

Case Report

Assessment of a *sargassum*-based liquid biofertilizer for enhanced banana cultivation in small-scale family farmsYaset Rodríguez-Rodríguez^a, Laura Isabel Soldevilla-Hernández^a, Miguel Ángel Guevara^{a,b}, Gustavo Gandini^c, Ulises Javier Jáuregui-Haza^{a,*}^a Instituto Tecnológico de Santo Domingo (INTEC), Avenida de Los Próceres #49, Los Jardines del Norte 10602, Santo Domingo, the Dominican Republic^b Instituto de Formación Docente (ISFODOSU), C/ Leonardo Da Vinci, Urb. Renacimiento, Santo Domingo, the Dominican Republic^c Asociación BANELINO. Ave. Miguel Crespo, Frente a INPONOR, Mao, 61000, the Dominican Republic

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ABSTRACT

The substantial influxes of pelagic *Sargassum* spp. along Caribbean coasts pose environmental challenges for the region's countries, but also offer opportunities for sustainable valorization. This research develops and evaluates a *Sargassum*-Based Liquid Biofertilizer (SBLB) produced via anaerobic fermentation, uniquely reducing heavy metal content (e.g., arsenic from 61.6 to 4.08 mg/kg) while retaining beneficial nutrients and microorganisms. This biofertilizer was applied to banana cultivation in Dominican small-scale family farms, SBLB enhanced leaf emission ($p < 0.05$ in two of three farms) compared to a traditional biofertilizer (BLB) and maintained Black Sigatoka disease below intervention thresholds, matching BLB performance. Chemical and microbiological analyses confirmed SBLB's safety and efficacy, positioning it as a sustainable alternative to chemical fertilizers. This approach mitigates *Sargassum* waste and supports organic farming, offering a replicable model for coastal agricultural systems.

1. Introduction

The recurring influx of large quantities of pelagic *Sargassum* spp. in the Caribbean Sea for over a decade has led to significant adverse consequences on industries such as tourism and fishing [1–3]. The decomposition of these macroalgae on the beaches of countries in the region, such as the Dominican Republic, leads to unpleasant odors, acid leachates, the release of toxic gases, and the degradation of coastal water quality, which severely impacts both the environment and public health [4,5]. These factors have a detrimental effect on the health of nearby communities, as well as marine and coastal ecosystems [6], by reducing oxygen levels in water and causing the death of marine organisms, including economically important species such as lobsters [5]. The social impact of this phenomenon is also evident, as it leads to diminished opportunities for recreational activities and the displacement of traditional fishing grounds [2,7–9]. Removing large amounts of *sargassum* from beaches and coastal areas is costly and time-consuming, placing a significant burden on local communities and businesses responsible for its management. Additionally, this process exerts further environmental strain, as the machinery involved can contribute to beach erosion and

compaction [5]. Beyond these immediate impacts, *Sargassum* accumulation has sparked growing interest in identifying valorization pathways that convert this environmental nuisance into a valuable resource.

While several potential applications have been proposed, including bioenergy, animal feed, bioplastics, and construction materials, many remain at an experimental or conceptual stage due to technical and economic barriers. Among these, the use of *Sargassum* as a raw material for biofertilizer production represents a particularly promising avenue, especially for low-income coastal agricultural systems seeking sustainable alternatives to chemical fertilizers.

The Dominican Republic's economy relies heavily on tourism, mining, agriculture, trade, and services [10]. However, the agriculture sector encounters various challenges, including increased chemical pesticides and fertilizers due to the intensification of food production. The use of these products can result in surface and groundwater contamination [11] and poses risks to both human health and the environment [12]. This issue is of growing concern in the country, as toxic compounds have been detected in certain crops recently [13]. Therefore, it is essential to explore sustainable alternatives that support agricultural production without jeopardizing the well-being of

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individuals and the environment.

Organic banana production is of significant importance to the Dominican Republic, positioning the country as one of the world's leading exporters of organic bananas [14,15]. This industry contributes substantially to the national economy and promotes sustainable agricultural practices [16] that safeguard both environmental and human health. Despite its success, organic banana farming faces challenges related to nutrient management and pest control without synthetic inputs, highlighting the need for innovative solutions to enhance productivity and sustainability [17]. A key alternative for modern and sustainable agriculture is the development of organic liquid fertilizers through anaerobic digestion [11,18], also known as liquid biofertilizers [19,20]. These can be produced through fermentation processes of various organic materials, undergoing decomposition by microorganisms such as bacteria and fungi [21,22]. Liquid biofertilizers (LBFs) offer a cost-effective and eco-friendly solution to these challenges, especially when derived from abundant and underutilized biomass such as Sargassum. Moreover, their use can contribute to the reduction of environmental pollution and the protection of human health.

Considering the numerous beneficial substances in the composition of sargassum [4,9,23], some research has explored its potential as a raw material for producing liquid biofertilizers [24–26]. The chemical composition of sargassum includes elements such as carbon, sodium, iron, nitrogen, phosphorus, potassium, magnesium, and calcium. These elements combine to form both organic and inorganic compounds, including water, carbohydrates (mainly polysaccharides such as alginates, cellulose, mannitol, fucoidan and laminarin), lipids (including fatty acids), proteins, vitamins and secondary metabolites, such as flavonoids, phenols, terpenes, saponins, carotenoids, and fucoxanthin [4].

The chemical composition of sargassum varies based on species, morphotype, location, season, and environmental conditions. Despite these variations, sargassum is considered valuable due to its richness in nutrients and useful materials, making it an attractive source for various industrial applications. However, there are concerns related to the presence of heavy metals in sargassum [27,28] and the products derived from it for use in agriculture and animal feed [4,29]. In particular, arsenic levels have raised alarms, necessitating safe processing methods that can mitigate toxicity while preserving beneficial components.

