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Effect of Green Waste Compost Soil on the Physicochemical and Biological Properties of Soil

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ABSTRACT: Numerous studies have demonstrated the potential of composted organic wastes not only as substitutes for peat as a growth substrate but also to stimulate plant growth and suppress soil-borne diseases. The major impediment to the use of green waste compost as substrates or biocontrol agents has been variation in physical and chemical characteristics of the soil. Therefore, in the present study leaves of five plants that are *Prosopis cineraria*, *Azadirachta indica*, *Albizia lebbek*, *Cassia fistula*, and *Holoptelea integrifolia* were used as green compost in equal ratio. The physicochemical and biological properties of compost soil with comparison to the untreated soil. The results of physical properties such as porosity, density, water holding capacity and chemical properties such as pH, moisture, organic matter, N, P, K and C:N characteristics of compost soil was highest as compared to the untreated soil. In biological analysis, two *Pseudomonas* species (*P. aeruginosa* and *P. viridiflava*) from untreated soil and three *Pseudomonas* species (*P. aeruginosa*, *P. syringae* and *P. viridiflava*) from compost soil were observed. The results have concluded that due to the application of green waste in agriculture land, the physicochemical and biochemical properties were boosted extremely which in turn is a good thing for the plant quality in terms of nutrition and yield.

KEYWORDS: Green waste, Compost soil, Physico-chemical properties, Biological properties

I. INTRODUCTION

Agriculture is of vital importance for the development of human societies and has an enormous impact in the soil functional system (Uusitalo *et al.*, 2019), since almost 40% of the total land area is used for food production through agriculture (Foley *et al.*, 2011). Currently, organic agriculture has been revived and represents a growing proportion in the world economy (Nain *et al.*, 2009), since the population seeks to obtain products which are “healthier” and free of chemicals harmful for humans and the environment (Lans, 2016). Conventional agriculture is characterized by the usage of chemicals during its production (Fan *et al.*, 2020) and side effects of chemicals on agricultural land and plant health. On the contrary, organic agriculture is based on the effective natural management of plant-soil nutrition cycling (Hellequin *et al.*, 2018) and makes use of natural organic compounds such as manure, compost and homemade fertilizers from animal waste such as chicken manure to compensate for nutrient deficiencies (Fan *et al.*, 2020).

Organic farming systems rely on the management of soil organic matter to enhance the chemical, biological, and physical properties of the soil, in order to optimize crop production. Soil management controls the supply of nutrients to crops, and subsequently to livestock and humans. Furthermore, soil processes play a key role in suppressing weeds, pests and diseases (Watson *et al.*, 2006).

Therefore, in the present study five plant leaves (*Prosopis cineraria*, *Azadirachta indica*, *Albizia lebbek*, *Cassia fistula*, and *Holoptelea integrifolia*) were used as green waste to prepare compost soil and identify physicochemical and biological properties with comparison to the normal soil.

II. MATERIAL AND METHODS

A. Collection Of Soil Sample

The suitable soil samples and green waste of five plants leaves including *Prosopis cineraria*, *Azadirachta indica*, *Albizia lebbek*, *Cassia fistula*, and *Holoptelea integrifolia* to prepare compost soil were collected from the various

farmer's field of different district of Rajasthan. Standard procedure was used to prepare compost soil (Hopkins *et al.*, 2009).

B. Physical characteristics

a) Color (Munsell color chart method)

Color is a qualitative characteristic of soil. The color of the compost soil was compared with untreated soil (Islam *et al.*, 2016). The color of both soils was independently measured in the laboratory under controlled lighting conditions, using Munsell soil color charts.

b) Particle size determination

ASTM D 422 - Standard Test Method was used for particle-size analysis of soils. The weight of each sieve, dried soil samples as well as the bottom pan was measured. All the sieves should be clean and assemble them in the ascending order of sieve numbers (4 sieves at top and 200 sieves at bottom). Place the pan below 200 sieves. Carefully pour the soil sample into the top sieve and place the cap over it. The sieve stack was placed in the mechanical shaker and shake for 10 minutes. Removed the stack from the shaker and carefully weighted and recorded the weight of each sieve with its retained soil. In addition, remember to weigh and recorded the weight of the bottom pan with its retained fine soil.

Data Analysis

Obtain the mass of soil retained on each sieve by subtracting the weight of the empty sieve from the mass of the sieve + retained soil, and record this mass as the weight retained on the data sheet. The sum of these retained masses should be approximately equals the initial mass of the soil sample. Calculate the percent retained on each sieve by dividing the weight retained on each sieve by the original sample mass. Calculated the percent passing (or percent finer) by starting with 100 percent and subtracting the percent retained on each sieve as a cumulative procedure.

