

*Original Research*

# High-Performance Liquid Chromatography Analysis of the Nutritional Quality of Tomato Fruits Harvested from Hybridized Bio-agents Assisted Microbial Remediation

**Sarah Alharthi<sup>1,2</sup>, Hilary Uguru<sup>3</sup>, Ovie I. Akpokodje<sup>4</sup>, Nashi K. Alqahtani<sup>5,6</sup>, Rokayya Sami<sup>7\*</sup>, Woroud A. Alsanei<sup>8</sup>, Awad A. Momen<sup>9</sup>, Hala M. Abo-dief<sup>9</sup>, Saad A. Al-Otaibi<sup>10</sup>, Mahmoud Helal<sup>11</sup>, Ahmed M. Abdulfattah<sup>12,13</sup>**

<sup>1</sup>Department of Chemistry, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

<sup>2</sup>Research Center of Basic Sciences, Engineering and High Altitude, Taif University, Taif, Saudi Arabia

<sup>3</sup>Department of Agricultural Engineering, Delta State University of Science and Technology, Ozoro, Nigeria

<sup>4</sup>Department of Civil and Water Resources Engineering, Delta State University of Science and Technology, Ozoro, Nigeria

<sup>5</sup>Department of Food and Nutrition Sciences, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia

<sup>6</sup>Date Palm Research Center of Excellence, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia

<sup>7</sup>Department of Food Science and Nutrition, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

<sup>8</sup>Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah 21589, Saudi Arabia

<sup>9</sup>Department of Science and Technology, Ranyah University College, Taif University, KSA

<sup>10</sup>Department of Biotechnology, Faculty of Science, Taif University, Taif 21974, Saudi Arabia

<sup>11</sup>Department of Mechanical Engineering, Faculty of Engineering, Taif University, P.O. 11099, Taif 21944, Saudi Arabia

<sup>12</sup>Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia

<sup>13</sup>Embryonic Stem Cell Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia

*Received: 20 October 2024*

*Accepted: 1 May 2025*

## Abstract

The increasing soil contamination by petroleum hydrocarbons has serious consequences for crop quality, eventually affecting public health and human nutrition. This study evaluates the remediation potential of bio-agents in petroleum-contaminated soil. The phytoremediation approach was facilitated by bio-absorbents (charcoal and rice husk ash, “RHA”) and bio-stimulants (plantain-based organic manure, “PBS”, algae-based organic manure, “ABS”, and seaweed extract-based organic manure, “SBS”). During the study, the total petroleum hydrocarbons (TPH), heavy metals, hydrocarbon-utilizing bacteria (HUB), and hydrocarbon-utilizing fungi (HUF) levels were measured by following

---

\*e-mail: rokayya.d@tu.edu.sa

the recommended American Society for Testing and Materials (ASTM) guidelines. Additionally, the High-Performance Liquid Chromatography (HPLC) approach determined the tomato fruit's nutritive qualities. The results illustrated that the appropriate combination of the treatment units substantially increased the tomato plant's phytoremediation, dietetic, and therapeutic qualities. It was observed that the TPH phytoremediation efficiencies were lowest in the setups that used only charcoal and RHA, at 16.80 and 19.76%, respectively. Outstandingly, the bio-agents extensively increased the HUB and HUF survival, in addition to substantial increments in the fruit's vitamin B, vitamin C, carotenoids, and phytochemical concentrations. This study's findings underscored the prospect of utilizing sustainable agricultural materials to address environmental contamination issues and improve crop dietetic and therapeutic characteristics.

**Keywords:** bio-additives, environmental degradation, HPLC, microbial remediation, tomato crop

## Introduction

Environmental pollution poses substantial challenges to food production and security. Environmental degradation linked to pollution tends to adversely affect human, crop, and animal productivity, resulting in a decline in food production [1-3]. Pollution hazards substantially retard plants' and animals' functionality, particularly in comestible and pharmaceutical plants, whose parts are used for dietary and medical purposes primarily due to their large volume of essential bioactive compounds [4]. Toxins and contaminants linked to domestic and industrial waste materials cause rapid soil and air quality deterioration. This leads to a decline in crop and animal yields and contamination of their products with poisonous residues. Contaminants build up in the food chain, potentially altering their nutritional and microbiological compositions, posing severe hazards to consumers' health, and causing serious ailments [5]. This hinders the improvement of human performance, food security, and agricultural productivity [6].

Studies have demonstrated that crude oil and its derivatives can significantly increase both heavy metal and petroleum hydrocarbon concentrations in both soil and water. Hydrocarbon contamination results in anoxic conditions, which weaken soil fertility, reduce microbial survival, and ultimately impede plant growth [5, 7, 8]. Hydrocarbons and heavy metals are persistent contaminants, causing longstanding challenges to both the bionetworks and human beings. The persistence of pollutants in the environment can be linked to their chemical stability, degradation resistance, and potential to accumulate within the food chain [1]. Heavy metal (HM) toxicity tends to disrupt the endocrine system, impair reproductive health, and lead to neurological disorders in humans and wildlife [3, 9]. As stated by Massányi [10], persistent exposure to toxic metals has health complications, such as diminished cognitive abilities, learning disabilities, behavioral problems, and even permanent brain damage. Petroleum hydrocarbons (PHs), potent environmental contaminants, consist of aliphatic and aromatic hydrocarbons. Typically, petroleum hydrocarbons are the major constituents in gasoline, diesel, lubricating oils, and other petroleum-

derived products. Remarkably, prolonged exposure to these pollutants has been associated with serious health issues, including respiratory complications, kidney failure, cancer, and neurological problems [11, 12].

Hydrocarbons have a major impact on crops' nutritional value. Certain hydrocarbons can disrupt plant metabolism, leading to the formation of toxic metabolites, which can adversely affect plant health and reduce their nutritional content [13, 14]. PHs interfere with crucial metabolic processes in plants, such as photosynthesis and respiration. This disruption causes oxidative stress, which can correlate to the buildup of reactive oxygen species (ROSs) and other potentially harmful compounds within the plant's body [15, 16]. Pollution from PH disrupts the plant's cellular structure, damages parenchyma tissue, and prevents root mitotic activity. Prolonged exposure to hydrocarbon pollution reduces plant biodiversity, negatively affecting crop yields, medicinal properties, and nutritional profiles [17]. Haider [18] reported that hydrocarbons reduce the essential nutrient content in plant tissues by disrupting key physiological processes. These compounds, like vitamins, antioxidants, and amino acids, are crucial for the plant's defense mechanisms and medicinal properties.

Previous studies have shown that toxic heavy metals have severe effects on crop growth and performance, as well as the utilization of their products [2,3]. HM poisoning interferes with the plant's chlorophyll formation, resulting in reduced growth and productivity. This situation adversely affects the plant's nutritional and medicinal properties, rendering it less appropriate for human consumption [19, 20]. The World Health Organization (WHO) and other regulatory bodies' safety guidelines specify that the maximum concentration of lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), chromium (Cr), and nickel (Ni) in the soil should not exceed 85, 0.8, 36, 50, 100, and 35 ppm, respectively. Similarly, WHO/FAO and Codex Alimentarius recommend that the maximum allowable Pb, Cr, Cd, Cu, Ni, and Zn concentrations in a plant's body should be 0.3, 0.25, 0.1, 73, 67.9, and 100 ppm, respectively [20]. Plants subjected to prolonged HM contamination usually have lower vitamin and antioxidant proportions

(concentrations), leading to lower medicinal and nutritive values. These actions largely compromise the plant's health benefits, resulting from the reduction of essential nutrients and bioactive compounds [4]. Toxic element (heavy metal) pollution has a significant sway on the potency of therapeutic plants, as these pollutants disrupt the plant's physiological, mechanical, and biochemical processes, resulting in a substantial decline in their medicinal efficacy [9, 21].

Health issues related to crude oil pollution emphasized the necessity to minimize human exposure to these substances, hydrocarbons, and heavy metals, especially in areas impacted by oil spills and regions where petroleum product effects have not been adequately remediated. To degrade or remove these contaminants from polluted sites, remediation techniques including bioremediation and phytoremediation are being studied and implemented [22-24]. These methods utilize biological processes and plant systems to rehabilitate contaminated soils and water, providing eco-friendly and cost-effective alternatives to chemical-based techniques [21]. The remediation method chosen for any contaminated environment depends on numerous factors, including the cost of remediating agents, the types of pollutants, and their concentration [13]. Certain bacteria and fungi, such as *Pseudomonas*, *Alcanivorax*, and *Mycobacterium*, possess the capability to metabolize complex PHs, degrading them into simpler and less toxic compounds. This biodegradation process is enhanced by the presence of organic materials (humus) and sufficient moisture in the soil, which provide the necessary nutrients and environment for microbial activity [23, 25]. Rice husk and its by-products support the phytoremediation of toxic elements and PHs and also improve the reproduction of hydrocarbon-utilizing bacteria (HUBs) and hydrocarbon-utilizing fungi (HUFs) [26]. Typically, bio-stimulants facilitate rapid plant growth, improving the efficiency of the phytoremediation approach. This is because the plants tend to have a greater capacity to absorb, accumulate, and/or degrade complex contaminants, such as crude oil, in polluted soils.