Given the factors above, particularly the urgent need to effectively manage the substantial influx of sargassum on the Dominican coasts and to address the unmet demands of the agricultural sector, this research proposes the development of a liquid biofertilizer derived from sargassum. Furthermore, the study aims to evaluate its impact on organic banana cultivation within family farming systems. The novelty of this research lies in demonstrating a replicable and scalable method to valorize Sargassum into a safe biofertilizer, characterized by low heavy metal content, improved nutrient profile, and verified microbiological safety. This is supported by the hypothesis that the Sargassum-based liquid biofertilizer will perform comparably to a traditional biofertilizer in promoting plant growth and managing disease pressure in organic banana crops, while simultaneously offering environmental benefits through the sustainable reuse of coastal seaweed biomass.

2. Materials and methods

2.1. Collection and sargassum preparation

The sampling of sargassum was carried out in October 2022 in Punta Cana (UTM Zone 19Q 568159.13 m E, 2050047.81 m N), Dominican Republic (Figure SI.1, supporting information). A composite sample of 5 kg was collected from five different points along the beach, ensuring a minimum distance of 50 m between each sampling point. The algae were gathered from the seawater before reaching the shore. Following collection, the algae were allowed to drain for 5 minutes through a 2 mm mesh to remove excess water before being packaged. Morphological analysis was then performed to identify the species and morphotypes of

sargassum present, following a method reported elsewhere [27].

Later, the sample was transported in a plastic bag to the Laboratory of Biotechnology of the *Instituto Tecnológico de Santo Domingo (INTEC)*, Dominican Republic. Upon arrival, it was immediately washed with potable water and subsequently distilled water, with debris (e.g., plastics, other algae) manually removed. Next, it was sun-dried for 10 hours per day over seven days. Afterward, the sargassum was further dehydrated in a laboratory oven (Thelco) at 70 °C for 24 hours. The dried sample was then pulverized and sieved, achieving a homogeneous particle size range between 0.6 and 2 mm, following the procedure established elsewhere [27]. A portion of the sample was preserved for chemical analyses, while a second portion was used to produce the liquid biofertilizer. To prevent microbial growth, the water activity of the dried sargassum was confirmed to be less than 0.6 aw using the ISO 18787 standard before storing it in sealed bags for future use [30].

2.2. Physicochemical analysis of the sargassum sample

The chemical analysis of the sargassum sample included the determination of alkali, alkaline earth and heavy metals, as well as total fats, proteins and carbohydrates. These analyses adhered to the methodology described elsewhere [28], incorporating both instrumental and classical methods of chemical analysis. For metal analysis, the samples underwent acid digestion before inductively coupled plasma optical emission spectrometry (ICP-OES). For the determination of heavy metals, alkali metals, and alkaline earth metals, the digestion of sargassum was carried out by preparing 500 mg of each sample in duplicate. Each sample was placed into a 75 mL Teflon vial, to which 7 mL of concentrated nitric acid (69 % HNO₃), 1.5 mL of hydrogen peroxide (H₂O₂), 0.5 mL of hydrofluoric acid (HF, 40–45 %), and 2 mL of deionized water were added. Digestion was performed using a PerkinElmer Titan MPS microwave digestion system. Residual HF was neutralized with boric acid (H₃BO₃), and the vials were returned to the sample preparation system. After filtering, the digested samples were transferred into 50 mL volumetric flasks and diluted to volume with deionized water. Subsequently, 5 mL aliquots were taken, further diluted, and adjusted to 50 mL with a 2 % w/v nitric acid solution prepared with deionized water. Metal quantification was conducted using a PerkinElmer Optima 8000 inductively coupled plasma optical emission spectrometer (ICP-OES). Calibration curves were established for each element using their respective emission spectra, and analyses were performed in duplicate [28].

Complementary proximate analysis was conducted to determine fat, protein, and carbohydrate content, following AOAC and colorimetric methods. For the analysis of total fats, acid hydrolysis was performed to liberate bound lipids, followed by extraction using a mixture of ethers (e.g., petroleum ether and diethyl ether) and subsequent gravimetric measurement. The total protein content was assessed using the Kjeldahl method on homogenized samples. This method involves the digestion of organic matter with concentrated sulfuric acid in the presence of catalysts like potassium sulfate and copper sulfate to convert organic nitrogen into ammonium sulfate. The resulting ammonium is then distilled as ammonia by steam distillation and titrated with hydrochloric acid. The nitrogen percentage was converted to protein percentage using a conversion factor specific to the protein matrix. The carbohydrate content was calculated by difference using the following formula:

$$\% \text{Carbohydrates} = 100 - (\% \text{Moisture} + \% \text{Protein} + \% \text{Lipid} + \% \text{Ash}) \quad (1)$$

Ash content was determined by subjecting the sample to high temperatures in a muffle furnace and weighing the residues (ashes) until a constant weight was achieved. To determine moisture content, the sample was subjected to a drying process (heating at 105 °C) until a constant weight was reached. The difference between the wet and dry weight of the sample was assumed to be the moisture content. Additionally, total fats were analyzed using nuclear magnetic resonance

(NMR) spectroscopy to confirm the gravimetric results. Carbohydrates were quantified using the phenol-sulfuric acid method described by (Dubois et al., 1956). Proteins were also subjected to elemental analysis to corroborate the results obtained from the Kjeldahl method.

