Rev. Int. Contam. Ambie. 40, 149-166, 2024
https://doi.org/10.20937/RICA.54917

MINERALIZATION OF MICROALGAL CARBON AND NITROGEN IN SODIC SOILS

Mineralización de carbono y nitrógeno microalgal en suelos sódicos

Ileana CASTRO-GONZÁLEZ[1], Héctor Iván BEDOLLA-RIVERA[1],
María de la Luz Xochilt NEGRETE-RODRÍGUEZ[1], Omar S. CASTILLO[2],
Dioselina ÁLVAREZ-BERNAL[3] and Eloy CONDE-BARAJAS[1]*

[1] Tecnológico Nacional de México/IT de Celaya, Antonio García Cubas 600, Celaya, C.P. 38010, Guanajuato, México.
[2] Universidad de Guanajuato, Mutualismo 303, Celaya, C. P. 38060 Guanajuato, México.
[3] Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Michoacán, Instituto Politécnico Nacional, Justo Sierra 28, Jiquilpan, C.P. 59510, Michoacán, México

*Author for correspondence: eloy.conde@itcelaya.edu.mx

(Received: October 2022; accepted: May 2023)

Key words: bioremediation, organic matter, microbial activity, dairy wastewater.

ABSTRACT

Sodic soils pose a challenge for the agricultural production due to their lack of nutrients, poor structure, low organic matter content, and susceptibility to erosion (water and wind). Their recovery is carried out by soil washing and applying calcium salts, which are sometimes unprofitable processes. A low-cost and environmentally friendly alternative to remedy adverse soil conditions is bioremediation using microorganisms or organic amendments. For this reason, this study intended to evaluate the effects of the addition of dry microalgal biomass on sodic soils and suggest its use as an organic amendment. The effect of the microalgal biomass was studied through the mineralization dynamics of carbon and nitrogen sources in short-term experiments. All experiments were performed at laboratory scale. Microalgal biomass was obtained from a consortium grown in dairy wastewater and subsequently dried and pulverized. Four different treatments of dry microalgal biomass were applied to 50 g of sodic soil, and high microbial activity was observed in the soil (obtaining a production of 240 mg $C\text{-}CO_2$/kg dry soil), along with the production of nitrates (presenting values 33.8-1.45 mg $N\text{-}NO_3^+$/kg dry soil) via the release of ammonia (obtaining 5.46 mg $N\text{-}NH_3^+$/kg dry soil), and mineralization of organic N into ammonium (producing 1071.92 mg $N\text{-}NH_4^+$/kg dry soil). The microalgal biomass as an organic amendment showed to be prone to mineralization and release of carbon and nitrogen sources, improving the microbial activity in a soil with sodicity problems.

Palabras clave: biorremediación, materia orgánica, actividad microbiana, agua residual láctea.

RESUMEN

Los suelos sódicos representan un reto para la industria agrícola debido a su falta de nutrientes, mala estructura, poca materia orgánica y susceptibilidad a sufrir erosión hídrica y eólica. Su recuperación se realiza mediante lavados y aplicación de sales de calcio, los cuales a veces resultan ser procesos poco rentables. Una alternativa de tratamiento de bajo costo y amigable con el ambiente es la aplicación de microorganismos y enmiendas orgánicas con la finalidad de mejorar las condiciones adversas del suelo. Por tal motivo, este estudio se propuso evaluar los efectos de la adición de biomasa microalgal seca en suelos sódicos y sugerir su uso como enmienda orgánica. Se estudió el efecto de la biomasa microalgal mediante dinámicas de mineralización de fuentes de carbono y nitrógeno en experimentos a corto plazo. Todos los experimentos se realizaron a escala de laboratorio. Microalgal biomass was obtained from a consortium grown in dairy wastewater and subsequently dried and pulverized. En frascos de vidrio con 50 g de suelo sódico, se aplicaron cuatro concentraciones distintas de biomasa microalgal seca, observándose en el suelo una actividad microbiana alta (producción de 240 mg C-CO_2/kg suelo seco), mineralización de nitratos (de 33.8 a 1.45 mg N-NO_3^- /kg suelo seco) vía liberación de amoniaco (5.46 mg N-NH_3^+/kg suelo seco) y mineralización del nitrógeno orgánico a amonio (1071.92 mg N-NH_4^+/kg suelo seco). La biomasa microalgal resultó ser una enmienda orgánica susceptible a la mineralización y con liberación de fuentes de carbono y nitrógeno, mejorando la actividad microbiana en el suelo con problemas de sodicidad.

INTRODUCTION

Soils naturally contain salts that participate in the cycling of minerals; however, when these salts increase, they cause problems for the structural properties and agricultural production of soil (Romano-Armada et al. 2020). The excessive salt accumulation in the soil and its relationship with the decrease in plant growth is known as soil salinization (Noori et al. 2021). Based on the electrical conductivity (EC), the exchange sodium percentage (ESP), and the sodium adsorption ratio (SAR) of the soil, soils can be classified into one of the following categories: sodic-saline with EC $>$ 4 dS/m, ESP $>$ 15%, SAR $>$ 13; saline with EC $>$ 4 dS/m, ESP $<$ 15%, SAR $<$ 13; sodic with EC $<$ 4 dS/m, ESP $>$ 15%, SAR $>$ 13; or regular with EC $<$ 4 dS/m, ESP $<$ 15%, SAR $<$ 13 (Osman 2018). Sodic soils are characterized by structural decline, clay dispersion, organic matter (OM) solubility, and nutrient deficit, among other adverse conditions (Noori et al. 2021). Because of high agricultural activity, sodicity in the soil has become a problem in Mexico and the world due to the excessive use of fertilizers, inadequate irrigation water, inadequate soil management, and lack of crop rotation (Bedolla-Rivera et al. 2020). The remediation of sodic soils is carried out by physical methods such as washing, where large quantities of water are applied in order to leach excess salt—provided the soil has adequate drainage (Osman 2018). Chemical methods are also used, such as the addition of lime, organic gypsum ($CaSO_4 \cdot 2H_2O$), and calcium chloride; this method allows calcium ions (Ca^{2+}) to replace sodium ions (Na^+), thus counteracting the dispersion of clays (Brusseau et al. 2019). An environmentally friendly strategy is bioremediation (biostimulation), which consists of using organisms, parts of organisms or their products to eliminate, contain or transform harmful substances in the environment, thus modifying adverse environmental conditions (Romano-Armada et al. 2020). In the case of sodic soils, OM contributes to the improvement of aeration and water holding capacity, moderation of climatic changes, stabilization of soil structure, aggregate formation, nutrient storage, pH regulation, and microbial activity (Datta et al. 2019). Examples of low-cost sources of OM that are easy to apply include organic amendments, organic fertilizers, and fruit peels (Wijitkosum 2020). Regarding biotechnological strategies, the use of dry microalgal biomass as an organic amendment in sodic soils has improved crop yields, fertility1, and nutrient supplies, by increasing the population of beneficial microorganisms and stabilizing soil structure and aggregates (Chatterjee et al. 2017).

Microalgae are unicellular, autotrophic and heterotrophic organisms, ranging in size from 0.5 to 200 μm. They are part of phytoplankton on aquatic and terrestrial surfaces in a wide variety of agroecosystems worldwide (Alvarez et al. 2021). Among the microalgae genera whose application has been associated to the improvement of soils with sodicity problems are Cyanobacteria, *Nostoc* (Alvarez et al. 2021), and *Nannochloropsis* (Ammar et al. 2022). In such soils, microalgae contribute to the formation and stabilization of soil aggregates, increasing the pore size and improving infiltration, water holding capacity, and aeration. Microalgae also provide a large number of diverse carbohydrates, proteins, enzymes, fatty acids, organic acids, vitamins, hormones, and other biomolecules, which contribute to plant protection against soil salinity or sodicity (Nichols et al. 2020).

In this regard, microalgae production in liquid cultures has been carried out in open ponds (natural or artificial) or closed photobioreactors. The former proves to be less expensive in large-scale biomass production, while the latter provides a controlled environment in which external contamination is avoided (Ammar et al. 2022). Photobioreactors optimize light use through tubular or flat-plate designs, control temperature, and culture medium conditions, and prevent extreme evaporation (Nichols et al. 2020). Likewise, autotrophic and mixotrophic cultures can be used in photobioreactors, providing the microalgae with adequate nutrients, light, and CO_2 for growth and reproduction (Daneshvar et al. 2019). Microalgae have also been characterized by their capacity to absorb large amounts of phosphorus (P) and nitrogen (N) during their production in liquid media; therefore, it has been beneficial to cultivate them in photobioreactors, in mixotrophic culture with wastewater (Daneshvar et al. 2019). While dairy wastewater (DWW) presents high concentrations of P and N, and—upon reaching the aquifer mantle—it may disrupt the balance of the ecosystem (Daneshvar et al. 2019); several studies have reported the use of DWW to produce different species of microalgae, for example *Scenedesmus quadricauda*, *Tetraselmis suecica* (Daneshvar et al. 2019), *Anabaena ambigua*, *Chlorella pyrenoidosa*, *Scenedesmus abundans* (Brar et al. 2019), and *Chlorella vulgaris* (Handayani et al. 2020).

As the use of DWW to produce microalgae has been evidenced and there is the need to gain knowledge about the application of organic amendments in soils, experiments were carried out in the present study in order to analyze the first stages of the addition of dry microalgal biomass (produced in DWW) as an organic amendment in sodic soils. Microorganisms present in the soil are responsible for processes that sustain soil fertility—such as aggregation and mineralization of OM—thus influencing biogeochemical and nutrient cycles (Alvarez et al. 2021). In addition, these microorganisms, together with plants, need rapidly assimilated sources of C and N for their utilization. In view of the above, this study aimed to obtain effective doses of microalgal biomass in terms of C and N mineralization. A direct relationship was hypothesized between the rate of addition of dry microalgal biomass (produced in DWW) and the increase in the rate of release of C compounds (production and accumulation of CO_2) and N mineralized from OM in the short term (hours).

MATERIALS AND METHODS

Microalgal biomass production

The microalgal consortium—obtained from the Department of Agroindustrial Engineering of the University of Guanajuato, Mexico—was subjected to an adaptation process under controlled conditions (pH 7.0, room temperature 25 ºC, constant aeration and photoperiod 16/8 h) for 30 days, placing the microalgal inoculum in flat-plate reactors (5 L), with 80:20 v/v Bold's Basal Medium (BBM) and inoculum (Barsanti and Gualtieri 2014). After the adaptation period, microalgal biomass production was performed, using 60% v/v BBM culture medium (Barsanti and Gualtieri 2014), 40% v/v DWW, in incubation periods of 10 days under mixotrophic conditions, with the availability of organic and inorganic C during the process. The 21 physical and chemical indicators evaluated for DWW characterization are listed in **table I.**

Microalgal growth

Growth and nutrient consumption kinetics were conducted during the production of microalgal biomass. The microalgal biomass concentration was established by spectrophotometry, following the methodology of García-Gozalbes et al. (2015), reported in g/L. The indicators pH, chemical oxygen demand (COD), soluble organic C (SOC), $N\text{-}NO_3^-$, and phosphates ($P\text{-}PO_4^-$) were determined as reported in **table II**. All indicators were evaluated in triplicate.

