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Combined Use of Sewage Sludge and Plant Growth-Promoting Rhizobia Improves Germination, Biochemical Response and Yield of Ridge Gourd (*Luffa acutangula* (L.) Roxb.) under Field Conditions

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Abstract: This research investigated the combined use of sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) for Ridge gourd (*Luffa acutangula* (L.) Roxb.) cultivated under field conditions. The different treatments of SS and PGPR such as 0% (soil as control), 5% SS, 5% SS + PGPR, 10% SS, and 10% SS + PGPR were applied to assess their impacts on seedling growth, biochemical response, and yield performance of Ridge gourd. The results showed that the highest seedling emergence (92.3 ± 2.1%), fresh biomass (9.6 ± 0.3 g), growth rate (1.4 ± 0.1 g/day), seedling length (15.5 ± 0.3 cm), root length (10.4 ± 0.3 cm), total chlorophyll (3.2 ± 0.1 mg/g), crop yield (13.8 ± 0.1 kg/plant), and average crop yield per harvest (2.8 ± 0.1 kg/plant) were observed in 10% SS + PGPR treatment. The enzyme activities of superoxide dismutase (SOD; µg/g) and catalase (CAT: µg/g) were significantly lowered after PGPR inoculation in higher SS treatments. The results of principal component (PC) and Euclidian clustered distance analyses showed a positive influence of SS dose on soil nutrient availability and Ridge gourd's growth, biochemical responses, and yield performance. Moreover, the elemental analysis showed that the bioaccumulation factor (BAF < 0.90) and health risk index (HRI < 0.40) of selected metal elements (Cd, Cr, Cu, Fe, Mn, and Zn) were within the permissible limits, indicating consumption of Ridge gourd fruits was safe. The outcomes of this study suggest the potential use of SS and PGPR for improved Ridge gourd production and contribution towards sustainable development goal (SDG) 12 on responsible consumption and production of vegetable crops.

Keywords: bioaccumulation; health risk; *Luffa acutangula*; metal elements; sewage sludge; target hazard quotient

1. Introduction

The rate of domestic waste discharges has recently grown dramatically as a result of an uncontrolled increase in the human population [1]. Sewage wastewater is amongst the top contributor of domestic wastes, which has become a difficult to manage problem globally [2]. It is estimated that nearly 359 billion cubic meters of wastewater are generated globally, with only 52% being sufficiently or partially treated while the remaining is disposed of without any treatment [3]. Sewage sludge (SS) is a solid or semi-solid substance generated from the different steps of sewage treatment plants (STPs). SS could create several environmental issues if not properly disposed of [4,5]. Generally, the management of SS occurs at different stages such as production (minimization), collection, treatment, and reuse [6]. Being rich in various organic and nutrient substances, SS is widely utilized for secondary purposes such as biogas, biochar, syngas, biofertilizer production, composting, building materials, among many others [7,8].

During the recent decades, the increasing use of SS in agriculture has appeared as one of the most effective methods of its management due to the presence of abundant nutrients [9]. Predominantly, nitrogen, phosphorus, potassium, and other microelements are the major constituents of SS that are useful for soil amendment [10]. Markowicz et al. [11] reported that SS mixing up to 15 t/ha was the most efficient composition for the reclamation of nutrient-deficient soil. SS application on lands helps in the mixing of various organic and inorganic nutrients, which further leads to improved crop yields [12,13]. However, the excessive mixing of SS, having a high nutrient load, may also damage soil health; thereby, affecting plant growth and reducing crop yields [14]. Moreover, the nutrients may contain metal elements that have relatively high density, which has been regarded as one of the major problems regarding SS utilization due to their toxicity at higher levels [15]. Therefore, it is necessary to monitor the migration of such elements from SS-treated soils to plant parts for human health benefits.

Ridge gourd (*Luffa acutangula* (L.) Roxb.) or Luffa is a multi-harvest vegetable crop grown in the South Asian region. It is commonly called "*Torai*" in rural areas of India. Non-ripened fruits of Ridge gourd having tender dark green color are cooked and eaten due to their high nutritional values [16]. The ripened fruits of Ridge gourd are widely used for various purposes, such as cleaning sponges and mattresses [17]. Being a multi-harvest crop, the biggest problem associated with Ridge gourd cultivation is the excessive required quantity of fertilizers, which makes it less profitable for the farmers [18]. Consequently, the yield and nutritional quality of Ridge gourd start decreasing if the required dose of fertilizers is not supplied timely and adequately [19]. Also, the repeated use of chemical fertilizers makes the soil unhealthy and harmful for microbial communities [20]. Therefore, SS application for Ridge gourd cultivation could be a potential technique to minimize chemical fertilizer input and improve soil health.

Plant growth-promoting rhizobia (PGPR) is a group of microorganisms that have distinct capabilities to assist the plant root systems in terms of efficient survival and nutrient deliverability [21]. Commercial-scale PGPRs commonly known as biofertilizers have shown substantial improvements in plant growth and crop yields, making agriculture more profitable [22,23]. A study has found that supplementation of PGPR was helpful for increased nutrient extraction by mustard green (*Brassica juncea*). Konkolewska et al. found that supplementation of PGPR was helpful for increased nutrient extraction by mustard green (*Brassica juncea*) [24]. Ke et al. also showed improved nutrient uptake by perennial ryegrass [25]. Moreover, Ipek et al. reported an increased yield and nutritional profile of strawberries under PGPR treatments [26]. Considering the role of PGPR in plant-nutrient delivery, it can be potentially used for Ridge gourd cultivation along with SS application for improved nutrient utilization. However, some of the toxic metal elements may also accumulate in edible parts of Ridge gourd, which might not be suitable for human consumption due to health concerns. Hence, biomonitoring of these metal elements needs proper attention, along with their potential health hazard.

Thus, this study aimed to assess the impacts of varying doses of SS and PGPR inoculation on germination, biochemical response, and productivity of Ridge gourd (*L. acutangula*) crop under multiple harvests. Further, the potential health risk of metal elements transferred into the Ridge gourd plant was studied using bioaccumulation and health risk assessment studies.

2. Materials and Methods

2.1. Experimental Materials

In the present study, the quality F1 hybrid seeds of Ridge gourd (*Luffa acutangula* (L.) Roxb.) (ES-KRITIKA) were procured from the Nufield Genetics Pvt. Ltd., Ahmedabad, Gujrat, India. This variety is widely grown in the Northern Indian plains to produce higher crop yields in the loamy soils. The plant growth-promoting rhizobia (PGPR) biofertilizer of 8×10^7 cfu/g microbial count each of *Bacillus subtilis* (MTCC 441) and *Pseudomonas fluorescence* (MTCC 103^T) was procured from the National Centre of Organic Farming (NCOF), Ghaziabad, India. Besides this, post-digested SS was obtained from 27 MLD Sewage Treatment Plant (STP) of Jagjeetpur, Haridwar, India (29°54′02.5″ N 78°08′26.6″ E), which is operated under the Clean Ganga Mission of Namami Gange Project, Government of India.

