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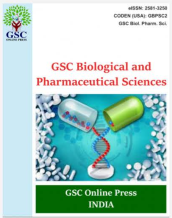

(Research Article)

Mitigating effects of Nigeria seaweed phytonutrients on biochemical and physiological conditions of *Lycopersicon esculentum* under heavy metal-induced abiotic stress

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Abstract

Abiotic stress has become a significant issue in recent years, affecting various physiological and biochemical aspects of crops and threatening food security. This study aimed to investigate the effects of seaweed on the biochemical and physiological conditions of Lycopersicon esculentum under heavy metal-induced abiotic stress. Lycopersicon esculentum plants were grown in greenhouse bags for four weeks and then exposed to 0.10M Pb Nitrate. They were divided into six groups: one control group sprayed with tap water and five experimental groups sprayed with different seaweed concentrations (0%, 20%, 40%, 60%, and 80%). Results showed that abiotic stress led to notable decreases in root length, shoot length, chlorophyll a, chlorophyll b, stomatal conductance, photosynthetic rate, Rubisco activity, and carotenoids by 48%, 47% 38%, 65%, 61%, 77%, 75%, and 68%, respectively. Seaweed treatment improved these indices, with the 80% seaweed group showing increases of 27%, 21%, 44%, 36%, 56%, and 48%, 54% after 21 days. Additionally, seaweed application enhanced antioxidant activities in the roots (SOD by 71%, CAT by 45%, POD by 51%, and APX by 92%) and in the leaves (SOD by 18%, CAT by 26%, POD by 34%, APX by 58%) compared to the control. The study concluded that seaweeds significantly mitigated oxidative stress in plants, boosting growth, antioxidant potential, mineral absorption, and osmotic protection. Nigeria seaweeds could be a promising tool to enhance food production by reducing oxidative stress in crops.

Keywords: Phytonutrients; Abiotic Stress; Phytohormones; Antioxidants; Seaweeds

1. Introduction

Seaweeds, also known as macroalgae, form a diverse and predominant group of photosynthetic organisms that play a crucial role in aquatic ecosystems. These marine plants belong to the kingdom *Thallophyta* and are considered essential components of the marine environment, particularly in coastal regions (Safinaz and Ragaa, 2013). Despite their immense potential, seaweeds remain an underutilized marine resource in Nigeria. Few research work exist as regards its bioactive contents, which perhaps may be one of the reasons why it is less utilized by majority of indigenous Africa people, even though they have been exploited for centuries as food sources, raw materials for industry, and in therapeutic and botanical applications.

Seaweed-based products are gaining prominence in agriculture due to their eco-friendly and cost-effective nature (Cai *et al.,* 2019). Seaweed extracts, rich in bioactive compounds, minerals, and nutrients, offer multiple benefits for plant growth, protection, and stress resistance. As concerns about the environmental hazards posed by synthetic fertilizers continue to rise, researchers are exploring alternative and sustainable agricultural practices. Seaweed-based fertilizers

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may serve as a promising solution to reduce reliance on chemical fertilizers, which often introduce toxic pollutants like heavy metals into the soil, leading to long-term environmental degradation (Rahman and Zhang, 2018)

Lead (Pb) contamination is a serious environmental concern, especially in agricultural soils. It can negatively impact plant health and development, including crop species such as tomatoes. Lead primarily enters the soil through industrial emissions, vehicular exhaust, and the use of lead-containing fertilizers or pesticides. When tomatoes are grown in lead-contaminated soil, the metal can be absorbed by the plant and affect various physiological, biochemical, and growth parameters (khan *et al.,* 2015).

Seaweeds present an opportunity to address these challenges through their application as organic fertilizers. Their rich content of essential nutrients, such as potassium, magnesium, and trace elements like iron, makes them valuable in improving soil health and plant resilience (ElBoukhari et al., 2020). The use of seaweed extracts as fertilizers can enhance soil fertility, promote sustainable agricultural practices, and contribute to better crop yields, even in contaminated environments (Zodape, 2001). Moreover, seaweed-based fertilizers help restore the ecological balance of soils degraded by the excessive use of synthetic chemicals.