Characterization of the microalgal biomass

The microalgal biomass was collected by flocculation with 1.0 g of chitosan (β-(1-4)-2-amino-2-desoxy-D-glucopyranose), 20% glacial acetic acid (CH_3COOH) and 80% drinking water (H_2O) v/v (Terkula Iber et al. 2022). Subsequently, 40 mL of flocculating agent per liter of microalgal culture was subjected to agitation, first at 200 rpm for 30 s, then at 100 rpm for 10 min, and finally it was left to stand for 10 min. Afterward, it was centrifuged in 50 mL Falcon tubes at 6000 rpm for 10 min, using a Thermo Scientific® Myspin 12 centrifuge. The precipitate was oven-dried at 80 ºC for 72 h, and then pulverized using an agate mortar. The biomass obtained was characterized using the 17 physical, chemical and nutritional indicators described in **table III**.

Sampling and physical chemical characterization of the soil

The samples of soil were obtained in Guanajuato, Mexico, at coordinates 20º60'22.22'' N, 101º02'30.56'' W, in compliance with the standards

TABLE I. INDICATORS FOR THE PHYSICAL AND CHEMICAL CHARACTERIZATION OF DAIRY WASTE WATER.

Indicator	Reference
TS TSS	(USEPA 2001)
pH EC	(Baird R.B. et al. 2017)
BOD_5 COD $P\text{-}PO_4^-$	(Hach 1999)
TOC	(Hach 2015)
TN	(Simonne et al. 1997)
$N\text{-}NO_3^-$	(Alef and Nannipieri 1995)
$N\text{-}NH_4^+$	(Keeney and Nelson 1983)
P B K Ca Mg Na Fe Cu Mn Zn	(Falciani et al. 2000)

TS: Total soluble solids (mg/L), TSS: Total suspended solids (mg/L), pH: Hydrogen potential, EC: Electric conductivity (dS/m), BOD_5: Biological oxygen demand at five days (mg /L), COD: Chemical oxygen demand (mg/L), $P\text{-}PO_4^-$: Phosphate (mg/L), TOC: Total organic C (%), TN: Total N (%), $N\text{-}NO_3^-$: Nitrate (mg/L), $N\text{-}NH_4^+$: Ammonium (mg/L), P: Phosphorus (mg /L), B: Boron (mg /L), K: Potassium (mg/L), Ca: Calcium (mg/L), Mg: Magnesium (mg/L), Na: Sodium (mg/L), Fe: Iron (mg/L), Cu: Copper (mg/L), Mn: Manganese (mg/L), Zn: Zinc (mg/L).

TABLE II. INDICATORS EVALUATED TO DETERMINE BIOMASS PRODUCTION AND NUTRIENT CONSUMPTION IN MICROALGAE GROWTH KINETICS.

Indicator	Reference
pH	(Thomas 1996)
COD $P\text{-}PO_4^-$ $N\text{-}NO_3^-$	(Hach 1999)
SOC	(Cook et al. 2017)

pH: Hydrogen potential, COD: Chemical oxygen demand (mg/L), $P\text{-}PO_4^-$: Phosphate (mg/L), $N\text{-}NO_3^-$: Nitrate (mg/L), SOC: Soluble organic C (μg SOC/L).

TABLE III. INDICATORS USED IN THE PHYSICAL, CHEMICAL AND NUTRITIONAL CHARACTERIZATION OF MICROALGAL BIOMASS.

Indicator	Reference
Ash	(Schulte and Hopkins 1996)
M	(Black 1965)
OM	(Schulte and Hopkins 1996)
TOC	(Schulte and Hopkins 1996)
TN	(Simonne et al. 1997)
$N\text{-}NO_3^-$	(Alef and Nannipieri 1995)
$N\text{-}NH_4^+$	(Keeney and Nelson 1983)
C/N	(Medina-Herrera et al. 2020)
P K Ca Mg Na Fe Cu Mn Zn	(Falciani et al. 2000)

Ash (%), M: Moisture (%), OM: Organic matter (%), TOC: Total organic C (%), TN: Total N (%), $N\text{-}NO_3^-$: Nitrate (mg/kg dried microalgae), $N\text{-}NH_4^+$: Ammonium (mg/kg dried microalgae), C/N: Carbon/Nitrogen ratio, P: Phosphorus (mg/kg dried microalgae), K: Potassium (mg/kg dried microalgae), Ca: Calcium (mg/kg dried microalgae), Mg: Magnesium (mg/kg dried microalgae), Na: Sodium (mg/kg dried microalgae), Fe: Iron (mg/kg dried microalgae), Cu: Copper (mg/kg dried microalgae), Mn: Manganese (mg/kg dried microalgae), Zn: Zinc (mg/kg dried microalgae).

described in ISO 18400-104:2018 (ISO 2018). The sampled soil was divided into three subplots of 600 m^2. In each area, a systematic zig-zag sampling pattern was followed starting from one corner of each subplot and performing excavations with an auger drill every 18 m, with 30 cm depth and 40 cm diameter. At each sampling point, 2 kg of soil was taken. In total, 30 kg of composite samples were taken per subplot. The samples were transported in plastic bags to the laboratory and stored at 4 ºC until analysis. The soil characterization was carried out with the determination of 34 physical and chemical indicators, as shown in **table IV**.

Short-term C and N mineralization dynamics of soil-microalgae systems

Experimental design

To monitor the mineralization dynamics of C and N sources from the dry microalgal biomass in the short

TABLE IV. INDICATORS APPLIED FOR THE PHYSICAL AND CHEMICAL CHARACTERIZATION OF THE SOIL.

Indicator	Reference	Indicator	Reference
M	(Page et al. 1982)	N_{min}	(Díez López 1999)
HC	(Ankeny et al. 1991)	CO_3^{-2}	(Chaney et al. 1982)
BD	(Blake and Hartge 1986)	P	(Olsen and Sommers 1982)
Texture	(Bouyoucos 1962)	B	(Falciani et al. 2000)
WHC	(Alef and Nannipieri 1995)	K	
pH	(Thomas 1996)	Ca	
EC	(Hendrickx et al. 2002)	Mg	
CEC	(Cottenie 1980)	Na	
ESP	(USDA 2017)	Fe	
SAR		Cu	
OM	(Read and Ridgell 1922)	Mn	
$N_{organic}$	(Cristóbal-Acevedo et al. 2011)	Zn	
TOC	(Walkley and Black 1934)	S	
TN	(Bremner 1996)	Ca/K	
C/N	(Medina-Herrera et al. 2020)	Mg/K	
$N\text{-}NO_3^-$	(Alef and Nannipieri 1995)	(Ca+Mg)/K	
$N\text{-}NH_4^+$		Ca/Mg	

M: Moisture (%), HC: Hydraulic conductivity (cm/h), BD: Bulk density (g/cm^3), Texture (clay, silt, sand), WHC: Water holding capacity (%), pH: Hydrogen potential, EC: Electric conductivity (dS/m), CEC: Cation exchange capacity (mEq/100 g dry soil), ESP: Exchangeable sodium percentage (%), SAR: Sodium adsorption ratio, OM: Organic matter (%), $N_{organic}$: Organic N (%), TOC: Total organic C (%), TN: Total N (%), C/N: Carbon/Nitrogen ratio, $N\text{-}NO_3^-$: Nitrate (mg/kg), $N\text{-}NH_4^+$: Ammonium (mg/kg), N_{min}: Mineral N (mg/kg), CO_3^{-2}: Carbonate (%), P: Phosphorus (mEq 100/g), B: Boron (mEq/100 g dry soil), K: Potassium (mEq/100 g dry soil), Ca: Calcium (mEq/100 g dry soil), Mg: Magnesium (mEq/100 g dry soil), Na: Sodium (mEq/100 g dry soil), Fe: Iron (mEq/100 g dry soil), Cu: Copper (mEq/100 g dry soil), Mn: Manganese (mEq/100 g dry soil), Zn: Zinc (mEq/100 g dry soil), S: Sulfur (mEq/100 g dry soil), Ca/K: Calcium/Potassium ratio, Mg/K: Magnesium/Potassium ratio, (Ca+Mg)/K: (Calcium+Magnesium)/Potassium ratio, Ca/Mg: Calcium/Magnesium ratio.

term, a completely randomized block (CRB) experimental design, five treatments, and three replicates (60 experimental units) were established. Treatments were designed based on adding dry microalgae as a function of $N\text{-}NH_4^+$ concentration, as follows: T0 (50 g soil + 0 mg microalgae [control]), T1 (50 g soil + 500 mg microalgae [25 mg $N\text{-}NH_4^+$/kg dry soil]), T2 (50 g soil + 1000 mg microalgae [50 mg $N\text{-}NH_4^+$/kg dry soil]), T3 (50 g soil + 2000 mg microalgae [100 mg $N\text{-}NH_4^+$/kg dry soil]), and T4 (50 g soil + 3000 mg microalgae [150 mg $N\text{-}NH_4^+$/kg dry soil]).

Soil pre-incubation

Prior to the mineralization dynamics, the soil was pre-incubated under controlled humidity conditions for a period of 7 days. Soil moisture was adjusted to a water holding capacity (WHC) of 40% at room temperature. The soil was placed in plastic containers together with a flask of distilled water to avoid desiccation, and amber flasks with 1.0 M sodium hydroxide (NaOH) were placed in order to capture the $C\text{-}CO_2$ produced by the microbial activity present in the soil (Conde et al. 2005).

C and N mineralization dynamics

The experimental units consisted of 1.0 L capacity glass jars with 100 mL distilled water. Two amber glass vials were placed inside the jars, one with 30 mL of 1.0 M NaOH, and the other with 20 mL boric acid (H_3BO_3 2% v/v) to evaluate the mineralization of C (C-CO_2) and the volatilization of N as ammonia (N-NH_3^+). Subsequently, glass flasks with a capacity of 125 mL were also placed inside the jars, to which a dry soil-microalgae mixture (WHC at 40%) was added according to the treatments used in this study (Gougoulias et al. 2018).