2.2. Experimental Design for Ridge Gourd Cultivation

2.2.1. Pre-Field Treatment Stage

The Ridge gourd cultivation experiments were conducted in arable land with no history of SS application, located in Kulheri Village of Saharanpur district, Uttar Pradesh, India (29°52′54.4″ N 77°16′18.0″ E). The field was priorly plowed on 20 March 2021 and thereafter left for 10 days. For the land preparation, the soil was dug ($20 \times 20 \times 20$ cm) to make pits for the planting sites of Ridge gourd and appropriate doses of SS were mixed. A liquid consortium was prepared by dissolving 10 g of PGPR biofertilizer in 100 mL of deionized water. Afterwards, a total of five different treatments, such as T1 (arable soil as control), T2 (5% SS *w*/*w* soil), T3 (5% SS *w*/*w* soil + 10 mL PGPR), T4 (10% SS *w*/*w* soil), and T5 (10% SS *w*/*w* soil + 10 mL PGPR) were applied to the pits. Given the soil volume and area, the percent ratio of SS sludge was calculated accordingly.

2.2.2. Pretreatment and Germination Stage

Before the sowing, the healthy seeds of Ridge gourd were placed on Whatman filter paper (No. 41) moistened with deionized water in a Petri plate for 24 h under room conditions. Then, one moistened seed was transplanted in the sterile polyethylene bags (250 g capacity) having 200 g soil obtained from the previously prepared pits. The bags were placed inside a room at a mean temperature of 25 °C for 7 days (16/8 h light/dark period). The bags were watered twice a day using a hand sprayer. The seedling growth was monitored in each of the five treatments ($n = 5 \times 12$) and finally, healthy seedlings were moved to their respective treatment pits (7 May 2021) prepared in the open fields. No additional fertilizer was buried on the surface of pits prepared for Ridge gourd cultivation.

2.2.3. Field Cultivation Stage

The seedlings were raised for 20 days, and bamboo plant logs were used as creeping stems. The creeping stems were supported thoroughly using a trellis prepared by steel wire (1.5 mm) (Uttam Fencing, Vagmine Enterprise, India) and polyethylene terephthalate (PET) plastic wire (1.2 mm) (Source India Industries, Jaipur, India). The vines were allowed to creep until the full net was covered. The vines were sprayed using a high-pressure sprayer machine to avoid dust and pests, periodically. The experiments lasted for 90 days under field conditions where average temperature and humidity were noted as 29 °C and 55% using a digital thermo-hygrometer (ApTechDeals HTC-1, Delhi, India). The plants were watered periodically after three days using the normal borewell water supply. In this, the defected vine parts (yellowed leaves, flowers, fruits, etc.) were carefully removed from time to time.

2.3. Chemical and Analytical Assessment

The arable soil and SS used in this study were analyzed for selected physicochemical and metal element properties (Figure 1). The pH and electrical conductivity (dS/m) were estimated using ESICO 1611 (India) digital multimeter. The contents of organic matter (OM: %), nitrate-nitrogen (NO_3-N; g/kg), and phosphate phosphorus (PO_4^{3-}-P: g/kg) were estimated as per standard protocols [23]. OC was determined by digesting the 0.5 g samples in 1 N $K_2Cr_2O_7$ and H_2SO_4 for 1 h followed by the addition of H_3PO_4, NaF, diphenylamine indicator, and finally titrated against $Fe(NH_4(SO_4))_2$ solution. Similarly, NO_3-N was estimated by the pheno-di-sulphonic acid method while using $CuSO_4$ as an extraction reagent. The contents of PO_4^{3-}-P were estimated by treating the samples against 0.002 N H_2SO_4. Moreover, six metal elements (Cd, Cr, Cu, Fe, Mn, and Zn) were also determined by using an inductively coupled plasma optical emission spectroscopy (ICP-OES: 7300 DV, Perkin Elmer, Waltham, MA, USA) instrument. The contents of metal elements in AS, SS, and Ridge gourd fruits were analyzed after oven drying at 105 °C for 1 h, followed by the di-acid digestion (2:1; HNO_3-$HClO_4$) method. All reagents were of analytical grade and procured from Merck Ltd. (India).

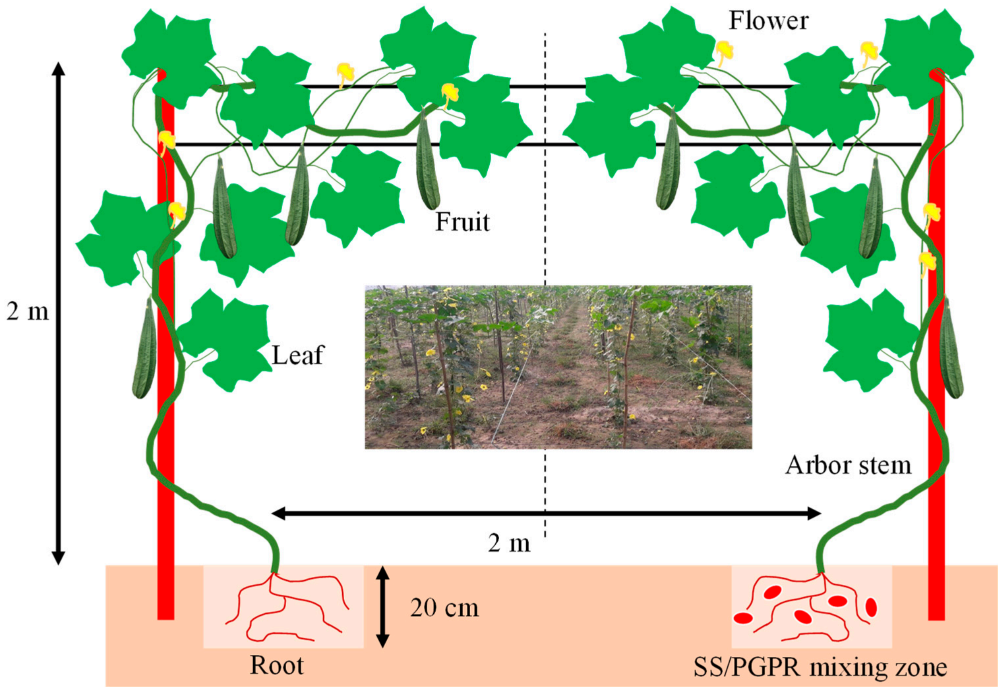

Figure 1. View of the experimental setup for Ridge gourd (*L. acutangula*) cultivation under sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) treatments.

