PLANT SCIENCE TODAY
Vol 12(sp1): 1-13
https://doi.org/10.14719/pst.8952

eISSN 2348-1900

RESEARCH ARTICLE

Enhancing soil health and crop growth using seaweed extracts of *Gracilaria* species in pulp and paper industry effluent-irrigated soil

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Received: 18 April 2025; Accepted: 19 July 2025; Available online: Version 1.0: 14 October 2025

Cite this article: Rizam R, Jayashree R, Dhevagi P, Chandrakumar K. Enhancing soil health and crop growth using seaweed extracts of *Gracilaria* species in pulp and paper industry effluent-irrigated soil. Plant Science Today. 2025; 12(sp1): 1-13. https://doi.org/10.14719/pst.8952

Abstract

Large amounts of toxic effluents that negatively impact soil health and plant development are released by the pulp and paper industry, making it a major worldwide polluter. To improve crop growth and reduce abiotic stress in soil irrigated with papermill effluents, this pot culture study explores the potential of seaweed extracts from *Gracilaria gracilis* and *Gracilaria edulis* as biostimulants. Recommended Dose of Fertilizer (RDF) was applied to soil samples together with different concentrations of seaweed extracts (2.5 %, 5.0 %, 7.5 % and 10 %). The parameters evaluated were soil pH, electrical conductivity, nutrient availability, enzyme activity, plant development metrics and the amount of carotenoid and chlorophyll. The findings showed a notable increase in soil fertility. The application of *Gracilaria* extracts increased the availability of nutrients, especially nitrogen (up to 145.55 kg/ha in T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF), phosphorus (up to 28.0 ppm in T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and potassium (up to 143.5 ppm in T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and lowering the pH of the soil (from 7.89 to 6.11). Significant improvement was observed in urease, phosphatase and dehydrogenase enzymatic activity. Concerning plant performance, 7.5 % *Gracilaria* extract produced the greatest results (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF). The height of Maize plants (up to 204.3 cm in T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF), the number of leaves (12 in T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) and the amount of chlorophyll (3.10 mg g^{-1} in T_{10}) increased. With a high carotenoid level of 0.86 mg/g in T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF), photosynthetic efficiency and stress tolerance were 10-15 % improved. According to the studys' findings, seaweed-based biostimulants, particularly at concentrations of 7.5 % seaweed extract from two species, offer an environmentally acceptable approach to mitigating abiotic stress, enhancing soil health and promoting crop growth in polluted areas. Using *Gracilaria* extracts in sustainable farming methods is suitable for alleviating environmental stress.

Keywords: abiotic stress mitigation; crop growth; *Gracilaria* sps; seaweed extract; soil

Introduction

The pulp and paper (PPI) industries produce enormous amounts of wastewater that are highly contaminated with a wide range of contaminants. These industries release 100 million kg of harmful pollutants into the environment annually (1). Pulp and Paper Industries have significant concentrations of various kinds of contaminants, both inorganic and organic. The Pulp and Paper industry is the sixth most polluting industry in the world, generating large-scale toxic effluent during paper manufacturing (2). Due to these pollutants, plants are affected by abiotic stress, including salt, dryness, freezing and extreme temperatures, which harm plant growth and development. In contrast, ideal environmental conditions have a significant impact on every aspect of plant life. By deactivating enzymes, denaturing proteins, rupturing membrane structures and generating harmful compounds such as Reactive Oxygen Species (ROS), these restrictions pose significant risks to plant growth and productivity. Globally, abiotic stressors result in significant losses in agricultural output (3, 4). Stressors, including heat, salinity and drought, have each been the focus of extensive research (4, 5). Salt stress is becoming a significant issue in regions where saline-sodic water is used for irrigation. A third of the worlds' food output is thought to come from irrigated areas, but salt stress affects around 20 % of the lands, or 45 million hectares, lowering the potential yield of annual crops by more than 50 % (6). Ionic toxicity and osmotic stress are two significant effects of salt stress on agricultural plants. Like other abiotic stressors, salt stress generates Reactive Oxygen Species (ROS), which harm a variety of biomolecules, including proteins, nucleic acids and lipids, thereby altering redox homeostasis (7). At the same time, salt concentrations can alter physiological and biochemical processes, limiting the development of the plants' root system and its aerial portion. Reduced water uptake by plant roots results from high salt concentrations in the soil because they lower the soils' water potential (8).

To alleviate this environmental stress on plants certain plant biostimulants are used to boost plant growth. Biostimulants are "materials, other than fertilizers, that boost plant growth when applied in low quantities" that were mentioned in scientific literature. Any material that benefits plants but isn't a nutrient, herbicide, or soil enhancer might be referred to as a "biostimulant" (9). By distinguishing biostimulants from other widely used classes of compounds applied to plants and crops, such as pesticides and fertilizers, it is feasible to define biostimulants in part by highlighting their differences (10). Over the past decade, the use of natural plant biostimulants has become increasingly significant. A potential defensive strategy involves the induction of plant defence mechanisms using polysaccharides or oligosaccharides isolated from seaweeds (11). It has been observed that seaweed metabolites protect plants from abiotic stressors. Active biomolecules with antiviral, antifungal, antiprotozoal and antibacterial properties are found in seaweed metabolites. The metabolites extracted from red, brown and green algae are often strong biochemicals with antibacterial properties (12). Applying seaweed extract directly or combining it with soil can increase yield and improve soil fertility. Field research has demonstrated that seaweed extract, when applied at comparatively low rates as foliar sprays or soil fertigation, increases yields for a variety of crops, including vegetables, sugarcane, strawberries and wine grapes (13). Extracts from *Gracilaria* sps. have been demonstrated to increase root growth and seedling vigour in crops such as rice by improving germination parameters (14, 15). Nutrients, including P, Ca, Mg and Fe, are abundant in *Gracilaria* species and are beneficial for plant development (16). It has been demonstrated that the application of *Gracilaria edulis* extract enhances the growth characteristics of crops such as maize and rice. Plant growth regulators, including cytokinins, gibberellins and phenylacetic acid, are responsible for the growth and development of crops. Dry matter accumulation in plants may be considerably increased by using an extract from *Gracilaria edulis*. For instance, when applied in conjunction with approved fertiliser dosages, a 5 % concentration of *Gracilaria edulis* sap enhanced dry matter in maize by 11 % compared to the control.

Extracts from *Gracilaria edulis* have been shown to enhance crop growth in several crops. This is especially evident in Maize, where the use of seaweed extracts considerably accelerated growth rates (17). *Gracilaria gracilis* extracts enhance seed germination rates and increase shoot length, which benefits plant growth. For instance, research discovered that a 0.5 % concentration of *G. gracilis* extract considerably lengthened tomato plant shoots by 45 % in the early stages and 14 % in the later stages. Extracts from seaweed, such as *Gracilaria gracilis*, can enhance plants' nutrient absorption. This is comparable to other seaweed extracts that have been demonstrated to improve crop absorption, such as maizes' uptake of nitrogen, phosphate and potassium (18). This study uniquely integrates environmental remediation with sustainable agricultural enhancement by evaluating the dual role of seaweed extracts of *Gracilaria gracilis* and *Gracilaria edulis* on (i) Crop growth and development and (ii) to alleviate abiotic stress on crop grown under papermill effluent irrigated soil.

Materials and Methods

Experimental details and sample collection

A pot culture experiment was initiated to assess the effect of seaweed extracts on alleviating the stress in paperboard effluent with Hybrid Maize as a test crop. Three replicates were used in a Completely Randomised Design. Soil samples were collected from the site with a longitude of 76°52'28.93" E and latitude of 11° 14'50.67" N (Paperboard PSPD LTD.) in the Coimbatore district that was irrigated by Paperboard effluent. After being shade-dried, the soil samples were sieved (0.2 mm) and kept in airtight containers for the analysis of physical and chemical properties. Seaweed has been collected from the Mandapam area of Ramanathapuram district. The seaweed was cleaned thoroughly in fresh water to remove debris and epiphytes, then it was shade-dried (at approximately 25-28 °C), ground using a blender and stored in a sealed bag with air at room temperature. It was then kept in cold storage at 4 °C. The seaweed extract of two *Gracilaria* sps. At the given concentration, the experimental details were applied, as the soil application was conducted at 20-day intervals (Table 1). The seeds of Maize were sown and plant and soil characteristics were analyzed on the 45th and 90th days.

Determination of the physico-chemical properties of soil irrigated by paperboard effluent

A glass electrode from a pH meter and a conductivity meter was used to measure the soils' pH and electrical conductivity (EC) using a water suspension ratio of 1:2.5 (w/v) (19). The soil physicochemical properties, such as the available potassium (K), phosphorus (P), nitrogen (N) and exchangeable calcium (Ex. Ca), exchangeable magnesium (Ex. Mg) and exchangeable sodium (Ex. Na) and exchangeable potassium (Ex. K) were calculated. The available N was determined using the alkaline permanganate technique (20). Available K was determined by combining neutral standard ammonium acetate with soil and measuring the filtrate using a flame photometer (Model FF-200D-I) (21). Available P was calculated using the sodium hydrogen carbonate ($NaHCO_3$) extract colourimetric technique (22). The exchangeable calcium and magnesium were determined using the versenate titration method and the exchangeable sodium and exchangeable potassium were determined by combining ammonium acetate with the soil and measuring the extract obtained using a flame photometer (19).

Further, the soil samples were analysed for soil enzyme activity. After extraction with aqua regia, the quantities of extractable heavy metals in soil samples were determined by Agilent 4210 Microwave Plasma Atomic Emission Spectroscopy

Table 1. Experimental details of the pot culture study to assess the potential of *Gracilaria* sps.