Although the phytoremediation capacities of many plants have been extensively studied, the use of tomato plants as phytoremediation agents has not been comprehensively explored. Consequently, this study investigates the effect of hybridized bio-stimulants and bio-absorbents on microbial remediation of crude oil-polluted soil. It utilizes High-Performance Liquid Chromatography (HPLC) to determine the nutritional content of the tomato fruit grown under these conditions. This approach facilitates understanding how bio-absorbents and bio-stimulants can enhance soil recovery while simultaneously preserving or even improving the crops' nutritional quality during the pollution remediation process.

## Materials and Methods

### Soil Sample

The contaminated soil used in the study was sourced from an oil spill site located in the creeks of Delta State, southern Nigeria, where a crude oil spill occurred in mid-2023. The topsoil used for this research was collected within a depth of 0 to 0.5 m, and about 500 kg of soil was collected from the polluted site based on the experimental design. This sampling depth was selected as it represents the potential root zone for the majority of arable crops.

### Bio-absorbent

Wood charcoal and rice husk ash (RHA) were the bio-absorbents used in this study to enhance the phytoremediation process. Notably, incorporating suitable bio-absorbents into phytoremediation helps boost plant growth and performance, leading to an overall increment in remediation efficiency.

### Bio-stimulant Preparation

#### *Bio-stimulant 1: Plantain Peel-based Organic Manure (PBS)*

This bio-stimulant was created by composting a blend of plantain peel by-products, cattle dung, and eggshells, mixed in a ratio of 6:3:1 (by mass) over a period of 2 months. Additionally, the by-products derived from plantain peels comprised 60% fresh plantain peels and 40% plantain peel ash (by mass).

#### *Bio-stimulant 2: Algae-based Organic Manure (ABS)*

The ABS was produced by composting a mixture of algae and cattle dung. The algae were harvested from a freshwater pond enriched with sawdust. The leaves were then dried and blended with cattle dung in a ratio of 30 to 70% by weight. The mixture was subsequently composted for 8 weeks using the aerated static pile composting technique.

#### *Bio-stimulant 3: Seaweed Extract-based Organic Manure (SBS)*

This bio-stimulant was prepared by composting a mixture of seaweed extract and cattle dung in a 20:80 ratio by weight, using the aerated static pile composting method for 2 months.

### Preliminary Remediation

Physical remediation through the use of charcoal blocks was used to weaken the toxicity of the crude oil on the tomato plant. The charcoal blocks were carefully inserted into the containers containing the contaminated

soil for 6 hours before they were removed and replaced with fresh ones. Before placing fresh charcoal inside the soil, the soil was thoroughly mixed to uniformly spread the oil in the soil and increase the crude oil absorption efficiency. This procedure was repeated twice a day for 7 days. The soil obtained from this preliminary remediation was tagged "PreT1".

After the initial physical remediation using charcoal (PreT1), 10 kg of dry rice husk was spread over the contaminated soil and then burnt on top of the soil to produce the second bio-absorbent, RHA. The burning of rice husks generates heat, which may help volatilize some of the lighter hydrocarbons present in the soil. Following the complete combustion of the rice husk, the resulting RHA was thoroughly mixed into the soil along with 3 liters of water. The mixture was left to acclimate for an additional 2 weeks, allowing the ash to integrate with the soil and support the remediation process. The soil obtained from this preliminary remediation was tagged "PreT2".

### Experimental Setup

The experimental design consisted of various treatments implemented in crude oil-polluted soil. The control group consisted of soil without any amendments: T1 (PreT1) and T2 (PreT2). The other treatments were as follows: T3 (PreT1 + PBS), T4 (PreT1 + ABS), T5 (PreT1 + SBS), T6 (PreT2 + PBS), T7 (PreT2 + ABS), and T8 (PreT2 + SBS).

The pre-remediated soil samples, labeled PreT1 and PreT2, were placed into plastic buckets with perforated bottoms, with each bucket containing 20 kg of soil. For treatments 3 to 8, 2 kg of the appropriate bio-stimulant was thoroughly mixed into the soil and allowed to stabilize for seven days prior to the transplantation of the tomato plants. This window period (7 days) aids the incorporation of the bio-stimulant nutrients into the soil, improving the soil's fertility, and enhances the optimization of plant performance and remediation effectiveness. Each treatment was done in triplicate, principally to guarantee statistical validity.

### Phytoremediation Procedure

The tomato plant (Cobra-26 F1) used for the phytoremediation was nursed in seed bags for a period of 28 days. Watering was done as needed, ensuring the soil was moist but not waterlogged. Weeding was carried out manually using handpicking to keep the plants free from competing weeds. Organic manure was incorporated into the soil 2 weeks before planting the tomato seeds, which aids in providing essential nutrients for healthy plant growth. After 28 days, the tomato seedlings were transplanted to the already prepared containers containing the pre-remediated soil samples. Initially, they were transplanted at a density of 5 seedlings per bucket, but after the tomato plants were

fully established, the number was reduced to 2 seedlings per container.

During the growth period, the control and all the treatments were subjected to consistent environmental conditions (light, temperature, humidity). Watering was done just to keep the soil moist; pests and weeds were controlled manually. The tomato fruits were collected for laboratory analysis at the pink maturity stage. After the phytoremediation program concluded, the soil from each bucket was transferred to a tray, sun-dried, and labeled with codes. Additionally, the tomato plants from the treatments were separated into 2 parts: the roots and the leaves, which were then dried in the sun and coded accordingly. All the specimens were transferred to the laboratory for chemical analysis.

### Quality Assurance and Quality Control

The chemicals and reagents employed to attain the goals of this research were of excellent grade and obtained from a reputable manufacturer, Merck KGaA, Darmstadt, Germany. Blank and standard specimens were scrutinized using the same procedure. The measurements were conducted in triplicate, producing a relative standard deviation of below 4%, while the recovery rates for the certified reference materials varied from 93.7% to 102.5% [27].

### Measurements and Data Collection

Chemical analyses were conducted on dried soil, plant roots, leaves, and fresh tomato fruits. The dried soil, roots, and leaves were processed using an electric grinder (model FW100, manufactured by Focus Technology Co., Ltd., China) and filtered through a 0.850 mm screen.

### Physicochemical Properties of the Soil

The soil's sample physiochemical properties were determined in accordance with ASTM-approved procedures after drying in the laboratory at room temperature ( $30 \pm 4^\circ\text{C}$ ,  $78 \pm 9\%\text{RH}$ ) [3, 5, 22].

### Heavy Metal (HM) Determination

The levels of HMs, which include iron (Fe), chromium, cadmium, lead, nickel, and copper, in the soil, roots, and leaf specimens were measured following ASTM-approved guidelines. A total of 10 g from each sieved sample was digested using a mixture of  $\text{HNO}_3$ ,  $\text{HCl}$ , and  $\text{H}_2\text{SO}_4$  in a ratio of 10:4:1, heated to  $95^\circ\text{C}$  until a clear product was attained. This product was strained into a measuring cylinder and then diluted with distilled water to obtain a 100 mL volume. An atomic absorption spectrophotometer (model AA-7800 series, produced in Japan) was employed to determine the concentration of each metal in the digested sample [3]. The concentration



of each metal was initially measured in milligrams per kilogram (mg/kg).

#### *HPLC Determination of Vitamin B and Carotenoid Profiles*

The tomato fruits' carotenoid and vitamin B profiles were measured using a High-Performance Liquid Chromatography (HPLC) machine (model LC-W100B, manufactured by Wincom Company Ltd. in China). This study uses a Luna C18 column (0.10 m × 4.6 mm I.D., 5 µm particle size) for the chromatographic analysis, coupled with a UV detector and a 20 µL injector loop. The mobile phase comprised methanol as the carrier, having a flow rate of 0.06 L/h, a constant temperature of 35°C, a run time of 12 minutes, and a wavelength of 295 nm [28].

#### *Spectrophotometric Determination of Vitamin C*

The vitamin C concentration in the tomato fruits (fresh weight; FW) was determined using a standard spectrophotometer (model: UV-5300, manufactured in India), following the recommended guidelines. A standardization curve was established by employing known concentrations of ascorbic acid, and the resulting absorbance was compared to a standard curve to ascertain the vitamin C level in the fruit. The results were recorded as mg/100 g of fresh weight (FW) of ascorbic acid, which was later converted to mg/kg (FW) using a conversion factor of 10.

#### *Phytochemical Properties Determination*

Gas Chromatography-Mass Spectrometry (GC-MS) was employed to identify the phytochemical properties present in the tomato fruit. The tomato fruit phytochemicals were extracted with the aid of methanol, and 2 µL of the specimen was injected into the system through the splitless injector. The GS employs helium as the carrier gas, flowing at 1.75 mL/min. The concentration of compounds can be quantified based on the peak areas in the chromatogram, often using calibration curves from standards.

#### *Total Petroleum Hydrocarbon (TPH)*

Gas Chromatography armed with the flame ionization detector (GC-FID) was utilized to assess the petroleum hydrocarbon (TPH) levels in the soil, tomato roots, and leaves. Using dichloromethane, TPH was extracted from ground samples (soil, plant roots, and leaves). The system used an HP-5 fused silica capillary column with these dimensions: 30 m × 0.32 mm × 0.25 µm. Helium, the carrier medium, flowing at a slow rate of 1.75 mL/min, ensuring optimal separation of the hydrocarbon compounds in the system, was configured with a detection limit greater than 0.0001. A 2 µL volume of the extract was introduced into the GC-FID system

in splitless mode at 250°C, which allows the entire sample to be directed into the column for maximum sensitivity, while the FID temperature was set to 300°C. The column temperature was initially maintained at 50°C for 2 minutes before it was increased to 250°C at a rate of 10°C/min. The final data was analyzed using Agilent software, processing the chromatographic data to determine the concentrations of TPH present in the samples [29].