2.4. Seedling Emergence, Biochemical Assay, and Productivity Assessment

The seedlings of Ridge gourd grown in germination bags were evaluated for selected morphological parameters such as seedling emergence (%), fresh biomass (g), growth rate (g/day), seedling length (cm), and root length (cm). Seedling emergence was calculated based on the percent germination out of total planted seeds. Fresh biomass was weighted using a digital scale (Samson HI-600K, Edapally, India) following official standardizing international nomenclature (SI: kg and g). The growth rate was expressed as a rate of biomass increase per day, while seedling and root lengths were measured using a calibrated scale (15 cm). Apart from the physical parameters, the Ridge gourd seedlings were also assessed for selected biochemical parameters such as superoxide dismutase (SOD: µg/g; Enzyme Commission Number (EC) 1.15.1.1), catalase (CAT: µg/g; EC 1.11.1.6), and total chlorophyll content (mg/g fresh weight basis: fwt.) as affected by the application of PGPR and SS. For the extraction, 1 g of biomass was mixed with 9 mL of deionized water followed by the addition of extraction solution and centrifuged at 4000 rpm for 15 min. The supernatant was further used for biochemical assay. SOD was estimated by using K-phosphate buffer solution as extraction reagent while taking the absorbance at 430 nm (UV-vis, Cary 60, Agilent Technologies, Santa Clara, CA, USA). CAT activity was estimated by using Na-hypochlorite solution and taking the absorbance at 240 nm [27]. Chlorophyll pigments in the seedling were estimated using 80% acetone extraction method and absorbance was taken at 645 and 663 nm. The chlorophyll contents (mg/g fwt.) were computed by using the given formula [23]:

$$\text{Chlorophyll } a = 12.7\ (A_{663}) - 2.69\ (A_{645})$$

$$\text{Chlorophyll } b = 22.9\ (A_{645}) - 4.68\ (A_{663})$$

$$\text{Total chlorophyll} = 20.2\ (A_{645}) + 8.02\ (A_{663})$$

The yield and productivity of Ridge gourd were monitored up to the first five harvests at an interval of each three days. The marketable Ridge gourd fruits of 2.5 cm diameter and tender dark green color were collected up to five harvests at an interval of 3 days and represented as effective crop yield.

2.5. Metal Element's Bioaccumulation and Health Risk Studies

The harvested Ridge gourd fruits under different PGPR and SS treatments were analyzed using the ICP-OES instrument to study the bioaccumulation of selected metal elements and their potential fate regarding the consumer's health. The bioaccumulation factor (BAF) index is widely used to study the migration of metal elements from the soil to the plant's aerial parts [28]. BAF of Ridge gourd cultivated on PGPR and SS treated soils was calculated using the following formula:

$$\text{BAF} = \text{Metal elements in fruit (mg/kg)}/\text{Metal elements in soil (mg/kg)}$$

Target Hazard Quotient (THQ) is a predictive tool that is used for the Health Risk Assessment (HRA) of hazardous elements in edible materials [29]. In the current study, the transfer of selected metal elements from SS-treated arable soils to Ridge gourd fruits was studied using the THQ tool to ensure the consumability of the crop [30]. The formula of THQ is given below:

$$\text{THQ} = 10^{-3} \times (\text{EE} \times \text{EA} \times \text{CF} \times \text{C})/(\text{BW} \times \text{ACP} \times \text{HRD})$$

where EE is the exposure efficiency (365 days/year), EA is the exposure age (70 years), CF is the consumption frequency (2.2 g/day), C is the concentration of metal element (mg/kg) in Ridge gourd fruit (fresh weight basis), BW is the average body weight of the vegetable consumer (70 kg for adult and 16 kg for child), and ACP is the average consumption period in days (25,550 days). In this, HRD is the metal elements reference doses of Cd, Cu, Cr, Fe, Mn, and Zn, viz., 5.0×10^{-4}, 4.2×10^{-2}, 3.0×10^{-3}, 7.0×10^{-1}, 1.4×10^{-2}, and 3.0×10^{-1} mg/kg/day, respectively [31]. Finally, HRI of metal elements intake through consumption of contaminated Ridge gourd fruit was simulated by using the following equation [32]:

$$\text{HRI} = \sum \text{THQ}\ (\text{Cd} + \text{Cr} + \text{Cu} + \text{Fe} + \text{Mn} + \text{Zn})$$

2.6. Software and Statistical Analysis

The data obtained in this study were analyzed using various software tools such as Microsoft Office Excel 2019, OriginPro (version 2021b), and SPSS (version 23). The data were subjected to various statistical tests such as one-way analysis of variance (ANOVA), coefficient of variance (CV), principal component analysis (PCA), and cluster analysis tests. The heatmaps were prepared using the "Clustered Heatmaps" addon of OriginPro (2021b) software package. The level of statistical significance for all tests was adjusted to Probability (p) < 0.05 (95% confidence interval).

3. Results and Discussion

3.1. Impact of SS on Arable Soil Properties

Table 1 provides the physicochemical, nutrient, and metal elements properties of AS, SS, and different treatment groups of SS such as 5, and 10%. The initial basic properties of AS and SS showed that SS was highly enriched with various organic and inorganic nutrients including metal elements. The pH (8.30 ± 0.05), EC (7.21 ± 0.12 dS/m), OM (23.90 ± 3.14%), NO_3-N (31.07 ± 1.380 g/kg), and PO_4-P (14.22 ± 1.10 g/kg) of SS was significantly ($p < 0.05$ higher as compared to AS which had pH of 7.28 ± 0.03 units, EC of 2.10 ± 0.08 dS/m, OM of 1.25 ± 0.03%, NO_3-N of 1.60 ± 0.10 g/kg, and PO_4-P of 1.25 ± 0.06 g/kg, respectively. The soil properties were also significantly ($p < 0.05$) changed after the SS mixing in both 5 and 10% treatment groups. Contrarily, the availability of all nutrients in AS was slowly increased with an increase in the SS mixing rate which signifies the usability of SS as an efficient nutrient supplement. PCA results showed that SS dose had a significant effect on increased nutrient availability (Table 2). The data was statistically transformed on two extracted components namely PC 1 and PC2 having eigenvalues of 3.12 and 0.84, while variances of 78.11 and 21.04%, respectively. The axial length of SS treatment in Figure 2a indicated the positive influence of the AS nutrient properties. Moreover, the analysis of soil physicochemical properties using the Euclidean distance-based neighboring cluster method revealed that metal elements possess more positive interaction (>0.5) with SS dose as compared to other soil nutrients (<0.5) parameters. The heatmap plot shown in Figure 2b indicates that Zn and Fe were closely related to each other in terms of their available concentration in the soil (mg/kg). Similarly, Mn showed close relation to Cu, while Cd with Cr. Overall, the amendment of SS on AS was helpful to increase its nutrient profile which can be a useful and low-cost fertilization alternative for Ridge gourd crop cultivation in the selected Indian region. Sewage sludge has been widely accepted for soil fertilization at the global level. Markowicz et al. [11] reported that a 15 t/ha rate of SS application was helpful to rehabilitate the degraded soils in Silesian Upland, Southern Poland. Marotrao et al. [33] also used SS (15 to 45 t/ha) for enhancing the soil mineral properties under the rice-wheat cultivation system. Another study by Marotrao et al. also used SS (15 to 45 t/ha) for enhancing the soil mineral properties under the rice-wheat cultivation They found that the soil metal elements under higher SS doses did not exceed the safe limits except for Cd. Thus, SS obtained from STP Jagjeetpur was rich in various nutrient parameters and can be recommended for AS fertilization, provided prior analysis of other toxic elements.