2.3. Fermentation process of the sargassum-based liquid biofertilizer (SBLB)

The fermentation methodology was adapted from previous research [24] and scaled up using 9-L fermenters in quadruplicate. (Fig. S1.2, supporting information). The procedure was as follows: 700 g of the prepared sargassum biomass, 834 mL of molasses, 68 mL of natural yogurt, 175 mL of UHT whole milk, and 70 g of yeast (*Saccharomyces cerevisiae*) were added to each fermenter. The volume in each fermenter was adjusted to 8 l using potable water, leaving 1 L of free space to prevent obstruction of the gas release hoses. All raw materials were thoroughly mixed, and the fermentation process began under anaerobic conditions, lasting a total of 28 days. The fermenters were maintained at ambient temperature and monitored daily. Gas release was ensured through hoses submerged in water, preventing oxygen entry.

2.4. Procedure for obtaining the reference liquid biofertilizer in BANELINO

To establish a comparative baseline, the performance of the SBLB was assessed against the liquid biofertilizer (BLB) routinely produced by the BANELINO Association. The field evaluation of SBLB was carried out on family farms affiliated with the BANELINO Association, where a liquid biofertilizer of their formulation is regularly used. Consequently, a comparative analysis was undertaken to assess the compositional differences between the BANELINO Liquid Biofertilizer (BLB) and the SBLB. The BLB is generated through the fermentation of molasses with a medium incorporating microorganisms from local forest mulch. The production of this bioproduct begins with the preparation of a microbial mother culture in a 200-L tank, comprising approximately 25 kg of forest mulch, 100 kg of rice bran, 25 kg of green grass, 20 L of molasses, and 10 L of milk. This mixture is sealed hermetically, allowing anaerobic fermentation for 30 days. Subsequently, the microbial community is transferred into perforated bags and introduced into a 4000-L plastic fermenter containing 100 L of molasses, with the remaining volume filled with water. This reference biofertilizer serves as the control treatment in field trials, reflecting standard local organic practices.

2.5. Physicochemical analysis of the sargassum-based liquid biofertilizer

The evaluation included physicochemical properties such as pH, electrical conductivity (EC), and salinity, as well as concentrations of alkali metals, alkaline earth metals, heavy metals, lipids, carbohydrates, and proteins. Metal analysis was conducted using the inductively coupled plasma optical emission spectrometry (ICP-OES) technique as described previously (see Section 2.2). Briefly, aliquots of the liquid biofertilizer were digested with nitric acid and analyzed using a PerkinElmer Optima 8000 instrument. Macronutrients were measured using gravimetric and colorimetric techniques. Carbohydrates were analyzed by the phenol-sulfuric acid method, proteins by Kjeldahl digestion, and lipids by ether extraction. All measurements were performed in duplicate.

2.6. Microbiological analysis of the sargassum-based liquid biofertilizer

Preparation of SBLB samples for yeast colony-forming unit (CFU) analysis adhered to the guidelines specified in NOM-110-SSA1-1994. Samples from the four bioreactors were diluted to a 10^{-4} concentration using sterile buffered peptone water (Himedia). Subsequently, duplicates of 0.1 mL from dilutions 10^{-1} to 10^{-4} were plated onto sterile Petri dishes using a micropipette. Then, 15 mL of sterilized potato

dextrose agar (PDA), maintained at 45 ± 1 °C in a water bath, were dispensed onto each plate. Thorough mixing of the medium and inoculum was ensured by gently swirling the plates in multiple directions. The Petri dishes were allowed to solidify on a cool horizontal surface. Control plates with 15 mL of medium were prepared to validate sterility. The plates were incubated inverted at 27 ± 1 °C for 3 days. Colonies on each plate were enumerated, and only plates containing 10 to 200 colonies were considered for calculating CFU per milliliter of the analyzed sample, using the following formula 2. Additional microbiological analyses were conducted at the *Instituto Dominicano de Investigaciones Agropecuarias y Forestales* (IDIAF) laboratory. The contents of fungi, bacteria, and actinomycetes were determined using growth tests on selective culture media.

$$\frac{CFU}{mL} = \frac{Colony\ count}{Volume\ plated} \times Dilution\ factor \quad (2)$$

2.7. Assessment of the implementation of sargassum-based liquid biofertilizer in banana farms

Field trials were conducted over three months to assess the effectiveness of the SBLB in improving soil fertility and the growth of banana plants. For this purpose, the BLB was used as the standard biofertilizer for the control group. The studies were conducted on three family plots. Two plots, belonging to Richard Núñez and Ramón Tejada respectively, are in the pilot zone of Montecristi. These soils are clayey and require good drainage systems; they have high concentrations of $CaCO_3$ and, in some cases, shallow soil depth due to reduced profiles. The third plot is in the Jaibón area, Laguna Salada, Valverde, owned by Ramón Medrano. These soils have severe salinity issues, but in this case, they have been managed with high applications of organic matter and the implementation of drainage systems. These soils are of alluvial-fluvial origin with a clay-loam texture, and profiles show alluvial sedimentation containing variable $CaCO_3$ content. Soil samples from the plots were subjected to microbiological analysis as described earlier (see Section 2.6). In each plot, 20 banana plants (the "first sucker" or "daughter" of the harvested plant) were selected. 300 mL of pure liquid biofertilizer was applied to the soil around each plant each 15 days, along with a foliar application of liquid biofertilizer diluted to 20 % in alternate week. Two variables were studied to determine the effect of the SBLB in the BANELINO organic banana production system: leaf emission rate and the evolutionary status of *Black Sigatoka* disease.