#### *Microbiological Analysis*

The hydrocarbon-utilizing bacteria (HUB) and hydrocarbon-utilizing fungi (HUF) populations in the soil samples were determined following standard guidelines as outlined by Achife [8].

#### *Data Analysis*

Data analysis of the laboratory results was performed using SPSS statistical software (version 20.0) to assess the effects of bio-stimulants and absorbents on the phytoremediation ability of the tomato plant. The means were distinguished using Duncan's Multiple Range Test (DMRT) at a significance level of  $p \leq 0.05$ .

#### *Bio-accumulation Factor (BCF)*

The BCF is used to determine the suitability of any crop for phytoextraction, and it is calculated using the formula shown in Equation (1) [3, 30].

$$BCF = \frac{\text{Conc of metal in the root}}{\text{Conc of metal in the soil}} \quad (1)$$

#### *Translocation Factor (TF)*

Translocation is a plant's capability to transfer accumulated contaminants from the soil through its root system to the shoot, where they are assimilated and managed within the plant's tissues. The translocation factor of a plant is calculated by using the expression given in Equation (2) [30].

$$Tf = \frac{\text{Conc of metal in the shoot}}{\text{Conc of metal in the root}} \quad (2)$$

## **Results and Discussion**

### *Soil Physiochemical Properties*

The results of the oil-impacted soil physiochemical attributes are presented in Table 1. In particular, it was observed that the oil spill tends to depreciate the soil's physiochemical parameters. The USDA soil classification depicts that the soil belongs to the sandy clay loam category. The low water holding capacity (21%) observed in the polluted soil can be linked to the

Table 1. Soil physiochemical properties.

Parameter		Value
Particle size distribution (%)	Sand	75.94
	Silt	18.22
	Clay	5.84
Soil texture classification*		Sandy Clay Loam
Soil organic matter (%)		5.17
Soil pH (H <sub>2</sub> O)		6.44
EC (dS/m)		5.73
Nitrogen (g/kg)		0.19
Water holding capacity (%)		21
Potassium (g/kg)		2.749

Note: \* (USDA – United States Department of Agriculture).

oily nature of the petroleum products, which formed protective layers around the soil grains. This tends to block the soil pores, thereby leading to a reduction in the soil's water absorption and retention capabilities [26, 31]. Interestingly, the results highlighted that the contaminated soil contained a large amount of potassium (2.749 g/kg) and a trace nitrogen level (0.19 g/kg). The soil electrical conductivity (EC) value of 5.73 dS/m indicates that the soil tends to be saline and negatively affects crop productivity [22, 32].

### Preliminary Remediation

Table 2 displays the outcomes of the preliminary remediation process utilizing bio-absorbents, which can be considered physical remediation. The findings depicted that both the charcoal and RHA had a substantial effect on the TPH and HM concentration of the soil ( $p \leq 0.05$ ). Following the physical remediation using only wood charcoal (PreT1), the concentrations of TPH, Pb, Cd, Cr, Cu, Ni, and Fe in the contaminated soil were reduced by 33.67, 20.63, 27.78, 32.12, 32.50, 25.10, and 23.38%, respectively. Furthermore, after conducting the second stage of physical remediation using rice husk (PreT2), the TPH, Pb, and Cd further depreciated by 18.88, 12.97, and 11.11%, respectively, while the soil's Cr, Cu, Ni, and Fe content appreciated by 5.58, 4.40, 5.57, and 6.36%, respectively. This finding aligns with the results reported by Shang [26] and Duwiejua [31] in their studies on the remediation potential of rice husk products. Notably, it was observed that the Pb, Cd, Cu, Zn, Cr, and Ni concentrations in the contaminated and preliminary remediated soils were within the permissible standards recommended by WHO and FAO.

Charcoal and rice husk are potent absorbents that help to reduce the bioavailability of toxic elements in the environment [26]. These absorbents' remediation capabilities can be linked to their physical and chemical

properties. Additionally, the heat generated by the rice husk tends to burn off volatile PHs from the soil (thermal degradation) and alter the chemical composition of the denser PHs in the process [13, 32]. Thermal degradation helps to break down larger hydrocarbon chains into smaller, more biodegradable compounds, facilitating subsequent bioremediation/phytoremediation processes. Moreover, burnt rice husk ash is rich in valuable nutrients, enhancing soil properties and supporting bioremediation. This could be attributed to the main reason the PreT2 soil sample has a lower TPH value when compared to the PreT1 soil sample [32, 33].

It was observed that the RHA caused a negligible increase in Cr, Cu, Ni, and Fe levels in the physically remediated soil. This may be attributed to HM discharge from the RHA into the soil. RHA is rich in silica (SiO<sub>2</sub>), calcium carbonate (CaCO<sub>3</sub>), phosphorus (P), and potassium (K), while also containing trace amounts of HMs [32]. The notable decrease in soil TPH levels following the addition of RHA can be linked to the ash's hygroscopic nature, adsorption capabilities, cementitious properties, and nutrient content, all of which contribute to the absorption and breakdown of hydrocarbons in the soil. The cementitious properties of rice husk ash (RHA) assist in binding hydrocarbons and other toxic metals in the soil, thereby effectively lowering their bioavailability, concentration, and leaching potential [33]. Additionally, the burnt rice husk produces ash rich in valuable nutrients, which enhances soil properties and supports the remediation process. This process stabilizes the soil, promotes the degradation of contaminants, and creates a conducive environment for beneficial microorganisms [32].

### Primary Phytoremediation

Table 3 presents the results of the phytoremediation potential of tomato plants under the various treatments. The TPH phytoremediation efficiency of T1, T2, T3, T4, T5, T6, T7, and T8 experimental units was 16.80, 19.76, 64.77, 69.18, 70.76, 68.03, 74.78, and 77.81%, respectively. Notably, the remediation agents had a significant effect ( $p \leq 0.05$ ) on the contaminated soil, as the control unit exhibited the highest TPH and HM values compared to the other experimental setups (T1 to T8). This indicates that the organic manure and the bio-stimulants (plantain peel, algae, and seaweed extract) effectively reduced the PH and HM concentrations in the soil, enhancing the tomato plant's capacity for phytoremediation. Additionally, the pyrolysis of rice husk ash in experimental units T2, T6, T7, and T8 will improve the physical properties of the soil, fostering healthier microbial populations that are vital for soil remediation [26, 32]. The research reflected that the tomato plant failed to thrive in the control setup, which may be attributed to crude oil toxicity. The toxic effects of crude oil cause physiological stress on the plant and disruption of nutrient absorption, thereby impairing

Table 2. The results of the preliminary remediation program.

Parameter	Contaminated (mg/kg)	PreT1 (mg/kg)	PreT2 (mg/kg)
TPH	1301.00 <sup>a</sup> ±18.73	863.00 <sup>b</sup> ±40.04	617.33 <sup>a</sup> ±18.72
Pb	3.78 <sup>c</sup> ±0.20	3.00 <sup>b</sup> ±0.13	2.51 <sup>a</sup> ±0.13
Cd	0.36 <sup>b</sup> ±0.05	0.26 <sup>a</sup> ±0.03	0.22 <sup>a</sup> ±0.03
Cr	13.79 <sup>b</sup> ±0.54	9.36 <sup>a</sup> ±1.46	10.13 <sup>a</sup> ±0.97
Cu	47.48 <sup>a</sup> ±2.58	32.05 <sup>b</sup> ±3.13	34.14 <sup>b</sup> ±1.93
Ni	12.75 <sup>a</sup> ±1.02	9.55 <sup>b</sup> ±0.96	10.26 <sup>b</sup> ±0.22
Fe	8172 <sup>a</sup> ±267	6262 <sup>b</sup> ±316	6781 <sup>b</sup> ±287

Note: Replication - 3, Mean±standard deviation, rows with the same common letter (superscript) indicate that the means are not significantly different at  $p \leq 0.05$  using DMRT, TPH - Total Petroleum Hydrocarbons, Pb – lead, Cd – cadmium, Cr – chromium, Cu – copper, Ni – nickel, Fe – iron, PreT1 – preliminary Treatment 1, PreT2 - preliminary Treatment 2.

their ability to thrive in a contaminated environment [17, 25, 34].

Generally, the remediation efficiency achieved when one or two agents were applied (T1 and T2) was lower than the efficiency obtained when multiple agents were hybridized (T3-T8). This highlights that combining different remediation agents creates a synergistic effect, resilience to variability, and a broader spectrum of action, enhancing pollutant degradation and immobilization [35, 36]. The decline in the TPH and HM concentrations observed in the T3 and T6 soil samples can be linked to the presence of plantain peel, plantain peel ash, and eggshell in the manure used for the remediation. Plantain peel ash has adsorptive properties that help trap contaminants and stabilize pollutants, while the plantain peels contain essential compounds and organic matter. These help to restore the soil's physical characteristics, nutritional status, and microbial environment [14]. The porous structure of eggshells improves soil structure by increasing aeration and water retention, which aids in the absorption of TPH and HMs. This reduces the mobility of these pollutants, limiting their spread and bioavailability in soil. Eggshells, which are primarily composed of calcium carbonate, enhance the bioremediation processes and help to restore soil health and engineering properties [37]. These results correlate with the findings on eggshells' bioremediation possibility, which Lim [38] documented in heavy metal-polluted soils.