Treatments	Paperboard effluent irrigated soil
T_1	Absolute Control
T_2	*Gracilaria* sp. 1 @ 2.5 % + RDF
T_3	*Gracilaria* sp. 1 @ 5.0 % + RDF
T_4	*Gracilaria* sp. 1 @ 7.5 % + RDF
T_5	*Gracilaria* sp. 1 @ 10 % + RDF
T_6	*Gracilaria* sp. 2 @ 2.5 % + RDF
T_7	*Gracilaria* sp. 2 @ 5.0 % + RDF
T_8	*Gracilaria* sp. 2 @ 7.5 % + RDF
T_9	*Gracilaria* sp. 2 @ 10 % + RDF
T_{10}	*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF

**Gracilaria* sp. 1- *Gracilaria gracilis, *Gracilaria* sp. 2- *Gracilaria edulis and RDF- Recommended dose of fertilizer*

(MP-AES). Enzymes include Urease, Dehydrogenase and Phosphatase. The urease is analysed using a high-throughput microplate technique for 24 hr, the soil is incubated at 37 °C with urea and citric acid buffer. Hypochlorite and sodium phenol are used to quantify ammonia release (23). The colourimetric approach, which utilises 2,3,5-triphenyltetrazolium chloride (TTC) as a substrate, is frequently employed to assess dehydrogenase activity in soil. Use a 2 mm mesh sieve for new soil. Combine 1 mL of 3 % TTC solution, 2.5 mL of Tris-HCl buffer (pH 7.6–7.8) and 6 g of soil. Incubate for 24 hr in the dark at 37 °C to prevent photodegradation. 10 mL of methanol are added to stop the process and extract TFF. Filter the solution after shaking it thoroughly. Measure the absorbance of the filtrate with a spectrophotometer at 485 nm (24). A colourimetric technique using p-nitrophenyl phosphate (pNPP) as the substrate is employed to assess the amount of phosphatase activity in soil. Use 1 g (dry weight equivalent) for each sample after sieving fresh soil using a 2 mm mesh. Combine the soil with 4 mL of buffer and 1 mL of 50 mM pNPP substrate. To increase the pH and stabilise the yellow colour, add 1 mL of 0.5 M NaOH to stop the process. Filter the supernatant after centrifuging the mixture. Make use of a spectrophotometer to measure absorbance at 410 nm (25).

Determination of the physico-chemical properties of plants irrigated by paperboard effluent

Plant samples taken from the papermill effluent-contaminated site were evaluated for total nitrogen (N), phosphorus (P) and potassium (K). Using semi-automatic Kjeldahl distillation equipment, the diacid extract (5:2 - H_2SO_4: $HClO_4$) technique was used to calculate total N (26). The vanadomolybdate colourimetric technique was used to quantify the total P from the Triacid extract (9:2:1 - HNO_3: H_2SO_4: $HClO_4$) using a Shimadzu UV-1800 UV-visible spectrophotometer. A flame photometer was used to measure the total K from the Triacid extract. The exchangeable calcium, exchangeable magnesium and exchangeable sodium were also analysed using the Ammonium Acetate Extraction Method. Approximately 600 mL of deionised water should be used to dissolve 77.08 g of ammonium acetate. Place 2 g of ground, air-dried plant or soil sample in a 50 mL polypropylene tube. To the sample, add 20 mL of the ammonium acetate solution. For 1 hr, shake the tube in a shaker. Use Whatman No. 1 filter paper to filter the mixture. Use Avio 550/560 Max systems of Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to determine the filtrates' calcium, magnesium and sodium content (27).

The plant samples' chlorophyll and carotenoids were analyzed. In a mortar with 80 % acetone, grind a known weight of the fresh plant sample (0.1 g) until the pigments are entirely removed. To remove debris, filter the extract into a centrifuge tube. To clarify the solution, centrifuge the extract for 10 min at 3000 rpm. The supernatant should be transferred to a cuvette. Use a spectrophotometer to measure absorbance at 645 nm for chlorophyll b and 663 nm for chlorophyll a (28). Using the extinction coefficients, the amount of chlorophyll was determined as per equations 1-3.

$$\text{Chlorophyll a } (\mu g\ mL^{-1}) = \frac{12.25\ E_{663.6} - 2.55\ E_{646.6}}{\text{Sample volume (ml)}} \quad \text{(Eqn. 1)}$$

$$\text{Chlorophyll b } (\mu g\ mL^{-1}) = \frac{20.31\ E_{646.6} - 2.55\ E_{663.6}}{\text{Sample volume (ml)}} \quad \text{(Eqn. 2)}$$

$$\text{Chlorophyll a + b } (\mu g\ mL^{-1}) = \frac{17.46\ E_{646.6} - 2.55\ E_{663.6}}{\text{Sample volume (ml)}} \quad \text{(Eqn. 3)}$$

where $E_{663.6}$ and $E_{646.6}$ stand for absorbances at 663.6 and 646.6 nm, respectively and a lower absorbance at 750 nm. Solvent extraction followed by spectrophotometric measurement is a quick method for determining the amount of carotenoid present in plant samples. Weigh a sample of plants (10 g). To acquire the extract, use acetone and centrifuge the sample. Using a separating funnel, move the carotenoid-rich phase to petroleum ether. To remove acetone, wash three times with ultrapure water. Petroleum ether is used as a blank to measure absorbance at 450 nm. Use the following formula to get the total amount of carotenoids (29).

$$\text{Carotenoids } (\mu g\ g^{-1}) = \frac{A \times V \times 10^4}{2592 \times P} \quad \text{(Eqn. 4)}$$

Where A is the absorbance, V is the volume of the extract and P is the sample weight. To collect biometric observations in the pot experiment, three plants (Maize) were randomly selected from the pots of each treatment at 90th days after sowing (DAS). Plant height and number of leaves were measured and the mean values were calculated using the SI system of units.

Statistical analysis of data

One-way analysis of variance was used for all data related to the treatments. Duncans' multiple range test was used to identify treatment differences at the $p < 0.05$ level. SPSS 18.0 for Windows was used to perform the statistical analyses (SPSS Inc., Chicago, IL, USA). Principal component analysis was performed on the parameters (30).

Result and Discussion

The initial physicochemical characteristics of the soil from the paperboard effluent-irrigated soil were analysed and are presented in Table 2. The pH of the soil was 7.89, indicating mildly alkaline conditions. While certain plants that prefer acidic soil may have problems absorbing nutrients, most agricultural

Table 2. Initial Physico-chemical characteristics of the soil from the paperboard effluent contaminated soil

S. No.	Parameters	Values
	Physico-chemical properties	
1.	pH	7.89
2.	Electrical conductivity (dS m^{-1})	0.46
3.	Available N (kg ha^{-1})	178.26
4.	Available P (kg ha^{-1})	24.79
5.	Available K (kg ha^{-1})	189.32
6.	Exchangeable Ca (cmol (p+) kg^{-1})	5.67
7.	Exchangeable Mg (cmol (p+) kg^{-1})	2.84
8.	Exchangeable Na (cmol (p+) kg^{-1})	1.72
9.	Exchangeable K (cmol (p+) kg^{-1})	2.34
	Heavy metals	
10.	Available chromium (ppm)	0.29
11.	Available lead (ppm)	0.86
12.	Available cadmium (ppm)	0.02
	Soil enzymes	
13.	Urease (µg of NH_4-N released g^{-1} of soil h^{-1})	8.68
14.	Phosphatase (µg PNPP g^{-1} of soil)	38.24
15.	Dehydrogenase (µg TPF g^{-1} of soil)	49.23

crops can thrive at this pH level. Electrical conductivity (0.46 dS m^{-1}), there is little chance of salt stress to plants because salinity is below an acceptable level for agriculture (31). Significant nitrogen availability (178.26 kg ha^{-1}) was shown by macronutrient analysis, indicating a sufficient supply of nitrogen for crop development without the need for intensive fertilization (32). The moderate range of available phosphorus (24.79 kg ha^{-1}) may be enough for the majority of crops, although high-demand crops may need supplementing. The exceptionally high potassium levels (189.32 kg ha^{-1}) suggest superior potassium stores that ought to promote robust plant development and stress tolerance (33). The exchangeable cations, which reflect the cation exchange capacity, indicate that calcium (5.67 cmol(p^+) kg^{-1}) is the most abundant cation. Magnesium (2.84 cmol(p^+) kg^{-1}), potassium (2.34 cmol(p^+) kg^{-1}) and sodium (1.72 cmol(p^+) kg^{-1}) are next in line. As is common in productive agricultural soils, this distribution indicates strong soil fertility and structure, with calcium predominating the exchange complex. A 2:1 calcium-to-magnesium ratio is ideal for soil physical characteristics and nutrient absorption (34). Low levels of contamination are indicated by the heavy metal analysis of the soil collected from the papermill effluent-irrigated soil. Lead (0.86 ppm), cadmium (0.02 ppm) and chromium (0.29 ppm) are all well below the legal limits for agricultural soils, indicating minimal environmental contamination and a reduced threat to crop growth and development. These low concentrations suggest that these potentially hazardous elements have either been effectively immobilized within the soil matrix or that human inputs have been minimized. Enzyme activity in soil sheds light on the dynamics of organic materials and microbial activity. 7.68 µg of NH_4-N emitted g^{-1} of soil h^{-1}, of urease, indicating a modest potential for nitrogen cycling. This will effectively reduce the salinity stress on plants (35). In addition to the modest quantities of accessible P, the phosphatase activity (38.24 µg PNPP g^{-1} of soil) indicates a fair potential for phosphorus mineralization. The comparatively high dehydrogenase activity (49.23 µg TPF g^{-1} of soil) suggests strong overall microbial activity and ideal circumstances for the breakdown of organic materials (36).