As the demand for organic food rises and concerns about soil degradation intensify, the use of seaweed-based fertilizers in modern farming practices is becoming increasingly important especially in the country like Nigeria. By harnessing the potential of seaweeds, agricultural systems can move toward more sustainable and environmentally responsible practices, ensuring food security for future generations. This study aimed to investigate the effects of Nigeria seaweed on the biochemical and physiological conditions of Lycopersicon esculentum under heavy metal induced abiotic stress.

2. Methodology

2.1. Chemicals

All reagents that was used for this research work was of analytical grade and most was sourced from Sartorius Stedim Biotech, Germany., Sigma chemical Co. Saint Quentin (France)., Sigma Company, St. Louis, MO), India.

2.1.1. Collection and preservation of Seaweeds

Seaweed was collected from Okitipupa, Ondo State, Nigeria and thoroughly rinsed with tap water to remove all epiphytes and sand particles. The cleaned seaweed was shade-dried for five days, then ground or cut into pieces and lyophilized. A 4 g portion of the lyophilized sample was transferred into a centrifuge tube and mixed with 15 mL of a MeOH/H2O solution (15:5 v/v). The mixture was subjected to sonication in an ultrasonic bath for 10 minutes to enhance extraction. After vortexing for 2 minutes, it was homogenized and extracted overnight (16 hours) at 20°C. The mixture was then centrifuged at 5000 rpm for 10 minutes. The residue underwent a second extraction with an additional 50 mL of solvent for 1 hour. Supernatants from both extraction steps were combined, filtered, and brought to a final volume of 100 mL. This filtrate was considered the 100% concentration. Five different concentrations (0%, 20%, 40%, 60%, and 80%) were then prepared for the study.

2.2. Analyses of samples

2.2.1. Proximate analyses: The proximate analyses of ash, fiber, fat, protein were determined for seaweeds using AOAC (2019). The protein contents were deter-mined using Kjeldalh method and the percentage of nitrogen was converted to crude protein by multiplying with a factor of 6.25. Fat content was determined by continuous solvent extraction method using Soxhlet apparatus. Crude fiber was determined gravimetrically. Total ash con-tent was determined by furnace incineration.

2.2.1. Analyses of minerals content using atomic absorption spectrophotometer (AAS) :

Mineral content was determined following AOAC (2019) guidelines using nitric-perchloric acid digestion. In brief, 1 g of each sample was digested with aqua regia (a mixture of nitric and perchloric acids in a 1:3 ratio) in a fume hood. After digestion, 10 mL of deionized water was added to ensure complete dissolution. The digested sample was then filtered using Whatman filter paper with a particle retention size of 2.5 µm, and the filtrate was diluted with 25 mL of deionized water. The mineral content was subsequently quantified using an Agilent 155A Atomic Absorption Spectrophotometer.

2.3. Experimental protocol

Tomato seeds (*Lycopersicon esculentum)* were sown in a mixture of peat and perlite at a 2:1 ratio (v:V) in multi-celled trays. Once the seedlings developed 2-3 true leaves, they were transplanted into 5 L pots (32 cm in diameter and 18 cm in height), with one seedling per pot. The pots were placed in greenhouse bags for four weeks. Afterward, the plants were exposed to 0.10M Pb Nitrate under controlled conditions: relative humidity of 60-65%, daytime temperature of 25 ± 2°C, and nighttime temperature of 18 ± 2°C. The protocols outlined below were followed.

- **So (H_20)** : No stressed (positive control) + H_20 Only
- **S_1 (metal induced stressed)**: Pb stressed tomatoes + H_20 Only
- **S_2 (20%)**: Pb stressed tomatoes + 20% seaweeds
- **S_3 (40%)**: Pb stressed tomatoes + 40% seaweeds
- **S_4 (60%)**: Pb stressed tomatoes + 60% seaweeds
- **S_5 (80%)**: Pb stressed tomatoes + 80% seaweeds

2.4. The Net Photosynthetic Rate, Chlorophyll Content, and Rubisco Activity in Lycopersicon esculentum.

Six healthy leaves were selected from each experimental group for analysis. The net photosynthetic rate of these leaves was measured using a Portable Photosynthesis System (PP Systems). During the measurements, the CO_2 concentration was maintained at 400 μmol CO_2/mol, while relative humidity was kept between 60-65% to ensure consistent environmental conditions. To assess the chlorophyll content of the leaves, ethanol extraction was employed. This method involves immersing leaf samples in ethanol to extract chlorophyll, which is then quantified to give an indication of the photosynthetic capacity and health of the plant. Additionally, the activity of ribulose-1,5-bisphosphate carboxylase (Rubisco), a crucial enzyme involved in the photosynthetic carbon fixation process, was measured. The leaf extract was incubated in an assay solution without ribulose-1,5-bisphosphate (RuBP) for 15 minutes at room temperature. This step was necessary to assess the enzyme's activity under controlled conditions, ensuring that the measurements reflect the enzyme's functionality in the absence of its substrate (RuBP). The Rubisco activity is an important indicator of the plant's ability to capture carbon dioxide and convert it into organic compounds during photosynthesis.