The seaweed extract displayed a strong ability to enhance the phytoremediation behaviors of the tomato crop; principally, the experimental groups integrating seaweed extract (T5 and T8) presented better results. This behavioral pattern can be linked to the capability of the seaweed to improve the soil nutrients and beneficial microbial performance, improving and boosting the plant's phytoremediation performance [39]. Additionally, apart from algae's high essential nutrient content, it also helps to improve the soil structure, carbon sequestration, and microbial activity [13], which can be attributed to their improved phytoremediation efficiency (T4 and T7).

Organic materials and sufficient moisture accelerate bio-substances' release, which promotes microbial survival and performance within the rhizosphere region. Notably, this enhances the microbial degradation ability of hydrocarbons and toxic elements (rhizodegradation) [34]. These microbial actions can be attributed to the higher remediation efficiency recorded in T6 to T8 soil samples. Interestingly, the results depicted that the Pb, Cd, and Cr concentrations in the tomato plant's body exceeded the maximum allowable limits approved by WHO and FAO, whereas the levels of Cu, Ni, and Fe remained within the recommended limits established by these organizations.

#### Bioaccumulation Factor (BCF) and Translocation Factor (TF) Analysis

Table 4 shows the BCF (soil-roots and soil-leaves) and TF values of the tomato plants, which vary significantly depending on the type of amendment incorporated into the contaminated soil. The one-way ANOVA results of the BCF and TF values revealed that no significant difference existed between the treatment results for TPH, Pb, Cd, Cr, Cu, Ni, and Fe ( $p < 0.05$ ). This result aligns with the findings of Shang [26], which indicate that a plant's phytoremediation ability is highly dependent on the specific soil treatments applied, significantly affecting the plant's capacity to both accumulate and translocate contaminants effectively. The BCF values of the treatments with higher percentages of organic materials (T6, T7, and T8) were less than 1, except for Cu, Cr, and Ni under Treatment 6. According to FAO/WHO Codex Alimentarius, a BAF value less than 1 is an indication that the plant is an excluder, while a BAF value greater than 1 is an indication that the plant is an accumulator [32, 33]. Typically, the results highlighted that soil contamination levels substantially impact tomato plants' bioaccumulation tendency.

This study's findings depicted that the prevalence of organic materials in the soil considerably inhibits contaminant accumulation in the plant's root

Table 3. TPH and HM concentrations in the remediated soil, tomato roots, and leaves (mg/kg).

	TPH	Pb	Cd	Cr	Cu	Ni	Fe
Soil							
Con	1266.00 <sup>b</sup> ±22.6	3.51 <sup>d</sup> ±0.23	0.33 <sup>a</sup> ±0.01	13.13 <sup>f</sup> ±0.2	44.89 <sup>c</sup> ±0.97	12.50 <sup>e</sup> ±0.19	8016.00 <sup>c</sup> ±34.18
T1	718.00 <sup>a</sup> ±4.58	2.43 <sup>c</sup> ±0.04	0.19 <sup>f</sup> ±0.02	7.19 <sup>de</sup> ±0.03	28.26 <sup>d</sup> ±0.65	7.14 <sup>cd</sup> ±0.10	5825.33 <sup>d</sup> ±22.05
T2	495.33 <sup>f</sup> ±6.66	2.25 <sup>b</sup> ±0.02	0.15 <sup>c</sup> ±0.02	7.40 <sup>c</sup> ±0.04	27.87 <sup>d</sup> ±0.35	7.28 <sup>d</sup> ±0.03	5772.00 <sup>d</sup> ±7.55
T3	304.00 <sup>a</sup> ±8.19	2.17 <sup>ab</sup> ±0.03	0.12 <sup>d</sup> ±0.02	6.15 <sup>ab</sup> ±0.13	25.98 <sup>b</sup> ±0.87	6.26 <sup>a</sup> ±0.05	5213.67 <sup>c</sup> ±2.52
T4	266.00 <sup>d</sup> ±5.00	2.18 <sup>ab</sup> ±0.02	0.10 <sup>c</sup> ±0.01	6.34 <sup>bc</sup> ±0.11	24.80 <sup>a</sup> ±0.29	6.77 <sup>b</sup> ±0.06	5246.33 <sup>c</sup> ±10.69
T5	252.33 <sup>d</sup> ±3.06	2.25 <sup>b</sup> ±0.01	0.06 <sup>b</sup> ±0.01	5.95 <sup>a</sup> ±0.09	25.94 <sup>b</sup> ±0.65	6.28 <sup>a</sup> ±0.06	5126.33 <sup>b</sup> ±51.33
T6	197.33 <sup>c</sup> ±2.52	2.18 <sup>ab</sup> ±0.02	0.06 <sup>b</sup> ±0.01	7.13 <sup>d</sup> ±0.09	27.20 <sup>cd</sup> ±0.66	7.01 <sup>c</sup> ±0.12	5069.00 <sup>b</sup> ±37.51
T7	155.67 <sup>b</sup> ±4.73	2.10 <sup>ab</sup> ±0.04	0.03 <sup>a</sup> ±0.01	5.95 <sup>a</sup> ±0.11	26.18 <sup>bc</sup> ±0.14	7.28 <sup>d</sup> ±0.09	4886.67 <sup>a</sup> ±61.21
T8	137.00 <sup>a</sup> ±4.58	2.07 <sup>a</sup> ±0.03	0.05 <sup>ab</sup> ±0.02	6.42 <sup>c</sup> ±0.16	24.84 <sup>a</sup> ±0.23	7.17 <sup>cd</sup> ±0.06	5068.00 <sup>b</sup> ±55.34
Roots							
T1	821.00 <sup>b</sup> ±2.65	2.71 <sup>b</sup> ±0.03	0.24 <sup>d</sup> ±0.02	6.98 <sup>c</sup> ±0.08	29.95 <sup>d</sup> ±0.08	6.92 <sup>c</sup> ±0.15	5291.67 <sup>c</sup> ±8.74
T2	506.33 <sup>a</sup> ±4.51	2.60 <sup>b</sup> ±0.05	0.14 <sup>c</sup> ±0.02	7.21 <sup>d</sup> ±0.04	30.24 <sup>d</sup> ±0.24	6.42 <sup>c</sup> ±0.04	5129.00 <sup>b</sup> ±3.46
T3	313.00 <sup>f</sup> ±3.61	2.87 <sup>b</sup> ±0.04	0.11 <sup>b</sup> ±0.02	6.94 <sup>c</sup> ±0.04	23.72 <sup>b</sup> ±0.44	4.79 <sup>a</sup> ±0.36	5319.33 <sup>d</sup> ±19.50
T4	336.33 <sup>c</sup> ±7.57	2.05 <sup>a</sup> ±0.04	0.15 <sup>c</sup> ±0.02	6.23 <sup>b</sup> ±0.03	20.53 <sup>a</sup> ±0.60	5.10 <sup>b</sup> ±0.08	5072.33 <sup>b</sup> ±108.03
T5	194.67 <sup>a</sup> ±7.09	1.78 <sup>a</sup> ±0.62	0.03 <sup>a</sup> ±0.01	6.15 <sup>b</sup> ±0.03	27.87 <sup>c</sup> ±1.00	5.30 <sup>b</sup> ±0.03	5687.00 <sup>c</sup> ±57.19
T6	172.67 <sup>c</sup> ±8.39	1.95 <sup>a</sup> ±0.02	0.04 <sup>a</sup> ±0.01	7.45 <sup>c</sup> ±0.07	30.49 <sup>d</sup> ±0.45	7.05 <sup>d</sup> ±0.06	4957.00 <sup>a</sup> ±35.04
T7	135.67 <sup>b</sup> ±7.57	1.85 <sup>a</sup> ±0.03	0.02 <sup>a</sup> ±0.02	5.24 <sup>a</sup> ±0.03	28.11 <sup>c</sup> ±0.26	4.83 <sup>a</sup> ±0.09	4721.33 <sup>a</sup> ±140.50
T8	122.00 <sup>a</sup> ±4.00	1.91 <sup>a</sup> ±0.04	0.04 <sup>a</sup> ±0.01	6.06 <sup>b</sup> ±0.05	23.07 <sup>b</sup> ±0.13	9.02 <sup>c</sup> ±0.18	5149.33 <sup>b</sup> ±63.17
Leaves							
T1	809.67 <sup>c</sup> ±7.09	2.18 <sup>c</sup> ±0.07	0.16 <sup>b</sup> ±0.02	7.74 <sup>c</sup> ±0.06	21.76 <sup>b</sup> ±0.37	7.25 <sup>d</sup> ±0.12	5140.00 <sup>c</sup> ±34.70
T2	500.00 <sup>d</sup> ±4.36	2.33 <sup>d</sup> ±0.13	0.18 <sup>b</sup> ±0.03	7.70 <sup>c</sup> ±0.16	34.92 <sup>c</sup> ±1.15	7.07 <sup>d</sup> ±0.06	4931.67 <sup>b</sup> ±90.65
T3	326.67 <sup>c</sup> ±12.66	2.71 <sup>c</sup> ±0.05	0.16 <sup>b</sup> ±0.04	7.23 <sup>c</sup> ±0.02	19.93 <sup>ab</sup> ±1.18	3.00 <sup>a</sup> ±0.12	5867.33 <sup>d</sup> ±21.22
T4	331.00 <sup>a</sup> ±4.58	2.03 <sup>b</sup> ±0.05	0.15 <sup>b</sup> ±0.02	6.04 <sup>b</sup> ±0.02	28.54 <sup>d</sup> ±0.79	7.14 <sup>d</sup> ±0.11	6072.67 <sup>c</sup> ±3.50
T5	206.67 <sup>b</sup> ±6.66	1.65 <sup>a</sup> ±0.04	0.05 <sup>a</sup> ±0.03	6.11 <sup>b</sup> ±0.05	25.48 <sup>c</sup> ±1.16	7.44 <sup>d</sup> ±0.96	6258.33 <sup>f</sup> ±38.28
T6	204.00 <sup>b</sup> ±5.57	2.16 <sup>c</sup> ±0.08	0.02 <sup>a</sup> ±0.01	7.99 <sup>f</sup> ±0.05	40.79 <sup>f</sup> ±1.98	6.01 <sup>c</sup> ±0.13	4281.33 <sup>a</sup> ±12.10
T7	169.33 <sup>a</sup> ±10.60	2.00 <sup>b</sup> ±0.02	0.05 <sup>a</sup> ±0.01	5.12 <sup>a</sup> ±0.03	33.93 <sup>c</sup> ±1.13	5.06 <sup>b</sup> ±0.09	5214.00 <sup>c</sup> ±4.58
T8	155.33 <sup>a</sup> ±14.36	2.11 <sup>bc</sup> ±0.08	0.06 <sup>a</sup> ±0.02	7.53 <sup>d</sup> ±0.06	18.61 <sup>a</sup> ±1.33	4.73 <sup>b</sup> ±0.04	4961.00 <sup>b</sup> ±79.90