The effect of *Gracilaria* sps. on the pH of the soil decreased for all treatments, as mentioned in Table 3. The first 45 days after surgery (DAS) had the highest pH values, which progressively reduced by 90 DAS. When seaweed (*Gracilaria* sp.) was applied in more quantities (7.5 % of seaweed extract of two species of *Gracilaria*), the pH of the soil was marginally higher than it was in the control. The greatest pH was found in Treatment T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) (7.59 at 45 DAS), followed by T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) showing a pH of 7.46 and 7.48 which is low pH compared to other treatments suggesting that seaweed had a buffering effect (37) and EC values showed a small decrease throughout time, ranging from 0.37 to 0.42 dS m^{-1} at 45 DAS and from 0.29 to 0.35 dS m^{-1} at 90 DAS. Treatments with greater *Gracilaria edulis* concentrations in addition to RDF (such as T_6 (*Gracilaria* sp. 2 @ 2.5 % + RDF) and T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) showed higher EC values, suggesting better nutritional availability. The pH range of 6.0 to 7.5 is ideal for most crops because it contains the highest concentrations of vital nutrients, including potassium, phosphate and nitrogen. Toxicities or nutritional shortages may arise outside of this range (7). The range of available nitrogen levels at 90 DAS was 135.52 kg ha^{-1} (T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) to 145.55 kg ha^{-1} (T_2 (*Gracilaria* sp. 1 @ 7.5 % + RDF), whereas at 45 DAS they were between 152.57 kg ha^{-1} (T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) and 160.61 kg ha^{-1} (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF). With standard errors of ± 3.52 and ± 3.64, the mean nitrogen levels were 165.58 kg ha^{-1} at 45 and 146.38 kg ha^{-1} at 90 DAS, respectively. Nitrogen retention was better in treatments with lower *Gracilaria* sp. concentrations (such as T_2 (*Gracilaria* sp. 1 @ 2.5 % + RDF and T_6 (*Gracilaria* sp. 2 @ 2.5 % + RDF) than in treatments with greater concentrations or combinations. By regulating growth and stress responses, this mechanism, which is controlled by ethylene (ET) and jasmonate (JA) signalling, improves tolerance to salt and cadmium (38). At 45 DAS, the amount of available phosphorus was 18.32 kg ha^{-1} (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and at 90 DAS, it was 15.11 kg ha^{-1} (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF). Phosphorus stabilizes cellular membranes and supports osmoregulation through proline accumulation during drought or salinity (39). During 45 DAS, the available potassium levels were 171.42 kg ha^{-1} (T_1 (Absolute Control) to 176.64 kg ha^{-1} (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and during 90 DAS, they were 150.85 kg ha^{-1} (T_1 (Absolute Control) to 161.09 kg ha^{-1} (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF). At 45 and 90 DAS, the mean potassium levels were 178.85 kg ha^{-1} and 157.31 kg ha^{-1}, respectively, with standard errors of ± 3.41 and ± 2.61.

Calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) exchangeable amounts in soil samples under various treatments and at two distinct intervals (45^{th} and 90^{th} DAS) are shown in Table 4. The treatments include the Recommended Dose of Fertilizers (RDF) in combination with different doses of *Gracilaria gracilis* and *Gracilaria edulis* includes 2.5 %, 5 %, 7.5 % and 10 %. At 45 and 90 DAS, the mean exchangeable Ca was 5.46 and 4.79 cmol(p+) kg^{-1}, respectively. This suggests that exchangeable Ca generally decreased with time for all treatments. The lowest values for 45 and 90 DAS were found in T_1 (Absolute Control) (5.18 and 4.62 cmol(p+) kg^{-1}, respectively). While Treatment T_6 (*Gracilaria* sp. 2 @ 2.5 % + RDF) recorded the greatest Ex. Ca at 90 DAS (4.90 cmol(p+) kg^{-1}), Treatment T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) recorded the highest Ex. Ca at 45 DAS (5.55 cmol(p+) kg^{-1}). For the differences between treatments to be considered statistically significant, $p > 0.05$ significance level must be more than 0.25 for 45 DAS and 0.19 for 90 DAS. A secondary messenger in stress signalling pathways is calcium. Stress causes changes in cytosolic Ca^{2+} levels, which activate calcium-dependent protein kinases (CDPKs) that control the expression of genes that respond to stress (40). The mean exchangeable magnesium decreased, reaching 2.74 cmol(p+) kg^{-1} at 45 DAS and 2.45 cmol (p+) kg^{-1} at 90 DAS. The lowest values at both time intervals (2.65 and 2.33 cmol(p+) kg^{-1}) were found in the control (T_1). Ex. Mg for treatment T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) was comparatively high at 45 and 90 DAS (2.81 and 2.57 cmol(p+) kg^{-1}, respectively). 45 and 90 DAS were related ($p > 0.05$) values of 0.09 and 0.13. At 45 DAS, the mean exchangeable Na was 1.65 cmol(p+) kg^{-1}; at 90 DAS, it was 1.44 cmol(p+) kg^{-1}. At both periods, the control (T_1) had lower values (1.55 and 1.32 cmol(p+) kg^{-1}) than the majority of treatments. At 45 and 90 DAS, respectively, treatments T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) had elevated Ex. Na levels. For 45 and 90 DAS, the $p > 0.05$ values are

Table 3. Effect of the seaweed extract of *Gracilaria gracilis and Gracilaria edulis* application on soil pH, EC and Macronutrients

Treatments	pH		EC (dS m^{-1})		Available N (kg ha^{-1})		Available P (kg ha^{-1})		Available K (kg ha^{-1})	
DAS	45	90	45	90	45	90	45	90	45	90
T_1 - Absolute Control	7.41 ± 0.09	6.11 ± 0.07	0.37 ± 0.00	0.29 ± 0.00	159.21 ± 0.02	139.99 ± 1.21	20.69 ± 0.27	14.33 ± 0.16	171.42 ± 0.19	150.85 ± 0.82
T_2 - *Gracilaria* sp. 1 @ 2.5 % + RDF	7.54 ± 0.00	6.22 ± 0.07	0.42 ± 0.00	0.35 ± 0.00	170.54 ± 1.90	153.49 ± 0.74	21.62 ± 0.09	17.84 ± 0.11	183.96 ± 2.05	161.09 ± 2.37
T_3 - *Gracilaria* sp. 1 @ 5.0 % + RDF	7.50 ± 0.04	6.20 ± 0.05	0.41 ± 0.00	0.33 ± 0.00	168.29 ± 1.11	151.46 ± 1.41	20.96 ± 0.09	17.29 ± 0.19	181.24 ± 1.63	159.49 ± 2.11
T_4 - *Gracilaria* sp. 1 @ 7.5 % + RDF	7.46 ± 0.02	6.15 ± 0.02	0.39 ± 0.00	0.30 ± 0.00	160.61 ± 0.68	145.55 ± 0.13	18.32 ± 0.13	15.11 ± 0.07	176.64 ± 2.23	155.44 ± 1.07
T_5 - *Gracilaria* sp. 1 @ 10 % + RDF	7.48 ± 0.02	6.17 ± 0.08	0.40 ± 0.0	0.34 ± 0.00	165.37 ± 2.39	148.83 ± 2.10	19.27 ± 0.23	15.90 ± 0.21	180.42 ± 1.79	158.77 ± 0.24
T_6 - *Gracilaria* sp. 2@ 2.5 % + RDF	7.62 ± 0.08	6.22 ± 0.09	0.41 ± 0.01	0.31 ± 0.00	169.53 ± 1.38	152.58 ± 1.97	22.17 ± 0.27	18.29 ± 0.03	179.96 ± 1.73	159.37 ± 1.39
T_7 - *Gracilaria* sp. 2@ 5.0 % + RDF	7.57 ± 0.09	6.16 ± 0.03	0.41 ± 0.00	0.33 ± 0.00	166.79 ± 1.95	150.11 ± 2.07	21.64 ± 0.27	17.85 ± 0.17	179.92 ± 2.00	156.33 ± 1.13
T_8 - *Gracilaria* sp. 2@ 7.5 % + RDF	7.48 ± 0.02	6.14 ± 0.02	0.38 ± 0.00	0.30 ± 0.00	161.37 ± 1.45	146.23 ± 1.41	19.79 ± 0.00	17.76 ± 0.03	174.34 ± 2.10	153.42 ± 0.51
T_9 - *Gracilaria* sp. 2@ 10 % + RDF	7.52 ± 0.05	6.20 ± 0.08	0.39 ± 0.01	0.32 ± 0.00	163.81 ± 1.58	147.43 ± 1.90	20.68 ± 0.24	17.06 ± 0.22	177.40 ± 1.65	157.11 ± 1.65
T_{10} - *Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2@ 7.5 % + RDF	7.59 ± 0.03	6.17 ± 0.03	0.42 ± 0.00	0.34 ± 0.00	169.52 ± 1.48	152.57 ± 1.15	21.53 ± 0.12	15.02 ± 0.09	183.25 ± 0.22	161.26 ± 1.50
Mean	7.51	6.18	0.399	0.321	165.50	148.82	20.66	16.64	178.85	157.31
SEd	0.14	0.16	0.009	0.007	3.52	2.96	0.30	0.32	3.41	2.69
P > 0.05	0.29	0.33	0.020	0.014	7.36	6.19	0.64	0.66	7.11	5.62