For the No.4 sieve:

Quantity passing = Total mass - Mass retained

The percent retained is calculated as;

% retained = Mass retained/Total mass

From this, the % passing = 100 - % retained

For the No. 10 sieve:

Quantity passing = Mass arriving - Mass retained

% retained = (Mass retained /Total mass) X 100

% Passing = 100 - % retained (No. 4 sieve)-% retained (No. 10 sieve)

c) Bulk Density

Filled a pre-weighed 100 ml graduated cylinder with air-dry soil. The soil was compacted in the cylinder by tapping the cylinder firmly 15 times on the palm of the hand. The volume of the packed soil in the cylinder was recorded. The weight of the cylinder and the soil was recorded. On a separate sample, determined the moisture content of the soil sample and calculated the oven-dry weight of the soil in the cylinder. Calculated bulk density by using following formula:

$$\text{Bulk Density (g/cm}^3\text{)} = \frac{\text{Weight of oven-dry soil (g)}}{\text{Volume of soil sample (cm}^3\text{)}}$$

C. Chemical characteristics

a) pH

20 gm of dried soils weighed individually into a beaker. 50 ml of distilled water was weighted and stirred with a glass rod thoroughly for about 5 minutes and keep for half an hour. Standardize the glass electrode using standard buffer of

pH = 7 and calibrate with the buffer pH = 4 or pH = 9.2. The electrodes dipped in the beakers containing the soil water suspension with constant stirring then wait 30 seconds and record the pH value (Singh *et al.*, 1999).

b) Moisture content

Gravimetric method of Black, 1965 was used to calculate moisture content of soil samples. Weight of aluminum tin was recorded. Place a soil sample of about 10g in the tin and record this weight as wet soil. The sample kept in the oven 105°C, and dried for 24 hours. Weigh the sample, and record this weight as weight of dry soil. Again the sample was oven dried for several hours, and determine the weight of dry soil. Repeat step until there was no difference between any two consecutive measurements of the weight of dry soil. The moisture content in dry weight basis was calculated using the following formula:

$$\theta_d (\text{weight of water}) = (\text{weight of wet soil}) - (\text{weight of dry soil}) / (\text{weight of dry soil})$$

c) Water Holding capacity

Heining (1963) method was used to calculate water holding capacity. Galvanized metal cylinders with a volume of 195cm³ were used. The base of each cylinder was covered with fine nylon fabric (3 threads/mm) and secured with a rubber band. The cylinders were filled with soil samples, individually and placed in a cold-water bath lined with capillary matting for 24h, by which time the samples were saturated. The cylinders were then placed on the sand surface in a sand tower for 24h. Then, still on the sand surface, a pressure of 50cm suction (equivalent to field capacity) was applied for 72h. Samples were weighed, dried at 103°C for 48h and then re-weighed. This weight denoted as field capacity. On the other hand, a known volume of fresh soil samples was weighed individually and soaked in distilled water overnight then placed in the apparatus and 220lb/in² pressure was applied for 14 days. The compost was weighed, dried at 103°C for 48h and re-weighed. This weight denoted as wilting point. The water holding capacity was measured using following formula:

$$\text{Water holding capacity} = \text{field capacity} - \text{wilting point}$$

d) Water soluble nutrients

1. Total Nitrogen (Kjeldahl, 1883)

10 g of soil weighed and transferred into an 800 ml Kjeldahl flask. Added 50 ml of sulphuric-salicylic acid mixture to the flask and swirled it to bring the sample quickly into intimate contact then kept it to stand overnight. Added 5 g of sodium thiosulphate gave heat gently for about 5 minutes with taking care to avoid frothing. When the flask gets Cool, 10 g of the sulphate mixture added and digest on the Kjeldahl apparatus gradually raising the temperature until the digest becomes clear. Further it was digested at full heat. Again, after cooling the flask, added 300 ml of distilled water and mixed. Slowly added 100 ml of concentrated sodium hydroxide by letting it run down the neck and settle in the bottom of the flask. Added a large piece of mossy zinc and a spoon of glass beads, connect the flask to the distillation unit, shake by swirling, turn on heat, and distil 150 ml into an Erlenmeyer flask containing 50 ml of 4 percent boric acid solution then added 10 drops of bromocresol green-methyl red indicator and titrate with the 0.05 M standard sulphuric acid solution to the first faint pink. Titrated a blank prepared in the same manner but without adding a soil sample. Following formula was used to calculate Kjeldahl N in percentage:

$$\text{Kjeldahl N (\%)} = (T-B) \times M \times 2.8/S$$

Here, T is ml of standard acid with sample titration, B is ml of standard acid with blank titration, M is molarity of sulphuric acid and S is weight of soil sample in gm.