Indicators analyzed during the dynamics of C and N mineralization

The dynamics of C and N mineralization in the soils were monitored during a period of 360 h in triplicate under the short-term experiments (STE) treatment model. The indicators analyzed were C-CO_2, pH, EC, N-NH_3^+, N-NH_4^+, and N-NO_3^- at 0, 72, 168, and 360 h. Total N (TN), total organic C (TOC), and OM were analyzed at the beginning of the mineralization dynamics of C and N. The determinations of pH, EC, N-NH_4^+, N-NO_3^-, TOC, OM, TN, N_{min}, and $N_{organic}$ were carried out following the same methodology mentioned for the physical and chemical characterization of the soil, which is shown in **table IV**.

Determination of N-NH_3^+ was performed by titration of H_3BO_3 2% v/v with sulfuric acid (H_2SO_4) 0.5 N (Beltrán-Hernández et al. 2007), reported in mg N-NH_3^+/kg dry soil. Soil extracts were made with potassium sulfate (K_2SO_4 0.5 M) and stored at -15 ºC until analysis (Conde et al. 2005). The evolution of C-CO_2 emission over time was determined according to the methodology of Alef and Nannipieri (1995), by titration of 1.0 M NaOH with hydrochloric acid (HCl 1.0 N and 0.1 N), reported as mg C-CO_2/kg dry soil.

Statistical analysis

Statistical analyses were performed using R statistical software version 3.6.2 (RCT 2019). It began with a Shapiro normality analysis, followed by a Kruskall-Wallis nonparametric analysis of variance, and subsequent Dunnett's mean contrast test with the Bonferroni adjustment method at a significance level $p \leq 0.05$. Correlations between the indicators analyzed were established using a Spearman correlation matrix (r), with those indicators with a Spearman correlation ($r \geq \pm 0.6$) being considered significant correlations (Zhang et al. 2016). Finally, a Friedman analysis was performed with a significant difference of $p \leq 0.05$ (Zimmerman and Zumbo 1993).

RESULTS

Characterization of dairy wastewater

The results of DWW characterization can be found in **table V**, where the indicators pH, COD, and biological oxygen demand at 5 days (BOD_5) presented values considered normal for DWW: pH of 4.7-11, COD of 80-95 000 mg/L, and BOD_5 of 40-48 000 mg/L (Daneshvar et al. 2019).

TABLE V. VALUES OF THE INDICATORS ANALYZED IN THE PHYSICAL AND CHEMICAL CHARACTERIZATION OF DAIRY WASTEWATER.

Indicator	Results	Units	Indicator	Results	Units
pH	10.90 ± 0.08	---	P	50.00 ± 6.20	mg/L
EC	2.80 ± 0.01	dS/m	K	100.00 ± 1.36	mg/L
TN	0.007± 0.002	%	Ca	90.00 ± 1.15	mg/L
TOC	0.10 ± 0.01	%	Mg	20.00 ± 2.65	mg/L
COD	2441.66 ± 23.40	mg/L	Na	700.00 ± 23.69	mg/L
BOD_5	269.66 ± 4.33	mg/L	Fe	2.29 ± 0.12	mg/L
TS	6874.00 ±112.26	mg/L	Cu	0.04 ± 0.03	mg/L
TSS	3619.00 ± 26.10	mg/L	Mn	0.69 ± 0.04	mg/L
N-NO_3^-	287.00 ± 29.36	mg/L	Zn	0.32 ± 0.02	mg/L
P-PO_4^-	40.00 ± 1.02	mg/L	B	0.34 ± 0.02	mg/L
N-NH_4^+	7.21 ± 0.74	mg/L	---	---	---

pH: Hydrogen potential, EC: Electric conductivity, TN: Total N, TOC: Total organic C, COD: Chemical oxygen demand, BOD_5: Biological oxygen demand at five days, TS: Total soluble solids, TSS: Total suspended solids, N-NO_3: Nitrate, P-PO_4^-: Phosphate, N-NH_4^+: Ammonium, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium, Na: Sodium, Fe: Iron, Cu: Copper, Mn: Manganese, Zn: Zinc, B: Boron.

Microalgal growth and microalgal biomass

The values for the indicators that were monitored during the microalgal growth kinetics are shown in **table VI**. The pH ranged from 8.6 to 9.2, being 9.02 the initial value and 9.10 the final value. An inverse trend was observed between the amount of biomass (which increased from 0.43 to 0.80 g/L) and the values of $N\text{-}NO_3^-$, $P\text{-}PO_4^-$, SOC, and COD, all of which decreased during the 10 days of growth as a consequence of the metabolism of the microalgal consortium.

Characterization of the microalgal biomass

The microalgal biomass presented TN values of 8.3%, TOC of 50.6%, a C/N ratio of 6.1, and 2.03% P (**Table VII**). Regarding the macronutrient content, the microalgal biomass presented 3000 mg/kg of dry microalgae for K, 23 900 mg/kg of dry microalgae for Ca, and 4600 mg/kg of dry microalgae for Mg. The presence of these elements is of great importance because, in their cationic form, they replace the Na^+ ions present in the soil aggregates, improving their structure (Leogrande and Vitti, 2019).

TABLE VI. VALUES OF THE INDICATORS DETERMINED IN BIOMASS PRODUCTION AND NUTRIENT CONSUMPTION IN MICROALGAL GROWTH KINETICS.

Day	DV					
	pH	Biomass	$N\text{-}NO_3^-$	$P\text{-}PO_4^-$	SOC	COD
0	9.02ab	**0.43c**	**62.63a**	**89.00a**	**276.58a**	**674.00a**
1	**8.60b**	0.45bc	---	---	---	---
2	8.93ab	0.50bc	38.70ab	79.13a	53.30ab	529.67ab
3	9.05ab	0.56bc	---	---	---	---
4	8.95ab	0.65ab	32.6ab	70.00ab	44.50ab	177.70abc
5	8.83ab	0.71ab	---	---	---	---
6	9.03ab	0.73ab	25.83bc	69.33ab	40.50bc	118.33bc
7	8.93ab	0.77ab	---	---	---	---
8	9.02ab	**0.80a**	16.67bc	64.33b	38.00bc	99.30bc
9	**9.17a**	**0.80a**	---	---	---	---
10	9.10ab	**0.80a**	**12.60c**	**62.15b**	**32.60c**	**95.50c**
X^2	23.00	29.15	16.25	13.87	16.02	15.44
p	0.010	0.001	0.006	0.016	0.006	0.008

DV: Dependent variable, X^2: Chi-square value, p: Significance value, pH: Hydrogen potential, Biomass: (g /L), $N\text{-}NO_3^-$: Nitrate (mg/L), $P\text{-}PO_4^-$: Phosphate (mg/L), SOC: Soluble organic C (μg /L), COD: Chemical oxygen demand (mg /L). Equal letters in the columns indicate no significant difference ($p \leq = 0.05$). Bold values indicate the maximum and minimum values of the analyzed indicator.

TABLE VII. VALUES OF INDICATORS USED IN THE PHYSICAL, CHEMICAL AND NUTRITIONAL CHARACTERIZATION OF MICROALGAL BIOMASS.

Indicator	Results	Units	Indicator	Results	Units
M	5.85 ± 0.31	%	K	3000.00 ± 135.39	mg/kg dried microalgae
Ash	12.70 ± 2.51	%	Ca	23900.00 ± 1142.87	mg/kg dried microalgae
OM	87.30 ± 0.15	%	Mg	4600.00 ± 264.75	mg/kg dried microalgae
TOC	50.60 ± 0.91	%	Na	0.24 ± 0.02	mg/kg dried microalgae
TN	8.30 ± 13.80	%	Fe	1.00 ± 0.12	mg/kg dried microalgae
P	2.03 ± 0.11	%	Cu	179.00 ± 3.42	mg/kg dried microalgae
C/N	6.10 ± 0.07	---	Mn	316.00 ± 4.91	mg/kg dried microalgae
$N\text{-}NO_3^-$	198.00 ± 17.42	mg/kg dried microalgae	Zn	303.00 ± 1.59	mg/kg dried microalgae
$N\text{-}NH_4^+$	2506.00 ± 10.26	mg/kg dried microalgae	---	---	---

M: Moisture, Ash, OM: Organic matter, TOC: Total organic C, TN: Total N, C/N: Carbon/Nitrogen ratio, $N\text{-}NO_3^-$: Nitrate, $N\text{-}NH_4^+$: Ammonium, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium, Na: Sodium, Fe: Iron, Cu: Copper, Mn: Manganese, Zn: Zinc.

Soil characterization

According to the physical and chemical characterization of the soil (**Table VIII**), it presented alkaline pH conditions (9.4), low EC ($0.37 < 2.0$ dS/m), and high ESP ($41 > 15\%$). The OM present was considered within the normal range for agricultural soils (4.95%) but with a high C/N ratio (47.9), which indicated an N deficit. Macro- and micronutrients and their various ratios were found in amounts considered low.

C and N mineralization dynamics

Figure 1 shows the results for C and N mineralization dynamics. The evolution of pH (**Fig. 1A**) ranged from 8.09 to 9.16, remaining in an alkaline range in all treatments. There were significant differences between T2, T3, and T4, with respect to T0 during all the C and N mineralization dynamics ($p \leq 0.05$), but no differences between T1 and T0 at 168 h ($p > 0.05$).

The EC indicator (**Fig. 1B**) presented values in the range of 0.37-1.04 dS/m, being T3 the one with the highest EC. Despite the increase in EC observed in T3, in general, the addition of microalgal biomass did not significantly ($p > 0.05$) increase the EC.

The dynamics of C-CO_2 emission (**Fig. 1F**) showed values in the range of 0-240 mg C-CO_2/kg dry soil, with an increase in C-CO_2 observed in all treatments as a function of the application rate and

TABLE VIII. VALUES OF THE INDICATORS APPLIED FOR THE PHYSICAL AND CHEMICAL CHARACTERIZATION OF THE SOIL.