Table 4. Effect of seaweed extract of *Gracilaria gracilis* and *Gracilaria edulis* application on soil micronutrients

Treatments	Ex. Ca (cmol (p+) kg^{-1})		Ex. Mg (cmol (p+) kg^{-1})		Ex. Na (cmol (p+) kg^{-1})		Ex. K (cmol (p+) kg^{-1})	
DAS	45	90	45	90	45	90	45	90
T_1 - Absolute Control	5.18 ± 0.06	4.62 ± 0.05	2.65 ± 0.02	2.33 ± 0.02	1.55 ± 0.02	1.32 ± 0.01	1.81 ± 0.02	1.01 ± 0.01
T_2 - *Gracilaria* sp. 1 @ 2.5 % + RDF	5.43 ± 0.08	4.85 ± 0.06	2.80 ± 0.03	2.56 ± 0.01	1.710.02 ±	1.50 ± 0.02	2.16 ± 0.03	1.91 ± 0.00
T_3 - *Gracilaria* sp. 1 @ 5.0 % + RDF	5.37 ± 0.07	4.79 ± 0.01	2.78 ± 0.03	2.54 ± 0.00	1.69 ± 0.00	1.49 ± 0.01	2.15 ± 0.00	1.85 ± 0.01
T_4 - *Gracilaria* sp. 1 @ 7.5 % + RDF	5.19 ± 0.00	4.64 ± 0.01	2.69 ± 0.04	2.37 ± 0.03	1.59 ± 0.02	1.38 ± 0.01	2.09 ± 0.02	1.86 ± 0.02
T_5 - *Gracilaria* sp. 1 @ 10 % + RDF	5.41 ± 0.04	4.83 ± 0.01	2.76 ± 0.03	2.43 ± 0.03	1.66 ± 0.00	1.47 ± 0.00	2.12 ± 0.03	1.88 ± 0.01
T_6 - *Gracilaria* sp. 2 @ 2.5 % + RDF	5.49 ± 0.05	4.90 ± 0.07	2.79 ± 0.02	2.46 ± 0.01	1.70 ± 0.01	1.51 ± 0.01	2.15 ± 0.02	1.91 ± 0.02
T_7 - *Gracilaria* sp. 2 @ 5.0 % + RDF	5.39 ± 0.03	4.81 ± 0.04	2.77 ± 0.04	2.44 ± 0.01	1.68 ± 0.01	1.49 ± 0.00	2.14 ± 0.02	1.90 ± 0.02
T_8 - *Gracilaria* sp. 2 @ 7.5 % + RDF	5.34 ± 0.03	4.77 ± 0.07	2.68 ± 0.02	2.36 ± 0.02	1.58 ± 0.01	1.39 ± 0.00	1.99 ± 0.03	1.76 ± 0.01
T_9 - *Gracilaria* sp. 2 @ 10 % + RDF	5.30 ± 0.08	4.73 ± 0.06	2.74 ± 0.01	2.51 ± 0.00	1.64 ± 0.00	1.44 ± 0.01	2.13 ± 0.03	1.89 ± 0.00
T_{10} - *Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF	5.55 ± 0.01	4.96 ± 0.06	2.81 ± 0.03	2.57 ± 0.02	1.71 ± 0.01	1.50 ± 0.01	2.26 ± 0.02	1.91 ± 0.00
Mean	5.46	4.79	2.74	2.45	1.65	1.44	2.09	1.78
SEd	0.11	0.09	0.04	0.06	0.03	0.03	0.03	0.03
P > 0.05	0.25	0.19	0.09	0.13	0.06	0.07	0.07	0.06

0.06 and 0.07, respectively. At 45 DAS, the mean exchangeable K was 2.09 cmol(p+) kg^{-1}; at 90 DAS, it was 1.78 cmol(p+) kg^{-1}. At 45 and 90 DAS, the control (T_1) had the lowest Ex. K values (1.81 and 1.01 cmol(p+) kg^{-1}). At 45 DAS (2.26 cmol(p+) kg^{-1}), Treatment T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) had the greatest Ex. K, while at 90 DAS (1.91 cmol(p+) kg^{-1}), Treatment T_6 showed the highest Ex. K. For 45 and 90 DAS, the $p > 0.05$ values are 0.07 and 0.06, respectively. Under stress, plant hormones such as abscisic acid (ABA) regulate ion transporters to preserve a desirable K^+/Na^+ ratio (41). These hormones are present in *Gracilaria* sps. and regulate plant growth and development.

At 45 and 90 days after sowing (DAS), the effects of applying seaweed extracts from *Gracilaria gracilis* (*Gracilaria* sp. 1) and *Gracilaria edulis* (*Gracilaria* sp. 2) on soil enzyme activities (Urease, Phosphatase and Dehydrogenase) are shown in Fig. 1-3. The impact of seaweed extract on soil urease activity on 45th and 90th days after sowing (DAS), across ten distinct treatments (T_1 to T_{10}) is depicted in the bar graph (Fig. 1). In comparison to the 90th DAS, the urease activity is consistently greater on the 45th DAS for all treatments. On the 45th DAS, in all the treatments, the urease activity varies between 8.5 µg and 8.7 µg of NH4-N released g^{-1} soil h^{-1}. The range of urease activity on the 90th DAS is around 7.4 µg to 7.7 µg of NH4-N emitted g^{-1} soil h^{-1}. The best treatment shows in T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) the values are 8.56 µg of NH4-N released g^{-1} soil h^{-1} and 8.57 µg of NH4-N released g^{-1} soil h^{-1} on 45th DAS and reduction has been found on the 90th DAS. Seaweed extracts function as biostimulants by promoting microbial activity and establishing optimal circumstances for advantageous bacteria. Preserving a wet environment that supports enzymatic processes indirectly increases urease activity (42). The impact of seaweed extract on soil phosphatase activity at 45 and 90 days after sowing (DAS) for ten distinct treatments (T_1-T_{10}) is depicted in Fig. 2. Across all treatments, phosphatase activity, expressed in µg PNPP g^{-1} of soil, often exhibits greater values at 45th DAS than 90th DAS. Although a decrease is present in all treatments, there are considerable differences in the absolute values of phosphatase activity across them. Notably, after 45th DAS, phosphatase activity was comparatively greater in treatments T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) than in the other treatments. The phosphatase activity at 45 DAS was between 33 µg PNPP g^{-1} (T_1 - Absolute Control) and 38 µg PNPP g^{-1} (T_2 - *Gracilaria* sp. 1 @ 2.5 % + RDF). The phosphatase activity at 90 DAS was between 30 and 34 µg PNPP g^{-1}. Phosphatases play a role in releasing phosphorus from organic molecules into a form accessible to plants (42). The impact of seaweed extract on soil dehydrogenase activity at 45 and 90 days after sowing (DAS) is depicted in Fig. 3. Dehydrogenase activity varied across treatments (T_1 to T_{10}) and was expressed in terms of µg TPF g^{-1} of soil. In comparison to their respective levels at 90 DAS, all treatments exhibited increased dehydrogenase activity at 45 DAS. At both periods, T_5 and T_6 showed the greatest dehydrogenase activity among the treatments, suggesting that seaweed extract significantly improved soil microbial activity that increased plant growth and development. On the other hand, at both periods, T_1 (control) had the lowest dehydrogenase activity. All treatments showed a decrease in dehydrogenase activity from 45 DAS to 90 DAS, which may indicate a gradual decrease in microbial metabolic activity. Dehydrogenase enzymes indicate microbial oxidative activity and general soil microbial health. Applying seaweed extracts increases dehydrogenase activity, which indicates better soil vitality and microbial respiration that induce plant health (43).

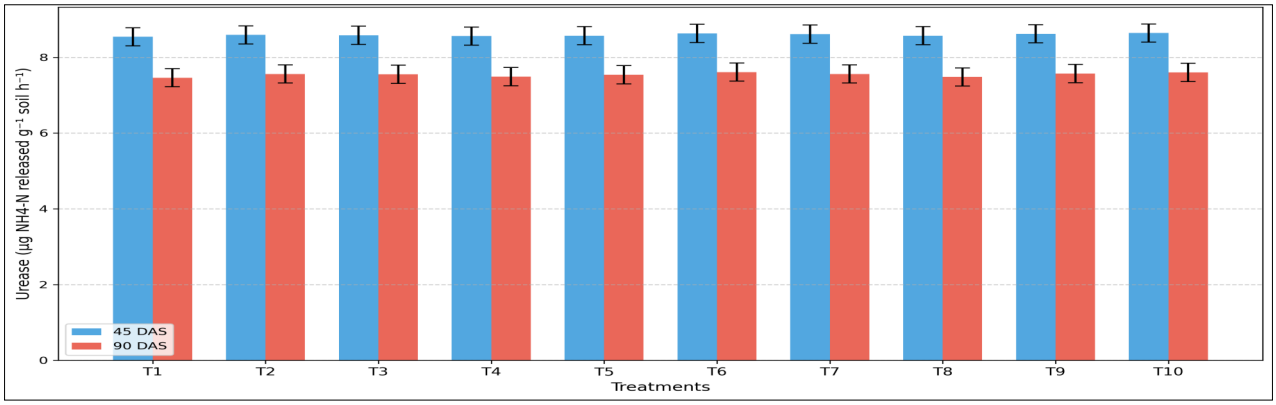

Fig. 1. Effect of seaweed extract on urease activity in soil irrigated with paperboard effluent at 45th and 90th DAS.