2. Available Phosphorous (Olsen's method; Drewry *et al.*, 2013)

5 gm soil sample weighed in 250 ml Erlenmeyer flask then added 100 ml of NaHCO₃ extracting solution to it. The flasks shake on a mechanical shaker for 30 minutes. The suspension filtered out through Whatman No. 40 filter paper. The suspensions shake by hand immediately before pouring it into the funnel and discarded the first 5 to 10 ml of filtrate if turbid. Transferred of 10.0 ml of filtrate to a 50 ml volumetric flask and acidify to pH 5 by adding 1.0ml of

2.5M H₂SO₄. Swirling has to be done carefully in the beginning, then vigorously to remove residual carbonates. Volume made up to 40 ml with distilled water; added 8 ml of the ammonium molybdate ascorbic acid solution and again the volume made up to 50 ml, mixed it well, and kept it stand for 10 minutes. The absorbance was read at 882 nm on the spectrophotometer. The color was stable for 24 hours and maximum intensity was obtained in 10 minutes. Determination of P concentration of the sample from a calibration curve relating the readings of absorption units to concentration in µg P/ml. For preparing standard, 0-20 ml P stock solution (5 µg P/ml) was used. Then 10 ml of the NaHCO₃ extracting solution, 1 ml of 2.5 M H₂SO₄ was added and developed color as described above. Phosphorous was calculated by using following formula:

$$P (\mu\text{g/g}) = P \mu\text{g/ml} \times 50\text{ml}/10 \text{ ml} \times 100\text{ml}/5\text{g soil}$$

3. Available Potassium (Knudsen *et al.*, 1982)

Accurately 5 gm of soil weighed and transferred into a 50 ml centrifuge tube. Added 20 ml of 1.0 M ammonium acetate solution to it and shake in a reciprocal shaker for 5 minutes. Centrifuge the tubes at 2000 rpm for 5 minutes or until the supernatant was clear. Decant the supernatant into a 100 ml volumetric flask. Steps repeated for three more times. Volume of the supernatant made up to 100 ml by adding ammonium acetate solution. Prepared a series of working K standard solutions in the range of 0 – 2 mmol/l of K from stock solution of 0.02 M KCl already prepared. For better results added LiCl in each standard to yield a final concentration of about 5 mmol/l of LiCl. Determination of K concentration in the extract is done by flame photometer.

$$\text{mmol of K}/100 \text{ g soil} = \text{Reading (mmol/l)} \times 100\text{ml}/1000\text{ml} \times 100\text{g/ wt. of soil (g)} = R \times 10/\text{wt. of soil (g)}$$

4. Organic matter content (Walkley and Black, 1934)

2 gm dried soil samples individually and transferred it to a 500 ml Erlenmeyer flask. Added 10 ml of 0.167 M K₂Cr₂O₇ by means of a pipette and 20 ml of concentrated H₂SO₄ by means of dispenser and swirled it gently to mix. Placed the flasks on an insulation pad and let it stand 30 minutes. Diluted the suspension with about 200 ml of water to provide a clearer suspension for viewing the endpoint then added 10 ml of 85% H₃PO₄, using a suitable dispenser, and 0.2 gm of NaF, using the “calibrated spatula” technique. The H₃PO₄ and NaF were added to complex Fe³⁺ which would interfere with the titration endpoint. 10 drops of ferroin indicator added then titrated the solution.

Titration

Titrate with 0.5 M Fe²⁺ to a burgundy endpoint. The color of the solution at the beginning was yellow-orange to dark green that depends on the amount of unreacted Cr₂O₇²⁻ remaining, which shifts to a turbid gray before the endpoint and then changes sharply to a wine red at the endpoint. Used of a magnetic stirrer with an incandescent light makes the endpoint easier to see in the turbid system (fluorescent lighting gives a different endpoint color). Alternatively, can be used a Pt electrode to determine the endpoint after titration. This will eliminate uncertainty in determining the endpoint by color change. Run a reagent blank following the above procedure without soil. The reagent blank is used to standardize the Fe²⁺ solution daily. Calculation was done by using following formula:

$$\% \text{ C (oxidizable organic C)} = (\text{B}-\text{S}) \times \text{M of Fe}^{2+} \times 12 \times 100/ \text{grams of soil} \times 4000$$

$$\% \text{ OM (organic matter)} = \% \text{ C} / 0.58 = \% \text{ C} \times 1.72$$

Here, B is ml of Fe²⁺ solution used to titrate blank, S is ml of Fe²⁺ solution used to titrate sample, and $12/4,000$ = milliequivalent weight of C in grams.

To convert easily oxidizable organic C to total C, divide by 0.77 (or multiply by 1.30)

D. Biological characteristics

a) Isolation of Bacteria from Samples

Nutrient agar (NA) media was prepared and both soil samples were spread on NA plate individually in triplicate form and kept the plates for incubation at 37°C for 24hrs. After incubation, some different types of bacterial colonies were

observed. These various bacterial colonies were pick out very carefully from each sample plate and sub-cultured on fresh NA plates. All sub-cultured plates were again incubated at 37°C for 24hrs to get individual bacterial colonies. These isolated bacterial pathogens were observed on the basis of morphological and biochemical characterizations.

b) Morphological Characterization

The isolated bacterial pathogens were initially identified by morphological studies (Gram's staining method and KOH test). Morphological studies were done based on color, shape, and opacity.