Indicator	Results	Units	Indicator	Results	Units
pH	9.4 ± 0.21		N-NO_3^-	6.05 ± 0.19	mg/kg dry soil
EC	0.37 ± 0.02	dS/m	N-NH_4^+	1.96 ± 0.21	
HC	0.5 ± 0.01	cm/h	N_{min}	8.01 ± 0.20	
H	11.7 ± 2.80	%	P-Olsen	0.13 ± 0.01	mEq/100 g dry soil
$N_{organic}$	98.6 ± 4.50		K	2.6 ± 0.13	
TOC	2.87 ± 0.26		S	0.09 ± 0.02	
OM	4.95 ± 0.55		Ca	18.58 ± 0.67	
TN	0.06 ± 0.02	mg N/kg dry soil	Mg	1.84 ± 0.37	
			Na	16.31 ± 0.07	
C/N	47.9 ± 2.61		Ca/K	7.15 ± 2.84	
BD	1.02 ± 0.03	g/cm^3	Mg/K	0.71 ± 0.03	
Texture	Clay		(Ca+Mg)/K	7.85 ± 0.80	
WHC	31 ± 8.44	%	Ca/Mg	10.10 ± 0.21	
Carbonate	5.19 ± 1.90		Fe	0.02 ± 0.01	mEq/100 g dry soil
ESP	41 ± 1.90		Cu	0.002 ± 0.001	
SAR	16.28 ± 4.30		Mn	0.01 ± 0.01	
CEC	39.4 ± 4.50	mEq/100 g dry soil	Zn	0.07 ± 0.007	
			B	0.22 ± 0.002	

pH: Hydrogen potential, EC: Electric conductivity, HC: Hydraulic conductivity, H: Moisture, $N_{organic}$: Organic N, TOC: Total organic C, OM: Organic matter, TN: Total N, BD: Bulk density, WHC: Water holding capacity, ESP: Exchangeable sodium percentage, SAR: Sodium adsorption ratio, CEC: Cation exchange capacity, N-NO_3^-: Nitrate, N-NH_4^+: Ammonium, N_{min}: Mineral N, P-Olsen: Phosphorus determined by the Olsen method, K: Potassium, S: Sulfur, Ca: Calcium, Mg: Magnesium, Na: Sodium, Ca/K: Calcium/Potassium ratio, Mg/K: Magnesium/Potassium ratio, (Ca+Mg)/K: (Calcium+Magnesium)/Potassium ratio, Ca/Mg: Calcium/Magnesium ratio, Fe: Iron, Cu: Copper, Mn: Manganese, Zn: Zinc, B: Boron.

over time. T0 (control) showed lower C-CO_2 emissions than the rest of the treatments.

The N-NH_4^+ indicator (**Fig. 1D**), showed values in the range of 35.18-1071.92 mg N-NH_4^+/kg dry soil, with an increase in N-NH_4^+ over time for all treatments, except for T0 (1.96 mg N-NH_4^+/kg dry soil). In the same context, T4 and T3 presented higher N-NH_4^+ values at most experimental time points

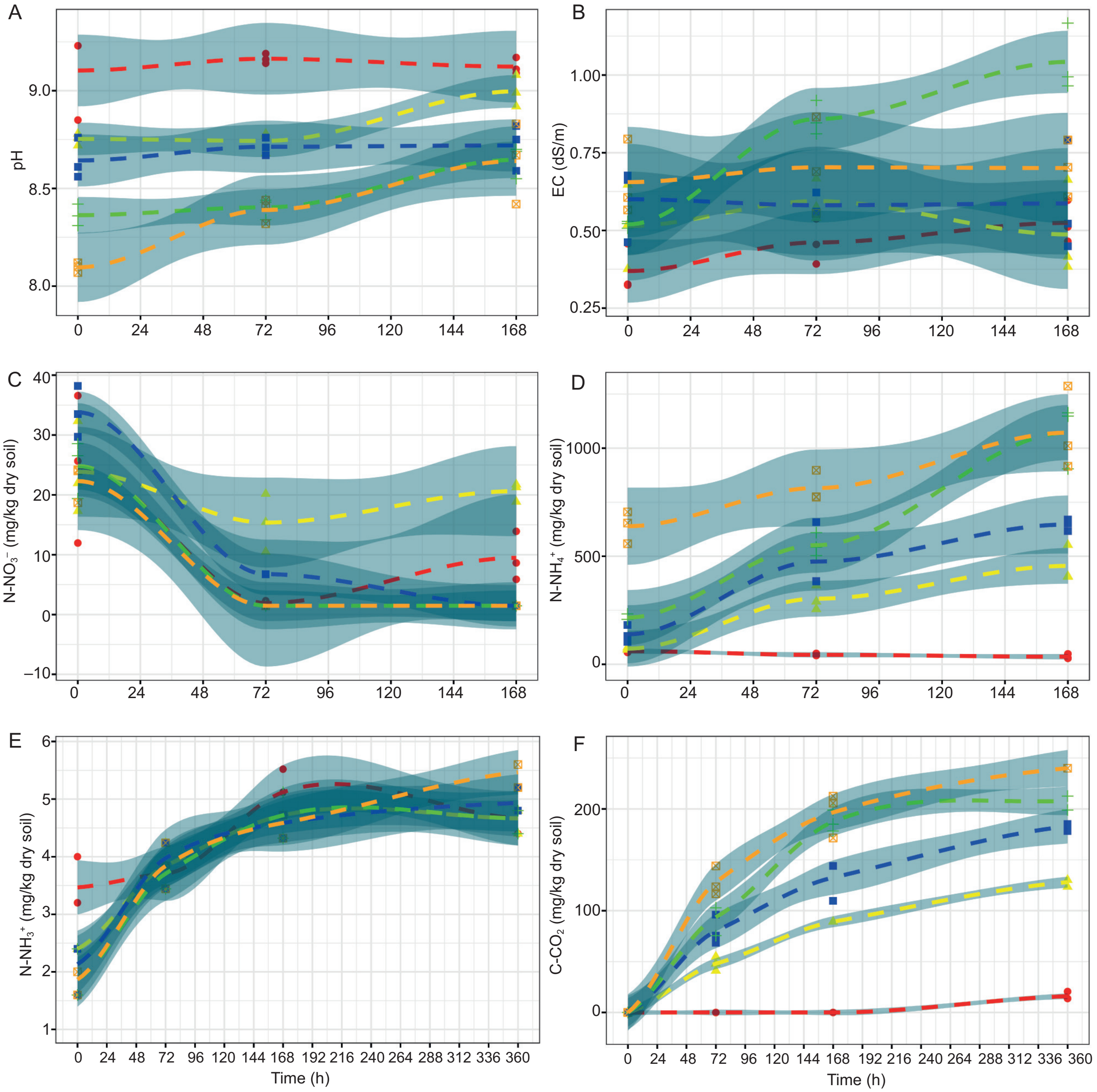

Fig. 1. Response of the physical and chemical indicators (A-F) evaluated during the 360 h mineralization dynamics for the different treatments tested. Red dotted line for T0 (50 g soil + 0 mg microalgae [control]), yellow dotted line for T1 (50 g soil + 500 mg microalgae [25 mg N-NH_4^+/kg dry soil]), blue dotted line for T2 (50 g soil + 1000 mg microalgae [50 mg N-NH_4^+/kg dry soil]), green dotted line for T3 (50 g soil + 2000 mg microalgae [100 mg N-NH_4^+/kg dry soil]), orange dotted line for T4 (50 g soil + 3000 mg microalgae [150 mg N-NH_4^+/kg dry soil]). Gray area refers to standard deviation with 95% confidence interval. A: pH; B: EC (electrical conductivity); C: N-NO_3^-; D: N-NH_4^+; E: N-NH_3^+; F: C-CO_2.

(1071 mg N-NH_4^+/kg dry soil for both). No loss of N-NH_3^+ by volatilization was observed with $p > 0.05$ (3.46-5.46 mg N-NH_3^+/kg dry soil), in **figure 1E**.

Regarding N-NO_3^- dynamics (**Fig. 1C**), values in the range of 33.8-1.45 mg N-NO_3^-/kg dry soil were observed, showing a decrease in N-NO_3^- in all treatments over time. There were no significant differences ($p > 0.05$) between T0 and the other treatments at any of the treatment time points, and the concentration decreased or remained equal in the following order: T1 > T0 > T2 = T3 = T4.

The values of TOC, TN, and C/N are shown in **figure 2**. The TOC results (**Fig. 2A**) were in the range of 0.41-2.95%, and its increase was directly proportional to the amount of microalgal biomass that had been added: T4 > T3 > T2 > T1 > T0. The TN results (**Fig. 2B**) presented values in the range of 508.41-2375.65 mg/kg dry soil, which implied an increase in TN at the beginning of the experiment, which was also directly proportional to the amount of microalgae added: T4 > T3 > T2 > T1 > T0. The variation of C/N (**Fig. 2C**) was in the range of 8.17–-20.44, and the concentration decreased in the following order: T2 > T3 > T1 > T4 > T0, highlighting the absence of significant differences ($p > 0.05$). In all treatments, the C/N ratios were below or close to 24, which is considered optimum for applying organic amendments (Ammar et al. 2022).