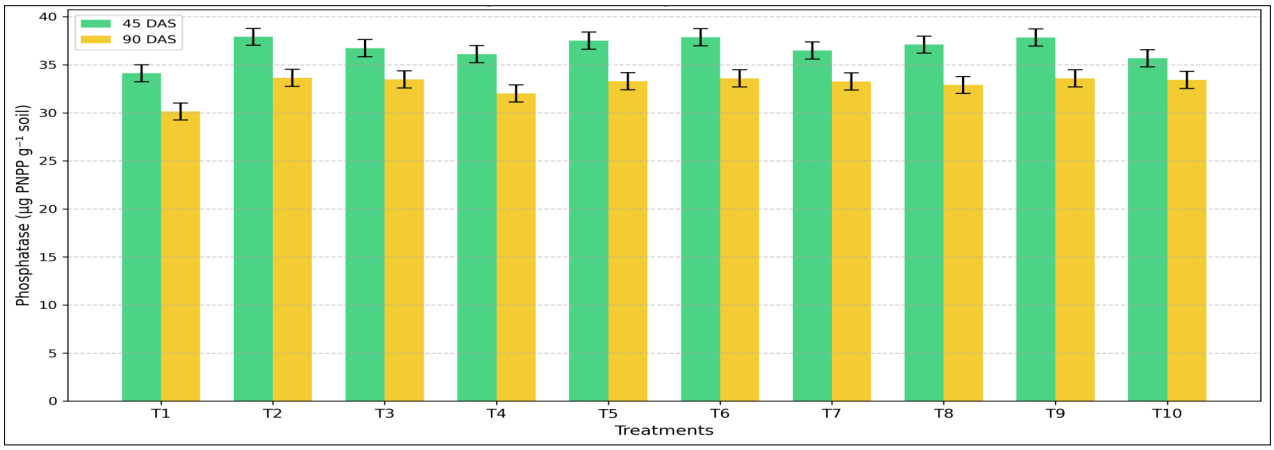

Fig. 2. Effect of seaweed extract on phosphatase activity in soil irrigated with paperboard effluent at 45th and 90th DAS.

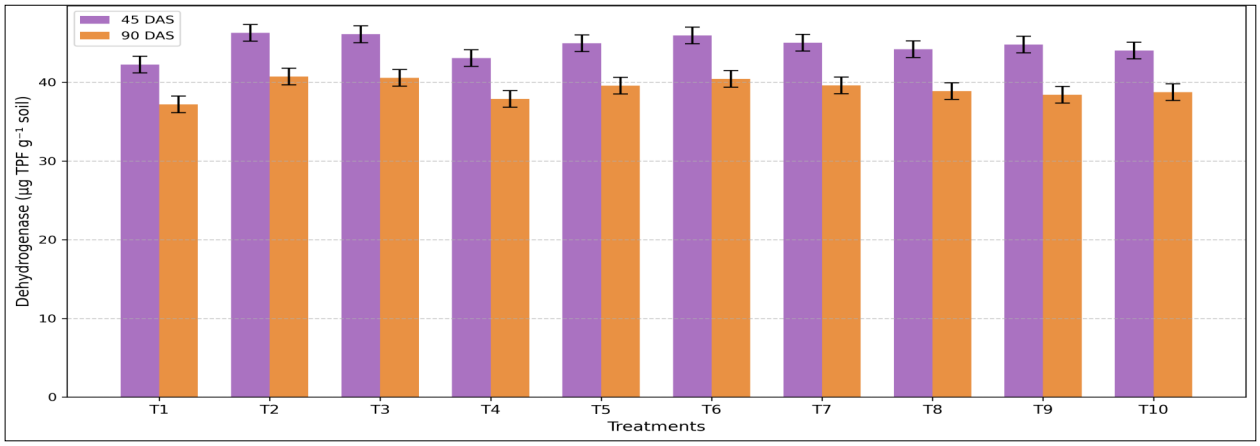

Fig. 3. Effect of seaweed extract on dehydrogenase activity in soil irrigated with paperboard effluent at 45th and 90th DAS.

PCA analysis in paperboard effluent irrigated soil

A principal Component Analysis (PCA) biplot, which shows the correlations between several variables and their contributions to the first two principal components (Dim1 and Dim2), is shown in Fig. 3a. Majority of the variability in the dataset is captured by Dim1, which accounts for 76.3 % of the overall variance. Dim2, which offers more but less important information, accounts for 9.5 % of the variation. Every variable is shown as a vector that starts at the plots' centre. The vectors' contributions to Dim1 and Dim2 are shown by their length and direction. The long vectors of variables like P, Na and K strongly coincide with this axis, indicating that they contribute significantly to Dim1.

Despite having less total effect since Dim2 has a smaller explained variance, variables like phosphatase and dehydrogenase contribute more to it. The vectors' color gradient corresponds to their cos2 values, which indicate how well they are represented on the PCA plot. The PCA dimensions better capture variables with larger cos^2 values (closer to 0.95, shown in red). Strong representation is suggested by the large cos^2 values of P and Na, for instance. Weaker representation is shown by variables like Ex..Ca and Urea having lower cos^2 values (nearer to blue).

Potential correlations or common qualities are suggested by strongly aligned or clustered variables together in similar orientations (e.g., K, Na, Ex). On the other hand, variables placed far apart might suggest opposing correlations. Majority of the data variability may be attributed to factors recorded along this axis, according to the large percentage of variation described by Dim1. Strong correlations between Dim1 and variables like P, Na and K suggest that these variables have a significant impact on the main patterns in the dataset. Dim2 picks up more details in the data, although it adds less variance (9.5 %). Dim2 has larger correlations with variables like phosphatase and dehydrogenase, indicating supplementary trends or factors that could supplement those that Dim1 measures. The plots' grouping of certain variables in similar orientations indicates possible common impacts or positive correlations. For instance, K and Na might have comparable roles or behave similarly in the dataset. On the other hand, variables that are orthogonally positioned (such as P vs others) could have different properties or conflicting effects. The cos^2 values gauge each variables' level of representation on the PCA biplot. High cos^2 values indicate strong representation for variables such as P, which makes them trustworthy interpretive indicators. For variables like urea, lower cos^2 values indicate less representation, necessitating care in evaluating their contributions.

In Table 5, the effects of seaweed extracts from *Gracilaria gracilis* (*Gracilaria* sp. 1) and *Gracilaria edulis* (*Gracilaria* sp. 2) on the total amount of nitrogen (N), phosphorus (P) and potassium (K) in plants at 45 and 90 days after sowing (DAS) are examined. At 45 and 90 DAS, the *G. gracilis* treatments (T_4-T_8) had a greater effect on the total N content than the control. Although it wasn't always the greatest, the N content was comparatively high at the maximum *G. gracilis* concentration of 10 % (T_5). Although the rise was not as significant as that shown with *G. gracilis* treatments, *G. edulis* treatments (T_6 (*Gracilaria* sp. 2 @ 2.5 % RDF) –T_9 (*Gracilaria* sp. 1 @ 10 % + RDF) Similarly, raised N content was raised in comparison to the control. While the overall N content was lower at 90 DAS than several other treatments, the combination treatment of *G. gracilis* and *G. edulis* (T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) had a high N content at 45 DAS. *G. gracilis* treatments (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) consistently displayed higher total P content than

Table 5. Effect of application of seaweed extract on *Gracilaria gracilis* and *Gracilaria edulis* on plants

Treatments	Total N (ppm)		Total P (ppm)		Total K (ppm)	
DAS	**45**	**90**	**45**	**90**	**45**	**90**
T_1 - Absolute control	227.9 ± 0.51	230.1 ± 0.50	17.2 ± 0.21	18.0 ± 0.01	128.8 ± 1.01	130.5 ± 0.18
T_2 - *Gracilaria* sp. 1 @ 2.5 % + RDF	246.5 ± 0.67	248.3 ± 2.09	21.5 ± 0.25	22.0 ± 0.04	130.8 ± 1.41	133.0 ± 0.04
T_3 - *Gracilaria* sp. 1 @ 5.0 % + RDF	247.2 ± 3.64	252.8 ± 1.29	22.5 ± 0.03	25.4 ± 0.33	132.3 ± 0.04	138.7 ± 1.46
T_4 - *Gracilaria* sp. 1 @ 7.5 % + RDF	249.1 ± 0.30	260.2 ± 2.03	24.3 ± 0.20	28.0 ± 0.17	136.0 ± 1.47	143.5 ± 1.25
T_5 - *Gracilaria* sp. 1 @ 10 % + RDF	251.2 ± 0.38	255.1 ± 1.84	26.2 ± 0.11	27.1 ± 0.38	139.6 ± 0.50	142.1 ± 1.92
T_6 - *Gracilaria* sp. 2 @ 2.5 % + RDF	227.5 ± 0.55	229.8 ± 1.73	15.8 ± 0.21	17.0 ± 0.18	130.2 ± 1.12	132.7 ± 1.16
T_7 - *Gracilaria* sp. 2 @ 5.0 % + RDF	229.0 ± 2.68	231.6 ± 3.13	17.9 ± 0.10	19.2 ± 0.18	131.9 ± 0.55	134.4 ± 1.62
T_8 - *Gracilaria* sp. 2 @ 7.5 % + RDF	235.6 ± 2.05	238.5 ± 1.15	18.2 ± 0.14	20.0 ± 0.19	135.1 ± 1.38	137.6 ± 1.49
T_9 - *Gracilaria* sp. 2 @ 10 % + RDF	239.2 ± 0.57	242.3 ± 0.73	20.4 ± 0.27	22.5 ± 0.25	138.3 ± 0.71	141.0 ± 0.89
T_{10} - *Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF	256.3 ± 0.46	250.0 ± 2.40	26.8 ± 0.06	23.5 ± 0.30	140.1 ± 1.18	135.2 ± 0.04
Mean	240.94	243.87	21.07	22.27	134.31	136.86
Sed	4.48	4.83	0.49	0.48	2.97	2.86
P > 0.05	9.35	10.08	1.02	1.02	6.20	5.98