In this research, the evolutionary status of Black Sigatoka in banana plants was evaluated by directly counting the total number of visible spots on leaves 3 to 7 of each plant, without using a specific severity scale. The characteristic spots, identified as dark brown to black lesions with irregular shapes and defined margins, were counted on both sides of the leaves. For each plant, the plant number, leaf number, and total number of spots were recorded. Evaluations were carried out over eight weeks by trained personnel, following a systematic pattern to avoid double counting and minimize bias [31]. To statistically compare the results of the field experiments, the means for each parameter in each family farm studied were compared pairwise. A Student's t-test was applied with a significance level of $\alpha = 0.05$.

3. Results and discussion

3.1. Identification and moisture content of sargassum

Three morphotypes of the two typical pelagic Caribbean sargassum species—*S. fluitans* (morphotype III), and *S. natans* (morphotypes I and VIII)—were identified, with a predominance of *S. fluitans* III. The identification was based on morphological differences at the level of stems and aerocysts, following methods reported in the literature [32–37]. This result confirms the species composition commonly reported during massive strandings in the region. Such strandings have been recorded

consistently since 2011, with significant ecological and logistical implications. The moisture content in the samples, determined by weight difference, was $86 \pm 3\%$, consistent with values of (81–88)% reported by other authors [4,38,39].

3.2. Chemical analysis of the sargassum

Table 1 presents the concentrations of heavy metals, alkali metals, and alkaline earth metals in the sargassum samples.

The outcomes derived from the analysis of three sargassum samples, along with the mean and sample standard deviation, are presented. Among these elements are alkali metals, alkaline earth metals, and heavy metals. Notably, among the metalloid, arsenic (As) exhibits the highest concentration at 61.6 mg/kg, followed by iron (Fe) at 42 mg/kg. The arsenic and boron levels exceed the permissible ranges for using them in agricultural soils (15–50 mg/kg for arsenic and 1 mg/kg for boron) and animal feed (2–40 mg/kg for arsenic). Given that iron stands as the fourth most abundant element in the Earth's crust, constituting 5.1 % of its total weight, and its soil content is estimated at 3.8 % [54, 55], it can be inferred that the iron concentrations found in *Sargassum* spp. do not pose a contamination concern for the soil. When compared to previous results of heavy metal determination in *Sargassum* spp. conducted in 2015 [27,56] and 2021 [28] on Dominican beaches, the values reported in our study fall within the ranges found by those authors. Similarly, they fall within the intervals obtained in the determination of sargassum on other Caribbean beaches [38,57,58]; and the Atlantic Ocean [59].

Among the alkali and alkaline earth metals, notable concentrations of sodium (10847 mg/kg), magnesium (4723 mg/kg), calcium (12448 mg/kg), and potassium (1016 mg/kg) are observed. Upon comparison with results from studies conducted in our country, the region, and the

Table 1

Chemical composition of *Sargassum* spp. biomass (dry basis). Concentrations of metals and macronutrients determined by ICP-OES and classical methods.

Parameter	Concentration (mg/kg)	Typical safety or reference range
Heavy metals and metalloids		
Arsenic (As)	61.6 ± 22.7	15–50 mg/kg (agricultural soil)
Cadmium (Cd)	0.26 ± 0.04	1–37 mg/kg
Lead (Pb)	0.11 ± 0.01	100–1000 mg/kg
Mercury (Hg)	<0.05	0.08 mg/kg
Chromium (Cr)	3.14 ± 0.49	50–280 mg/kg
Nickel (Ni)	2.80 ± 0.3	100–139 mg/kg
Copper (Cu)	2.40 ± 0.06	60–200 mg/kg
Zinc (Zn)	2.00 ± 0	70–600 mg/kg
Selenium (Se)	1.73 ± 0.32	–
Vanadium (V)	1.85 ± 0.34	130 mg/kg
Cobalt (Co)	0.37 ± 0.05	25–50 mg/kg
Tin (Sn)	0.22 ± 0.04	5 mg/kg
Molybdenum (Mo)	0.15 ± 0.03	5 mg/kg
Aluminum (Al)	10.00 ± 0.6	–
Iron (Fe)	42.00 ± 3.0	–
Macronutrients and essential elements		
Sodium (Na)	$10,847.2 \pm 9974.4$	–
Calcium (Ca)	$12,448 \pm 1611.6$	–
Magnesium (Mg)	4723 ± 438	–
Potassium (K)	1016 ± 258	–
Phosphorus (P)	275 ± 35.6	–
Boron (B)	89.2 ± 8.0	1 mg/kg (soil limit)
Silicon (Si)	20 ± 10	–
Barium (Ba)	10.68 ± 1.93	750 mg/kg
Strontium (Sr)	850.6 ± 129.75	–
Titanium (Ti)	0.9 ± 0.1	–
Macronutrients (proximate analysis)		
Total carbohydrates	54.0 ± 2.7 g/100 g	11.7–75.5 g/100 g (literature range)
Total proteins	5.4 ± 0.2 g/100 g	2.5–15.4 g/100 g
Total lipids	<1.0 g/100 g	0.3–4.6 g/100 g

Note: Values represent mean $\pm$ standard deviation for three replicates. Reference ranges compiled from multiple sources [40–53,61–68].

Atlantic Ocean, these concentrations are within the ranges reported by other researchers [27,28,38,56,57,60]. Particularly noteworthy are the high values of strontium (850.6 mg/kg), an element that had not been previously reported in sargassum by other researchers. Ultimately, none of the studied samples exhibited detectable levels of silver, beryllium, bismuth, lithium, mercury, antimony, and thallium.