Note: Con - control, replication -3, Mean±standard deviation, T1 – Treatment 1, T2 – Treatment 2, T3 – Treatment 3, T4 – Treatment 4, T5 – Treatment 5, T6 – Treatment 6, T7 – Treatment 7, T8 – Treatment 8, columns with the same common letter (for the same category) indicate that the means are not significantly different at  $p \leq 0.05$  using DMRT, (NOTE: Treatments 1 – 8 are special amendments that contain different additives at different concentration “quantity”).

system, ultimately leading to phytovolatilization and phytodegradation mechanisms. Humus facilitates soil microorganisms' productivity and performance, resulting in the rapid degradation of the contaminants' concentrations [13, 24]. Interestingly, the experimental outcomes highlighted that at very low organic material volume (T1), the tomato crop displayed the highest BCF value, while a high humus proportion in the experimental groups led to crops with lower BCF values. These situations might be linked to the crop's ability to absorb and degrade contaminants in the presence of

higher humus levels, subsequently leading to effective phytoremediation [40].

Furthermore, the results reflected that the TPH and Pb demonstrated the minimum TF values in comparison to other pollutants investigated in this research. This reveals that the tomato has a lower efficiency in translocating these contaminants (Cd, Cr, Cu, Ni, and Fe) from the root to the shoot system. Kafle [35] stated that, in a situation where the TF is greater than 1, there is an indication that the remediating plant has lower competence in conveying toxic materials from the



Table 4. The bioaccumulation factor and translocation factor of heavy metals in tomato roots and leaves.

	TPH	Pb	Cd	Cr	Cu	Ni	Fe
	BCF (soil-roots)*						
T1	1.14	1.12	1.26	0.97	1.06	0.97	0.91
T2	1.02	1.16	0.93	0.97	1.09	0.88	0.89
T3	1.03	1.32	0.92	1.13	0.91	0.77	1.02
T4	1.26	0.94	1.50	0.98	0.83	0.75	0.97
T5	0.77	0.79	0.50	1.03	1.07	0.84	1.11
T6	0.88	0.89	0.67	1.04	1.12	1.01	0.98
T7	0.87	0.88	0.67	0.88	1.07	0.66	0.97
T8	0.89	0.92	0.80	0.94	0.93	1.26	1.02
p-value	0.7571 ns						
	BCF (soil-leaves)*						
T1	1.13	0.90	0.84	1.08	0.77	1.02	0.91
T2	1.01	1.04	1.20	1.04	1.25	0.97	0.89
T3	1.07	1.25	1.33	1.18	0.77	0.48	1.02
T4	1.24	0.93	1.50	0.95	1.15	1.05	0.97
T5	0.82	0.73	0.83	1.03	0.98	1.18	1.11
T6	1.03	0.99	0.33	1.12	1.50	0.86	0.98
T7	1.09	0.95	1.67	0.86	1.30	0.70	0.97
T8	1.13	1.02	1.20	1.17	0.75	0.66	1.02
p-value	0.4299ns						
	TF *						
T1	0.99	0.80	0.67	1.11	0.73	0.97	0.97
T2	0.99	0.90	1.29	1.07	1.15	0.88	0.96
T3	1.04	0.94	1.45	1.04	0.84	0.77	1.10
T4	0.98	0.99	1.00	0.97	1.39	0.75	1.20
T5	1.06	0.93	1.67	0.99	0.91	0.84	1.10
T6	1.18	1.11	0.50	1.07	1.34	1.01	0.86
T7	1.25	1.08	2.50	0.98	1.21	0.66	1.10
T8	1.27	1.10	1.50	1.24	0.81	1.26	0.96
p-value	0.1158 ns						

Note: The BCF and TF values were calculated from the average heavy metal values found in the soil, roots, and leaves. \*\* - significant at  $p < 0.05$ , ns – not significant at  $p < 0.05$ .

roots to the leaves. Generally, the biomaterials (organic materials) play a pivotal role in the tomato plant's ability to shift the pollutants from the lower plant's section to the upper section. It is obvious in the findings displayed by the T6, T7, and T8 experimental groups' results, as their results depicted that the TF values documented were greater than 1 ( $TF > 1$ ). Notably, the T6 unit demonstrated a hyperaccumulation approach to Cu and Ni metals, as both the BCF and TF results surpassed 1, specifying phytoextraction success of these deadly

elements. According to Ref [40], a condition that results in a TF value greater than 1 designates an effective phytoextraction procedure. Interestingly, this research's outcomes depicted that the tomato plant facilitated phytostabilization of TPH and Pb under treatments 1 and 2, as indicated by a BCF greater than 1 and a TF less than 1, which helps to hinder the further spread of these pollutants within the ecosystem, making the plant effective for stabilizing contaminated soils.

Table 5. HUB and HUF loads of the various treatments of tomato fruits ( $\times 10^4$  cfu/g).

Code	HUB	HUF
Control	1.87 <sup>a</sup> ±0.03	0.69 <sup>a</sup> ±0.01
T1	3.43 <sup>d</sup> ±0.01	2.87 <sup>c</sup> ±0.13
T2	3.55 <sup>d</sup> ±0.04	2.25 <sup>b</sup> ±0.24
T3	5.16 <sup>e</sup> ±0.15	4.91 <sup>c</sup> ±0.08
T4	5.62 <sup>f</sup> ±0.12	4.88 <sup>c</sup> ±0.07
T5	6.23 <sup>g</sup> ±0.02	6.72 <sup>f</sup> ±0.11
T6	3.11 <sup>c</sup> ±0.06	4.51 <sup>d</sup> ±0.42
T7	3.04 <sup>b</sup> ±0.01	2.93 <sup>c</sup> ±0.01
T8	2.91 <sup>b</sup> ±0.04	3.66 <sup>d</sup> ±0.01

Note: Replication = 3, Mean±standard deviation, HUB - hydrocarbon-utilizing bacteria, HUF - hydrocarbon-utilizing fungi, columns with the same common letter indicate that the means are not significantly different at  $p \leq 0.05$  using DMRT.

### Microbial Population

Table 5 displays the microbiological conditions of the soil, revealing that bio-materials significantly influence the HUB and HUF population in the contaminated soil. The HUB load in the Control, T1, T2, T3, T4, T5, T6, T7, and T8 soil samples measured  $1.87 \times 10^4$ ,  $3.43 \times 10^4$ ,  $3.55 \times 10^4$ ,  $5.16 \times 10^4$ ,  $5.62 \times 10^4$ ,  $6.23 \times 10^4$ ,  $3.11 \times 10^4$ ,  $3.04 \times 10^4$ , and  $2.91 \times 10^4$  cfu/g, respectively. Furthermore, the population of HUF in the control, T1, T2, T3, T4, T5, T6, T7, and T8 soil samples was  $0.69 \times 10^4$ ,  $2.87 \times 10^4$ ,  $2.25 \times 10^4$ ,  $4.91 \times 10^4$ ,  $4.88 \times 10^4$ ,  $6.72 \times 10^4$ ,  $4.51 \times 10^4$ ,  $2.93 \times 10^4$ , and  $3.66 \times 10^4$  cfu/g, respectively. Remarkably, the results show that the populations of HUF and HUB declined significantly as the amount of organic materials in the treatment plans (T6, T7, and T8) increased. This suggests that the initial application of excess bio-stimulants and organic materials promotes the rapid degradation of petroleum contaminants during the early stages of remediation. However, as the bioremediation process progresses, the availability of PHs diminishes, which limits the substrate for microbial activity. Consequently, this decline in available hydrocarbons leads to a reduction in the population performance of the HUB and HUF.