the control at both time points; the effect seems to be somewhat concentration-dependent, with higher concentrations generally resulting in greater P content; treatments with *G. edulis* (T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) also increased P content relative to the control, although the increase was not as significant as that of the *G. gracilis* treatments; and a potential interaction effect was suggested by the fact that the combined *G. gracilis* and *G. edulis* treatment (T_{10} *Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) produced a high P content at 45 DAS but declined to a value lower than other treatments at 90 DAS. In general, the total K content increased from 45 to 90 DAS. K content was enhanced by *G. gracilis* treatments (T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) in comparison to the control. Though not as much as the *G. gracilis* treatments, the *Gracilaria edulis* treatments (T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) also raised the K content in comparison to the control. At 45 DAS, the combined seaweed extract application to crop (T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) had a high K level; however, by 90 DAS, the concentration had dropped. Studies suggested that seaweed extracts, which are high in bioactive chemicals, improve plant defense systems and nutrient intake, fostering development even under stressful environments (44).

The effects of seaweed extracts from *Gracilaria gracilis* (*Gracilaria* sp. 1) and *Gracilaria edulis* (*Gracilaria* sp. 2) on exchangeable calcium, exchangeable magnesium and exchangeable sodium in plants at 45 and 90 days after sowing (DAS) are investigated in Table 6. The exchangeable calcium (Ex. Ca) concentration in plants exhibited considerable variation between treatments at both 45 and 90 days after sowing (DAS), with the greatest Ex. Ca content was found in treatment T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) at 45 DAS (3140 ppm) and in treatment T_7 (*Gracilaria* sp. 2 @ 5.0 % + RDF) at 90 DAS (3130 ppm). These findings suggest that using seaweed extracts-specifically, *Gracilaria* sp. increases calcium uptake, which is essential for plant development and food absorption, at a rate of 7.5 %. Calcium plays an essential role in photosynthesis, nutrient transport and cell wall construction, contributing to improved biomass generation (45). Exchangeable magnesium (Ex. Mg) content also varied significantly across treatments. The highest magnesium levels were reported in treatment T_5 (*Gracilaria* sp. 1 at 10 % + RDF) at both 45 DAS (231 ppm) and 90 DAS (236 ppm). This implies that greater levels of *Gracilaria* sp. extract enhance magnesium absorption, which is critical for chlorophyll production, enzyme activity and overall plant productivity. A magnesium deficit can significantly affect biomass accumulation and photosynthetic efficiency, underscoring the significance of a sufficient magnesium supply (46). The use of seaweed extracts enhanced the exchangeable sodium (Ex. Na) concentration while maintaining it within acceptable bounds for plant development. Treatment T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) had the greatest salt levels at 45 DAS (157 ppm) and 90 DAS (152 ppm). The salt content of the substrate affects sodium buildup, which can alter the osmotic equilibrium in plants. Moderate sodium levels can improve stress tolerance, but too much salt can cause ionic toxicity, which can limit development (47).

Gracilaria gracilis (*Gracilaria* sp. 1) and *Gracilaria edulis* (*Gracilaria* sp. 2) seaweed extracts were analyzed for their impact on Maize metrics for growth at different Recommended Dose of Fertilizer (RDF) treatment levels. Plant height and leaf count were among the growth characteristics measured at 4, 45 and 90-day intervals in Fig. 4. Plant height was higher in treatments containing seaweed extracts than in the absolute control (T_1) at

Table 6. Effect of application of seaweed extract of *Gracilaria gracilis* and *Gracilaria edulis* on plants

Treatments	Ex. Ca (ppm)		Ex. Mg (ppm)		Ex. Na (ppm)	
	45	90	45	90	45	90
T_1 - Absolute control	2955 ± 30.28	2950 ± 17.11	214 ± 0.55	216 ± 1.86	140 ± 1.26	139 ± 0.92
T_2 - *Gracilaria* sp. 1 @ 2.5 % + RDF	2995 ± 43.19	3010 ± 16.28	219 ± 1.05	222 ± 3.07	144 ± 0.65	143 ± 0.73
T_3 - *Gracilaria* sp. 1 @ 5.0 % + RDF	3025 ± 10.00	3065 ± 35.92	222 ± 2.27	233 ± 2.94	149 ± 1.70	145 ± 0.96
T_4 - *Gracilaria* sp. 1 @ 7.5 % + RDF	3140 ± 7.55	3120 ± 25.31	230 ± 1.66	238 ± 0.72	157 ± 0.80	152 ± 0.64
T_5 - *Gracilaria* sp. 1 @ 10 % + RDF	3070 ± 41.51	3100 ± 30.74	231 ± 1.80	236 ± 1.28	151 ± 1.00	148 ± 1.16
T_6 - *Gracilaria* sp. 2@ 2.5 % + RDF	2980 ± 27.76	3000 ± 26.14	217 ± 1.43	220 ± 1.85	143 ± 0.77	140 ± 0.80
T_7 - *Gracilaria* sp. 2@ 5.0 % + RDF	2905 ± 16.58	3130 ± 11.29	221 ± 0.27	224 ± 2.15	145 ± 1.79	142 ± 0.21
T_8 - *Gracilaria* sp. 2@ 7.5 % + RDF	3130 ± 18.81	3060 ± 22.07	225 ± 1.89	229 ± 1.44	148 ± 1.11	145 ± 0.70
T_9 - *Gracilaria* sp. 2@ 10 % + RDF	3055 ± 20.19	3085 ± 41.71	229 ± 02.8	232 ± 0.42	152 ± 0.64	150 ± 1.85
T_{10} - *Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF	3091 ± 32.51	2934 ± 8.82	235 ± 2.61	226 ± 2.72	147 ± 0.57	155 ± 1.72
Mean	3634.60	3046.00	224.30	227.60	147.60	145.90
Sed	75.60	59.79	4.16	4.79	1.96	3.72
P > 0.05	157.71	124.73	8.68	9.99	4.10	7.77

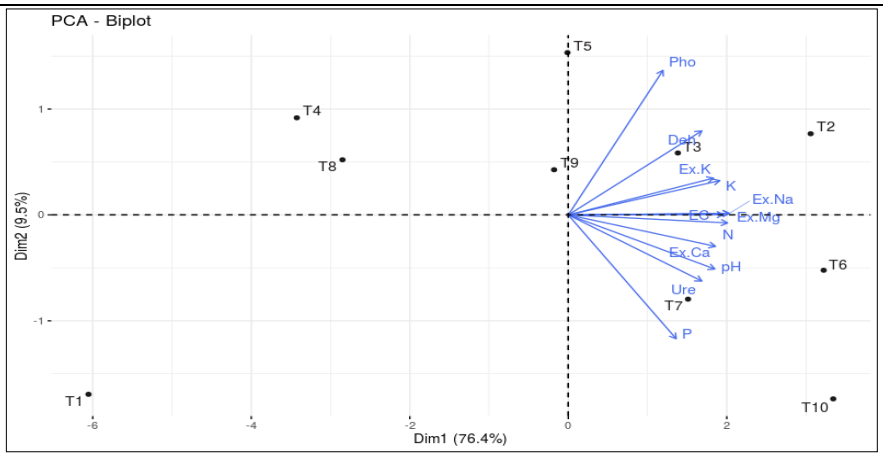

Fig. 4. Principal component analysis (PCA) biplot showing nutrient contributions along with treatments.

all intervals. The maximum plant height, for instance, was attained by T_8 (*Gracilaria* sps. 2 @ 7.5 % RDF) on day 90 (204.3 cm), followed by T_5 (*Gracilaria* sp. 1 @ 10 % RDF) at 196.8 cm. Significant improvement was also seen by the combined treatment (T_{10} *Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % RDF), which reached 187.6 cm on day 90. Higher seaweed extract treatment doses resulted in a gradual increase in the number of leaves. Treatments T_5 (*Gracilaria* sp. 1 @ 10 % + RDF), T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) and T_9 (*Gracilaria* sp. 2 @ 10 % + RDF) achieved the maximum leaf count of 12 by day 90. The average plant height for all treatments reached 188.10 cm on day 90, while the average number of leaves increased at 11.30. Abiotic stressors, such as drought or nutrient deficits, that interfere with metabolic processes affect growth and development in Maize. Under stressful conditions, seaweed extracts improve plant height and leaf count, demonstrating that they counteract these effects through metabolic reprogramming (48). Combined applications of *Gracilaria* sp. 1 and *Gracilaria* sp. 2 (e.g., T_{10} treatment) demonstrated synergistic effects on growth parameters, suggesting that different species of seaweed may complement each other in enhancing maize resilience to abiotic stress.