2.5. Analyses of phytohormones contents, using high performance liquid chromatography.

5g of the lyophilized sample was transferred into centrifuge tube and mixed with 15mL of MeOH/H2O (15:5 v/v) in ultrasonic bath for 10 min. Sonication was applied to support the extraction process. After being vortexed for 2min, it was then homogenized and extracted overnight (16 h) at 20^0C. After extraction, the mixture was centrifuged at 5000 rpm for 10 min. The residue was extracted once again with 50mL of additional solvent for 1 h. Supernatants in both fractions were combined and filtered. The final extract was subjected to further purification and enrichment procedures by solid-phase extraction processes.

2.6 Analysis of antioxidant activity: The fresh leaves and shoots of seaweed samples were homogenized in an extraction solution following the protocol outlined by Liu *et al*.,(2014). After homogenization, the mixture was centrifuged, and the supernatant obtained was used to assess the activities of key antioxidant enzymes, including catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). Enzyme activities were measured using spectrophotometry at specific wavelengths: CAT activity was determined at 240 nm, POD activity at 436 nm, and SOD activity at 560 nm. These spectrophotometric measurements followed the methodology described by Liu et al. (2014) for the accurate quantification of enzyme activities in biological samples.

2.7 Statistical analysis

Statistical analysis was done using a one-way analysis of variance (ANOVA) while results were presented as means ± standard error of the mean (SEM). Significant differences were assessed at a 95% level of significance between control and treated groups. P values less than 0.05 were considered significant

3. Results

3.1. Proximate analyses of Nigeria seaweeds

- *Proximate analysis:* Results from proximate analysis (Fig. 1) showed that Seaweeds had appreciable presence of protein (17.87%), fat (1.89%), fiber (5%), and ash (7%)

- *Mineral composition:* The mineral contents of seaweeds using AAS machine shows the appreciable amount of fe content (% d.w) with 61 followed by magnesium with values of 8.9 with least value of Nitrogen (0.67)
- *Bioactive contents:* As represented in Fig. 3. The seaweeds contains variable amount phytohormones which follows the descending order Absicic acid > Ethylene > Auxins> Gibberellins> Zeatin > Indole-3-acetic acid > Kinetin