Table 1. Soil properties (mean ± standard deviation, n = 3) used for Ridge gourd (*L. acutangula*) cultivation under different sewage sludge and plant growth-promoting rhizobia (PGPR) treatments.

Parameter	Arable Soil	Sewage Sludge	Treatments	
			5% SS	10% SS
pH	7.28 ± 0.03	8.30 ± 0.05 *	7.35 ± 0.05 *	7.68 ± 0.02 *
EC (dS/m)	2.10 ± 0.08	7.21 ± 0.12 *	2.70 ± 0.20 *	3.16 ± 0.24 *
OM (%)	1.25 ± 0.03	23.90 ± 3.14 *	2.18 ± 0.09 *	2.70 ± 0.10 *
NO_3-N (g/kg)	1.60 ± 0.10	31.07 ± 1.38 *	2.06 ± 0.10 *	3.19 ± 0.15 *

Table 1. *Cont.*

Parameter	Arable Soil	Sewage Sludge	Treatments	
			5% SS	10% SS
PO_4^{3-}-P (g/kg)	1.25 ± 0.06	14.22 ± 1.10 *	2.10 ± 0.13 *	3.04 ± 0.09 *
Cd (mg/kg)	0.27 ± 0.01	2.71 ± 0.23 *	0.69 ± 0.04 *	0.94 ± 0.03 *
Cr (mg/kg)	3.28 ± 0.13	11.53 ± 0.46 *	4.42 ± 0.12 *	5.25 ± 0.08 *
Cu (mg/kg)	5.46 ± 0.21	50.24 ± 2.71 *	11.61 ± 0.10 *	16.33 ± 0.30 *
Fe (mg/kg)	14.11 ± 1.03	39.31 ± 0.99 *	20.72 ± 0.83 *	29.01 ± 2.11 *
Mn (mg/kg)	8.28 ± 0.51	29.01 ± 0.12 *	10.52 ± 0.48 *	13.43 ± 0.87 *
Zn (mg/kg)	4.15 ± 0.36	110.17 ± 10.55 *	16.44 ± 3.14 *	39.03 ± 2.03 *

*: Significantly different from the control group at probability (p) < 0.05.

Table 2. Principal component analysis (PCA) results of interaction between arable soil, sewage sludge, and metal elements content.

Parameters	Principal Components	
	PC 1	PC 2
pH	−0.18	1.00
EC	−1.32	0.08
OM	−1.22	−0.42
NO_3-N	−1.07	−0.53
PO_4^{3-}-P	−1.36	−0.22
Cd	−1.84	−0.15
Cr	−0.90	0.20
Cu	0.98	−0.22
Fe	3.04	1.53
Mn	0.74	0.72
Zn	3.14	−1.97
Eigenvalue	3.12	0.84
Variance (%)	78.11	21.04

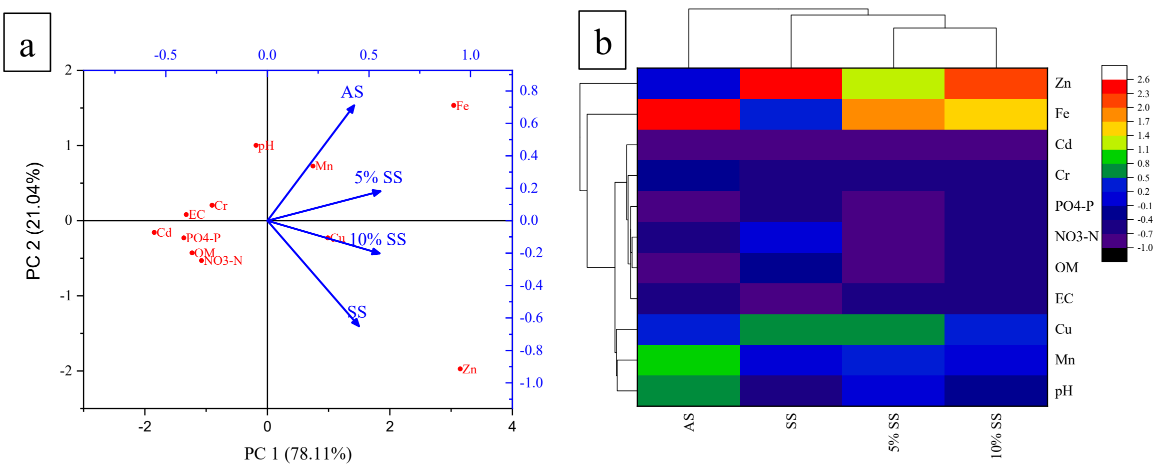

Figure 2. (**a**) PCA biplot and (**b**) clustered heatmap for the interaction of sewage sludge (SS) on soil nutrient availability.

3.2. Impact of PGPR and SS on Ridge Gourd Seedling Germination

The result depicted in Table 3 revealed that the germination of Ridge gourd seedlings was significantly ($p < 0.05$) affected by SS and PGPR treatments. The increasing order of selected parameters seedlings germination were recorded in an order of T1 < T2 < T3 < T4 < T5. In this, the highest seedling emergence was obtained using 10% SS + PGPR i.e., 92.3 ± 2.1%. Moreover, the highest seedling biomass, growth rate, seedling length, and root length were recorded as 9.6 ± 0.3 g, 1.4 ± 0.1 g/day, 15.5 ± 0.3 cm, and 10.4 ± 0.3 cm, respectively.

Figure 3 shows the effect of SS and PGPR on Ridge gourd seedling morphology. Plumular hook formation is largely associated with soil structure. This hook structure is favorable to seedlings for soil penetration as it protects the fragile apical growth point from injury. In the 10% and 10% SS + PGPR treatments, the soil structure was loosened so the Plumular hook formation was not seen in these treatments. However, it is possible that increased nutrient availability also affected the ethylene phytochrome formation in the Ridge gourd plant which might affect its growth and senescence. A thick hypocotyl might be due to the rapid growth rate of seedlings which differentiated it from other treatments and achieved more growth within a short period. Besides this, the SOD and CAT enzyme activities were maximally induced from 5 to 10% SS treatments i.e., 96.1 ± 5.3 and 85.2 ± 7.2 µg/g, respectively. However, it was slightly reduced while using PGPR in both 5 and 10% SS doses. On the other hand, the total chlorophyll content of Ridge gourd seedlings gradually increased with an increase in SS and PGPR dose. The maximum chlorophyll recorded was 3.2 ± 0.1 mg/g fwt. in 10% SS + PGPR treatment (Table 3). PCA results provided in Figure 4a, and Table 4 showed that the vector length of SS and PGPR treatments were positively correlated with all morphological and biochemical parameters of Ridge gourd. Also, the heatmap and cluster analysis confirmed relatively positive Euclidean distances in higher SS doses.