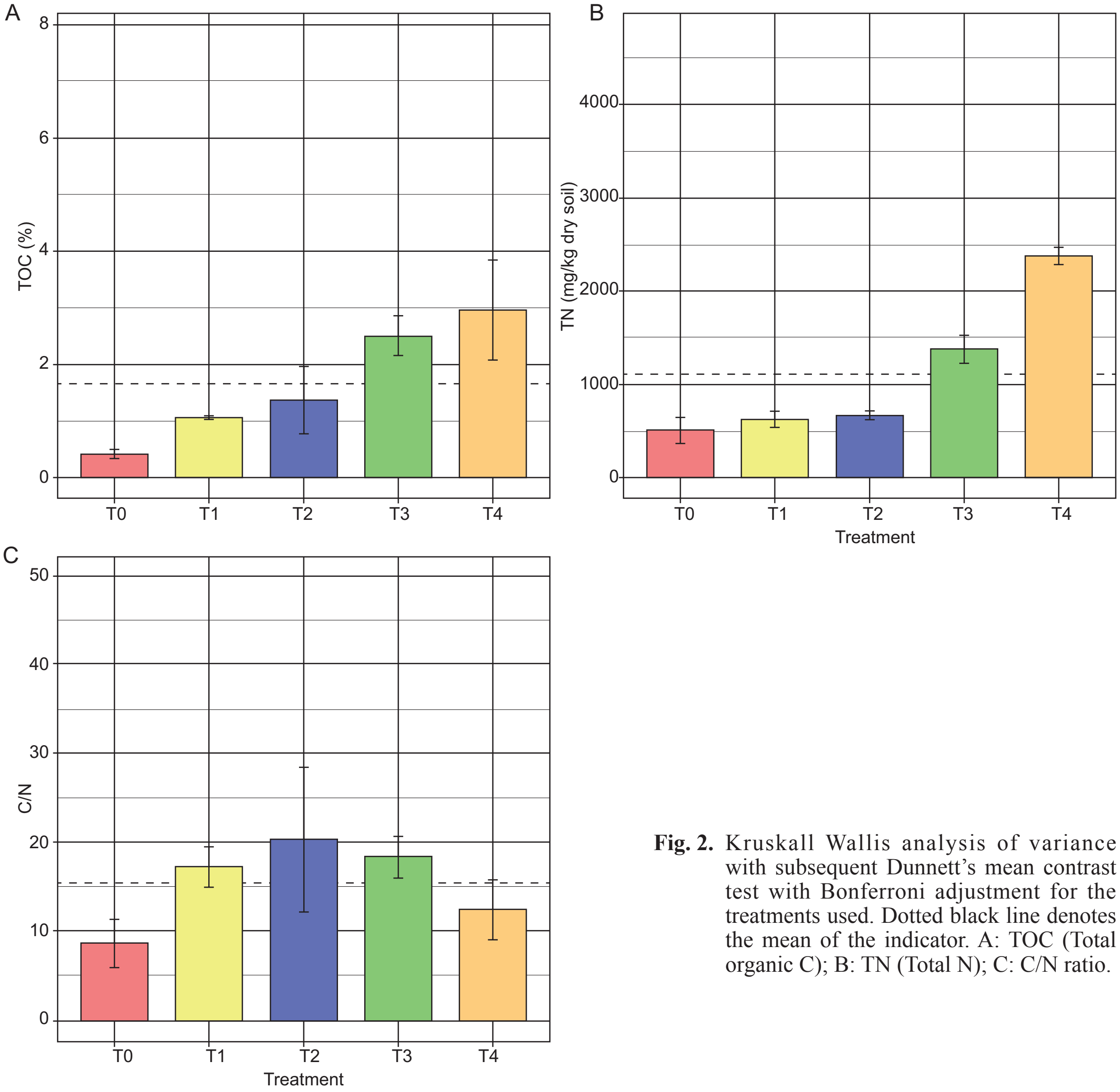

Fig. 2. Kruskall Wallis analysis of variance with subsequent Dunnett's mean contrast test with Bonferroni adjustment for the treatments used. Dotted black line denotes the mean of the indicator. A: TOC (Total organic C); B: TN (Total N); C: C/N ratio.

DISCUSSION

Microalgal biomass production

The production of microalgae in wastewater is of great relevance worldwide due to the high content of nutrients present in wastewater, which facilitates the growth of microalgal consortia. During their growth, these consortia undergo bioremediation processes, metabolizing large amounts of N and P (Brar et al. 2019, Talapatra et al. 2021). However, the physical and chemical characteristics of DWW may vary, and this will influence the production of microalgal biomass. For example, *Chlorella vulgaris* grown in DWW reached 1.2 g/L of biomass in 18 days (Handayani et al. 2020), whereas *Scenedesmus quadricauda* and *Tetraselmis suecica* in DWW reached 0.39 g/L and 0.51 g/L in 12 days, respectively (Daneshvar et al. 2019), and *Chlorella pyrenoidosa* and *Scenedesmus abundans* in DWW reached 0.043 g/L in 312 h and 0.019 g/L in 6 days (Brar et al. 2019). Therefore, the biomass obtained in the present work of 0.8 g/L in 10 days (**Table VI**) is within the range of values reported in previous studies.

Furthermore, during their growth, microalgae consume $N\text{-}NO_3^-$ and $P\text{-}PO_4^-$, which are important compounds to eliminate from wastewater before its final disposal. Regarding $N\text{-}NO_3^-$ removal, values of 89.52% have been reported for *Anabaena ambigua*, 88.91% for *C. pyrenoidosa*, and 84.72% for *S. abundans* after 25 days in DWW (Brar et al. 2019). Concerning $P\text{-}PO_4^-$ removal, values of 87.83% have been reported for *A. ambigua*, 79.02% for *C. pyrenoidosa*, and 86.51% for S. abundans after 25 days in DWW (Brar et al. 2019). In the case of *S. quadricauda* and *T. suecica*, values of 71.20% and 41.63%—respectively—have been reported for the removal of $P\text{-}PO_4^-$ in a period of 12 days (Daneshvar et al. 2019). In the present study, in 10 days the percentage of $N\text{-}NO_3^-$ removal by the microalgal consortium was 79.88% (going from 62.63 to 12.60 mg/L) and the $P\text{-}PO_4^-$ removal was 30.17% (going from 89 to 62.15 mg/L) (**Table VI**). N and P removal has been related to microalgal biomass production because these elements are vital for protein and nucleic acid biosynthesis (Patel et al. 2020). Specifically, microalgae perform the assimilation process of $N\text{-}NO_3^-$, reducing it to $N\text{-}NH_4^+$, which is finally incorporated into amino acids (Daneshvar et al. 2019, Handayani et al. 2020). In addition, P is used to generate compounds such as astaxanthin and polyunsaturated fatty acids through orthophosphate assimilation (Brar et al. 2019).

Another important indicator is the chemical oxygen demand (COD). COD removal is an indicator directly related to the mixotrophic cultivation of microalgae, where, apart from utilizing $C\text{-}CO_2$, they can metabolize more organic compounds (Brar et al. 2019). In other studies, Tetraselmis indica showed 77.03% COD removal in 10 days (Talapatra et al. 2021) and C. vulgaris 92% removal in 10 days (Handayani et al. 2020). The microalgal consortium analyzed in this study presented a percentage of COD removal between the values previously mentioned, with 85.8% (going from 674 to 95.5 mg/L) in 10 days as well (**Table VI**).

P and N contents in microalgal biomass have been reported to be 1.3% and 6.6%, respectively (Lage et al. 2018). In the case of the microalgal biomass obtained in this study, the values of the indicators N and P exceeded what had been reported by 0.73% and 1.70%, respectively (**Table VII**). Likewise, there are reports of organic fertilizers with 0.7% P and 1.09% N (Mahapatra et al. 2018). Therefore, the use of microalgal biomass as an organic amendment would be a good alternative, since it supplies the soil with a higher amount of P and N than an organic fertilizer.

Finally, when applying microalgal biomass to soils, high contents of C and N and a low C/N ratio of < 25 (**Table VII**), can favor microbial activity (Liyanage et al. 2022), which is essential for the improvement of soil quality, increasing the C of microbial biomass (CMB) and improving the nutrient cycling and enzyme production (Alvarez et al. 2021). Moreover, the contents of macro- and micronutrients found in this study (**Table VII**) suggests that microalgal biomass could be a complementary amendment to traditional chemical fertilizers, since the latter do not include this type of micronutrients in their formulation (Yilmaz and Sönmez 2017).

Sampled soil and dynamics of C and N mineralization

The sampled soil in this study could be classified as sodic, with values corresponding to: EC < 4 dS/m, ESP > 15%, SAR > 13 (Osman 2018, Brusseau et al. 2019) (**Table VIII**). Such values of EC, ESP, and SAR compromise soil structure, reducing its WHC, as well as its ability to supply nutrients to microorganisms and crops. Besides, the growth and development of microorganisms and plants can be affected by the high C/N ratio (Brusseau et al. 2019, Medina-Herrera et al. 2020). In sodic soils, a high Na^+ concentration increases osmotic stress in plants and microorganisms, affecting both systems' correct development and activities (Srivastava 2020, Noori et al. 2021). In addition, a deficit of nutrients, such as Mg, K, P, Fe, and B (**Table VIII**), can affect the growth of the plant cover, preventing it from carrying out essential functions such as photosynthesis (Villegas Hurtado et al. 2016). In this regard, organic amendments can solve this deficit due to their macro- and micronutrient contents, which are available to improve soil fertility (Sulok et al. 2021). Concerning the results of the dynamics of C and N mineralization, a lower pH (**Fig. 1A**) in the treatments with respect to T0 can be explained by their high microbial activity, since microbes produce organic acids during the mineralization of additional sources of C and N due to their metabolism (Leogrande and Vitti 2019). The increase in pH at 168 h in treatments T2 and T4 was related to the mineralization processes of nitrogenous compounds, carbonates, and silicates (Castro-Alonso et al. 2019, Oviedo 2020). When the initial pH of the soil is alkaline, it is beneficial if the applied amendment does not promote an increase in soil pH, an effect reported in the application of compost, *Gliricidia*, charged biochar, tea waste, and raw biochar (Liyanage et al. 2022), as well as organic amendments made from residues of *Phaseolus vulgaris* L. and *Cajanus cajan* (Abera et al. 2012), all of which increased the soil pH. Likewise, the importance of avoiding an increase in soil pH lies in the fact that alkaline values (pH > 8) promote N-NH_3^+ release and inactivation of enzymes, disfavoring the activity of actinomycetes and bacteria (whose ideal pH is 6.5-8), which is important in nutrient cycling (Zhao et al. 2018, Neina, 2019). In contrast, not increasing the pH favors the mineralization of the added OM, as well as that of the soil, thanks to the breaking of its bonds with clays and its high solubility, thus increasing the mineral N and the release of C-CO_2 (Neina 2019).

The values of the EC indicator increased, possibly due to the rapid decomposition of OM and the release of salts contained in the added microalgal biomass. The increase in EC has been considered a disadvantage in organic amendments (Noori et al. 2021); therefore, monitoring this indicator is suggested for applying microalgal biomass as an amendment to not considerably affect the soil with the effects of salinity. In this regard, the increase in EC concentration in T3 (**Fig. 1B**) could be an atypical form of accumulation or release of salts in the soil sample. However, it remained at an EC value < 4 dS/m, which is expected not to affect the mineralization of added OM (Osman 2018). With respect to C-CO_2 release (**Fig. 1F**), the addition of microalgal biomass biostimulated microbial development and metabolism, which could be observed in the decrease in N-NO_3^- and the increase in C-CO_2 production. This could probably lead to an increase in soil quality indicators of the microbial biomass, such as C and N, microbial diversity, and enzymatic production (Alvarez et al. 2021).

Regarding N-NH_4^+, the results shown in **figure 1D** suggest that the addition of microalgal biomass promoted ammonification processes. The added N could be exploited by microorganisms present in the soil for the development of cellular structures and production of intra- and extracellular enzymes (Liyanage et al. 2022). As for N-NH_3^+ volatilization (**Fig. 1E**), it involves chemical reactions where N-NH_4^+—in the presence of high concentrations of OH^- ions—releases protons, resulting in the production of N-NH_3^+ and water molecules. Such reactions are maintained in equilibrium when the pH is neutral, however, at an alkaline pH and above, the release of N-NH_3^+ is favored, with maximum volatilization peaks at pH 8.6; in contrast, when the pH decreases, the opposite reaction is favored (Neina 2019). On the other hand, the organic N applied and mineralized (via production of N-NH_4^+ and N-NO_2^-) into N-NO_3^- (**Fig. 1C**) can be quickly taken advantage of by the microorganisms through either biotic immobilization or abiotic immobilization, thus avoiding its loss via leaching processes, which explains the decrease in N-NO_3^- in the period evaluated in this study. Similarly, according to the pH values obtained (9.16-8.09), N-NO_3^- could also be used by denitrifying bacteria, which grow at pH values of 6.5 to 8.3, and from this compound, they can release N_2 or accumulating NO_2^- and producing N_2O (Neina 2019). In the same context, an alkaline pH in the soil, if there is no increase in N-NO_3^- concentration over time during mineralization dynamics, could result in low activity by nitrifying microorganisms (Zhao et al. 2018).