The quantitative analysis of carbohydrates, lipids, and proteins in the sargassum sample is presented in Table 2. In previous studies conducted in the Dominican Republic, data regarding protein and fat concentrations in analyzed *sargassum* samples were not documented. When comparing our findings with other investigations from our geographical region, it is evident that the protein content falls within the range of values reported by other authors, ranging from 2.5 % to 15.4 % [61–67], as well as the carbohydrate content, which is within the reported range of 11.7 %–75.5 % [38,61,62,64,66–68]. Regarding total fats, the observed values reside at the lower limit of what has been reported by other authors, ranging from 0.3 % to 4.6 % [38,61,62,64,65,67,68], or even lower. These values facilitate the assessment of sargassum for potential use in agriculture and animal feed, provided that the established standards for microcontaminants are met.

3.3. Fermentation process of the sargassum-based liquid biofertilizer

Fig. 1(left) illustrates the pH variation throughout the fermentation process, representing the average value from the four fermenters. Upon analysis, a typical trend is observed, marked by a decrease in pH in the first four days of the process. This decline can be attributed to the formation of lactic and acetic acids from the fermentation of molasses and milk. Subsequently, it is followed by an increase in pH, likely resulting from the transformation of algal biomass in the presence of acids in the medium (which facilitate its degradation), stabilizing its value within the range of 5–5.2. This pH value aligns with the range of other liquid biofertilizers (4.13–7.29) [69–73].

Similarly, Fig. 1 (right) depicts the variation in electrical conductivity (average value of the four fermenters) for the fermentation process, ranging between 27.7 and 30.5 mS/cm. This value is slightly higher than those reported by other authors (8.4–28.1) mS/cm; [69,71]. When analyzing it, in the course of examination, a reduction in the amount of dissolved electrolytes in the medium was evident, suggesting that chemical transformations commenced from the first day. Following this, an increase in this property is observed from day 6 onwards, maintaining relative stability starting on day 10. This pattern is like that shown in Fig. 3. The slight variations observed between days may be influenced by the type of static fermentation used. Bubbling inside the fermenters may have caused a modest mixing of the ferment; however, this proved insufficient to achieve homogeneity in the samples alone.

The average values of pH and electric conductivity of the SBLB were 5.08 ± 0.08 and 30.30 ± 0.43 respectively, with a relative error of less than 2 %, which shows the robustness of the fermentation processes carried on. As previously mentioned, the pH levels fall within the established range reported for other liquid biofertilizer [70–73]. Moreover, the average conductivity is slightly below the maximum value reported by other authors, which stands at 28.1 mS/cm [69,71].

3.4. Chemical analysis of the sargassum-based liquid biofertilizer

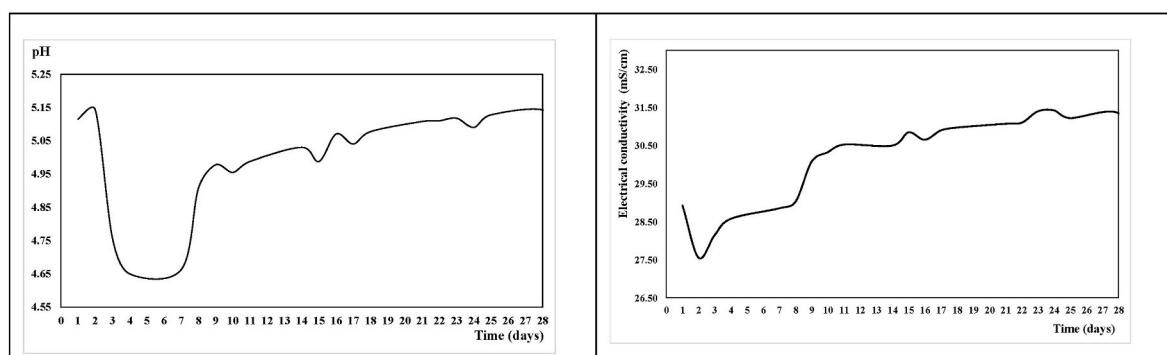
Table 2 provides an overview of macroelement concentrations in both the sargassum liquid biofertilizer and its corresponding solid waste. Additionally, this includes the characterization of the traditional liquid biofertilizer produced by the BANELINO Association, a novel information introduced in this study. A comparative column is incorporated to illustrate the range of the evaluated parameters published by other researchers for different liquid biofertilizers [69–75]. It is noteworthy that, for both the sargassum liquid biofertilizer and its solid waste, the reported values represent the average measurements obtained from the four fermenters.

Table 2

The concentration of macroelements in sargassum liquid biofertilizer, its solid residue, and traditional liquid biofertilizer produced in the BANELINO Association.

Parameters	Sargassum liquid biofertilizer	Sargassum liquid biofertilizer solid residue	BANELINO Association liquid biofertilizer	Other liquid biofertilizers reported in literature ^a
N, %	0.18 ± 0.02	0.59 ± 0.03	0.04–0.07	0.04–3.7
P, %	0.16 ± 0.03	0.1 ± 0.01	0.031–0.054	0.007–1.9
K, %	0.31 ± 0.06 ^b	0.3 ± 0.02	0.03–0.1	0.04–2.6
	0.996 ± 0.05 ^c			
Ca, %	0.79 ± 0.04 ^b	0.74 ± 0.05	0.03–0.18	0.04–0.81
	0.128 ± 0.006 ^c			
Mg, %	1.04 ± 0.02 ^b	0.57 ± 0.02	0.02–0.03	0.013–0.6
	0.094 ± 0.005 ^c			
Total Carbohydrates (g/100g)	2.3 ± 0.2	22 ± 1.1	–	–
Total Lipids (g/100g)	<1.0	<1.0	–	–

Note:

^a [69–75].^b Elements quantified by Atomic Absorption Spectroscopy (AAS).^c Elements quantified by Inductively Coupled Plasma with Optical Emission Spectrometry (ICP-OES).**Fig. 1.** Variation of pH (left) and electric conductivity (right) during the fermentation process over time.