1. Gram's staining

A thin smear of isolated bacterial culture was prepared on a slide and then gram staining was performed by following Hildebrand and Scroth, 1972 method. The slide was air-dried and fixed it using heat. The bacterial smear was flooded with crystal violet and left for 1minute. The slide was washed properly with deionized water. The slide was flooded with gram's iodine solution and allowed to stand for 30 s, thereafter the slide was washed completely with deionized water. For decolorization, the slide was flooded with 95% ethyl alcohol and washed with distilled water. Further, the smear was covered with safranin for 20 seconds and washed with distilled water. In the end, air-dried the slide was, and then observation was done under a compound microscope (10-40X). Gram-positive bacteria show blue color and gram-negative bacteria show red color under a compound microscope.

2. KOH test

Gram-positive and negative bacteria can also differentiate by the KOH procedure, as described by Chandra and Mani, 2011. More inoculum of isolated bacterial culture was added into a drop of 3% aqueous solution of KOH on a cleaned glass slide and mixed properly by inoculation loop. The glass slide was cleaned with 70% ethanol. It was stirred completely up to approximately 1 minute and then the loop was lifted slowly to observe the string. The string was seen within the first 30 seconds after mixing in the KOH solution. Gram-positive bacteria are unable to change the viscosity of the potassium hydroxide solution and gram-negative bacteria convert potassium hydroxide solution into mucoid in appearance.

c) Biochemical Characterization

1. Catalase test

The catalase activity in isolated bacteria was identified by using Facklam and Elliott, 1995. NA slant was inoculated with isolated bacterial pathogens. Thereafter the tubes were incubated at 37°C for 24 h. After incubation 3-4 drops of 3% hydrogen peroxide were added to the growth of each slant. The culture was observed for the presence or absence of gas bubbles. Catalase positive produced copious bubbles with bacterial culture and negative has a lack of bubbling.

2. Oxidase test

Modified Kovac's, 1956 procedure was used to detect cytochrome c oxidase potential in bacterial isolates. Took 3 pieces of filter paper for each pathogenic bacterium and labeled them properly. Each isolated culture was rubbed by a sterile inoculation loop on the filter paper moist with Kovács oxidase reagent. Thereafter filter paper was observed to check the color of the smear after 15 to 30 seconds of rubbing. Oxidase-positive bacteria show dark blue color within 5 to 10 seconds and oxidase-negative bacteria have no change in color.

3. Indole production test

Indole test was done by using a modified MacFaddin, 2000 method. 1% tryptone broth was prepared and sterilized by using an autoclave. The tryptone broth tubes were inoculated individually with each isolated bacteria and incubated at 37°C for 48h. 5 drops of Kovac's reagent were added into each tube after 48h of incubation. The tubes were shaken gently after 10-minute intervals. Thereafter the tubes were left for a few minutes to prepare a layer of

reagent on the top. The observation was done based on color. Indole positive bacteria form a cherry red-colored ring on the surface of the media and indole negative bacteria forms a yellow ring.

4. Citrate utilization test

Jawetz *et al.*, 1989 method with some modification was used to citrate utilization test. Simon's citrate agar media was prepared and sterilized by using an autoclave machine at 121°C temperature, and 15 lbs pressure for tentatively 15 minutes, and also sterilized required glasswares. Slants were prepared by pouring 5ml of media into each tube. After slants preparation, each tube was inoculated with isolated bacterial culture and then incubated for 48hrs. at 37°C. At the end of the incubation period based on growth and color, the tubes were observed. In citrate positive result, the color of media changes from green to bluish, and in negative result color remains the same as the control.

5. Cetramide test

A modified method according to Brown and Lowbury (1965) was used to observe cetramide positive and negative results. Cetrimide Agar Media was prepared and heated to mix the medium properly. After proper dissolving, media was autoclaved for sterilization. 5 ml sterilized media was carefully poured into each tube followed by prepared slants. Thereafter each tube was inoculated with isolated bacterial culture, followed by incubation for 48h at 37°C. After 48 h of incubation, all tubes were properly observed based on color and growth. The dark blue-green color of bacterial growth was observed as cetrimide positive bacteria and no growth was denoted as cetrimide negative bacteria.

Confirmatory test

6. Gelatin hydrolysis

Gelatin hydrolysis tests were done by using the modified Lelliott and Stead, 1987 method. Gelatin media was prepared and autoclaved at 15 lbs pressure (121°C) for 15mins. Performed stab inoculation using an inoculating loop, from each isolated bacterial culture into labeled test tubes. All inoculated and un-inoculated (control) tubes were incubated at 37°C for 4 to 7 days. After incubation, place the tubes in the refrigerator at 4°C at least for 30 min. or until the control tube solidifies. The inoculated tubes were observed for liquefaction. The liquefied media showed positive results and solidified media showed negative results.

7. Arginine Dihydrolase Broth test

Modified Lelliott and Stead, 1987 was used for the arginine dihydrolase broth test. Arginine Dihydrolase Broth medium was prepared and heated if necessary to dissolve the medium completely. 5ml of broth was distributed into each tube. Thereafter all tubes were sterilized by autoclaving at 15 lbs pressure (121°C) for 15mins. All tubes were allowed to cool in an upright position. Then each tube was inoculated with isolated bacterial culture and incubated for 48hat 37°C. At the last, the tubes were observed based on growth and color. Control has appeared in purple clear to slightly opalescent gel forms in tubes as butts. Purple-colored converted into light yellow color referred to as positive result and no change in color noted as negative results.

