biomasses as direct alternatives to conventional fertilizers based only on their properties, it is important to evaluate them under soil and plant conditions. Further research will therefore aim at field experiments using the types of microalgal biomass as soil enhancers, with the major focus on the influence on soil properties, nutrient content, soil moisture content, the growth of plants, and the productivity of crops. Through comparison of the three types of biomasses both separately and in combinations if needed, their agricultural potential and optimum application rate would be defined.

Acknowledgements

The writers would like to show their deep appreciation to the Faculty of Sciences in Agadir and the Faculty of Applied Science in Ait Melloul, both belonging to Ibn Zohr University (Agadir, Morocco), and to the Laboratory of Physicochemistry of Inorganic and Organic Materials in Rabat, Morocco, for making available laboratory facilities and materials. In addition, the authors sincerely appreciate the financial assistance that has made the work possible.

REFERENCES

1. Agüero-Quiñones, R., De La Cruz-Noriega, M., Rojas-Villacorta, W. (2025). Electric potential of *Chlorella* sp. microalgae biomass in microbial fuel cells (MFCs). *Bioengineering*, *12*(6), 635.
2. Akgül, F. (2019). Effect of Spirulina platensis (Gomont) geitler extract on seed germination of wheat and barley. *Alinteri Journal of Agriculture Science*, *34*(2), 148–153.
3. Al Dayel, M. F., El Sherif, F. (2021). Evaluation of the effects of *Chlorella vulgaris*, *Nannochloropsis salina*, and *Enterobacter cloacae* on growth, yield and active compound compositions of Moringa oleifera under salinity stress. *Saudi Journal of Biological Sciences*, *28*(3), 1687–1696.
4. Aljabri, H., Cherif, M., Siddiqui, S. A., Bounnit, T., Saadaoui, I. (2023). Evidence of the drying technique's impact on the biomass quality of *Tetraselmis subcordiformis* (Chlorophyceae). *Biotechnology for Biofuels and Bioproducts*, *16*(1), 85.
5. Alvarez, A. L., Weyers, S. L., Goemann, H. M., Peyton, B. M., Gardner, R. D. (2021). Microalgae, soil and plants: A critical review of microalgae as renewable resources for agriculture. *Algal Research*, *54*, 102200.
6. Aly, M. S., Esawy, M. A. (2008). Evaluation of Spirulina platensis as bio stimulator for organic farming systems. *Journal of Genetic Engineering and Biotechnology*, *6*(2), 1–7.
7. Anitha, L., Bramari, G. S., Kalpana, P. (2019, December). Bioalgalization—a novel approach for soil amendment to improve fertility. In *International Conference On Computational And Bio Engineering* (pp. 557–568). Cham: Springer International Publishing.
8. Arif, M., Li, Y., El-Dalatony, M. M., Zhang, C., Li, X., Salama, E. S. (2021). A complete characterization of microalgal biomass through FTIR/TGA/CHNS analysis: An approach for biofuel generation and nutrients removal. *Renewable Energy*, *163*, 1973–1982.
9. Bleakley, S., Hayes, M. (2021). Functional and bioactive properties of protein extracts generated from Spirulina platensis and Isochrysis galbana T-Iso. *Applied Sciences*, *11*(9), 3964.
10. Bouaik, H., Tabal, A., Barakat, A., El Harfi, K., Aboulkas, A. (2021). Optimal parameters and structural composition of bio-oil and biochar from intermediate pyrolysis of red algal biomass. *Comptes Rendus. Chimie*, *24*(S1), 85–99.
11. Bouramdane, A. A. (2023). Quelle est la Relation entre l'Agriculture et le Changement Climatique. *Énergie/Mines Carrières*, *20*.
12. Boureima, S. (2021). L'impact de la dégradation du sol sur la production agricole et la sécurité alimentaire au Burkina Faso.
13. Bule, M. H., Ahmed, I., Maqbool, F., Bilal, M., Iqbal, H. M. (2018). Microalgae as a source of high-value bioactive compounds. *Front. Biosci*, *10*(2), 197–216.
14. Cebrián-Lloret, V., Metz, M., Martínez-Abad, A., Knutsen, S. H., Ballance, S., López-Rubio, A., & Martínez-Sanz, M. (2022). Valorization of alginate-extracted seaweed biomass for the development of cellulose-based packaging films. *Algal Research*, *61*, 102576.
15. Chanthini, K. M. P., Rajan, P. K., Abdel-Megeed, A., Senthil-Nathan, S. (2024). Mechanistic perceptions of the elicitorial resources from the marine kingdom-A paradigm shift in plant defense against insect pests. *Biocatalysis and Agricultural Biotechnology*, *58*, 103141.
16. Chen, W. H., Chu, Y. S., Liu, J. L., Chang, J. S. (2018). Thermal degradation of carbohydrates, proteins and lipids in microalgae analyzed by evolutionary computation. *Energy Conversion and Management*, *160*, 209–219.

17. Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., De Gelder, L. (2016). The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. *Journal of applied phycology*, *28*(4), 2367–2377.
18. Da Silva Veiga, P. A., Cerqueira, M. H., Gonçalves, M. G., da Silva Matos, T. T., Pantano, G., Schultz, J.,..., Mangrich, A. S. (2021). Upgrading from batch to continuous flow process for the pyrolysis of sugarcane bagasse: Structural characterization of the biochars produced. *Journal of Environmental Management*, *285*, 112145.
19. Dessì, F., Mureddu, M., Ferrara, F., Fermoso, J., Orsini, A., Sanna, A., Pettinau, A. (2021). Thermogravimetric characterisation and kinetic analysis of *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae for pyrolysis, combustion and oxy-combustion. *Energy*, *217*, 119394.
20. Díaz, L. E., Gonzalez, J. D., Morales-Gonzalez, M. P., Garzón-Castro, C. L. (2024). Harnessing the power of microalgae consortia for sustainable crop production : case study on lettuce *(Lactuca sativa* L.). *Journal of Applied Phycology*, *36*(6), 3273–3286.
21. Díaz, S., Romero, F., Suárez, L., Ríos, R., Alemán, M., Venuleo, M., Ortega, Z. (2024). Characterization of microalgae biomass-based composites obtained through rotational molding. *Polymers*, *16*(13), 1807.
22. Dineshkumar, R., Kumaravel, R., Sampathkumar, P. (2017). Cultivation of efficient marine microalgae and their biochemical composition and its antibacterial activity against human pathogens. *Journal of Aquaculture & Marine Biology*, *5*(4), 00127.
23. Eissa, M. A., Alotaibi, M. O., Alotibi, M. M., Aljuaid, A., Aldayel, T. H., Ghoneim, A. M. (2025). A novel biostimulant–biochar strategy for improving soil quality and salinity tolerance in medicinal mint (*Mentha longifolia* L.). *Soil Systems*, *9*(2), 58.
24. El Moussaoui, H., Idardare, Z., Bouqbis, L. (2024). Effect of integrated soil fertility management on water retention, productivity and physiological parameters of Alfalfa (*Medicago sativa*) without and under deficit irrigation. *Scientia Horticulturae*, *327*, 112816.
25. El-Shazoly, R. M., Yousef, S., Hifney, A. F., Abdel-Wahab, D. A. (2025). Effectiveness of algae as a low-cost alternative input to stimulate *Sesamum Indicum* L. growth and productivity for sustainable purposes. *Journal of Soil Science and Plant Nutrition*, 1–20.
26. Garbowski, T. (2024). The effect of *Chlorella vulgaris* (Chlorellales: Chlorellaceae) on the fertility of sandy soils and on the composition of soil leachates. *Journal of Soil Science and Plant Nutrition*, *24*(4), 6496–6506.
27. Gharib, F. A. E. L., Osama, K., Sattar, A. M. A. E., Ahmed, E. Z. (2024). Impact of *Chlorella vulgaris*, *Nannochloropsis salina*, and *Arthrospira platensis* as bio-stimulants on common bean plant growth, yield and antioxidant capacity. *Scientific Reports*, *14*(1), 1398.
28. Gholami, T., Pirsaheb, M., Seifi, H., Alsultany, F. H., Salavati-Niasari, M. (2025). Thermal analysis as a tool for the environmental assessment of agricultural chemical residues: a systematic review. *RSC advances*, *15*(19), 15408–15416.
29. Gonçalves, J., Freitas, J., Fernandes, I., Silva, P. (2023). Microalgae as biofertilizers: a sustainable way to improve soil fertility and plant growth. *Sustainability*, *15*(16), 12413.
30. Hamed, S. B., Hamed, M. B. B., Kassouar, S., Abi Ayad, S. M. E. A. (2016). Physicochemical analysis of cellulose from microalgae *Nannochloropsis gaditana*. *African Journal of Biotechnology*, *15*(24), 1201–1206.
31. Hayes, M., Mora, L., Marzocchella, A., Kuchendorf, C. M., Ackermann, B., Aurino, E. (2025). Functional and Potential Bioactive Health Benefits of Selected Microalgal Hydrolysates Assessed In Silico and In Vitro. Hayes, M., Mora, L., Marzocchella, A., Kuchendorf, C. M., Ackermann, B., Aurino, E. (2025). Functional and potential bioactive health benefits of selected microalgal hydrolysates assessed in silico and in vitro. Marine Drugs *23*(2).
32. Hernandez, J. P., de-Bashan, L. E., Rodriguez, D. J., Rodriguez, Y., Bashan, Y. (2009). Growth promotion of the freshwater microalga Chlorella vulgaris by the nitrogen-fixing, plant growth-promoting bacterium Bacillus pumilus from arid zone soils. *European journal of soil biology*, *45*(1), 88–93.
33. Hu, Z., Zhang, C., Shu, Q. (2025). Study on the adsorption characteristics of spirulina dry powder biomass for rare earth element praseodymium (III): Adsorption isotherms, kinetics, and thermodynamics analysis. *Separations*, *12*(8), 195.
34. Jacinto, G. S. S., Cruz, G., Cabral, A. A., Bezerra, G. V. P., Garcia, R. R. P., Magalhaes, U. N., Gomes, W. C. (2023). Biotechnological investigation of *Pediastrum boryanum* and *Desmodesmus subspicatus* microalgae species for a potential application in bioenergy. *Algal Research*, *75*, 103266.
35. Kapoore, R. V., Wood, E. E., Llewellyn, C. A. (2021). Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices. *Biotechnology Advances*, *49*, 107754.
36. Khamila, S., D'Adamo, S., Eppink, M. H., Wijffels, R. H., Boboescu, I. Z. (2025). Structural and biochemical characterization of *Nannochloropsis oceanica* IMET1 cell wall and its implications for enzyme-assisted extraction. *Algal Research*, *90*, 104119.
37. Khanra, S., Mondal, M., Halder, G., Tiwari, O. N., Gayen, K., Bhowmick, T. K. (2018). Downstream