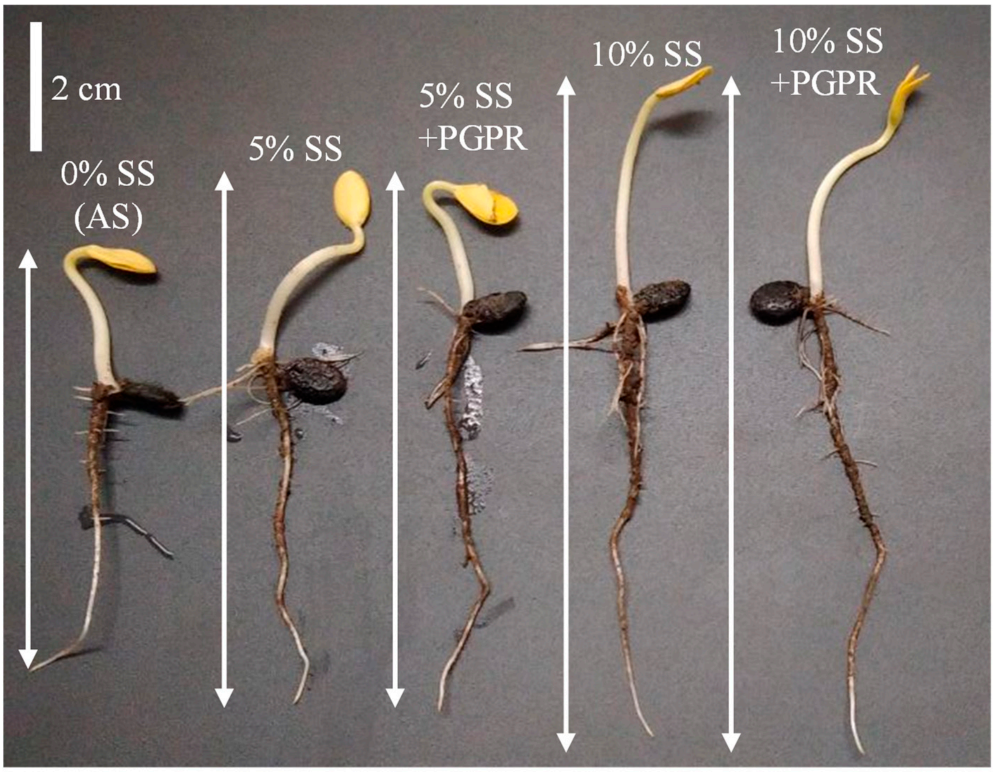

Figure 3. Effect of sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) amendment on seedling growth of Ridge gourd (*L. acutangula*).

It is evidenced that increased bio-availability of nutrients helps in the rapid germination of plants [34]. Seedlings rapidly respond to the easy nutrient availability and texture of soil [35]. In this experiment, the applied SS helped in altering the soil properties which might be beneficial for the Ridge gourd seedlings in terms of rapid adaptation and quicker growth. Similarly, PGPR supplementation assists in survival under stressed conditions such as excess nutrients, heat, cold, drought, pest, and pathogen attacks [36]. The increased nutrient availability as a result of SS mixing also affected the biochemical response of Ridge gourd seedlings which was evidenced from the SOD and CAT enzyme activities. However, PGPR supplementation in those treatments aided in minimizing the SOD and

CAT activities which might be due to amelioration of nutrient stress within the rhizosphere zone of Ridge gourd. SOD acts as a defense border for oxidative stress in plants caused by both physical and chemical factors while CAT acts as an important biomolecule that plays important roles in H_2O_2 scavenging, fatty acid oxidation, plant defense, aging, cellular deterioration, etc. [37]. The levels of SOD and CAT might increase under higher nutrient availability; however, their impacts can be minimized if beneficial microbes such as PGPR, vascular arbuscular mycorrhizae, etc. are inoculated within the rhizosphere region which creates secondary pathways for nutrient delivery through microbial catalysis [38,39].

Table 3. Effect (mean ± standard deviation, *n* = 3) of sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) treatment on germination and biochemical response of Ridge gourd (*L. acutangula*) seedlings (25 °C for 7 days).

Parameter	Treatments				
	Arable Soil	**5% SS**	**5% SS + PGPR**	**10% SS**	**10% SS + PGPR**
Seedling emergence (%)	70.3 ± 2.8	78.4 ± 1.5 *	81.9 ± 2.3 *	86.5 ± 3.5 *	92.3 ± 2.1 *
Seedling fresh biomass (g)	6.7 ± 0.1	7.5 ± 0.2 *	8.6 ± 0.3 *	9.1 ± 0.2 *	9.6 ± 0.3 *
Seedling growth rate (g/day)	1.0 ± 0.0	1.1 ± 0.0 *	1.2 ± 0.1 *	1.3 ± 0.1 *	1.4 ± 0.1 *
Seedling length (cm)	7.1 ± 0.1	11.3 ± 0.1 *	11.3 ± 0.1 *	15.1 ± 0.5 *	15.5 ± 0.3 *
Root length (cm)	4.6 ± 0.1	7.4 ± 0.1 *	8.2 ± 0.1 *	10.6 ± 0.3 *	10.4 ± 0.3 *
SOD (µg/g)	51.2 ± 4.3	74.1 ± 3.9 *	65.9 ± 2.8 *	96.1 ± 5.3 *	90.2 ± 6.2 *
CAT (µg/g)	24.3 ± 2.0	63.5 ± 7.0 *	60.0 ± 3.5 *	85.2 ± 7.2 *	78.1 ± 5.8 *
Total chlorophyll (mg/g)	2.4 ± 0.1	2.9 ± 0.1 *	3.2 ± 0.20 *	3.0 ± 0.2 *	3.2 ± 0.1 *

*: Significantly different from the control group at probability (p) < 0.05.

Table 4. Crop yield (mean ± standard deviation, *n* = 3) of Ridge gourd (*L. acutangula*) cultivation under different sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) treatments.

Parameter	Treatments				
	Arable Soil	**5% SS**	**5% SS + PGPR**	**10% SS**	**10% SS + PGPR**
Total crop yield (kg/plant)	8.1 ± 0.2	9.6 ± 0.3 *	10.6 ± 0.3 *	12.6 ± 0.1 *	13.8 ± 0.1 *
Average crop yield per harvest (kg/plant)	1.6 ± 0.1	1.9 ± 0.1 *	2.1 ± 0.1 *	2.5 ± 0.1 *	2.8 ± 0.1 *
CV (%)	9.5	14.3	14.9	3.8	5.1

*: Significantly different from the control group at probability (p) < 0.05; CV: coefficient of variation.