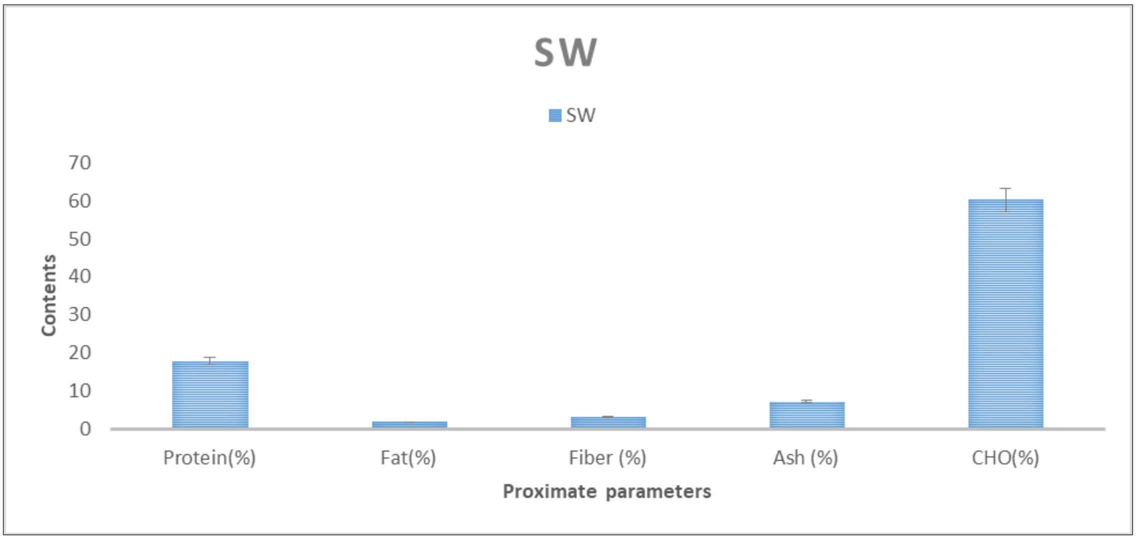

Figure 1 Preliminary proximate analysis of seaweeds

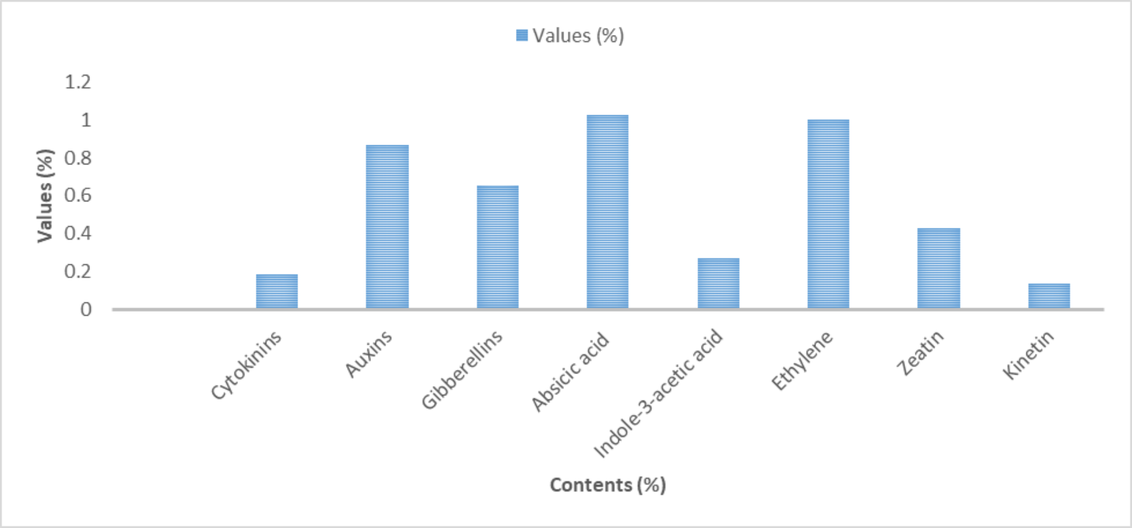

Figure 2 Bioactive components of seaweeds extract

The table 1 shows how different concentrations of seaweed fertilizer affect the photosynthetic efficiency and growth of tomato plants under metal stress. The control group (So, H_2O) had the highest net photosynthetic rate at 11.07 µmol/m^2. In contrast, the metal-induced stress group (S1) showed a dramatic reduction to 2.70 µmol/m^2, indicating that heavy metal exposure severely impaired photosynthesis. As seaweed fertilizer concentrations increased (S2–S5), the net photosynthetic rate improved, with the highest rate observed at 80% seaweed concentration (9.77 µmol/m^2). Similar to photosynthetic rate, chlorophyll a and b content was highest in the control group (2.36 mg/g and 1.98 mg/g, respectively). Heavy metal stress reduced chlorophyll content (0.89 mg/g for chlorophyll a and 0.75 mg/g for chlorophyll b in S1). Rubisco enzyme activity, was reduced under heavy metal stress (3.65 µmol/CO_2/g/min) compared to the control (5.37 µmol/CO_2/g/min). Seaweed application gradually restored Rubisco activity, with the highest activity observed at 60% seaweed concentration (5.00 µmol/CO_2/g/min). Root and shoot lengths were significantly reduced under heavy metal stress (8.16 cm and 1.69 cm, respectively), compared to the control (17 cm and 3.2 cm).