These findings are consistent with those reported by Zhang [41] and Xu [23], who studied petroleum-contaminated soil remediated with petroleum-degrading bacteria immobilized on biochar. The populations of HUB and HUF in petroleum-contaminated soil typically peak during the active bioremediation phase. Following this period, the populations of hydrocarbon-utilizing bacteria gradually decline due to the depletion of hydrocarbons, which serve as their primary food source [8, 42].

## Nutritional Components of the Tomato Fruit

### Vitamin Levels and Carotenoid Profile

The results of the vitamin levels and carotenoids present in the tomato fruits (FW) harvested from the experimental groups are presented in Table 6. It was noted that the remediation program had a significant effect on the vitamin B, C, and total carotenoid content of the tomato fruits ( $p \leq 0.05$ ). The lower vitamin and carotenoid concentrations observed in the tomato fruits harvested from T1 and T2 experimental units could be attributed to the elevated concentrations of TPH and HMs present in the soil throughout the tomato growing period (Table 3). Alengebawy [19] noted that high HM levels interfere with a plant's aptitude to absorb nutrients from the growing medium and carry out metabolic processes, which can impede the production of essential compounds such as vitamins and carotenoids.

Furthermore, the contaminated soil treated with a combination of charcoal, burnt rice husk, and organic manure (T6, T7, and T8) produced fruits with higher levels of vitamins and carotenoids compared to soil treated with the combination of only charcoal and organic manure (T3, T4, and T5). This showed that the burned rice husk enhanced the phytoremediation process by increasing nutrient availability and supporting plant metabolic functions, which ultimately led to improved nutrient synthesis and accumulation in the fruits. Rice husk ash can enhance soil nutrients and foster more suitable environmental conditions for the microbial breakdown of hydrocarbons and other pollutants [24]. Also, the fruits collected from the soils treated with plantain peel-based organic manure (T3 and T6) demonstrated significantly higher amounts of vitamins and carotenoids compared to those treated with algae and seaweed extract-based organic manure (T4, T6, T7, and T8). This depicts that the plantain peel-based organic manure provides a favorable soil nutrient profile, which enhances the tomato's ability to produce and accumulate these essential compounds in the fruits. Plantain peels and eggshells have abundant basic nutrients such as potassium, phosphorus, nitrogen, and calcium, which play a crucial role in promoting stress tolerance, plant growth, and overall performance [36].

This study's vitamins B and C are lower than the values reported by Refs [43, 44] but were within the range of values documented by Mellidou [45]. The discrepancy noted in the results may be due to the effects of field practices on the development of tomato fruits. These practices encompass toxicity from PHs and HMs, as well as the impact of the soil amendments employed in tomato cultivation. Exposure to PHs and HMs can lead to physiological stress, reducing the ability of the plants to synthesize essential vitamins. PHs can disrupt a plant's ability to absorb nutrients, leading to deficiencies in essential nutrients like antioxidants and vitamins [46].

Table 6. Vitamin content and carotenoid profile of tomato fruits (mg/kg FW).

	Vitamin B profile					
	B1	B2	B3	B6	B9	B12
T1	0.041 <sup>a</sup> ±0.009	0.029 <sup>a</sup> ±0.006	0.001 <sup>a</sup> ±0.001	0.046 <sup>a</sup> ±0.005	0.23 <sup>a</sup> ±0.002	0.022 <sup>a</sup> ±0.001
T2	0.057 <sup>ab</sup> ±0.012	0.040 <sup>ab</sup> ±0.011	0.002 <sup>ab</sup> ±0.002	0.050 <sup>ab</sup> ±0.003	0.024 <sup>a</sup> ±0.002	0.018 <sup>a</sup> ±0.002
T3	0.063 <sup>abc</sup> ±0.006	0.058 <sup>bc</sup> ±0.011	0.004 <sup>abc</sup> ±0.002	0.060 <sup>bcd</sup> ±0.003	0.063 <sup>bc</sup> ±0.002	0.029 <sup>b</sup> ±0.003
T4	0.080 <sup>bc</sup> ±0.010	0.066 <sup>bc</sup> ±0.016	0.005 <sup>bcd</sup> ±0.002	0.063 <sup>cd</sup> ±0.011	0.063 <sup>bc</sup> ±0.008	0.035 <sup>bc</sup> ±0.004
T5	0.063 <sup>abc</sup> ±0.006	0.078 <sup>c</sup> ±0.017	0.007 <sup>cd</sup> ±0.002	0.056 <sup>abc</sup> ±0.009	0.055 <sup>b</sup> ±0.003	0.030 <sup>b</sup> ±0.003
T6	0.078 <sup>bc</sup> ±0.024	0.063 <sup>bc</sup> ±0.012	0.007 <sup>cd</sup> ±0.002	0.056 <sup>abc</sup> ±0.006	0.056 <sup>b</sup> ±0.005	0.037 <sup>cd</sup> ±0.005
T7	0.072 <sup>abc</sup> ±0.019	0.057 <sup>bc</sup> ±0.019	0.008 <sup>d</sup> ±0.001	0.071 <sup>d</sup> ±0.007	0.072 <sup>d</sup> ±0.003	0.042 <sup>de</sup> ±0.003
T8	0.093 <sup>c</sup> ±0.031	0.079 <sup>c</sup> ±0.014	0.007 <sup>cd</sup> ±0.003	0.062 <sup>bcd</sup> ±0.004	0.064 <sup>c</sup> ±0.005	0.045 <sup>c</sup> ±0.006
	Carotenoid profile					
	Lycopene	Phytoene	β-Carotene			
T1	6.033 <sup>a</sup> ±0.100	2.480 <sup>a</sup> ±0.551	1.140 <sup>a</sup> ±0.090			
T2	7.093 <sup>a</sup> ±0.208	2.120 <sup>a</sup> ±0.178	1.073 <sup>b</sup> ±0.131			
T3	11.657 <sup>b</sup> ±0.964	5.743 <sup>c</sup> ±0.454	2.793 <sup>c</sup> ±0.221			
T4	16.673 <sup>c</sup> ±1.991	3.913 <sup>b</sup> ±0.142	3.160 <sup>bc</sup> ±0.207			
T5	12.790 <sup>b</sup> ±1.725	3.977 <sup>b</sup> ±0.310	3.063 <sup>bc</sup> ±0.103			
T6	23.280 <sup>de</sup> ±3.387	8.447 <sup>c</sup> ±1.628	3.430 <sup>c</sup> ±0.547			
T7	25.650 <sup>e</sup> ±1.545	9.683 <sup>f</sup> ±0.674	4.627 <sup>d</sup> ±0.386			
T8	21.260 <sup>d</sup> ±0.291	7.107 <sup>d</sup> ±0.388	5.097 <sup>d</sup> ±0.194			
	Vitamin C					
T1	134.683 <sup>a</sup> ±13.54					
T2	138.617 <sup>a</sup> ±11.42					
T3	149.630 <sup>b</sup> ±11.62					
T4	152.027 <sup>b</sup> ±13.38					
T5	167.597 <sup>c</sup> ±12.56					
T6	172.477 <sup>c</sup> ±12.74					
T7	172.923 <sup>c</sup> ±14.48					
T8	182.067 <sup>d</sup> ±13.63					

Note: Replication = 3, Mean±standard deviation, columns with the same common letter (for the same category) indicate that the means are not significantly different at  $p \leq 0.05$  using DMRT.

### Phytochemical Properties

Remarkably, Table 7 presents the phytochemical properties of the tomato fruits sampled from the various experimental units. Out of the 24 compounds identified in the fresh fruits, catechin, butein, tangeretin, coumaric acid, ferulic acid, naringenin, apigenin, and kaempferol were the most predominant. These compounds have potent antioxidant, anti-inflammatory, and phytochemical properties and play crucial roles in enhancing tomato fruits' nutritional and therapeutic qualities [47]. Apigenin, butein, and kaempferol are

powerful flavonoids with antioxidant, anti-inflammatory, and anticancer properties. They also contribute to cardiovascular health and offer neuroprotective benefits [48]. Ferulic and coumaric acids have numerous medicinal benefits, promoting cardiovascular and skin health and contributing to cancer prevention [49]. The subpar phytochemical properties observed in the T1 to T4 experimental groups' tomato fruits can be linked to the elevated TPH levels found in both the soil and plant tissues (Table 2). High levels of PH concentration inhibit plant growth and phytochemical properties (flavonoids, phenolics, and antioxidants) by causing

Table 7. The phytochemical properties of tomato fruits (mg/kg FW).