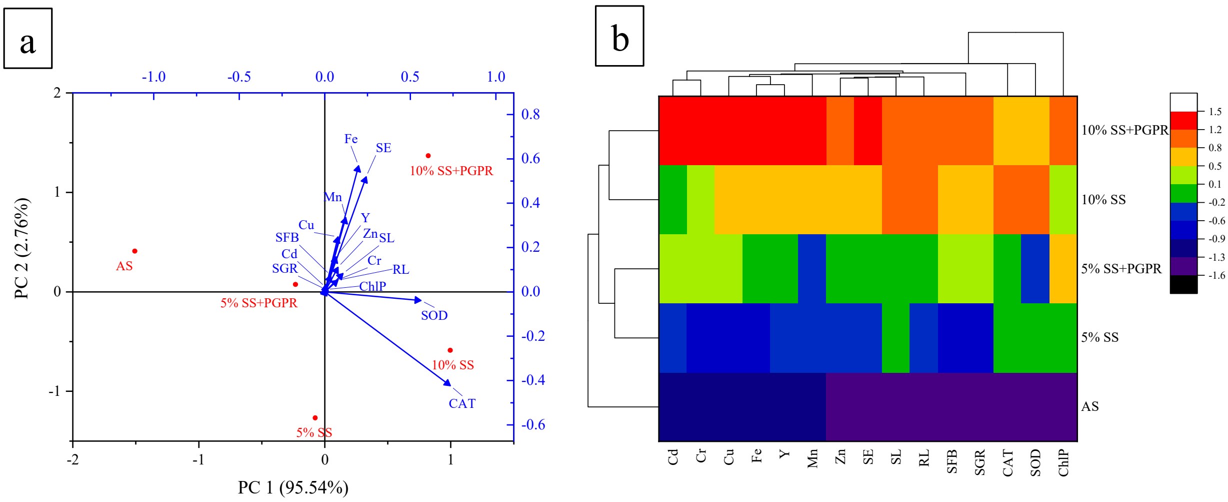

Figure 4. (**a**) PCA biplot and (**b**) clustered heatmap for the interaction of sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) on metal element contents and biochemical response of Ridge gourd (*L. acutangula*).

Previously, Cristina et al. [40] studied the impact of biogas digestate obtained from SS on the seed germination potential of garden grass (*Lepidium sativum*). Their findings suggested that the SS amendment was helpful to accelerate germination most efficiently at a 2.5% rate. Similarly, Silva et al. [41] also conducted laboratory pot experiments to study the biochemical effects of SS application on soybean (*Glycine max*). They reported that both SOD and CAT activities were positively correlated with the applied SS dose (0, 50, and 100%). The SOD and CAT activities reached the maximum in 100% SS i.e., 1.12 and 6.39 µmol/min/µg, respectively. Therefore, these results are in line with the findings of the current study, which recommends the sustainable use of SS and amelioration against oxidative stresses by the use of PGPR biofertilizers.

3.3. Impact of SS and PGPR on Ridge Gourd Crop Productivity

The fruits of Ridge gourd were harvested once they reached a maximum diameter of 2.5 cm and tender dark green. Table 4 shows the crop yield of Ridge gourd obtained in different SS and PGPR treatments. The crop yield in the first two harvests was typically lesser, which gradually reached the maximum in the 4th harvest and again decreased in the 5th harvest. The yield was significantly ($p < 0.05$) higher in SS and PGPR treatment groups as compared to normal AS treatment, where CV values were observed below 14.9%. Figure 5a shows the total crop yield (kg/plant), while Figure 5b shows the per harvest crop yield (kg/plant) in different SS and PGPR treatment groups. After five harvests, the maximum average crop yield per harvest (2.8 ± 0.1 kg/plant) total crop yield (13.8 ± 0.1 kg/plant) was attained using 10% SS + PGPR treatment. The crop yield typically depends on the availability of suitable soil texture, nutrients, and composition of beneficial microorganisms, and the minimal impact of intrinsic and extrinsic stresses [42]. The rooting of Ridge gourd is considered as one of the vigor associations between its plant and soil system. Generally, a perfect soil-root association promotes the increased growth of aerial plant tissues such as leaves, stems, flowering, and fruit numbers [12]. Mixing of SS helps in efficient mineralization of AS, which might be a reason behind increased Ridge gourd production.

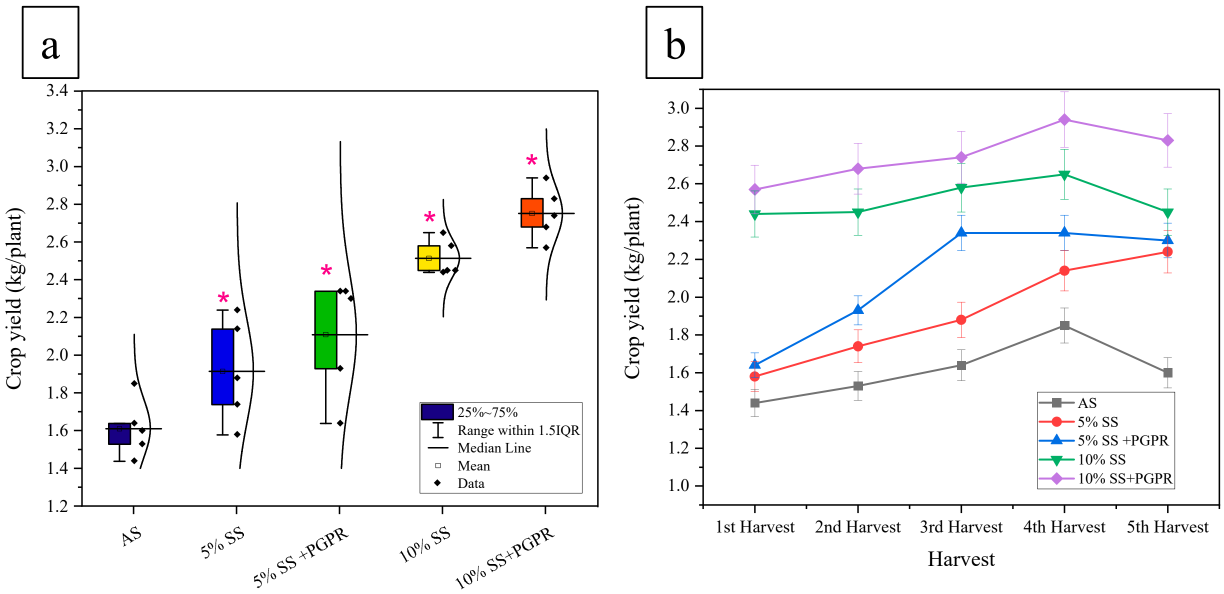

Figure 5. Effect of sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) amendment on (**a**) total and (**b**) per harvest crop yield of Ridge gourd (*L. acutangula*); * indicates significantly different from AS as control group.