Antioxidant capacity: The figure 4 and 5 shows the antioxidant capacity of tomatoes roots and shoots under metal-induced stress, CAT activity decreased from 21 µg/g in the control to 8 µg/g in S1. The addition of seaweed fertilizer increased CAT activity, with the highest at 80% seaweed concentration (17.4 µg/g). Ascorbate Peroxidase (APX) Content content showed a similar trend, with a reduction under metal stress (21.0 µg/g in S1 compared to 51 µg/g in the control). Seaweed application boosted APX activity, with a 92% increase in S5 compared to the control. Superoxide Dismutase (SOD) Content content was similarly reduced under metal stress (1.44 µg/g in S1 compared to 3.2 µg/g in the control), but seaweed treatment increased SOD activity, with a 71% improvement at the highest concentration (2.17 µg/g in S5).Peroxidase (POD) Content was severely reduced under stress (0.10 µg/g in S1). However, seaweed treatment gradually restored POD activity, with an 85% increase in S5 compared to the control.Similar trends were observed for antioxidant enzyme activities in the shoots:

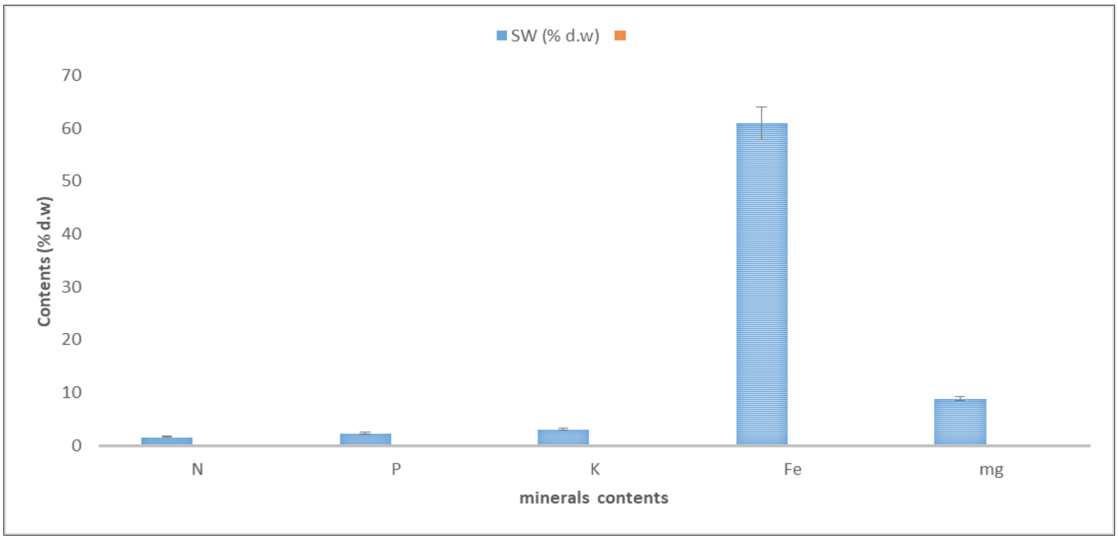

Figure 3 Elemental Composition of Nigeria Seaweeds (% d.w)

Table 1 Effects of varied proportions of seaweeds fertilizer on photosynthetic capacity of the leaves and some morphological features of L*ycopersicon esculentum*

Group	**Net photosynthetic rate (µmol/m$^{2)}$**	**Chlorophyll content a (mg/g)**	**Chlorophyll content b (mg/g)**	**Rubisco enzyme activity ((µmol/Co$_2$)/g.min**	**Root length (cm)**	**Shoot length (cm)**	**Stomata conductance µS/cm**
So (H$_2$0)	11.07±0.08	2.36±0.03	1.98±0.05	5.37 ±0.06	17±0.01	3.2	874
S$_1$(metal induced stressed)	2.70 ±0.05	0.89±0.03	0.75±0.02	3.65±0.02	8.16±0.05	1.69	533.14
S$_2$ (20%)	5.35±0.03	1.37±0.07	0.91±0.08	3.85±0.04	9.03±0.03	1.81	544.89
S$_3$ (40%)	7.83±0.02	1.49±0.04	1.48±0.03	4.11±0.04	10.14	2.01	588.00
S$_4$ (60%)	8.14±0.05	1.63±0.01	1.60±0.04	5.00±0.09	11.34	2.33	597.04
S$_5$ (80%)	9.77±0.08	1.96±0.02	1.71±0.02	4.398±0.07	11.93	2.66	606.56