For basal respiration (**Fig. 2**), as well as for C and mineralizable N concentrations, it was confirmed that the values of these indicators increase in soils treated with microalgal biomass, which corresponds to what had been reported by Aytenew and Bore (2020). The advantage of applying microalgal biomass to the soil was thus highlighted because C and N appear in bioavailable forms to be used immediately by microorganisms, a situation sometimes not observed with other amendments such as composts, in which C can be found in the form of lignocellulose or molecules that are difficult to degrade and be used by microorganisms (Sulok et al. 2021). Moreover, the utilization of the N applied to the soil by the microbial biomass is evidenced by the decrease of N-NO_3^-, the increase of N-NH_4^+ and the release of C-CO_2 (**Fig. 1**).

Since the indicators analyzed during the short-term C and N mineralization dynamics did not present a normal distribution, non-parametric analyses

(Spearman correlation and Friedman analyses) were performed. **Figure 3** shows the Spearman correlation analysis, with the highest positive association between N-NH$_4^+$ and C-CO$_2$ (0.85), and the lowest positive association for N-NH$_3^+$ and N-NH$_4^+$ (0.26), the latter confirming the reversible chemical reaction between both compounds. The existence of a positive correlation of 0.43 between pH and N-NH$_3^+$—indicators which are noteworthy—would indicate that, at a higher pH, soils tend to lose N by volatilization, a characteristic of sodic soils. The fact that the addition of microalgal biomass did not increase the pH reduced the N loss from the soil by volatilization. In addition, there are positive correlations between the C-CO$_2$ indicator with the N-NH$_4^+$ and N-NH$_3^+$ indicators, and a negative one with the N-NO$_3^-$ indicator, which would support what was mentioned in previous paragraphs about possible ammonification and denitrification processes carried out by the microorganisms present in the soil (Liyanage et al. 2022), since both microbiological processes are confirmed by the negative relationship (0.56) between N-NH$_4^+$ and N-NO$_3^-$. This negative relationship also confirms the absence of nitrifying bacteria mentioned in previous paragraphs and explains why N-NH$_4^+$ is not being transformed into N-NO$_3^-$, and thus the transformation rates between the two indicators differ.

A Friedman analysis was performed for the various treatments with respect to the indicators analyzed

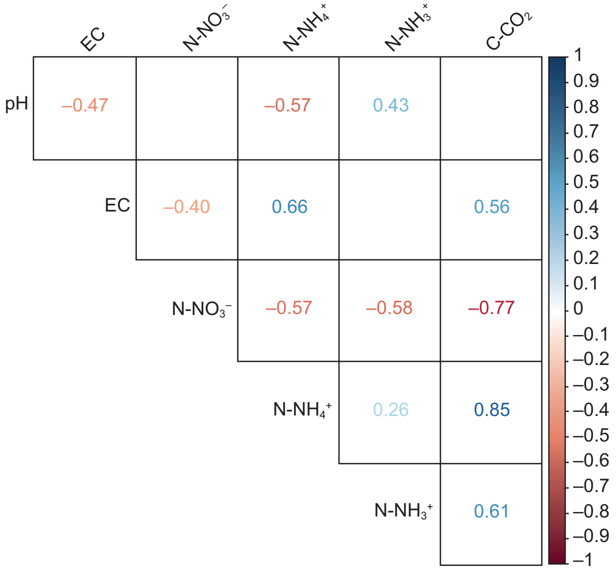

Fig. 3. Spearman correlation matrix for analyzed indicators (+1: Perfect positive association, -1: Perfect negative association, 0: No association). EC: electrical conductivity.

against time and the results of this analysis are shown in **figure 4**. An increase in the concentration of the TOC, TN, and C/N indicators can be observed as a function of the treatment. It is worth noting that T0 presented a very marked area in reference to the pH and N-NH$_3^+$ indicators; as the dose of microalgal biomass added increases, the area expands towards the other indicators. This would indicate an adjustment in relation to the nutritional conditions of the soil, allowing for the development of microorganisms and nutrient cycling processes (Alvarez et al. 2021).

Likewise, as can be seen in **figure 4**, all treatments presented significant differences in pH; where the highest alkalinity was observed in T0 and the pH decreased as a function of the dose of microalgal biomass applied. The above result could be related to the production of organic acids by microbial activity, which was favored by the increase in the application dose of the organic amendment (Leogrande and Vitti 2019). Also, the initial concentration of Ca^{2+} present in the microalgal biomass could help to lower the pH by replacing part of the H$^+$ present in the soil solution, as well as reacting with bicarbonates (Ca(HCO$_3$)$_4$), precipitating calcium carbonates (CaCO$_3$), and releasing H$^+$ (Noori et al. 2021).

There were differences between T1 and T2 when compared to the other treatments in terms of N-NO$_3^-$, showing a tendency to decrease with increasing doses of organic amendment application (**Fig. 4**). This suggests that, due to the low concentration of available N in the soil, microorganisms could be using the available N-NO$_3^-$ and maintaining the N immobilization processes by the microbial biomass (Leogrande and Vitti 2019, Liyanage et al. 2022). The above is supported by the Spearman correlation matrix shown in **figure 3**, specifically by the negative association between N-NO$_3^-$ and the N-NH$_4^-$ and C-CO$_2$ indicators.

In the case of N-NH$_4^-$ (**Fig. 4**), it was observed that the greater the addition of microalgal biomass, the greater the production of this compound. Its release through ammonification processes allows it to be absorbed by plants via a simple route with low energy consumption (Liyanage et al. 2022). It should be noted that microalgal biomass has been reported to be a quick-use amendment for crops. Furthermore, there was no significant difference in N volatilization via N-NH$_3^+$ production, which prevented the release of greenhouse gasses (Chávez-García and Siebe 2019).

Finally, in **figure 4**, the increase in C-CO$_2$ concentration as a function of the increase in the application rate of microalgal biomass could be due to the biostimulation of microbial activity in soils when organic

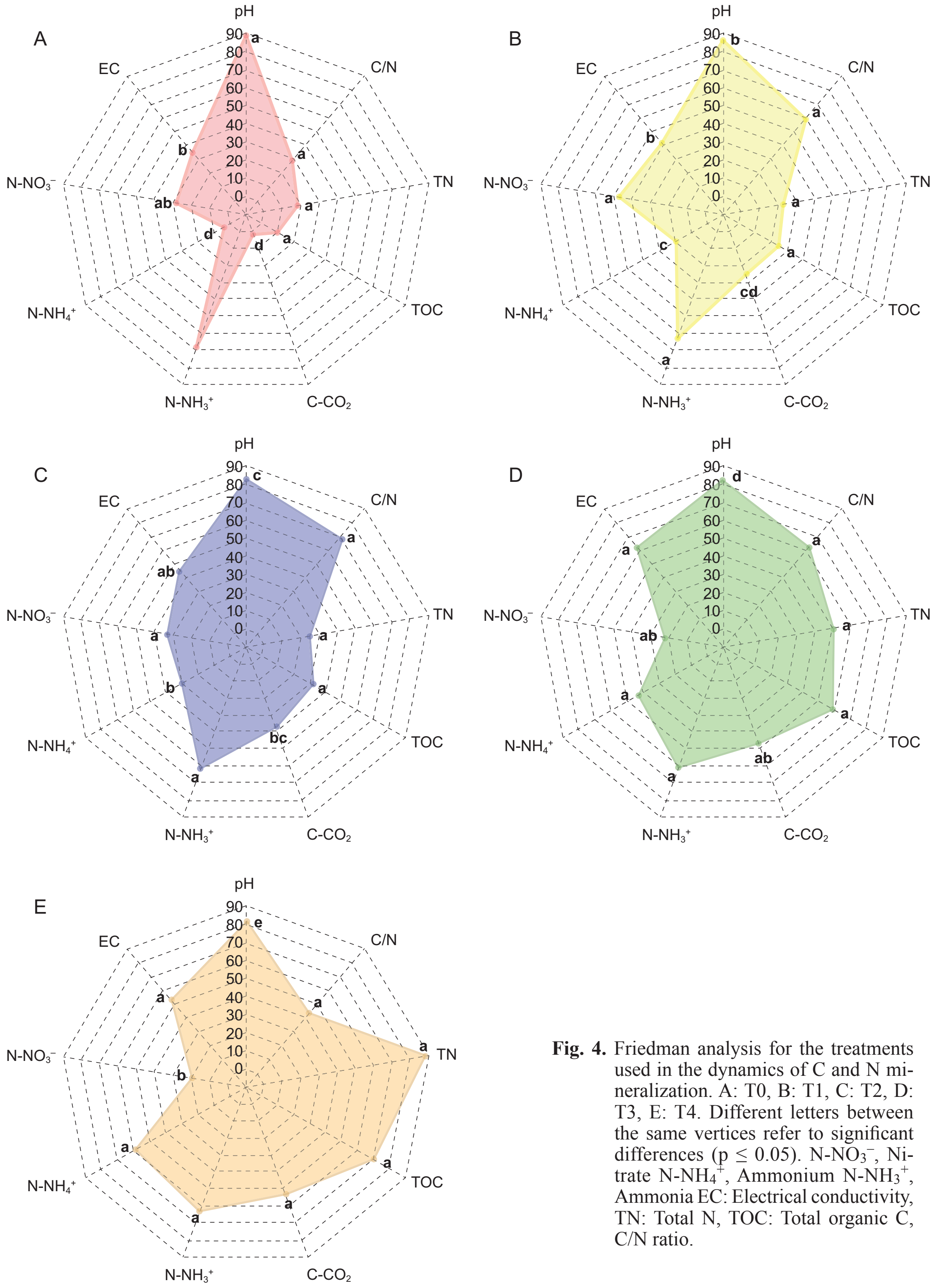

Fig. 4. Friedman analysis for the treatments used in the dynamics of C and N mineralization. A: T0, B: T1, C: T2, D: T3, E: T4. Different letters between the same vertices refer to significant differences ($p \leq 0.05$). N-NO$_3^-$, Nitrate N-NH$_4^+$, Ammonium N-NH$_3^+$, Ammonia EC: Electrical conductivity, TN: Total N, TOC: Total organic C, C/N ratio.

amendments are applied as sources of rich and more available C and N (Medina-Herrera et al. 2020). This biostimulation is the basis for improving soil conditions because it contributes to nutrient cycling, since the microbial biomass generated will become part of the available OM and the C from the microbial biomass will be available for use (Alvarez et al. 2021).