Gracilaria gracilis (*Gracilaria* sp. 1) and *Gracilaria edulis* (*Gracilaria* sp. 2) seaweed extracts' effects on several biochemical parameters in plants at 45 and 90 days after sowing (DAS) are examined in the findings shown in Fig. 5-8. In contrast to absolute control (T_1), the treatments involve varying amounts of seaweed extracts combined with the required dosage of fertilizers (RDF). The application of seaweed extracts increased the amount of chlorophyll a and b. T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) had the greatest Chl. a level (1.98 mg/g) at 90 DAS. Likewise, T_{10} at 90 DAS also had the greatest Chl. b level (1.12 mg/g). The biostimulant action of seaweed extracts, which encourages nutrient absorption and improves photosynthetic efficiency, may be responsible for this rise in chlorophyll concentration. Similar results were found in *Ascophyllum nodosum* extract significantly increased the amount of chlorophyll in *Asparagus* plants (43). The trend of total chlorophyll concentration was comparable to that of individual chlorophyll components. At 90 DAS, T_{10} had the highest total chlorophyll content (3.10 mg/g). Additionally, carotenoid content responded favorably to the application of seaweed extract. At 90 DAS, T_{10} had the greatest carotenoid concentration (0.86 mg/g). Carotenoids contribute to the overall photosynthetic efficiency and are essential in preventing photo-oxidation of chlorophyll. The observed rise indicates that plants treated with seaweed extracts have improved stress tolerance. By reducing oxidative damage, seaweed extracts increase carotenoid levels, which in turn promote photoprotection and stress resistance in crops (49).

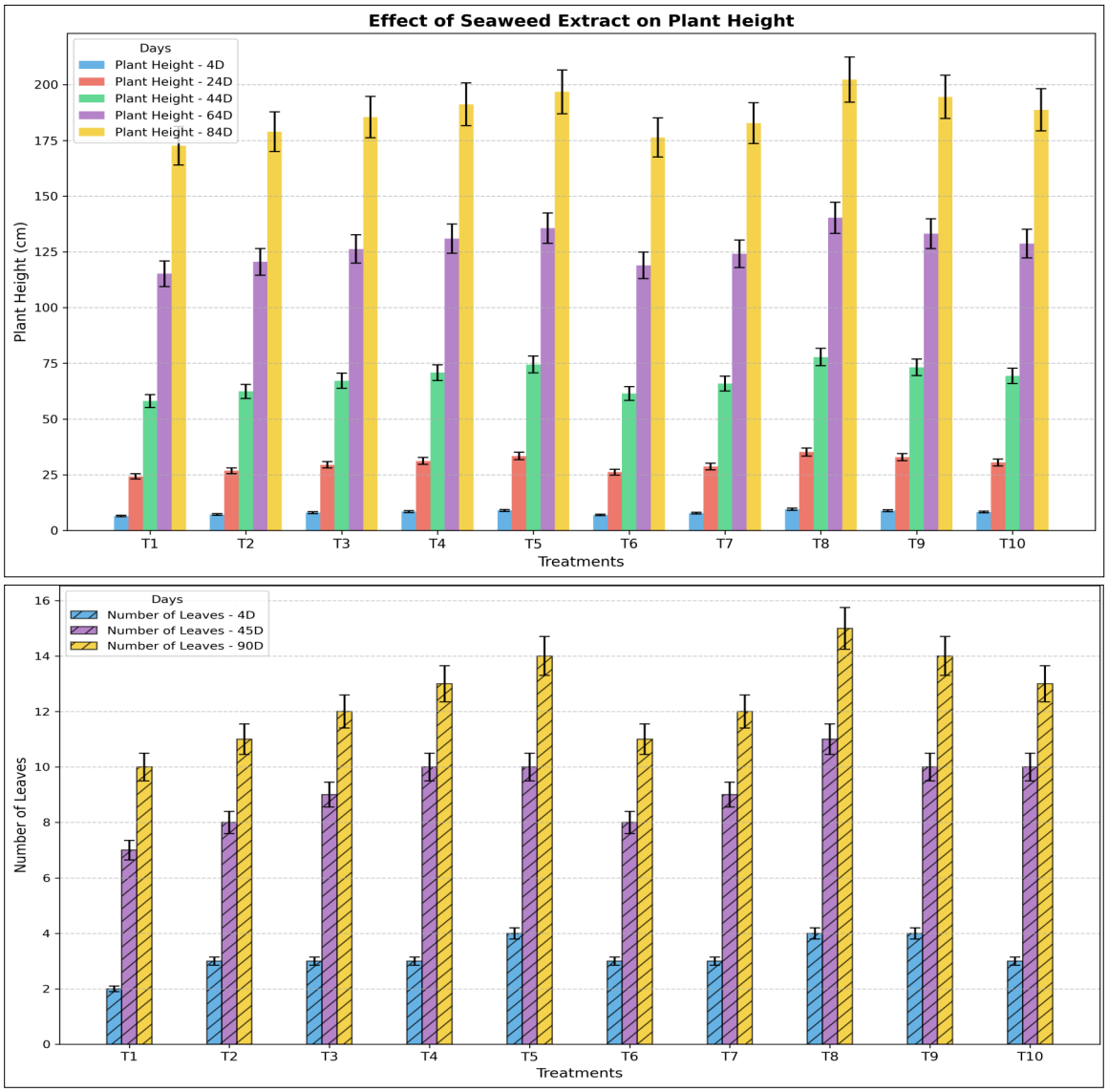

Fig. 5. Effect of seaweed extract and rdf on: **a.** plant height and **b.** number of leaves at various growth stages.

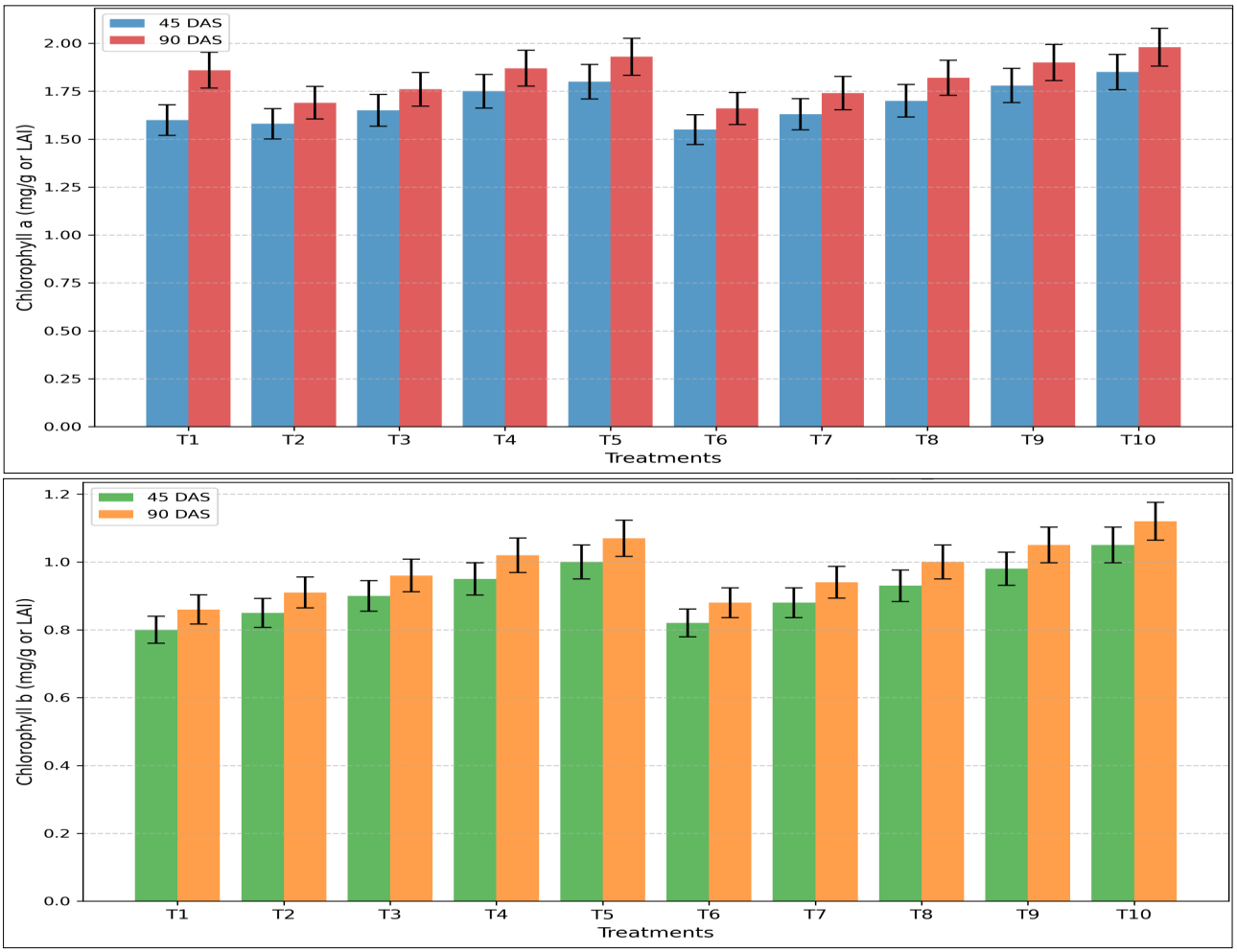

Fig. 6. Effect of seaweed extract on: **a.** chlorophyll a and **b.** chlorophyll b levels.