8. Carbohydrate fermentation test

Modify Hemraj *et al.*, 2013 method was performed to identify carbohydrate fermentation activity in isolated bacterial culture. 16.1 grams above reagents dissolved in 990 ml distilled water and heated for properly dissolving all reagents in distilled water. This prepared broth was autoclaved (121°C, 15 lbs pressure for 15 minutes) for sterilization. Thereafter aseptically added 10 ml separately sterilized carbohydrate (glucose) solution to give a final concentration of 0.5% and mixed completely. Each tube containing a specific carbohydrate broth medium was labeled and inoculated with isolated bacterial culture and incubated at 37°C for 48h. All sample tubes then were compared with control based on color change from purple color (transparent) to yellow color (translucent) [due to production of acid]. The purple color of the broth medium changed into a yellow color that indicated acid production by isolates due to fermentation of glucose referred to as positive bacteria. No change in color represented negative bacteria.

III. RESULT AND DISCUSSION

A. Physical Characteristics

Physical properties of compost soil and untreated soil was evaluated in which soil having four different physical properties where porosity is that part of the bulk volume of soil that is not occupied by either mineral or organic matter but is open space occupied by either gases or water. Table 1 shows different physical properties of untreated soil and compost soil. Soil color is largely determined by the organic matter content, drainage conditions, degree of oxidation, and in some cases, presence of specific minerals. The color of untreated soil of Mandawar was brown and both in Behror and in Thanagazi soil dark brown color were found. However, the color of the soil changed when green waste was added to it for composting. The compost soil color of Mandawar soil was dark brown, pale brown color of Behror soil and Thanagazi having brown color soil.

The soil porosity of Mandawar soil was 45.7%, 48.66% was in Behror soil and Thanagazi soil found 45.39% soil porosity whereas the compost soil of Mandawar, Behror and Thanagazi soil porosity was found 52.91%, 61.8% and having 50.3%. In the study of **Ahn et al., 2008** the soil porosity values ranged from 60.69 to 72.47% for different compost types. The lowest value of the porosity (60.69%) was found for cattle manure compost and the highest value of the porosity (72.47%) was found for sugar cane plants residues compost. The porosity depends on bulk density and moisture content of compost. The porosity of compost decreased from 72.47 to 60.69% when the bulk density increased from 420 to 655 kg/m³ and moisture content increased from 25.6 to 32.1%. **Wallace et al., 2019** aimed to determine if the incorporation of municipal compost (MC) can increase the quantity (total volume) and concentration (total volume per unit volume) of soil pores that hold readily available water (defined as macro-mesopores of 30–3 μm diameter) and plant available water (defined as mesopores of 30–0.3 μm diameter). The MC was screened to three different maximum particle sizes – MC4 (<4 mm), MC2 (<2 mm) and MC0.25 (<0.25 mm) – and incorporated into repacked soil cores at five different rates: 0, 5, 25, 50 and 80% wt/wt. Incorporation of MC0.25 increased the concentration and quantity of macro-mesopores and mesopores at significantly lower application rates than MC4 and MC2.

Another property was soil density in which Mandawar untreated soil density was 198 kg/m³, 210 kg/m³ Behror soil density and Thanagazi soil having 173 kg/m³ soil density. In case of compost soil, maximum soil density was found in Behror compost soil of 270 kg/m³ followed by Mandawar with a density of 234 kg/m³, and Thanagazi soil density being 218 kg/m³. Likewise, **Yadav et al., 2020** conducted a field experiment at Instructional Farm, Rajasthan College of Agriculture, Udaipur during consecutive kharif season of the year 2016 and 2017 and the result revealed that nutrient management practices decreased bulk density in soil during both the individual years and in pooled data for two years. The minimum bulk density (1.29 Mg m⁻³) at 0–15 cm depth were recorded under the 100% vermicompost. In the study conducted by **Somerville et al., 2018** at three sites in Melbourne, Australia, three different soil types (two different loamy coarse sands and a coarse sandy loam) were compared with a sludge compost application of 50% v/v. All three soils had a reduced bulk density at both 3 months (15–26% reduced) and 15 months (14–25% reduced) post compost application. In another study at northern California, **Curtis and Claassen, 2009** found that the bulk densities of four soils having different parent materials were reduced by 19–21% from a compacted control with the incorporation of 540 mg ha⁻¹ of yard waste compost to 50 cm deep in the soil profile.