Previously, no study was available on the use of SS for Ridge gourd cultivation; however, it was widely used for the cultivation of other crops. Muhammad et al. [43] studied the effect of sewage wastewater irrigation on the yield of Ridge gourd and found significant improvement in crop yield. Out of them, giant miscanthus cultivation was done by Dubis et al. [44] using varying doses of SS (equivalent to 100–160 kg/N/ha) and observed 32–34% higher yields. Uzinger et al. [45] conducted a study on the impact of combined use of biochar, composted SS, and PGPR for improved production of maize (*Zea mays*) crops. They reported that the nutrient profile of soil was significantly improved after biochar and SS application, while PGPR treatment was helpful inefficient nutrient delivery to maize plants which yield 2.7 times more crop yield.

3.4. Metal Elements Bioaccumulation and Health Risk Assessment

The metal elements analysis of Ridge gourd plant tissues using the ICP-OEM instrument is presented in Table 5. The mean metal elements content in the first five crop harvests significantly increased ($p < 0.05$) from 0 to 10% SS treatments. Furthermore, the PGPR amendment again improved the metal elements uptake as compared to normal SS treatments. The level of the selected metal elements was found to be in the order of Cd < Cr < Cu < Zn < Mn < Fe. The highest level of metal elements was determined in the root parts followed by the arbor stem and fruits. In particular, the maximum content of Cd, Cr, Cu, Fe, Mn, and Zn in fruit parts was observed as 0.61 ± 0.01, 1.18 ± 0.03, 9.23 ± 0.04, 24.10 ± 1.98, 12.33 ± 0.24, and 9.18 ± 0.67 mg/kg in 10% SS + PGPR treatment, respectively. Similarly, the Ridge gourd arbor parts had maximum Cd, Cr, Cu, Fe, Mn, and Zn contents of 0.78 ± 0.02, 1.57 ± 0.04, 9.81 ± 0.09, 27.81 ± 1.29, 12.70 ± 1.03, and 10.02 ± 0.20 mg/kg in the same treatment, respectively. Besides this, the maximum contents of Cd, Cr, Cu, Fe, Mn, and Zn were noted as 0.87 ± 0.01, 1.64 ± 0.02, 11.43 ± 0.37, 32.20 ± 2.39, 15.62 ± 1.04, and 10.45 ± 1.11 mg/kg, respectively. Overall, the mean content Cd, Cr, Cu, Fe, Mn, and Zn in the whole Ridge gourd plant was noted as 0.76 ± 0.04, 1.46 ± 0.02, 10.16 ± 1.38, 28.04 ± 3.98, 13.55 ± 0.54, and 9.88 ± 0.27 mg/kg, respectively. The higher levels of metal elements in root parts might be due to early exposure to them and the first route of elemental transfer to upper body parts of the Ridge gourd. The PCA biplot in Figure 4a and vector length in Table 6 also showed a positive relation between SS + PGPR doses and metal elements accumulation in Ridge gourd fruits. Moreover, cluster analysis using the Euclidean distance model showed that Cd and Cr; Cu and Fe; Zn and Mn had similar accumulative intensities based on their concentrations (Figure 4b).

Table 5. Contents of metal elements (mean ± standard deviation, $n = 3$) accumulated by different plant tissues of Ridge gourd (*L. acutangula*) grown under different sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) treatments.

Tissue	Treatments	Metal Element Content (mg/kg)					
		Cd	Cr	Cu	Fe	Mn	Zn
Fruit	AS	0.08 ± 0.00	0.40 ± 0.01	2.07 ± 0.06	6.76 ± 0.47	1.65 ± 0.02	2.61 ± 0.01
	5% SS	0.20 ± 0.01 *	0.52 ± 0.11 *	3.51 ± 0.02 *	10.18 ± 0.32 *	4.02 ± 0.15 *	5.52 ± 0.30 *
	5% SS + PGPR	0.34 ± 0.01 *	0.81 ± 0.05 *	5.99 ± 0.03 *	14.55 ± 1.08 *	4.65 ± 0.08 *	5.90 ± 0.35 *
	10% SS	0.33 ± 0.01 *	0.81 ± 0.04 *	7.01 ± 0.09 *	19.83 ± 2.20 *	9.46 ± 0.30 *	7.99 ± 0.29 *
	10% SS + PGPR	0.61 ± 0.01 *	1.18 ± 0.03 *	9.23 ± 0.04 *	24.10 ± 1.98 *	12.33 ± 0.24 *	9.18 ± 0.67 *
Arbor stem	AS	0.11 ± 0.00	0.43 ± 0.01 *	2.25 ± 0.02	7.07 ± 0.76	1.71 ± 0.07	4.18 ± 0.03
	5% SS	0.25 ± 0.01 *	0.84 ± 0.01	4.52 ± 0.11 *	11.33 ± 1.00 *	4.75 ± 0.18 *	5.77 ± 0.39 *
	5% SS + PGPR	0.42 ± 0.02 *	0.99 ± 0.02 *	6.49 ± 0.10 *	14.90 ± 1.75 *	5.66 ± 0.28 *	6.39 ± 0.55 *
	10% SS	0.34 ± 0.01 *	1.31 ± 0.02 *	7.71 ± 0.21 *	21.47 ± 2.12 *	10.05 ± 1.42 *	9.07 ± 0.37 *
	10% SS + PGPR	0.78 ± 0.02 *	1.57 ± 0.04 *	9.81 ± 0.09 *	27.81 ± 1.29 *	12.70 ± 1.03 *	10.02 ± 0.20 *

Table 5. *Cont.*

Tissue	Treatments	Metal Element Content (mg/kg)					
		Cd	Cr	Cu	Fe	Mn	Zn
Root	AS	0.28 ± 0.02	0.45 ± 0.01	2.42 ± 0.15	8.53 ± 0.29	2.20 ± 0.11	4.36 ± 0.02
	5% SS	0.46 ± 0.01 *	0.92 ± 0.01 *	6.90 ± 0.28 *	14.51 ± 0.73 *	5.73 ± 0.38 *	5.89 ± 0.08 *
	5% SS + PGPR	0.47 ± 0.02 *	1.11 ± 0.02 *	7.97 ± 0.08 *	17.31 ± 0.67 *	6.80 ± 0.43 *	7.43 ± 0.19 *
	10% SS	0.77 ± 0.01 *	1.36 ± 0.03 *	9.67 ± 0.22 *	26.41 ± 1.45 *	11.88 ± 1.18 *	9.75 ± 0.24 *
	10% SS + PGPR	0.87 ± 0.01 *	1.64 ± 0.02 *	11.43 ± 0.37 *	32.20 ± 2.39 *	15.62 ± 1.04 *	10.45 ± 1.11 *
Full plant	AS	0.16 ± 0.01	0.43 ± 0.03	2.25 ± 0.03	7.45 ± 0.92	1.85 ± 0.12	3.72 ± 0.01
	5% SS	0.30 ± 0.01 *	0.76 ± 0.05 *	4.98 ± 0.13 *	12.01 ± 1.01 *	4.83 ± 0.26 *	5.73 ± 0.09 *
	5% SS + PGPR	0.41 ± 0.03 *	0.97 ± 0.04 *	6.82 ± 0.35 *	15.59 ± 1.25 *	5.70 ± 0.18 *	6.57 ± 0.16 *
	10% SS	0.48 ± 0.05 *	1.16 ± 0.04 *	8.13 ± 1.30 *	22.57 ± 0.78 *	10.46 ± 0.69 *	8.94 ± 0.28 *
	10% SS + PGPR	0.76 ± 0.04 *	1.46 ± 0.02 *	10.16 ± 1.38 *	28.04 ± 3.98 *	13.55 ± 0.54 *	9.88 ± 0.27 *

*: Significantly different from the control group at probability (p) < 0.05; AS: arable soil as control.