When comparing the concentration of essential macronutrients for use as fertilizers in both the liquid biofertilizer (N, P, and K) and the solid waste, it is evident that nitrogen levels are higher in the residue than in the liquid biofertilizer. Conversely, phosphorus and potassium exhibit higher concentrations in the liquid biofertilizer compared to the waste. It should be noted that the amount of potassium in the liquid biofertilizer (Table 2) surpasses that of the initial sargassum (Table 1) by approximately ninefold, which may be attributed to the presence of this element in the liquid phase of the ferment from the other raw materials used to produce the liquid biofertilizer. The increase in the quantity of potassium is particularly advantageous, given its important role in plant growth [76].

In the case of magnesium (Mg) and calcium (Ca), their concentrations remain comparable in both the liquid biofertilizer and solid waste. Remarkably, the content of carbohydrates and total proteins is higher in the residue than in the liquid biofertilizer fraction. Therefore, it becomes pertinent to explore, in future studies, the potential applications of the solid waste from the process of obtaining sargassum liquid biofertilizer in agriculture (e.g. compost and animal feed), if the content of heavy metals and other possible contaminants is analyzed in the solid residue. Likewise, it should be noted that both the sargassum liquid biofertilizer and its residue exhibit lower carbohydrate and protein contents than the initial sargassum (Table 1). The mass balance indicates that, during the liquid biofertilizer production process, a portion of the carbohydrates and proteins undergo degradation.

Table 2 shows the variation ranges of macronutrients across four distinct industrial batches of BANELINO liquid biofertilizer. In each instance, it is observed that the macronutrient levels in the sargassum liquid biofertilizer surpass those obtained for the four batches of the BANELINO liquid biofertilizer. Finally, a comparative analysis was conducted to juxtapose the macronutrient content of our sargassum

liquid biofertilizer with that of other liquid biofertilizers derived from cattle and pig excreta [69,71] and from various algae species [70, 72–75]. Notably, the content of nitrogen, phosphorus, potassium and calcium in sargassum liquid biofertilizer is within the ranges of values published for other liquid biofertilizers. However, the magnesium content exceeds the typical values reported for this element in biomass. Regarding protein content, the sargassum liquid biofertilizer exhibits a lower value compared to that reported by Ref. [75]. Reference values reported for carbohydrates and proteins in liquid biofertilizers were not found in the available literature.

As can be observed from Table 2, there are significant differences in the K, Ca, and Mg values obtained using the two methods: atomic absorption and ICP-OES. This suggests that interlaboratory validation of chemical analysis methods is necessary to ensure more reliable results when working with complex matrices such as algae-based biofertilizers, where interferences can be significant.

3.5. Alkali metals, alkaline earth metals, and heavy metals in liquid biofertilizer samples

Table 3 shows results corresponding to alkali metals, alkaline earth metals, and heavy metals concentration in the Sargassum liquid biofertilizer. Out of the elements examined, fifteen fall below the detection limit of the utilized method, indicating that numerous metallic elements analyzed were not detectable by the technique in the final product. Notably, ten elements that were reported in the starting sargassum biomass (Table 1), Al, Ba, Cd, Co, Cr, Ni, Pb, Se, Sr, and V, were not detected in the liquid biofertilizer, suggesting their retention in the residual solid phase of the process.

A total of six elements initially present in sargassum were identified in the liquid biofertilizer, namely arsenic, copper, iron, manganese,

Table 3

Heavy metal concentrations in liquid biofertilizers are presented in parts per million (ppm).

Metals	Concentration in liquid biofertilizer (ppm)			Soil concentration limits in agriculture ^b
	SBLF	BANELINO Association	Other liquid biofertilizers reported in the literature ^a	
Aluminum (Al)	<0.1	–	57	
Antimony (Sb)	<0.1	–	–	
Arsenic (As)	4.08 ± 0.24	–	–	15–50
Barium (Ba)	<0.1	–	–	750
Beryllium (Be)	<0.02	–	–	
Cadmium (Cd)	<0.1	–	0.08–0.2	1–37
Cobalt (Co)	<0.1	–	0.1	25–50
Copper (Cu)	2.64 ± 0.13	0.6–1.2	0.1–0.9	60–200
Chrome (Cr)	<0.3	–	–	50–280
Strontium (Sr)	<0.1	–	–	
Iron (Fe)	14.16 ± 0.71	5.3–145.1	1.6–55	
Manganese (Mn)	3.96 ± 0.2	1.5–14	0.5–35	
Mercury (Ag)	<0.05	–	–	0.08
Nickel (Ni)	<0.3	–	–	100–139
Silver (Ag)	<0.01	–	–	20
Lead (Pb)	<0.1	–	<0.01	100–1000
Selenium (Se)	<0.1	–	–	
Sodium (Na)	2060 ± 103	–	60–280	
Thallium (Tl)	<0.05	–	–	
Vanadium (V)	<0.1	–	–	130
Zinc (Zn)	5.42 ± 0.27	0.4–1.2	0.15–7.5	70–600

Note:

^a [69–72,74,75].

^b [40–50,52,53,77,78].

sodium and zinc. Arsenic, one of the most controversial metals when evaluating the applicability of sargassum due to its elevated content within the algae, exhibited a concentration of 4.08 mg/l in the liquid biofertilizer. This value is approximately fifteen times lower than the concentration determined in the initial sargassum sample (Table 1) and falls below the maximum limit of 41 mg/kg permitted by the [79] and the established maximum limit of 10 mg/kg by the FAO for products intended for use in agricultural soils. The presence of arsenic has not been analyzed in other liquid biofertilizer studies in our knowledge.