The water holding capacity of untreated soil low as compared to the compost soil. Mandawar, Behror and Thanagazi untreated soil had 46.28% capacity, 49.85% and 41.92% whereas the compost soil had a water holding capacity of 48.09%, 52.1%, and 45.8%, respectively. **Rehman et al., 2020** produced biochar from animal manure through the pyrolysis process at different temperatures, and studied its effect on the water-holding capacity of soil. The water-holding capacity of sandy loamy soils was found to be increased by 1.5% by mass for each 1% biochar addition in the soil. **Schmid et al., 2017** had the highest reported compost rate (6.04 cm) and the deepest incorporation depth (30 cm) that produced a significant change in water retention. The shallowest incorporation depth was to yield a significant change in water retention which was 5–10 cm. **Bouajila and Sanaa, 2011** reported that organic amendments allowed better water infiltration. Their result showed that the application of 120 t/ha household wastes compost and manure improved water infiltration (549.25 and 596.46 cm, respectively) when compared with control (332.16 cm).

Table 1: Physical characteristics of untreated soil and compost soil

Physical properties	Mandawar		Behror		Thanagazi	
	Untreated soil	Compost soil	Untreated soil	Compost soil	Untreated soil	Compost soil
Color	Brown	Dark brown	Dark brown	Pale brown	Dark brown	Brown
Porosity (%)	45.7	52.91	48.66	61.8	45.39	50.3
Density (kg/m³)	198	234	210	270	173	218
Water holding capacity (%)	46.28	48.09	49.85	52.1	41.92	45.8

B. Chemical Characteristics

Chemical analysis of soil and compost soil such as pH, moisture, organic matter content, water soluble nutrients and C:N content were determined and are defined in Table 2. The pH of Mandawar, Behror and Thanagazi soil was 7.7, 7.8 and 7.49, respectively. Moisture content in Mandawar soil was 32%, 28% in Behror soil and in Thanagazi soil it was found to be 34%. Organic carbon content observed in Mandawar, Behror and Thanagazi soil was 0.43, 0.51% and 0.20%. Water soluble nutrients like N, P and K, in Mandawar, Behror and Thanagazi soil was estimated in which N content was 114.1 kg/ha, 118.73 kg/ha and 120.9 kg/ha, P content in was 31 kg/ha, 34.6 kg/ha and 39.5 kg/ha, whereas K content found was 265.33 kg/ha, 194.33 kg/ha and 231.2 kg/ha. The C: N content of Mandawar, Behror and Thanagazi soil was 16.65, 19.1 and 13.33, respectively.

Likewise, the pH of compost soil from Mandawar, Behror and Thanagazi was estimated and found to be 7.3, 6.8 and 7.2, respectively. Moisture content in Mandawar compost soil was 52.18%, 48% in Behror compost soil and in Thanagazi compost soil it was found to be 56.7%. Organic carbon content observed in Mandawar, Behror and Thanagazi compost soil was 0.61%, 0.68% and 0.57%. Water soluble nutrients like N, P and K was also determined Mandawar, Behror and Thanagazi compost soil where the N content was 139 kg/ha, 151.9 kg/ha and 174.65 kg/ha while P content was 42 kg/ha, 56 kg/ha and 78.8 kg/ha, and the K content was 294.4 kg/ha, 202 kg/ha and 267 kg/ha, respectively. The C: N content in Mandawar compost soil was 23.88, 25.9 in Behror compost soil and Thanagazi compost soil had 20.7 content.

Similarly, in 2019, Pandit *et al.* reported pH change occurring through various composting methods with and without biochar on soil fertility and maize growth. Three types of compost, i.e., conventional compost, aerobic compost and bokashi fermentation with premixed or co-composted biochar (Comp.conv + BC_{co-comp}, Comp.aer + BC_{co-comp} and Bok + BC_{co-comp}), post-mixed biochar (Comp.conv + BC_{post-mix}, Comp.aer + BC_{post-mix} and Bok + BC_{post-mix}) and without biochar (Comp.conv, Comp.aer and Bok) were applied in two different dosages (40 g and 120 g per pot equivalent to 20 t ha⁻¹ and 60 t ha⁻¹, respectively). Aerobic co-composted biochar (Comp.aer + BC_{co-comp}) had the highest pH (7.9 ± 0.1) and bokashi fermentation (Bok) showed the lowest pH (pH 4.89 ± 0.04). By contrast, bokashi in the presence of biochar (Bok + BC_{co-comp}) was neutral (pH 7.20 ± 0.02). Similarly, in another study by Cooper *et al.*, 2020 conducted a field experiment with control, compost only, biochar only, and a mixed compost-biochar application (co-composted and only mixed) at low and high application rates (9–70 t ha⁻¹) in southern Germany. After six years of agricultural use, surface (0–10 cm) and subsurface (10–30 cm) soil samples were analyzed for pH, microbial biomass carbon