CONCLUSIONS

Since microalgal biomass is a source of N and C, it is viable to use it as a short-term organic amendment to improve the microbial activity in soils with sodicity problems on a laboratory scale. Moreover, this non-conventional organic amendment is a low-cost and environmentally friendly alternative to traditional soil remediation methodologies because wastewater is taken advantage of to produce microalgae that will not only be used to improve the conditions of sodic soils but, during their growth, they will also help to reduce the contamination of the wastewater in which they are cultivated. The treatments in this study established a direct relationship between the applied doses of dry microalgal biomass and the increase in soil concentrations of the indicators CO_2, N_{min}, TOC, and TN. For this reason, this investigation may serve as the foundations for further field research on the benefits of adding microalgal biomass to sodic soils, benefits which in this laboratory study have been reflected in the release of $C\text{-}CO_2$ and C and N sources.

ACKNOWLEDGMENTS

To the Consejo Nacional de Ciencia y Tecnología (CONACyT) for its support with the grant awarded as part of the calls for proposals “Estancias Posdoctorales por México modalidades 1 y 2” and “Estancias Posdoctorales por México 2022(1)”.

REFERENCES

Abera G., Wolde-Meskel E. and Bakken L.R. (2012). Carbon and nitrogen mineralization dynamics in different soils of the tropics amended with legume residues and contrasting soil moisture contents. Biology and Fertility of Soils 48 (1), 51-66. https://doi.org/10.1007/s00374-011-0607-8

Alef K. and Nannipieri P. (1995). Methods in applied soil microbiology and biochemistry. Elsevier Inc. London, UK, 576 pp. https://doi.org/10.1016/B978-0-12-513840-6.X5014-9

Alvarez A.L., Weyers S.L., Goemann H.M., Peyton B.M. and Gardner R.D. (2021). Microalgae, soil, and plants: A critical review of microalgae as renewable resources for agriculture. Algal Research 54, 102200. https://doi.org/10.1016/j.algal.2021.102200

Ammar E.E., Aioub A.A.A., Elesawy A.E., Karkour A.M., Mouhamed M.S., Amer A.A. and EL-Shershaby N.A. (2022). Algae as bio-fertilizers: Between current situation and future prospective. Saudi Journal of Biological Sciences 29 (5), 3083-3096. https://doi.org/10.1016/j.sjbs.2022.03.020

Ankeny M.D., Ahmed M., Kaspar T.C. and Horton R. (1991). Simple field method for determining unsaturated hydraulic conductivity. Soil Science Society of America Journal 55 (2), 467-470. https://doi.org/10.2136/sssaj1991.03615995005500020028x

Aytenew M. and Bore G. (2020). Effects of organic amendments on soil fertility and environmental quality: A review. Journal of Plant Sciences 8 (5), 112-119. https://doi.org/10.11648/j.jps.20200805.12

Baird R.B., Eaton A.D. and Rice E.W. (2017). Standard methods for the examination of water and wastewater. 23rd ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington D.C., USA, 1796 pp.

Barsanti L. and Gualtieri P. (2014). Algae: Anatomy, biochemistry, and biotechnology. 2nd ed. CRC Press, Boca Raton, USA, 361 pp. https://doi.org/10.1201/b16544

Bedolla-Rivera H.I., Xochilt Negrete-Rodríguez M. de la L., Medina-Herrera M. del R., Gámez-Vázquez F.P., Álvarez-Bernal D., Samaniego-Hernández M., Gámez-Vázquez A.J. and Conde-Barajas E. (2020). Development of a soil quality index for soils under different agricultural management conditions in the central lowlands of Mexico: Physicochemical, biological and ecophysiological indicators. Sustainability 12 (22), 9754. https://doi.org/10.3390/su12229754

Beltrán-Hernández R.I., Luna-Guido M.L. and Dendooven L. (2007). Emission of carbon dioxide and dynamics of inorganic N in a gradient of alkaline saline soils of the former lake Texcoco. Applied Soil Ecology 35 (2), 390-403. https://doi.org/10.1016/j.apsoil.2006.07.005

Black C.A. (1965). Method of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy: Soil Science Society of America, Madison, USA, 160 pp.

Blake G.R. and Hartge K.H. (1986). Particle density. In: Methods of soil analysis: Part 1 Physical and mineralogical methods, 5.1. 2nd ed. (A. Klute, Ed.). American Society of Agronomy: Soil Science Society of America, Madison, USA, pp. 377-382. https://doi.org/10.2136/sssabookser5.1.2ed.c14

Bouyoucos G.J. (1962). Hydrometer method improved for making particle size analyses of soils. Agronomy Journal 54 (5), 464-465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Brar A., Kumar M. and Pareek N. (2019). Comparative appraisal of biomass production, remediation, and bioenergy generation potential of microalgae in dairy wastewater. Frontiers in Microbiology 10, 678. https://doi.org/10.3389/fmicb.2019.00678

Bremner J.M. (1996). Nitrogen-total. In: Methods of soil analysis: Part 3 Chemical methods, 5.3. (D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston and M.E. Sumner, Eds.). American Society of Agronomy: Soil Science Society of America, Madison, USA, pp. 1085-1121. https://doi.org/10.2136/sssabookser5.3.c37

Brusseau M.L., Glenn E.P. and Pepper I.L. (2019). Reclamation and restoration of disturbed systems. In: Environmental and pollution science. 3rd ed. (M.L. Brusseau, I.L. Pepper and C.P. Gerba, Eds.). Elsevier Inc., London, UK, pp. 355-376. https://doi.org/10.1016/B978-0-12-814719-1.00020-3

Castro-Alonso M.J., Montañez-Hernandez L.E., Sanchez-Muñoz M.A., Macias Franco M.R., Narayanasamy R. and Balagurusamy N. (2019). Microbially induced calcium carbonate precipitation (MICP) and its potential in bioconcrete: Microbiological and molecular concepts. Frontiers in Materials 6, 126. https://doi.org/10.3389/fmats.2019.00126

Chaney R.C., Slonim, S.M. and Slonim S.S. (1982). Determination of calcium carbonate content in soils. In: Geotechnical properties, behavior, and performance of calcareous soils. (K.R. Demars and R.C. Chaney, Eds.). ASTM International, West Conshohocken, PA, pp. 3-15. https://doi.org/10.1520/STP28907S

Chatterjee A., Singh S., Agrawal C., Yadav S., Rai R. and Rai L.C. (2017). Role of algae as a biofertilizer. In: Algal green chemistry. (R.P. Rastogi, D. Madamwar and A. Pandey, Eds.). Elsevier, Amsterdam, Netherlands, pp. 189-200. https://doi.org/10.1016/B978-0-444-63784-0.00010-2

Chávez-García E. and Siebe C. (2019). Rehabilitation of a highly saline-sodic soil using a rubble barrier and organic amendments. Soil and Tillage Research 189, 176-188. https://doi.org/10.1016/j.still.2019.01.003

Conde E., Cardenas M., Ponce-Mendoza A., Luna-Guido M.L., Cruz-Mondragón C. and Dendooven L. (2005). The impacts of inorganic nitrogen application on mineralization of ^{14}C-labelled maize and glucose, and priming effect in saline-alkaline. Soil Biology and Biochemistry 37 (4), 681-691. https://doi.org/10.1016/j.soilbio.2004.08.026

Cook S., Peacock M., Evans C.D., Page S.E., Whelan M.J., Gauci V. and Kho L.K. (2017). Quantifying tropical peatland dissolved organic carbon (DOC) using UV-visible spectroscopy. Water Research 115, 229-235. https://doi.org/10.1016/j.watres.2017.02.059

Cottenie A. (1980). Soil and plant testing as a basis of fertilizer recommendations. FAO soils bulletin, 38/2. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 64-65.

Cristóbal-Acevedo D., Álvarez-Sánchez M.E., Hernández-Acosta E., Améndola-Massiotti R., Cristóbal-Acevedo D., Álvarez-Sánchez M.E., Hernández-Acosta E. and Améndola-Massiotti, R. (2011). Concentración de nitrógeno en suelo por efecto de manejo orgánico y convencional. Terra Latinoamericana 29 (3), 325-332. https://www.terralatinoamericana.org.mx/index.php/terra/article/view/1181/1408

Daneshvar E., Zarrinmehr M.J., Koutra E., Kornaros M., Farhadian O. and Bhatnagar A. (2019). Sequential cultivation of microalgae in raw and recycled dairy wastewater: Microalgal growth, wastewater treatment, and biochemical composition. Bioresource Technology 273, 556-564. https://doi.org/10.1016/j.biortech.2018.11.059

Datta A., Setia R., Barman A., Guo Y. and Basak N. (2019). Carbon dynamics in salt-affected soils. In: Research developments in saline agriculture (J.C. Dagar, R.K. Yadav and P.C. Sharma, Eds.). Springer, Singapore, Singapore, pp. 369-389. https://doi.org/10.1007/978-981-13-5832-6_12

Díez López J.A. and Antonio J. (1999). Optimización de la fertilización nitrogenada: Procedimientos de análisis del suelo, toma de muestra y elección del tipo de fertilizante. Edafología 6, 73-84. https://digital.csic.es/handle/10261/2156

Falciani R., Novaro E., Marchesini M. and Gucciardi M. (2000). Multi-element analysis of soil and sediment by ICP-MS after a microwave-assisted digestion method. Journal of Analytical Atomic Spectrometry 15 (5), 561-565. https://doi.org/10.1039/b000742k

García-Gozalbes C.C., Arbib Z. and Perales-Vargas-Machuca J.A. (2015). Cinéticas de crecimiento y consumo de nutrientes de microalgas en aguas residuales urbanas con diferentes niveles de tratamiento. Tecnología y Ciencias del Agua 6 (1), 49-68. https://www.revistatyca.org.mx/ojs/index.php/tyca/article/view/1207

Gougoulias N., Papapolymerou G., Karayannis V., Spiliotis X. and Chouliaras N. (2018). Effects of manure enriched with algae chlorella vulgaris on soil chemical properties. Soil and Water Research 13 (1), 51-59. https://doi.org/10.17221/260/2016-SWR

Hach (1999). Wastewater and biosolids analysis manual. Digestion and selected methods for determining metals, minerals, and other related parameters. Hach Company [online]. https://www.hach.com/asset-get.download.jsa?id=7639984470 01/10/2022

Hach (2015). Hach Method 10267: Spectrophotometric measurement of total organic carbon (TOC) in finished drinking water. Hach Company [online]. https://www.hach.com/asset-get.download-en.jsa?id=52145150104 01/10/2022

Handayani T., Mulyanto A., Priyanto F.E. and Nugroho R. (2020). Utilization of dairy industry wastewater for nutrition of microalgae Chlorella vulgaris. Journal of Physics: Conference Series 1655, 012123. https://doi.org/10.1088/1742-6596/1655/1/012123

Hendrickx J.M.H., Das B., Corwin D.L., Wraith J.M. and Kachanoski R.G. (2002). Solute content and concentration - indirect measurement of solute concentration - nonintrusive electromagnetic induction. In: Methods of soil analysis: Part 4 (J.H. Dane and G.C. Topp, Eds.). Soil Science Society of America, Inc., Madison, USA. pp. 1275-1282. https://doi.org/10.2136/sssabookser5.4

ISO (2018). ISO 18400-104:2018. Soil quality-sampling-part 104: Strategies. International. Organization for Standardization. Geneva, Switzerland, 131 pp.