Copper was detected in slightly higher amounts than in the starting sargassum (Table 1), presumably due to the contribution of some of the raw materials used for the fermentation. Its concentration surpasses that found in the BANELINO liquid biofertilizer and other liquid biofertilizers [69–72,74]. It is pertinent to highlight that copper is acknowledged as a micronutrient for crops and, in this case, is present at levels considerably lower than those deemed hazardous for agricultural soils [45,78].

In contrast, iron, manganese, sodium and zinc exhibited reduced concentration in the liquid biofertilizer compared to the sargassum employed in its production. The levels of Fe, Mn and Zn present in the SBLB align to those reported in BANELINO liquid biofertilizer and other liquid biofertilizers [69–72,74,75]. However, the amount of sodium present in the SBLB is much higher when compared to other liquid biofertilizers obtained from sargassum (60–280) ppm; [71,72,75].

Monitoring the sodium content is crucial due to its association with soil salinity, and few terrestrial plants can thrive in highly saline soils [80]. Therefore, future research should focus on assessing the effect of sodium levels in sargassum-based biofertilizers on soil salinity and their potential implications for banana cultivation.

As can be seen, none of the heavy metals detected in SBLB exceed the permissible values for their use in agricultural soils, affirming the safety of its application in family farming. However, it is advisable to plan studies to evaluate the possibility of the long-term accumulation of these elements in the soil.

3.6. Microbiological analysis of the sargassum-based liquid biofertilizer

The microbiological composition of biofertilizers is a critical factor influencing their effectiveness and safety in agricultural applications. The average yeast colony-forming units (CFU) per milliliter in the analyzed liquid biofertilizer samples was $(1.75 \pm 0.21) \times 10^7$ CFU/mL. This high yeast population density indicates a robust fermentation process, suggesting that yeasts played a significant role in the decomposition of organic matter and the stabilization of the biofertilizer over the 30-day fermentation period. Yeast produces various metabolites, including vitamins and enzymes, which can enhance plant growth and soil health [81].

Cultivation of the liquid biofertilizer on potato dextrose agar (PDA) medium led to the identification of several fungal species: *Colletotrichum* sp., *Sepedonium* sp., *Cladosporium* sp., and *Geotrichum* sp. It is noteworthy that these fungi are not considered pathogenic to plants or humans under normal environmental conditions [82]. Instead, some of these genera have been associated with beneficial effects in soil ecosystems. For example:

- *Cladosporium* sp.: Commonly found in soil and decaying organic matter, species of this genus can contribute to the decomposition process and nutrient cycling [83].
- *Geotrichum* sp.: Known for their ability to decompose pectin and other polysaccharides, they can enhance the breakdown of organic materials in the soil [84].

The presence of these non-pathogenic fungi suggests that the biofertilizer could positively influence soil microbial diversity and activity, potentially leading to improved soil structure and fertility [85].

Additionally, Table SI.1 (supporting information) presents comprehensive data on the total counts of bacteria, actinomycetes, and fungi in both the liquid biofertilizer samples and the solid residues remaining after fermentation. The bacterial counts were significantly high, which is beneficial since many bacteria in biofertilizers are known to promote plant growth through nitrogen fixation, phosphorus solubilization, and the production of growth hormones [86]. The actinomycetes presence is advantageous, as they are involved in the complex organic compounds decomposition and have antagonistic effects against soil-borne pathogens [81]. The rich microbial diversity observed in the SBLB indicates its potential as a bio-inoculant that can enhance soil health and plant growth. The high yeast and bacterial populations suggest that the biofertilizer can introduce beneficial microorganisms into the soil, promoting nutrient availability and suppressing pathogenic microbes through competitive exclusion [87].

Moreover, the absence of pathogenic fungi is critical for guaranteeing the safe application of biofertilizers in agriculture. The identified fungal species are generally saprophytic and contribute to organic matter decomposition, further supporting soil fertility [88]. While the microbiological profile is promising, it is essential to ensure that the biofertilizer does not harbor any harmful microorganisms that could pose risks to crops, soil health, or human safety. Regular microbiological assessments should be conducted to monitor the presence of any potential pathogens, especially if the fermentation conditions or raw materials change [89].

Furthermore, the high microbial activity in the biofertilizer may lead to rapid nutrient mineralization when applied to the soil, making nutrients readily available for plant uptake [87]. However, this also necessitates careful management to prevent nutrient leaching and to align application rates with crop nutrient requirements.

3.7. Evaluation of liquid biofertilizer application in organic banana crops

Fig. 2 depicts the leaf emission trends after eight weeks across the three farms under investigation. Weekly leaf emission is important as it dictates the pace of bunch appearance. The outcomes of SBLB application are juxtaposed with those of BLB. The leaf emission indexes between the two treatments did not attain statistical significance in Richard Nuñez's plot, implying an equal contribution of both formulations to leaf emission. However, a significant difference was observed in Ramón Medrano's and Ramón Tejada's plots, which favor the Sargassum liquid biofertilizer use. The superior results obtained with the sargassum biofertilizer in two of the three farms can likely be attributed to the nutrients contributed by sargassum and the presence of biostimulant molecules provided by the seaweed.

Regarding nutrient contribution, the sargassum contains many carbohydrates, primarily polysaccharides. The most prominent polysaccharides in these brown algae are fucoidans, alginates, and laminarins, which are recognized for their antimicrobial and antioxidant effects [90,91]. Its protein content is also considered high [92] and of good quality, as it typically includes all essential amino acids [93,94]. Additionally, sargassum provides essential nutrients such as potassium, calcium, sodium, magnesium, and phosphorus, along with several trace elements like iron, zinc, copper, and manganese as shown in the sargassum and biofertilizer characterization here, and other authors have reported it [93,95] all of which support the vegetative development of banana plants in this study.