Parameter	Treatments							
	1	2	3	4	5	6	7	8
Resveratrol	0.642	0.683	0.703	0.711	0.755	0.792	0.816	0.822
Apigenin	3.521	3.417	3.852	4.110	4.350	4.594	4.926	4.802
Piperic acid	2.187	2.094	2.512	2.663	2.810	3.121	3.261	3.744
Catechin	10.210	13.772	13.951	15.014	16.290	17.811	18.462	18.993
Daidzein	1.084	1.127	1.392	1.241	1.259	1.466	1.703	1.605
Vanillic acid	1.109	1.103	1.342	1.496	1.440	1.652	1.712	1.776
Butein	6.112	7.318	8.216	9.103	9.380	10.231	10.619	12.024
Naringenin	2.851	2.963	3.712	4.228	4.303	4.198	5.091	4.744
Luteolin	1.121	1.165	1.201	1.234	1.221	1.447	1.408	1.632
Kaempferol	2.119	2.105	2.243	2.255	2.279	2.308	2.296	2.364
Epicatechin	1.314	1.421	1.328	1.502	1.526	1.632	1.598	1.771
Epigallocatechin	0.274	0.325	0.411	0.451	0.826	0.951	0.854	0.886
Quercetin	1.241	1.35	1.422	1.506	1.510	1.554	1.723	1.647
Gallocatechin-3-gallate	0.195	0.175	0.201	0.211	0.208	0.254	0.232	0.247
Robinetin	0.497	0.533	0.452	0.660	0.932	0.872	0.104	0.923
Myricetin	1.162	1.341	1.802	2.477	2.832	3.319	3.532	3.961
Artemetin	0.398	0.401	0.385	0.409	0.414	0.397	0.431	0.442
Nobiletin	0.382	0.372	0.392	0.385	0.398	0.473	0.406	0.436
Tangeretin	4.941	4.663	5.217	6.055	6.214	6.712	6.945	7.102
Naringin	0.281	0.258	0.338	0.482	0.543	0.684	0.667	0.839
Lunamarin	0.520	0.381	0.405	0.723	0.964	0.994	1.052	0.983
Cinnamic acid	0.195	0.162	0.235	0.269	0.273	0.271	0.283	0.292
Coumaric acid	2.287	2.502	3.107	3.413	3.665	3.820	3.984	3.806
Ferrulic acid	11.982	13.244	15.931	18.219	21.584	22.651	25.121	25.887

ROS, phytochemical alterations, and physiological stress within the plant [18, 50]. This may lead to reduced antioxidant levels and nutritional value of the tomato fruits.

The research findings indicate that remediation therapies considerably influence the phytochemical properties of the fruit. Soils treated with larger quantities of organic materials (T6 to T8) generally exhibited higher phytochemical properties concentrations than those remediated with smaller amounts of organic materials (T1 to T5). This highlights that organic materials not only aid the phytoremediation process, but they also enhance the formation of phytochemical properties within plant tissues. Organic amendments not only improve soil nutrient availability but also stimulate microbial activity and root exudates. This is an indication that these procedures considerably assisted in increasing the plant's ability to produce

essential bioactive compounds, which aid environmental remediation, enhance crop nutrition, and medicinal applications [26, 51].

Fascinatingly, this study's findings exposed the possibility of utilizing plantain peels as a suitable substitute for seaweed extract or algae in forming vital bio-stimulants, with high remediation and nutrient improvement efficacies. Decaying plantain peels have high humus, P, and K proportions, resulting in an enhancement in the soil structure and fertility level. Intriguingly, this will increase the tomato's phytoremediation ability and phytochemical properties [14]. Charcoal and RHA, used additionally in the remediation process, are known high absorption agents, which will assist immensely in absorbing harmful substances during the remediation process [34].



## Conclusions

This study was conducted to appraise bio-materials' usefulness in environmental control and crop productivity. An experimental investigation was undertaken on the potential of using charcoal, rice husk ash (RHA), seaweed extract, algae, and plantain peels as bio-additives to increase tomato performance and petroleum remediation ability. Results from the laboratory tests revealed that hybridizing charcoal, rice husk ash (RHA), seaweed extract, algae, and plantain peels absolutely increased tomato medical attributes and phytoremediation potential. Furthermore, the findings emphasized that organic materials improve beneficial microbial performance in the soil specimens, primarily through promoting HUB and HUF functionality, further enhancing petroleum degradation in the soil. Additionally, the HPLC results revealed that bio-agents markedly increased the tomato fruits' vitamin and carotenoid profiles. These results portrayed crucial roles these natural admixtures (charcoal, RHA, algae, and plantain peels) played in facilitating the phytoremediation procedures and enhancing the crop's dietary and therapeutic qualities. Distinctively, this research was conducted within 5 months, focusing solely on tomato plants; it is recommended that the potential of additional crops and the effects of long-term application of bio-agents on soil microbial communities and nutrient dynamics be explored further.

## Funding

This research was funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2024-79).

## Acknowledgment

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-79).

## References

- MITRA S., CHAKRABORTY A.J., TAREQ A.M., EMRAN T.B., NAINU F., KHUSRO A., IDRIS A.M., KHANDAKER M.U., OSMAN H., ALHUMAYDHI F.A., SIMAL-GANDARA J. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *Journal of King Saud University – Science*. **34** (3), 101865, **2022**.
- VASILACHI I.C., STOLERU V., GAVRILESCU M. Analysis of Heavy Metal Impacts on Cereal Crop Growth and Development in Contaminated Soils. *Agriculture*. **13** (10), 1983, **2023**.
- UGURU H., ESSAGHAH AE., AKWENUKE OM., AKPOKODJE OI., ROKAYYA SAMI, MAHMOUD H., ROQAYAH H.K. Environmental Impact of Wasteyard Leachate Pollution, It's Health Risks with Some Microbial and Ecological Implications. *Journal of Biobased Materials and Bioenergy*. **17**, 270, **2023**.
- ZHANG A., CAO H., JIANG Y., CHEN Y., LIU J., ZHOU X., ZHOU A., GAO Y., LI J. Refining health risk assessment for determining arsenic and cadmium safety limits in an herbal medicine and its cultivation soils. *Journal of Cleaner Production*. **331**, 129837, **2022**.
- CHINEDU E., CHUKWUEMEKA C.K. Oil Spillage and Heavy Metals Toxicity Risk in the Niger Delta, Nigeria. *Journal of health & pollution*. **8** (19), 180905, **2018**.
- AKPAN E.E. Environmental Consequences of Oil Spills on Marine Habitats and the Mitigating Measures—The Niger Delta Perspective. *Journal of Geoscience and Environment Protection*. **10** (6), 191, **2022**.
- SU Q., YU J., FANG K., DONG P., LI Z., ZHANG W., LIU M., XIANG L., CAI J. Microbial Removal of Petroleum Hydrocarbons from Contaminated Soil under Arsenic Stress. *Toxics*. **11** (2), 143, **2023**.
- ACHIFE C., JOSHUA U., BALA J., OYELEKE S. Microbial population of soil and water around petroleum depot Suleja, Nigeria, and their hydrocarbon utilisation. *International Journal of Life Sciences and Biotechnology*. **4** (1), 90, **2021**.
- HLIHOR R.M., ROȘCA M., HAGIU-ZALESCHI L., SIMION I.M., DARABAN G.M., STOLERU V. Medicinal Plant Growth in Heavy Metals Contaminated Soils: Responses to Metal Stress and Induced Risks to Human Health. *Toxics*. **10** (9), 499, **2022**.
- MASSÁNYI P., MASSÁNYI M., MADEDDU R., STAWARZ R., LUKÁČ N. Effects of Cadmium, Lead, and Mercury on the Structure and Function of Reproductive Organs. *Toxics*. **8** (4), 94, **2020**.
- ZAMANIAN Z., SEDAGHAT Z., MEHRIFAR Y. Harmful Outcome of Occupational Exposure to Petrol: Assessment of Liver Function and Blood Parameters among Gas Station Workers in Kermanshah City, Iran. *International Journal of Preventive Medicine*. **9**, 100, **2018**.
- PATEL A.B., SHAIKH S., JAIN K.R., DESAI C., MADAMWAR D. Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. *Frontiers in Microbiology*. **11**, 562813, **2020**.
- BALA S., GARG D., THIRUMALESH B.V., SHARMA M., SRIDHAR K., INBARAJ B.S., TRIPATHI M. Recent Strategies for Bioremediation of Emerging Pollutants: A Review for a Green and Sustainable Environment. *Toxics*. **10** (8), 484, **2022**.
- CHUKU O.S., AMADI N. Effects of different levels of plantain peel waste on the amelioration of crude oil polluted soil. *Applied Sciences Research Periodicals*. **1** (4), 53, **2023**.
- MASLENNIKOV P.V., CHUPAKHINA G.N., SKRYPNIK L.N., FEDURAEV P.V., MELNIK A.S. Assessment of the Antioxidant Potential of Plants in Urban Ecosystems under Conditions of Anthropogenic Pollution of Soils. *Russian Journal of Ecology*. **49** (5), 384, **2018**.
- SKRYPNIK L., MASLENNIKOV P., NOVIKOVA A., KOZHIKIN M. Effect of crude oil on growth, oxidative stress and response of antioxidative system of two rye (*Secale cereale* L.) Varieties. *Plants*. **10** (1), 157, **2021**.
- TRUSKEWYCZ A., GUNDRY T.D., KHUDUR L.S., KOLOBARIC A., TAHA M., ABURTO-MEDINA A., BALL A.S., SHAHSAVARI E. Petroleum hydrocarbon contamination in terrestrial ecosystems-fate and microbial responses. *Molecules (Basel, Switzerland)*. **24** (18), 3400, **2019**.