Table 6. Principal component analysis (PCA) results of interaction between metal element accumulation and of Ridge gourd (*L. acutangula*) response grown under different sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) treatments.

Parameters	Principal Components	
	PC 1	PC 2
Cd	0.01	0.02
Cr	0.01	0.03
Cu	0.07	0.24
Fe	0.19	0.56
Mn	0.12	0.33
Zn	0.07	0.11
Crop yield (kg/plant)	0.06	0.15
Seedling emergence (%)	0.24	0.51
Seedling fresh biomass (g)	0.03	0.07
Seedling growth rate (g/day)	0.01	0.01
Seedling length (cm)	0.10	0.08
Root length (cm)	0.07	0.05
SOD (μg/g)	0.56	−0.03
CAT (μg/g)	0.73	−0.42
Total chlorophyll (mg/g)	0.01	0.01
Eigenvalue	1015.61	29.38
Variance (%)	95.54	2.75

The BAF studies showed that metal elements were significantly migrated from soil to edible parts (fruits) of Ridge gourd. The BAF values above 1 indicate a strong phytoaccumulation behavior of plants which might possess potential health risks to the consumer [46]. In this study, the BAF values did not exceed 1; however, it gradually increased from 5% SS treatment reported maximum in 10% SS + PGPR treatment (Figure 6a). More specifically, Mn showed the highest BAF value of 0.90, followed by Cu (0.80), and Cd (0.07). Besides this, the health risk assessment using THQ showed that HRI values in both adult and child groups did not exceed the values of 1. Nevertheless, SS and PGPR amendments showed a positive influence on HRI values. Comparatively, the child group (HRI < 0.40) was found to be more susceptible to contaminated Ridge gourd as compared to the adult human group (HRI < 0.10) (Figure 6b). Therefore, the HRI values above 1 indicate that consumption of contaminated crops may create a serious health hazard in humans [32].

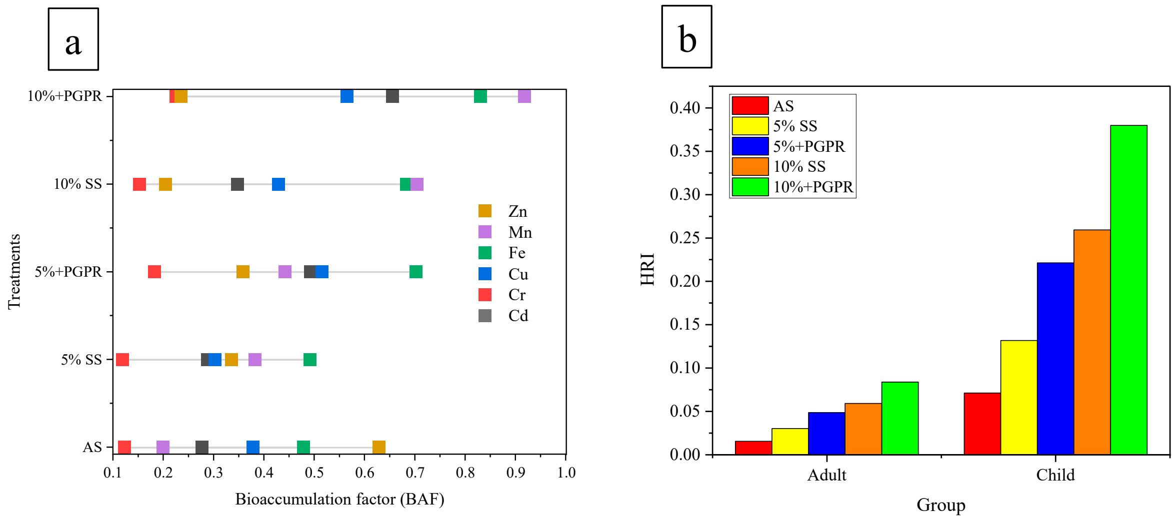

Figure 6. Effect of sewage sludge (SS) and plant growth-promoting rhizobia (PGPR) amendment on (**a**) metal element's bioaccumulation factor (BAF) and (**b**) health risk index (HRI) in Ridge gourd (*L. acutangula*) fruits.

Metal elements act as essential minerals in plant physiology and biochemistry, which assist in plant growth, metabolism pathways, and survival against oxidative stresses [47]. Some toxic metal elements might get accumulated within the plant cells as a substitute for other elements, and create toxicity equilibrium within the rhizosphere region [48]. In this process, metal elements availability will induce the plant to uptake them through various mechanisms. Moreover, the presence of beneficial microbes can also improve the uptake of these elements through secondary pathways such as microbial fixation [49]. In this study, the SS mixing increased the availability of metal elements in soil, which was further taken by Ridge gourd plants. Moreover, further inoculation of PGPR also improved the elemental uptake. Since the BAF and HRI values did not exceed the permissible limits, the harvested Ridge gourd crop can be safely recommended for marketable and edible purposes.

To date, no study is available on Ridge gourd cultivation on SS and PGPR amended soils. However, a study on Ridge gourd cultivation by Khan et al. [50] showed that the levels of twelve metal elements (Cr, Mn, Ni, Cd, Co, Cu, Pb, Zn, Fe, Se, As, and Mo) were significantly increased after domestic wastewater irrigation on cultivated soils. Substantial values of HRI were reported for As, Mo, Mn, and Pb, which could pertain to health risks in humans. Similarly, Khan et al. [51] determined the HRI of metal elements present in the Ridge gourd crop cultivated on different irrigation regimes. The HRI and pollution index levels in consumable parts of Ridge gourd were considerably high, indicating potential health risk.

4. Conclusions

The combined use of SS and PGPR was helpful to increase the seedling success, biochemical response, and crop yield of Ridge gourd. The most efficient growth and productivity of Ridge gourd were obtained using 10% SS and PGPR inoculation. The bioaccumulation and health risk studies also confirmed that the contents of selected metal elements (Cd, Cr, Cu, Fe, Mn, and Zn) were within the acceptable limits i.e., BAF and HRI < 1, signifying the safe consumption of the harvested Ridge gourd. Therefore, the present study suggests the combined use of SS and PGPR for improved vegetable production and efficient nutrient recycling, along with the generation of imperative agro-economy. Further studies on soil-microbe-plant interactions under combined SS and PGPR application along with the monitoring of other toxic metal elements are strongly recommended.

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