processing of microalgae for pigments, protein and carbohydrate in industrial application: A review. *Food and bioproducts processing*, *110*, 60–84.

38. Kholssi, R., Marks, E. A., Miñón, J., Montero, O., Debdoubi, A., Rad, C. (2019). Biofertilizing effect of *Chlorella sorokiniana* suspensions on wheat growth. *Journal of Plant Growth Regulation*, *38*(2), 644–649.
39. Kritikaki, A., Karmali, V., Vathi, D., Bartzas, G., Komnitsas, K. (2025). Advanced characterization of biochars produced from three different organic-based feedstocks and their potential applications. *Circular Economy and Sustainability*, 1–24.
40. Ladjal-Ettoumi, Y., Douik, L. H., Hamadi, M., Abdullah, J. A. A., Cherifi, Z., Keddar, M. N.,..., Nazir, A. (2024). Physicochemical and functional properties of spirulina and chlorella proteins obtained by iso-electric precipitation. *Food Biophysics*, *19*(2), 439–452.
41. Ladjal-Ettoumi, Y., Hamadi, M., Douik, L. H., Cherifi, Z., Nazir, A. (2024). Physicochemical, functional, and nutraceutical properties of Spirulina and Chlorella biomass: A comparative study. *Algal Research*, *81*, 103561.
42. Lima, I. E. S., Gonzalez, W. A., Teixeira, V. L. (2024). Structural characterization of biomass from *Crassiphycus birdiae* in natura and residual. *Journal of the Brazilian Chemical Society*, *35*(8), e-20240041.
43. Liu, Y., Wang, Y., Cao, L., Huang, Z., Zhou, Y., Fan, R., Li, C. (2023). Preparation and characterization of intracellular and exopolysaccharides during cycle cultivation of *Spirulina platensis*. *Foods*, *12*(5), 1067.
44. Lothenbach, B., Scrivener, K., Snellings, R. (Eds.). (2016). *A practical guide to microstructural analysis of cementitious materials*. CRC Press.
45. Ma, M., Ren, Y., Xie, W., Zhou, D., Tang, S., Kuang, M.,..., Du, S. K. (2018). Physicochemical and functional properties of protein isolate obtained from cottonseed meal. *Food chemistry*, *240*, 856–862.
46. Marcilla, A., Gómez-Siurana, A., Gomis, C., Chápuli, E., Catalá, M. C., Valdés, F. J. (2009). Characterization of microalgal species through TGA/FTIR analysis: Application to nannochloropsis sp. *Thermochimica Acta*, *484*(1–2), 41–47.
47. Martín-Juárez, J., Markou, G., Muylaert, K., Lorenzo-Hernando, A., Bolado, S. (2018). Breakthroughs in bioalcohol production from microalgae: solving the hurdles. *Microalgae-Based Biofuels and Bioproducts.*
48. Mashhadi, E., Safaei-Ghomi, J. (2024). Sulfonated magnetic spirulina nanobiomaterial as a novel and environmentally friendly catalyst for the synthesis of dihydroquinazolin-4 (1H)-ones in aqueous medium. *Scientific Reports*, *14*(1), 2296.