On the other hand, the biostimulant effect can be considered because sargassum has a vast metabolite profile that includes various groups such as terpenes, polyphenols, sterols, phlorotannins, phytosterols, and glycolipids [96]. Among these metabolites are phytohormones such as cytokinins, auxins, gibberellins, and ethylene [97,98]. These substances play critical roles in coordinating plant growth and development and in responding to biotic and abiotic stressors [99–101]. This rich metabolite composition makes sargassum an ideal substrate for fermentation and enhances its potential as an agricultural bioinput.

The presence of *Black Sigatoka* serves as an indicator of the presence of the fungus *Mycosphaerella fijiensis*, or its evolutionary state. Biologically, plants with adequate nutrition or the presence of microorganisms

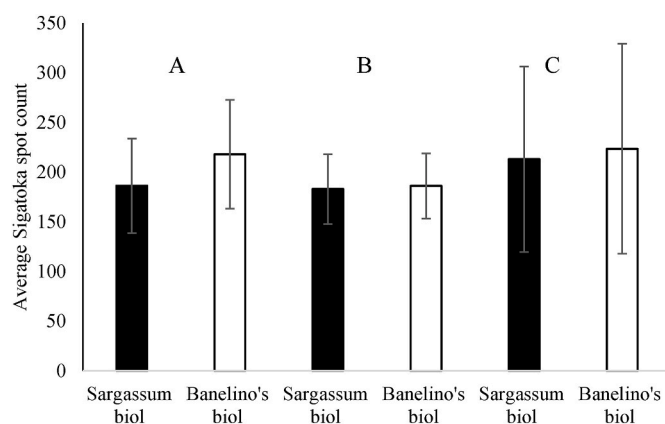


Fig. 3. Amount of Black Sigatoka spots per leaf at the end of the trial (8 weeks) in banana plants cultivated with SBLB (black bars) and BLB (white bars). A- Richard Núñez farm, B- Ramón Medrano's farm, C- Ramón Tejada's farm.

in the soil or at the vascular level (endogenous) result in reduced attack or increased plant adaptation. Fig. 3 illustrates the evolutionary state of Black Sigatoka after eight weeks of study, expressed in the quantity of Sigatoka spots per banana plant leaf. Statistical analysis reveals no significant difference in disease state progression between the use of SBLB or BLB. It is noteworthy that, throughout the study period, the disease state remained below the threshold for chemical treatment intervention (below 300 spots per leaf).

The results obtained during the 8 weeks confirm that SBLB meets the expectations of a liquid biofertilizer, delivering outcomes comparable to traditional liquid biofertilizer in terms of its positive effects on leaf emission and the control of *Black Sigatoka*'s evolutionary state. Although the differences between the two biofertilizers studied were not statistically significant in controlling Black Sigatoka, the fact that both were able to keep the pest under control at levels that do not lead to disease development is important and confirms the biological control value of both biofertilizer formulations in banana cultivation.

4. Conclusions

This study demonstrates that Sargassum spp., an abundant marine biomass in the Caribbean, can be effectively valorized through anaerobic fermentation to produce a safe, nutrient-rich liquid biofertilizer. The resulting product showed acceptable levels of essential macronutrients and significantly reduced concentrations of heavy metals, including arsenic, while maintaining a diverse and beneficial microbial community. Its application on small-scale organic banana farms showed agronomic performance comparable to that of a conventional biofertilizer, supporting healthy plant development and disease control without adverse effects.

These findings suggest that liquid biofertilizers based on Sargassum represent a sustainable alternative for organic farming systems, offering both environmental and agricultural benefits. Future research should explore the long-term impact of sodium accumulation in soils, optimize solid waste management, and assess applicability to other crops and agroecological contexts. Integrating this approach into coastal agricultural practices could contribute to circular economy strategies and help mitigate the environmental burden of Sargassum influxes in the Caribbean.

CRedit authorship contribution statement

Yaset Rodríguez-Rodríguez: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Isabel Soldevilla-Hernández:** Writing – original draft, Visualization,

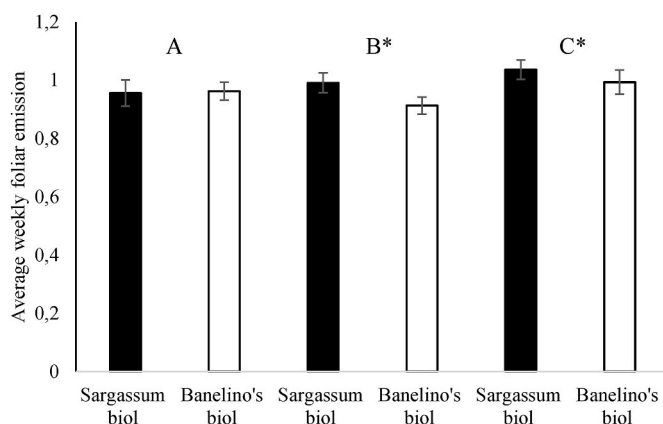


Fig. 2. Average leaf emission index at the end of the trial (8 weeks) in organic banana crops with SBLB (black bars) and BLB (white bars). A- Richard Núñez farm, B- Ramón Medrano's farm, C- Ramón Tejada's farm. *Statistical differences in the use of the two biofertilizers were analyzed at a significance level of $\alpha = 0.05$.

Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Miguel Ángel Guevara:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gustavo Gandini:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Ulises Javier Jáuregui-Haza:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ulises Jauregui-Haza reports financial support was provided by the FAO letter of agreement INTEC-FAO-UNJPDOM024UNJ-2022.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cscee.2025.101252>.

Data availability

Data will be made available on request.

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