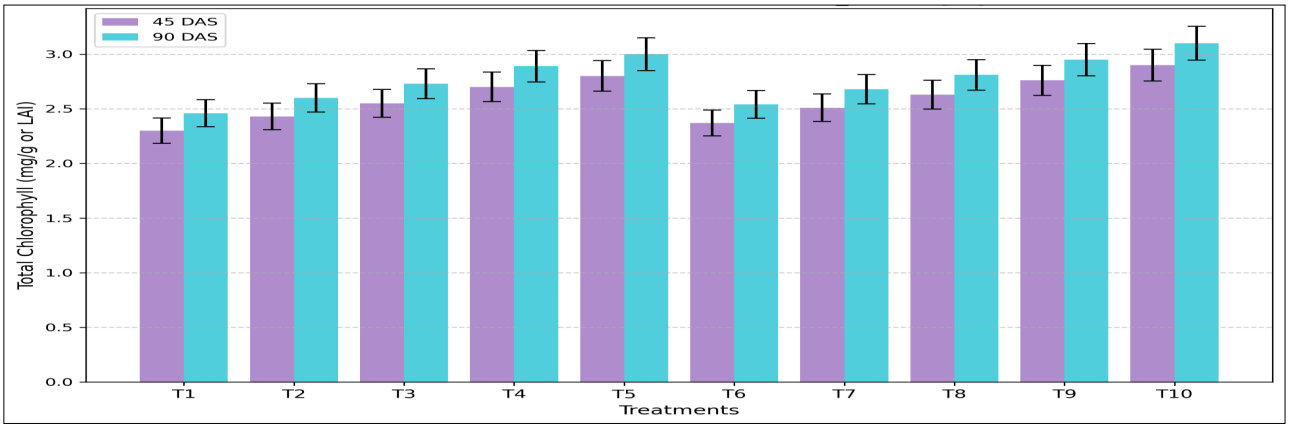

Fig. 7. Effect of seaweed extract on total chlorophyll levels.

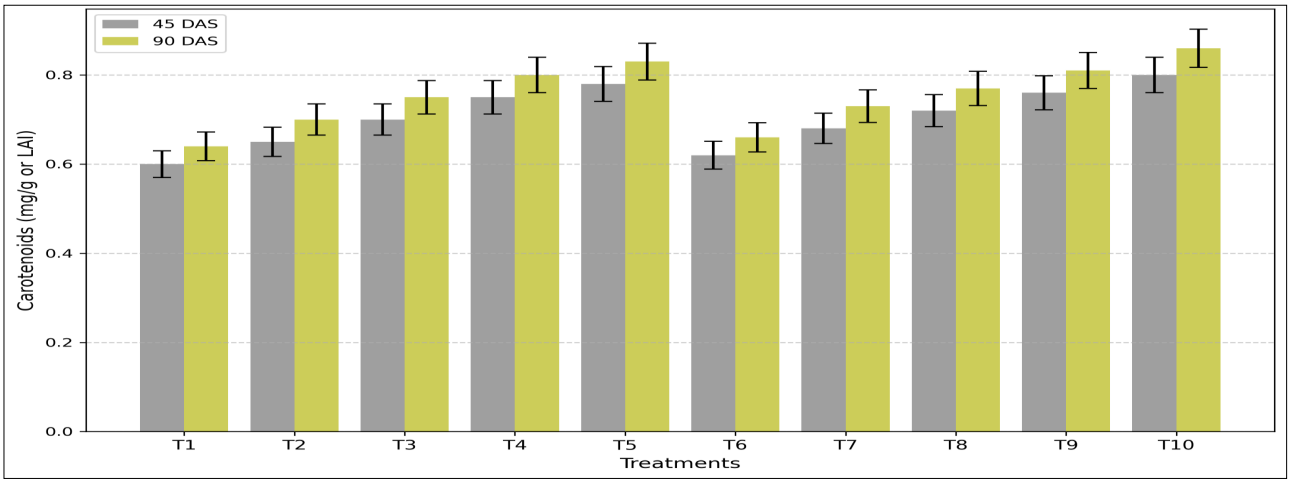

Fig. 8. Effect of seaweed extract on carotenoid content.

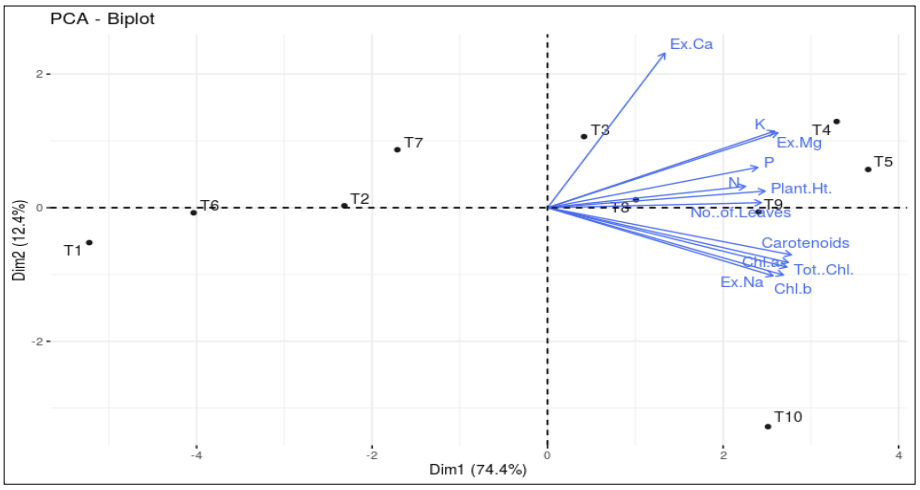

Fig. 9. Principal component analysis (PCA) biplot showing relationships between variables and treatments.

PCA analysis in the crop grown in paperboard effluent irrigated soil

The PCA biplot in Fig. 9, shows the principal component analysis of treatments (T_1 to T_{10}) and how they relate to different characteristics, including pigment concentrations (carotenoids, Chl. a, Chl.b, Tot.Chl.), plant height (plant ht.), number of leaves (no. of leaves) and nutrient content (Ex. Ca, Ex. Mg, Ex. Na, K, P, N). Of the entire variance, 74.4 % can be explained by the first principal component (Dim1) and 12.4 % by the second principal component (Dim2). Dim1 is very favorably connected with traits like Ex. Ca, Ex. Mg, K, P, N, plant height and no. of leaves. Carotenoids, chlorophyll a, chlorophyll b and total chlorophyll are pigments that also play a major role in Dim1. On Dim1s' negative side, T_1 (Absolute Control) is isolated. T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_5 (*Gracilaria* sp. 1 @ 10 % + RDF) are situated on Dim1s' positive side, whereas Dim2 is somewhat positive. For the majority of characteristics, T_6 (*Gracilaria* sp. 2 @ 2.5 % + RDF) and T_2 (*Gracilaria* sp. 1 @ 2.5 % + RDF) cluster close to the origin, suggesting moderate levels. T_7 (*Gracilaria* sp. 2 @ 5.0 % + RDF) is near the origin along Dim1 but in the positive quadrant of Dim2. T_3 (*Gracilaria* sp. 1 @ 5.0 % + RDF) strongly resembles features such as Ex. Mg and Ex. Ca. Near characteristics like plant height and pigments are grouped together by T_8 (*Gracilaria* sp. 2 @ 7.5 % + RDF) and T_9 (*Gracilaria* sp. 2@ 10 % + RDF). T_{10} (*Gracilaria* sp. 1 @ 7.5 % + *Gracilaria* sp. 2 @ 7.5 % + RDF) is firmly situated on Dim1s' positive side. Dim1s' significant contribution (74.4 %) indicates that it is the component that predominantly influences the majority of characteristics. Treatments positioned positively along Dim1 (e.g., T_4, T_5) have a strong correlation with traits like pigments and nutritional content (Ex. Ca, Ex. Mg, K). The isolation of T_1 suggests a unique profile with low values for the majority of the characteristics. Treatments such as T_4 (*Gracilaria* sp. 1 @ 7.5 % + RDF) and T_5 (*Gracilaria* sp. 1 @ 10 % + RDF) are appropriate for increasing pigment concentrations and nutritional content since they show high values for features that are favorably connected with Dim1. Treatments close to the origin, such as T_6 and T_2, have balanced trait values without sharp differences. The combination of T_8 (*Gracilaria* sp.2 @ 7.5 % + RDF) and T_9 (*Gracilaria* sp. 2 @ 10 % + RDF) indicates that these treatments improve characteristics associated with plant growth, such as the number of leaves and plant height. The grouping of characteristics, such as pigments (carotenoids, chlorophyll a and chlorophyll b), indicates that they are connected and influenced by treatments similarly.

Conclusion

The findings show that seaweed extracts, collected from the Ramanathapuram district, especially those from *Gracilaria gracilis* and *Gracilaria edulis*, hold great promise for improving plant development and reducing abiotic stress in crops cultivated in soil irrigated with paper mill effluent. In addition to increasing soil enzyme activities, including urease, dehydrogenase and phosphatase, the application of these extracts enhanced the physicochemical characteristics of the soil, such as nutrient availability and increased enzyme activity. In maize, these improvements enhanced biometric metrics, including plant height and leaf number, increased chlorophyll and carotenoid content and improved nutrient absorption. Additionally, the extracts showed that they might increase microbial activity and buffer the effects of salt stress, improving crop growth conditions. This study highlights the effectiveness of seaweed-based biostimulants as long-term solutions for improving agricultural productivity and soil health in contaminated areas, while reducing dependence on chemical inputs.

Acknowledgements

The authors are grateful to the ITC, PSPD, LTD, for funding this study.

Authors' contributions

RR was responsible for conceptualization, methodology, validation, formal analysis, data collection and interpretation, original draft writing and concluding the results and discussion. JR contributed to technical data analysis, validation, topic finalization and article correction. DP participated in manuscript correction and validation. CK was involved in editing and final correction of the article.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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