18. HAIDER F.U., EJAZ M., CHEEMA S.A., KHAN M.I., ZHAO B., LIQUN C., SALIM M.A., NAVEED M., KHAN N., NÚÑEZ-DELGADO A., MUSTAFA A. Phytotoxicity of petroleum hydrocarbons: Sources, impacts and remediation strategies. *Environmental Research*. **197**, 111031, **2021**.
19. ALENGBAWY A., ABDELKHALEK S.T., QURESHI S.R., WANG M.Q. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics*. **9** (3), 42, **2021**.
20. ANGON P.B., ISLAM MD.S., KC S., DAS A., ANJUM N., POUDEL A., SUCHI S.A. Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. *Heliyon*. **10** (7), **2024**.
21. YAN A., WANG Y., TAN S.N., MOHD YUSOF M.L., GHOSH S., CHEN Z. Phytoremediation: a promising approach for vegetation of heavy metal-polluted land. *Frontiers in Plant Science*. **11**, 139, **2020**.
22. SHARMA J.K., KUMAR N., SINGH N.P., SANTAL A.R. Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. *Frontiers in Plant Science*. **14**, 1076876, **2023**.
23. XU X., LIU W., TIAN S., WANG W., QI Q., JIANG P., GAO X., LI F., LI H., YU H. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis. *Frontiers in Microbiology*. **9**, 2885, **2018**.
24. MUKHERJEE S., SARKAR B., ARALAPPANAVAR V.K., MUKHOPADHYAY R., BASAK B.B., SRIVASTAVA P., MARCHUT-MIKOŁAJCZYK O., BHATNAGAR A., SEMPLE K.T., BOLAN N. Biochar-microorganism interactions for organic pollutant remediation: Challenges and perspectives. *Environmental Pollution*. **308**, 119609, **2022**.
25. AZIZ Z.S., JAZZA S.H., DAGEEM H.N., BANOON S.R., BALBOUL B.A., ABDELZAHER M.A. Bacterial biodegradation of oil-contaminated soil for pollutant abatement contributing to achieve sustainable development goals: A comprehensive review. *Results in Engineering*. **22**, 102083, **2024**.
26. SHANG X., WU S., LIU Y., ZHANG K., GUO M., ZHOU Y., ZHU J., LI X., MIAO R. Rice husk and its derived biochar assist phytoremediation of heavy metals and PAHs co-contaminated soils but differently affect bacterial community. *Journal of Hazardous Materials*. **466**, 133684, **2024**.
27. UGURU H., EFEOGHENE E.A., AKPOKODJE O.I., SAMI R., BAAKDAH F., PAREEK S. Exposure to airborne pollutants in urban and rural areas: levels of metals and microorganisms in PM10 and gaseous pollutants in ambient air. *Air Quality, Atmosphere & Health*. **18** (2), 317, **2024**.
28. ADEJUMO O.E., POPOOLA E.A., BAMIRO O.A., DAODU J.O., OLAITAN O.J. Physicochemical characteristics and HPLC determination of Alpha-Tocopherol in eighteen edible vegetable oils marketed in Nigeria. *Dhaka University Journal of Pharmaceutical Sciences*. **20** (1), 49, **2021**.
29. OMOKPARIOLA D.O., NDUKA J.K., KELLE H.I., MGBEMENA N.M., IDUSERI E.O. Chemometrics, health risk assessment and probable sources of soluble total petroleum hydrocarbons in atmospheric rainwater, Rivers State, Nigeria. *Scientific Reports*. **12** (1), 11829, **2022**.
30. MILETIĆ Z., JARIĆ S., JONJEV M., MITROVIĆ M., PAVLOVIĆ D., MATIĆ M., PAVLOVIĆ P. Phytoremediation potential of invasive plant species for potentially toxic elements along the Sava River upstream. *Heliyon*. **10** (13), e33798, **2024**.
31. DUWIEJUAH A.B., ABUBAKARI A.H., QUAINOO A.K., AMADU Y. Review of biochar properties and remediation of metal pollution of water and soil. *Journal of Health and Pollution*. **10** (27), 142, **2020**.
32. YIN M., LI X., LIU Q., TANG F. Rice husk ash addition to acid red soil improves the soil property and cotton seedling growth. *Scientific Reports*. **12** (1), 1704, **2022**.
33. LI Z., ZHENG Z., LI H., XU D., LI X., XIANG L., TU S. Review on rice husk biochar as an adsorbent for soil and water remediation. *Plants (Basel, Switzerland)*. **12** (7), 1524, **2023**.
34. ALI M.H., KHAN M.I., NAVEED M., TANVIR M.A. Microbe-assisted rhizoremediation of hydrocarbons and growth promotion of chickpea plants in petroleum hydrocarbons-contaminated soil. *Sustainability*. **15** (7), 6081, **2023**.
35. KAFLE A., TIMILSINA A., GAUTAM A., ADHIKARI K., BHATTARAI A., ARYAL N. Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Environmental Advances*. **8**, 100203, **2022**.
36. OKUNLOLA F.O., ABOYEJI C.M., ADEKIYA A.O., EJUE W.S., AREMU C., ERERE A., OLAJIDE O.P., ADEWUMI A.E., OWOOJUONA O.M. Potentials of plantain peel and Tithonia diversifolia leaves as soil amendments in enhancing performance and nutritional contents of tomato (*Solanum lycopersicum*). *Heliyon*. **9** (4), e14737, **2023**.
37. RAHEEM L.S., KHADIM H.J. Microbial-induced carbonate precipitation using eggshells and scallop shells as recycled materials. *Case Studies in Chemical and Environmental Engineering*. **10**, 100867, **2024**.
38. LIM J.E., AHMAD M., USMAN A.R.A., LEE S.S., JEON W.T., OH S.E., YANG J.E., OK Y.S. Effects of natural and calcined poultry waste on Cd, Pb and As mobility in contaminated soil. *Environmental Earth Sciences*. **69** (1), 11, **2012**.
39. ALI O., RAMSUBHAG A., JAYARAMAN J. Biostimulant properties of seaweed extracts in plants: implications towards sustainable crop production. *Plants (Basel, Switzerland)*. **10** (3), 531, **2021**.
40. MOCEK-PLÓCINIĄK A., MENCEL J., ZAKRZEWSKI W., ROSZKOWSKI S. Phytoremediation as an Effective Remedy for Removing Trace Elements from Ecosystems. *Plants (Basel, Switzerland)*. **12** (8), 1653, **2023**.
41. ZHANG B., ZHANG L., ZHANG X. Bioremediation of petroleum hydrocarbon-contaminated soil by petroleum-degrading bacteria immobilized on biochar. *RSC Advances*. **9** (60), **2019**.
42. DAS N., CHANDRAN P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology Research International*. **2011**, 941810, **2011**.
43. ALI M.Y., SINA A.A.I., KHANDKER S.S., NEESA L., TANVIR E.M., KABIR A., KHALIL M.I., GAN S.H. Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. *Foods*. **10** (1), 45, **2020**.
44. DAPHNEE N.T.R., DULCE CARLOS ANTUNES M., MARGARIDA CORTÊS VIEIRA M. Potential of Carotenoids from Fresh Tomatoes and Their Availability in Processed Tomato-Based Products. *IntechOpen*. **2022**.
45. MELLIDOU I., KEULEMANS J., KANELLIS A.K., DAVEY M.W. Regulation of fruit ascorbic acid

- concentrations during ripening in high and low vitamin C tomato cultivars. *BMC Plant Biology*. **12** (1), 512, **2012**.
46. ODUKOYA J., LAMBERT R., SAKRABANI R. Understanding the Impacts of Crude Oil and its Induced Abiotic Stresses on Agrifood Production: A Review. *Horticulturae*. **5** (2), 47, **2019**.
47. SHILPA V.S., SHAMS R., DASH K.K., PANDEY V.K., DAR A.H., AYAZ MUKARRAM S., HARSÁNYI E., KOVÁCS B. Phytochemical properties, extraction, and pharmacological benefits of naringin: A Review. *Molecules* (Basel, Switzerland). **28** (15), 5623, **2023**.
48. ULLAH A., MUNIR S., BADSHAH S.L., KHAN N., GHANI L., POULSON B.G., EMWAS A.H., JAREMKO M. important flavonoids and their role as a therapeutic agent. *Molecules* (Basel, Switzerland). **25** (22), 5243, **2020**.
49. SOVA M., SASO L. Natural sources, pharmacokinetics, biological activities and health benefits of hydroxycinnamic acids and their metabolites. *Nutrients*. **12** (8), 2190, **2020**.
50. OROCIO-CARRILLO J.A., RIVERA-CRUZ M. DEL C., JUÁREZ-MALDONADO A., BAUTISTA-MUÑOZ C. DEL C., TRUJILLO-NARCÍA A., GONZÁLEZ-GARCÍA Y., CÁRDENAS-VILLEGAS S. Crude oil induces plant growth and antioxidant production in *Leersia hexandra* Sw. *Plant, Soil and Environment*. **70** (2), 72, **2024**.
51. KUMAR S., DIKSHA SINDHU S.S., KUMAR R. Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Current Research in Microbial Sciences*. **3**, 100094, **2022**.