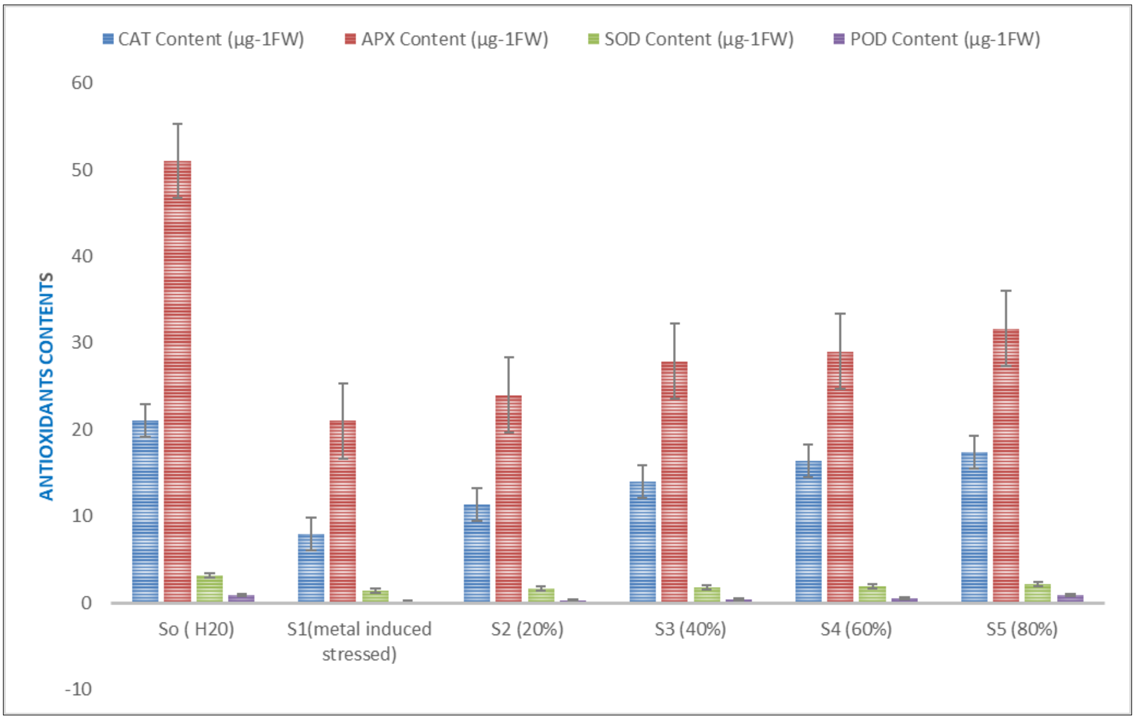

Figure 4 Effects of varied proportions of seaweeds fertilizer on Antioxidants capacity of heavy metals induced root of *Lycopersicon esculentum*

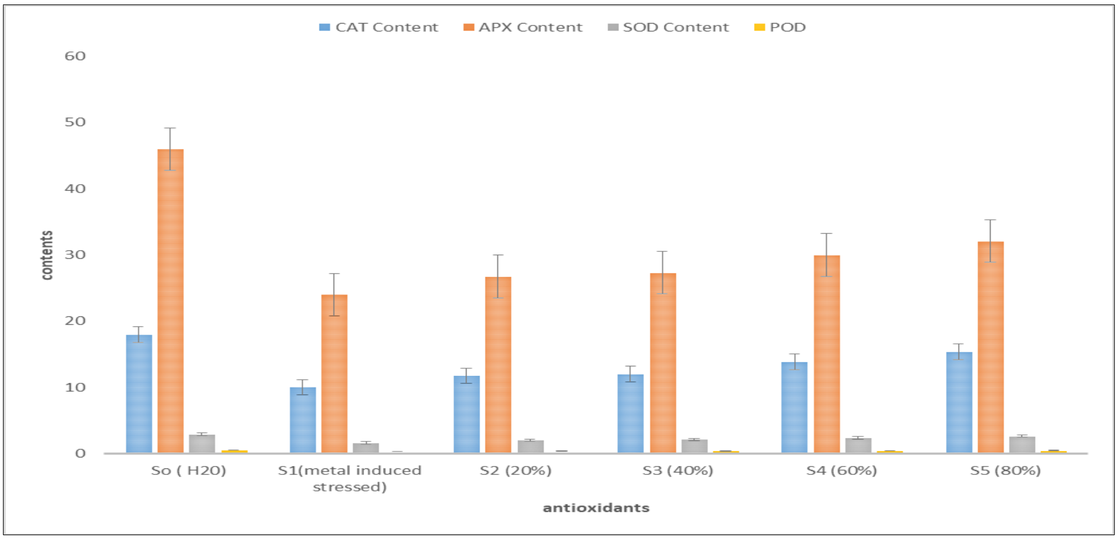

Figure 5 Effects of varied proportions of seaweeds fertilizer on Antioxidants capacity of heavy metals induced shoot of *Lycopersicon esculentum*