Keeney D.R. and Nelson D.W. (1983). Nitrogen-Inorganic forms. In: Methods of soil analysis: Part 2 Chemical and microbiological properties, 9.2.2, 2nd ed. (A.L. Page, Ed.). American Society of Agronomy, Inc., Soil Science Society of America, Madison, USA, pp. 643-698. https://doi.org/10.2134/agronmonogr9.2.2ed.c33

Lage S., Gojkovic Z., Funk C. and Gentili F. (2018). Algal biomass from wastewater and flue gases as a source of bioenergy. Energies 11 (3), 664. https://doi.org/10.3390/en11030664

Leogrande R. and Vitti C. (2019). Use of organic amendments to reclaim saline and sodic soils: A review. Arid Land Research and Management 33 (1), 1-21. https://doi.org/10.1080/15324982.2018.1498038

Liyanage M., Hanafi M.M., Sulaiman M.F., Ismail R., Gunaratne G., Dharmakeerthi S., Rupasinghe G. and Mayakaduwa A. (2022). Consequences of nitrogen mineralization dynamics for soil health restoration of degraded tea-growing soil using organic amendments. Chilean Journal of Agricultural Research 82 (2), 199-210. http://dx.doi.org/10.4067/S0718-58392022000200199

Mahapatra D.M., Chanakya H.N., Joshi N.V., Ramachandra T.V. and Murthy G.S. (2018). Algae-based biofertilizers: A biorefinery approach. In: Microorganisms for green revolution. Microorganisms for sustainability. (D.G. Panpatte, Y.K. Jhala, H.N. Shelat and R.V. Vyas, Eds.). Springer, Singapore, Singapore, pp. 177-196. https://doi.org/10.1007/978-981-10-7146-1_10

Medina-Herrera M. del R., Rodríguez M. de la L.X.N., Vázquez F.P.G., Bernal D.Á. and Conde-Barajas E. (2020). La aplicación de lodos residuales afecta, a corto plazo, la biomasa microbiana y su actividad en suelos sódicos. Revista Internacional de Contaminación Ambiental 36 (3), 577-591. https://doi.org/10.20937/RICA.53425

Neina D. (2019). The role of soil pH in plant nutrition and soil remediation. Applied and Environmental Soil Science 2019, 1-9. https://doi.org/10.1155/2019/5794869

Nichols K. (2020). Microalgae as a beneficial soil amendment. Myland Company [online] https://myland.ag/wp-content/uploads/MyLand-Microalgae-White-Paper-nov2020.pdf 01/10/2022

Noori Z., Delavar M.A., Safari Y. and Alavi-Siney S.M. (2021). Reclamation of a calcareous sodic soil with combined amendments: Interactive effects of chemical and organic materials on soil chemical properties. Arabian Journal of Geosciences 14 (3), 166. https://doi.org/10.1007/s12517-021-06485-w

Osman K.T. (2018). Saline and sodic soils. In: Management of soil problems (K.T. Osman, Ed.). Springer International Publishing, Cham, Switzerland. pp. 255-298. https://doi.org/10.1007/978-3-319-75527-4_10

Oviedo C.V.R. (2020). Precipitación de carbonatos inducida microbiológicamente como alternativa para remediar entornos naturales contaminados con metales y metaloides tóxicos. M. Sc. Thesis. Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Química. Bogotá, Colombia, 122 pp.

Olsen S.R. and Sommers L.E. (1982). Phosphorous. In: Methods of soil analysis. Part, 2. Chemical and microbiological properties. 2nd ed. (A.L. Page, R.H. Miller and D.R. Keeney, Eds.). American Society of Agronomy, Inc., Soil Science Society of America, Madison, USA, pp. 403-430.

Page A.L. Miller, R.H. Keeney, D.R. Baker, D.E. Ellis, and R. Rhoades J.D. (1982). Methods of soil analysis. American Society of Agronomy, Inc., Soil Science Society of America, Madison, USA. 1159 pp.

Patel A.K., Joun J. and Sim S.J. (2020). A sustainable mixotrophic microalgae cultivation from dairy wastes for carbon credit, bioremediation, and lucrative biofuels. Bioresource Technology 313, 123681. https://doi.org/10.1016/j.biortech.2020.123681

RCT (2019) R: A language and environment for statistical computing. R Core Team. R Foundation for Statistical Computing [online] https://www.R-project.org/ 01/10/2022

Read J.W. and Ridgell R.H. (1922). On the use of the conventional carbon factor in estimating soil organic matter. Soil Science 13 (1), 1-6. https://journals.lww.com/soilsci/citation/1922/01000/on_the_use_of_the_conventional_carbon_factor_in.1.aspx

Romano-Armada N., Yañez-Yazlle M.F., Irazusta V.P., Rajal V.B. and Moraga N.B. (2020). Potential of bioremediation and PGP traits in streptomyces as strategies for bio-reclamation of salt-affected soils for agriculture. Pathogens 9 (2), 117. https://doi.org/10.3390/pathogens9020117

Schulte E.E. and Hopkins B.G. (1996). Estimation of soil organic matter by weight loss-on-ignition. In: Soil organic matter: Analysis and interpretation. (F.R. Magdoff, M.A. Tabatabai and E.A. Hanlon Jr., Eds.). American Society of Agronomy, Inc., Soil Science Society of America, Madison, USA, pp. 21-31. https://doi.org/10.2136/sssaspecpub46.c3

Simonne A.H., Simonne E.H., Eitenmiller R.R., Mills H.A. and Cresman C.P. III. (1997). Could the Dumas method replace the Kjeldahl digestion for nitrogen and crude protein determinations in foods? Journal of the Science of Food and Agriculture 73 (1), 39-45. https://doi.org/10.1002/(SICI)1097-0010(199701)73:1<39::AID-JSFA717>3.0.CO;2-4

Srivastava N. (2020). Reclamation of saline and sodic soil through phytoremediation. In: Environmental concerns and sustainable development. Volume 2: Biodiversity, soil and waste management. (V. Shukla and N. Kumar Eds.). Springer Singapore, Singapore, pp. 279-306. https://doi.org/10.1007/978-981-13-6358-0_11

Sulok K.M.T., Ahmed O.H., Khew C.Y., Zehnder J.A.M., Jalloh M.B., Musah A.A. and Abdu A. (2021). Chemical and biological characteristics of organic amendments produced from selected agro-wastes with potential for sustaining soil health: A laboratory assessment. Sustainability 13 (9), 4919. https://doi.org/10.3390/su13094919

Talapatra N., Gautam R., Mittal V. and Ghosh U.K. (2021). A comparative study of the growth of microalgae-bacteria symbiotic consortium with the axenic culture of microalgae in dairy wastewater through extraction and quantification of chlorophyll. Materials Today: Proceedings. 80, 2268-2273. https://doi.org/10.1016/j.matpr.2021.06.227

Terkula Iber B., Azman Kasan N., Torsabo D. and Wese Omuwa J. (2022). A review of various sources of chitin and chitosan in nature. Journal of Renewable Materials 10 (4), 1097-1123. https://doi.org/10.32604/jrm.2022.018142

Thomas G.W. (1996). Soil pH and soil acidity. In: Methods of soil analysis: Part 3 chemical methods, 5.3. (D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston and M. E. Sumner, Eds.). American Society of Agronomy, Inc., Soil Science Society of America, Madison, USA, pp. 475-490. https://doi.org/10.2136/sssabookser5.3.c16

USDA (2017). Soil survey manual. Soil Science Division. United States Department of Agriculture Agriculture. Handbook No. 18, Soil Survey Staff. Washington D.C., USA, 603 pp.

USEPA (2001). EPA-821-R-01-015. Total, fixed, and volatile solids in water, solids, and biosolids. United States Environmental Protection Agency. Method 1684. Washington D.C., USA, 16 pp.

Villegas Hurtado D.A., Dussán Currea S.L. and Miranda Lasprilla D. (2016). Efecto de la deficiencia de N, P, K, Mg, Ca y B sobre la acumulación y distribución de la masa seca en plantas de guayaba (Psidium guajava L.) var. Ica Palmira II en fase de vivero. Revista Colombiana de Ciencias Hortícolas 10 (1), 40-52. https://doi.org/10.17584/rcch.2016v10i1.4277

Walkley A. and 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.

Wijitkosum S. (2020). Applying rice husk biochar to revitalize saline sodic soil in Khorat Plateau area - A case study for food security purposes. In: Biochar applications in agriculture and environment management. (J.S. Singh and C. Singh, Eds.). Springer International Publishing, Cham, Switzerland, pp. 1-31. https://doi.org/10.1007/978-3-030-40997-5_1

Yilmaz E. and Sönmez M. (2017). The role of organic/bio-fertilizer amendment on aggregate stability and organic carbon content in different aggregate scales. Soil and Tillage Research 168, 118-124. https://doi.org/10.1016/j.still.2017.01.003

Zhang W.-Y., Wei Z.-W., Wang B.-H. and Han X.-P. (2016). Measuring mixing patterns in complex networks by Spearman rank correlation coefficient. Physica A: Statistical mechanics and its applications, 451, 440-450. https://doi.org/10.1016/j.physa.2016.01.056

Zhao Q., Tang J., Li Z., Yang W. and Duan Y. (2018). The influence of soil physico-chemical properties and enzyme activities on soil quality of saline-alkali agroecosystems in western Jilin Province, China. Sustainability 10 (5), 1529. https://doi.org/10.3390/su10051529

Zimmerman D.W. and Zumbo B.D. (1993). Relative power of the Wilcoxon test, the Friedman test, and repeated-measures ANOVA on ranks. The Journal of Experimental Education 62 (1), 75-86. https://doi.org/10.1080/00220973.1993.9943832