49. Metson, A.J., (1957). Methods of chemical analysis for soil survey samples. *Soil Sci. 83*(3), 245.
50. Meysami, M., Rabie, A., Najafabadi, R. A., Meysami, A., Isfahani, T. (2025). Comparative thermal and structural analysis of biochar from rapeseed meal and Fraxinus excelsior sawdust. *Results in Engineering*, 106397.
51. Molino, A., Iovine, A., Casella, P., Mehariya, S., Chianese, S., Cerbone, A.,..., Musmarra, D. (2018). Microalgae characterization for consolidated and new application in human food, animal feed and nutraceuticals. *International Journal of Environmental Research and Public Health*, *15*(11), 2436.
52. Monteiro, C. M., Castro, P. M., Malcata, F. X. (2012). Metal uptake by microalgae: underlying mechanisms and practical applications. *Biotechnology progress*, *28*(2), 299–311.
53. Navalho, S., Ferrer-Ledo, N., Barbosa, M. J., Varela, J. (2025). Nannochloropsis lipids and polyunsaturated fatty acids: Potential applications and strain improvement. *Marine Drugs*, *23*(3), 128.
54. Nyoni, B., Duma, S., Shabangu, S. V., Hlangothi, S. P. (2020). Comparison of the slow pyrolysis behavior and kinetics of coal, wood and algae at high heating rates. *Natural Resources Research*, *29*(6), 3943–3955.
55. Parmar, P., Kumar, R., Neha, Y., Srivatsan, V. (2023). Microalgae as next generation plant growth additives: Functions, applications, challenges and circular bioeconomy based solutions. *Frontiers in Plant Science*, *14*, 1073546.
56. Patel, U., Kumar, N., Kaur, S. (2025). Drying characteristics of thin layer hot air drying for preparation of ready-to-reconstitute protein enriched spongy snack (khaman). *Discover Chemistry*, *2*(1), 1–14.
57. Paterson, S., Gómez-Cortés, P., de la Fuente, M. A., Hernández-Ledesma, B. (2023). Bioactivity and digestibility of microalgae *Tetraselmis* sp. and *Nannochloropsis* sp. as basis of their potential as novel functional foods. *Nutrients*, *15*(2), 477.
58. Patil, V. V., Singh, M., Kandasubramanian, B. (2025). Utilization of *Spirulina Algae* powder for efficient removal of lubricating oil from aqueous media. *Cleaner Water*, 100120.
59. Redmile-Gordon, M., Gregory, A. S., White, R. P., Watts, C. W. (2020). Soil organic carbon, extracellular polymeric substances (EPS), and soil structural stability as affected by previous and current land-use. *Geoderma*, *363*, 114143.
60. Renganathan, P., Gaysina, L. A., Holguín-Peña, R. J., Sainz-Hernández, J. C., Ortega-García, J., Rueda-Puente, E. O. (2024). Phycoremediated microalgae and cyanobacteria biomass as biofertilizer for sustainable agriculture: A holistic biorefinery approach to promote circular

bioeconomy. *Biomass*, *4*(4), 1047–1077.