Where

- **So (H_20)** : No stressed (positive control) + H_20 Only
- **S_1(metal induced stressed)**: Pb stressed tomatoes + H_20 Only
- **S_2 (20%)**: Pb stressed tomatoes + 20% seaweeds
- **S_3 (40%)**: Pb stressed tomatoes + 40% seaweeds
- **S_4 (60%)**: Pb stressed tomatoes + 60% seaweeds
- **S_5 (80%)**: Pb stressed tomatoes + 80% seaweeds

4. Discussion

The proximate composition of seaweeds reveals that they are a valuable source of nutrients, particularly protein, fiber, fat, and ash. Seaweeds are composed of approximately 17.87% protein, making them a potential alternative protein source for improving plant nutrition. This high protein content aligns with findings from previous research, which has highlighted the nutrient-dense nature of seaweeds. Their ability to enhance soil fertility and promote plant growth makes them a promising component in sustainable agriculture. The fiber content, measured at 5%, indicates seaweeds' potential to improve soil structure and water retention when applied as an organic fertilizer. The results are in accordance with the reports of Tabarsa *et al.,* (2012) and Sakthivel and Pandima Devi (2015) but the values obtained are higher than the report of Radha, (2018) which obtained the values of 1.41 and 1.49% respectively. While the fat content in seaweeds is relatively low at 1.89%, it still plays a role in the overall nutritional profile. Fats serve as an energy source for various biological functions in plants, contributing to their metabolic processes. The ash content, which constitutes 7% of the seaweed composition, further points to their mineral richness. This is important because it supports the mineral analysis that highlights the nutritional value of seaweeds, particularly for agricultural purposes.

Seaweeds are also a significant source of essential minerals. Among the minerals present, iron (Fe) is found in the highest concentration, making up 61% of the seaweed's dry weight. Iron plays a critical role in the synthesis of chlorophyll and electron transport during photosynthesis. This high iron content explains why seaweed fertilizers can improve the photosynthetic capacity of plants, as observed in studies involving Lycopersicon esculentum (tomatoes). Magnesium, which constitutes 8.9% of the seaweed's mineral content, is another essential nutrient necessary for photosynthesis, as it forms the central atom of the chlorophyll molecule.

Seaweeds are also rich in various bioactive compounds, including phytohormones, which play important roles in plant growth and stress response. These phytohormones are present in descending concentrations, starting with abscisic acid, followed by ethylene, auxins, gibberellins, zeatin, indole-3-acetic acid, and kinetin. Each of these phytohormones contributes to different aspects of plant development and stress management. These supports the findings of Mori *et al.,* (2017) who shows the presence of some phytohormones in seaweeds as well as in terrestrial plants. For instance, abscisic acid is well-known for enhancing plants' ability to tolerate environmental stresses such as drought and salinity. Ethylene is involved in regulating processes such as fruit ripening and leaf senescence. Auxins and gibberellins, on the other hand, promote cell elongation and division, which leads to improved root and shoot development. These effects are evident in studies where seaweed-treated tomato plants (S2-S5) showed enhanced growth in both root and shoot length.

The application of seaweed fertilizers has demonstrated notable positive effects on the physiological and biochemical processes of Lycopersicon esculentum. For instance, seaweed fertilizer has been shown to mitigate the adverse effects of metal stress on the net photosynthetic rate. In the absence of seaweed treatment, metal-stressed tomato plants experience a dramatic decline in photosynthetic rate, dropping as low as 2.70 $\mu mol/m^2$. However, as seaweed concentration increases, the photosynthetic rate improves significantly, with the highest rate of 9.77 $\mu mol/m^2$ observed when 80% seaweed is applied. This recovery in photosynthesis can be attributed to the enhanced synthesis of chlorophyll and the increased activity of the Rubisco enzyme, both of which are influenced by the mineral and phytohormonal components of seaweed.