(C_{mic}), organic carbon (OC) and cation exchange capacity (CEC). Biochar significantly increased OC and pH, while compost significantly increased OC, pH, C_{mic} , and CEC six years after application. Biochar also significantly increased OC storage (10–32 times higher) and all aggregate fractions by 56–62 %, 32–47 %, and 29–32 % for the 2–0.25 mm, 0.25–0.053 mm, and < 0.053 mm fraction, respectively, while compost increased OC storage only in the 10–30 cm soil depth in the soil fractions > 0.053 mm. **Roghianianet et al., 2012** determined the effects of Municipal Waste Compost (MWC) on soil chemical properties and corn plant responses in pot experiment. They found that the amount of available N, P, K and micronutrient concentrations in soil increased as the result of waste compost application. The increases were significant for all concentrations, especially for 60 Ton ha⁻¹ treatment. They also tested the concentrations of N, P, K and micronutrient elements in the dry matter of the aerial part of the plant. The result revealed that N, P and K content and concentration of micronutrients in plant increased with increase of compost concentration. Also, study of **Petric et al., 2012** showed that the addition of organic fraction of municipal solid waste + poultry manure + mature compost in soil increased the aeration of the composting pile, and optimized the moisture, C/N ratio, pH and porosity of the pile. **Guo et al., 2012** also reported that the addition of pig manure in soil aided in adjusting the high moisture content and low C/N ratio.

Table 2: Chemical characteristics of untreated soil and compost soil

Physical properties		Mandawar		Behror		Thanagazi	
		Untreated soil	Compost soil	Untreated soil	Compost soil	Untreated soil	Compost soil
pH		7.7	7.3	7.8	6.8	7.49	7.2
Moisture (%)		32	52.18	28	48	34	56.7
Organic carbon content (%)		0.43	0.61	0.51	0.68	0.20	0.57
Water soluble nutrients (kg/ha)	N	114.1	139	118.73	151.9	120.9	174.56
	P	31	42	34.6	56	39.5	78.8
	K	265.33	294.4	194.33	202	231.2	267
C:N		16.65	23.88	19.1	25.9	13.33	20.7

C. Biological Characteristics

The addition of compost to soils affects soil microorganisms directly by providing a source of nutrients and indirectly by changing chemical and physical soil properties. Addition of compost from various feed stocks has been shown to increase the abundance of Gram-positive bacteria (**Sutton-Grier et al., 2010**). In the current study, bacteria were isolated from the untreated soil and compost soil. The isolated bacteria were identified on the basis of several biochemical tests such as Gram's staining, KOH, Catalase, Oxidase, Citrate, Indole and Cetrinide tests which were further subjected to confirmatory test such as Gelatin hydrolysis, Arginine dihydrolase test and Carbohydrate utilization test for more precise identification of bacterial species. Cetrinide agar is a selective medium which was used to