61. Rohman, G. A. N., Aziz, M. A., Nawaz, A., Elgzoly, M. A., Hossain, M. M., Razzak, S. A. (2024). High-performance biochar from Chlorella pyrenoidosa algal biomass for heavy metals removal in wastewater. *Separation and Purification Technology*, *341*, 126870.
62. Selem, E. (2019). Physiological effects of Spirulina platensis in salt stressed Vicia faba L. plants. *Egyptian Journal of Botany*, *59*(1), 185–194.
63. Sharma, A. K., Jaryal, S., Sharma, S., Dhyani, A., Tewari, B. S., Mahato, N. (2025). Biofuels from microalgae: a review on microalgae cultivation, biodiesel production techniques and storage stability. *Processes*, *13*(2), 488.
64. Shedeed, Z. A., Gheda, S., Elsanadily, S., Alharbi, K., Osman, M. E. (2022). *Spirulina platensis* biofertilization for enhancing growth, photosynthetic capacity and yield of *Lupinus luteus*. *Agriculture*, *12*(6), 781.
65. Singh, J. K., Chaurasia, B., Dubey, A., Noguera, A. F., Gupta, A., Kothari, R.,..., Allah, E. F. A. (2021). Biological characterization and instrumental analytical comparison of two biorefining pretreatments for water hyacinth (*Eicchornia crassipes*) biomass hydrolysis. *Sustainability. 13,* 245.
66. Sočo, E., Domoń, A., Azizi, M., Pająk, D., Cieniek, B., Michel, M. M., Papciak, D. (2025). Efficiency of A. platensis (S) in the treatment of model wastewater containing Ni (II) and Pb (II). *Materials*, *18*(15), 3639.
67. Tian, S. L., Khan, A., Zheng, W. N., Song, L., Liu, J. H., Wang, X. Q., Li, L. (2022). Effects of *Chlorella extracts* on growth of *Capsicum annuum* L. seedlings. *Scientific Reports*, *12*(1), 15455.
68. Trinh, C. T., Tran, T. H., Bui, T. V. (2017, September). Effects of plant growth regulators on the growth and lipid accumulation of *Nannochloropsis oculata* (droop) Hibberd. In *AIP conference proceedings 1878*(1), 020017. AIP Publishing LLC.
69. Uroić Štefanko, A., Leszczynska, D. (2020). Impact of biomass source and pyrolysis parameters on physicochemical properties of biochar manufactured for innovative applications. *Frontiers in Energy Research*, *8*, 138.
70. Varkolu, M., Gundekari, S., Palla, V. C. S., Kumar, P., Bhattacharjee, S., Vinodkumar, T. (2025). Recent advances in biochar production, characterization, and environmental applications. *Catalysts*, *15*(3), 243.
71. Walkley, A., Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science*, *37*(1), 29–38.
72. Wang, J., Yin, Y. (2018). Fermentative hydrogen production using pretreated microalgal biomass as feedstock. *Microbial Cell Factories*, *17*(1), 22.
73. Wang, S. N., Ge, S. H., Zhuang, L. L., Zhang, J. (2024). Multiple pathways for the enhancement of wheat growth by *Chlorella vulgaris*. *Journal of Plant Growth Regulation*, *43*(2), 550–562.
74. Wang, Z., Chen, J., Tan, J., Lu, Z., Wang, X., Li, J. (2022). The bio-immobilization of Pb (II) induced by the Chlorella–montmorillonite composite in the Ca (II) environment. *Frontiers in Environmental Science*, *10*, 983430.
75. Xiao, R., Zheng, Y. (2016). Overview of microalgal extracellular polymeric substances (EPS) and their applications. *Biotechnology advances*, *34*(7), 1225–1244.
76. Zeller, M. A., Hunt, R., Jones, A., Sharma, S. (2013). Bioplastics and their thermoplastic blends from Spirulina and Chlorella microalgae. *Journal of Applied Polymer Science*, *130*(5), 3263–3275.
77. Ziyue, T., Chen, W., Chen, X., Xia, M., Chen, Y., Yang, H.,..., Xiaorui, L. (2023). Thermal behavior and kinetic mechanism of microalgae and model compounds. *Fuel*, *344*, 128037.