In addition to improving photosynthetic capacity, seaweed fertilizers positively affect chlorophyll content. Chlorophyll levels, particularly chlorophyll a and b, are greatly reduced in metal-stressed plants, but seaweed treatment restores these levels in a dose-dependent manner. At higher concentrations, such as 80% seaweed, the chlorophyll content approaches near-normal levels. This can be explained by the abundant iron and magnesium present in seaweeds, which are essential for chlorophyll production and overall photosynthetic function.

Rubisco enzyme activity, a key factor in carbon fixation during photosynthesis, also benefits from seaweed application. The enzyme's activity increases alongside the concentration of seaweed, with the highest activity observed in plants treated with 80% seaweed. This enhanced enzyme activity directly correlates with the improvements in photosynthetic rate and overall plant health.

Root and shoot growth, which are typically inhibited by metal stress, show substantial recovery when treated with seaweed fertilizers. At higher seaweed concentrations, such as 60% and 80%, both root and shoot lengths increase significantly. The growth-promoting effects of seaweed are likely due to the presence of bioactive compounds like auxins and gibberellins, which stimulate cell elongation and division.

Stomatal conductance, which controls gas exchange in plants, is another physiological process that benefits from seaweed treatment. Seaweed application helps to recover stomatal conductance in metal-stressed plants, improving their ability to regulate water loss and carbon dioxide uptake. This recovery is likely influenced by abscisic acid, a phytohormone known for regulating stomatal closure in response to stress.

Seaweed fertilizers also boost the antioxidant capacity of tomato plants under heavy metal stress. In both roots and shoots, seaweed treatment increases the activity of key antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and peroxidase (POD). These enzymes are essential for detoxifying reactive oxygen species (ROS) generated by metal stress, thereby protecting plants from oxidative damage. For example, catalase activity in roots rises from 8.0 μg/g in stressed plants to 17.4 μg/g in plants treated with 80% seaweed. A similar pattern is observed in shoots, where catalase activity increases from 10 μg/g to 15.4 μg/g. The same trend applies to APX, SOD, and POD, all of which show elevated activity in both roots and shoots as seaweed concentration increases. These findings are supported by literature on various plant species, such as *Citrullus lanatus* (Nawaz et al., 2018) and *Stevia rebaudiana* (Simlat *et al.,* 2018), under environmental stress. Thus, seaweed application can reduce oxidative damage and repair cellular membranes disrupted by salinity by balancing ROS levels. Antioxidant enzymes play a critical role in the plant defense system against both biotic and abiotic stress conditions.

The results of these studies clearly demonstrate the significant benefits of seaweed application in improving the physiological and biochemical responses of plants under stress. By enhancing photosynthesis, promoting root and shoot growth, and boosting antioxidant defenses, seaweed serves as a highly effective organic bio-fertilizer. Its rich content of essential minerals, phytohormones, and bioactive compounds makes it a valuable tool for improving crop performance, particularly in stressful environmental conditions such as heavy metal contamination. Seaweed fertilizers hold promise for sustainable agricultural practices, offering a natural means of enhancing plant health and productivity.

5. Conclusion

The results clearly indicate that seaweed fertilizer mitigates the detrimental effects of heavy metal stress in *Lycopersicon esculentum.* Seaweed application enhanced the photosynthetic capacity by improving chlorophyll content, Rubisco activity, and stomatal conductance, while also promoting root and shoot growth. Additionally, seaweed significantly boosted the plant's antioxidant defense system by increasing the activities of key enzymes such as CAT, APX, SOD, and POD. These enzymes play crucial roles in scavenging ROS, reducing oxidative stress, and improving overall plant resilience. The improvements observed at higher seaweed concentrations (60% and 80%) suggest that seaweed fertilizer is a potent bio-stimulant that can alleviate metal stress and promote plant growth through enhanced photosynthetic performance and antioxidant defense. In conclusion, seaweed fertilizer has a protective effect on tomato plants subjected to heavy metal stress by enhancing both physiological (photosynthesis and growth) and biochemical (antioxidant activity) responses. This makes seaweed an effective and sustainable solution for improving crop performance under stressful environmental conditions.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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