identify bacteria of the genus *Pseudomonas*. As a result, the isolated bacteria from Mundawar, Behror and Thanagazi untreated soil and compost soil were *P. aeruginosa*, *P. viridiflava*, and *P. syringae*, respectively and are mentioned in Table 3 and 4. In 2016, Van Hofwegen *et al.* did the isolation of bacterial strains from vermicompost helped to identify the microbes that enhances soil fertility and also help in crop improvement. On the basis of morphological, microscopic and biochemical studies, eight bacterial strains were primarily identified viz. *Actinomycesraelli* (BIFV-A), *Azotobacter* (BIFV-B), *M. Luteus* (BIFV-C), *B. Cereus* (BIFV-D), *Azotobacter* (BIFV-E), *B. subtilis* (BIFV-F), *P. aeruginosa* (BIFV-G) and *Enterobacter* (BIFV-H). The biochemical test results were interpreted by the reaction that taken place in form of the change in colour. In the Indole test, the strain BIFV A, BIFV B, BIFV C, BIFV D, BIFV E, BIFV F, BIFV G and BIFV H were unable to produce indole from tryptone both giving negative result. In case of citrate agar test, BIFV B, BIFV C, BIFV D, BIFV E, BIFV F, BIFV G were producing blue colour which showed positive result and utilization of citrate as the sole source of carbon. Likewise, Stella *et al.*, 2010 isolated four strains of microorganisms which were free-living diazotrophs from compost made of empty fruit bunches and chicken dung and consequently labelled as NC 2, NC 4, NC 10 and NC 11 and all four isolates were catalase positive. Similarly, to differentiate isolates between Gram positive and Gram negative, the KOH test was used. NC 2 and NC 10 were identified as Gram-positive bacteria whereas NC 11 was identified as Gram-negative. Vashist *et al.*, 2012 selected eight morphotypes from microscopic examinations (labeled as A1 A2 B1 B2 E1 F1 G1 and H1) from compost pile made up of soil and plant materials. The results from conducted biochemical tests (Gram's staining, catalase, oxidase and indole test) stated that some of the isolates were gram-negative (A1, A2, B1, E1) and rest gram-positive (B2, F1, H1, G1). Bubble formation on catalase test confirmed a positive reaction on four isolates (A1, B1, G1, and H1). The Bactident oxidase Test strips in oxidase test turned purple to confirm isolate A1 positive. Positive citrate result on isolates (B1, E1, F1, G1, and H1) was established from the formation of a blue color change on the slants and for a positive indole test, indole reacts with Kovac's reagent to produce red coloured ring. Carbohydrate test was also conducted where showed that the liquid in the test tubes turned yellow and bubbles were formed inside the tubes for all bacterial isolates. A drop in pH and a gas production results from the production of the acid and oxygen when lactose ferments in the media. Anaukwu *et al.*, 2015 performed the isolation of *Pseudomonas monteilii* from spent-oil contaminated soil sample with cetrimide agar in a study and similar motive was shared in the study of Onwosi and Odibo *et al.*, 2013 who isolated *Pseudomonas nitroreducens* from petroleum contaminated soil sample using the same medium. Rashedi *et al.*, 2005 also isolated *Pseudomonas aeruginosa* from oil well using cetrimide agar. Although cetrimide agar is a selective medium for isolation of *Pseudomonas*, the isolation of *Citrobacter murlinae* with cetrimide agar was performed by de Ther – allonne, 2003 who observed that strains of *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Providencia*, *Alcaligenes* and *Aeromonas* may also grow on cetrimide agar, causing a slight yellowing of the medium. However, there has been no work reported till date for the cetrimide test used for identification of *Pseudomonas* species from normal soil and compost soil in any literature. Hence, for the first time cetrimide test have been reported for the identification of *Pseudomonas* species from the soil and compost soil in the current work. In 2014, Johnson *et al.* isolated *Peptoniphilus stercorisuis* sp. nov., from a swine manure which corresponded to positive reactions for arginine dihydrolase. Manero *et al.*, 1999 performed arginine dihydrolase test on *Enterococcus* species isolated from pig manure which included *E. asini*, *E. avium*, *E. moladoratus*, *E. sulfureus*, *E. columbae*, *E. raffinosus*, *E. cecorum* and *E. saccharolyticus* being negative to the test and some other species like *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. malodoratus* and *E. mundtii* were positive. Raja *et al.*, 2012 studied the carbohydrate utilization test on *Bacillus* and *Pseudomonas* sp. which were isolated from compost of spoiled vegetables and fruits. Samples were collected from local market and Super Market in and around Madurai, Tamilnadu, India. The organisms were analyzed for the utilization of 12 carbon sources like Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Mellibiose, Sucrose, L-Arabinose, Mannose, Inulin, Sodium Gluconate, Glycerol, Salicin, Glucosamine, Dalicitol, Inositol, Sorbitol, Manitol, Adonitol, α Methyl D-Glucoside, Ribose, Rhamnose, Cellobiose, Melezitose, α Methyl D-mannoside, Xylitol, ONPG, Esculin, D-Arabinose, Citrate, Malanate and Sorbose. Similarly, Prasanna *et al.*, 2019 designed a study to evaluate the maize straw to integrated management of composting and vermicomposting. Fifteen cellulolytic bacterial strains were isolated from soil by using serial dilutions and pour plate technique. In carbohydrate utilization tests the results showed that 11 isolates utilized glucose, 6 isolates utilized galactose and 7 isolates used lactose.



Table 3: Biological characteristics of untreated soil

Soil sample	Mandawar soil	Behror soil	Thanagazi soil
Gram staining	-	-	-
Catalase	+	+	+
KOH	+	+	+
Oxidase	+	-	+
Citrate	+	+	+
Indole	-	-	-
Cetramide	+	-	+
Gelatin test	+		+
Arginine test	+		+
Carbohydrate utilization test		-	
Isolated bacteria	<i>P. aeruginosa</i>	<i>P. viridiflava</i>	<i>P. aeruginosa</i>

Table 4: Biological characteristics of compost soil

Compost soil sample	Mandawar				Behror						Thanagazi				
	Incubated	Normal			Incubated			Normal			Incubated		Normal		
		1	1	2	3	1	2	3	1	2	3	1	2	1	2
Gram staining	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
KOH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	+	+	+	-	+	+	-	-	+	-	-	-	-	



Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cetramide	-	+	+	+	-	+	+	-	-	+	-	-	-	-
Gelatin test		+	+	+		+	+			+				
Arginine test		+	+	+		+	+			+				
Carbohydrate utilization test	-				-			-	+		-	-	-	-
Isolated bacteria	PV	PA	PA	PA	PV	PA	PA	PV	PS	PA	PV	PV	PV	PV

Abbreviations: PA- *Pseudomonas aeruginosa*, PV- *Pseudomonas viridiflava*, PS- *Pseudomonas syringae*

IV. CONCLUSION

Organic matter stimulates soil physical properties. The application of green waste compost improves the efficiency of physical, chemical and biological properties of plant and soil, so that the use of inorganic fertilizer can be reduced. This approach will make it possible to reduce fertilizer consumption and to decrease the contamination risks, with the extra benefit of improving soil microbiological properties and reusing organic wastes. Therefore, it can be concluded that the application of green waste composts is an effective and very encouraging method to increase agricultural sustainability and reduce the use of environmentally dangerous chemical products commonly used. Further studies are needed to improve the microbial cultivability and understand the influence of organic and conventional management on the diversity of the natural microbiota of agricultural soil.

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