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RESEARCH ARTICLE

Conversion of organic biomedical waste into potential fertilizer using isolated organisms from cow dung for a cleaner environment

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Abstract

Management of organic biomedical waste is a global quandary, and it is becoming difficult to confront day by day. Conversion of organic biomedical waste into fertilizer is of great concern. In the present research, organic biomedical waste samples (blood swabs, dressing swabs, and used cotton) were collected then after cow dung was collected in sterile container and immediately transported to the laboratory and screened for any gastrointestinal infection by using routine microscopy for intestinal parasitic infection, routine bacterial culture, and fecal occult blood for any intestinal bleeding. Then after, the pure culture of organisms and fungus were prepared, and further samples were subjected to degradation for 288 h by using various organisms and fungus. Then after, the specific quantity of biomedical waste was subjected for incineration. The physicochemical parameters of biomedical waste samples were analyzed. Then treated samples were mixed with soil to confirm a role as potential fertilizer. Then after, tomato plantation was done and phytochemical parameters of tomato plant were analyzed. This study states that organic biomedical waste produces a sanitary and stable fertilizer.

Keywords Biomedical waste · Total dissolved solids · Chemical oxygen demand · Dissolved oxygen · Electric conductivity · Distilled water · Neem and tobacco extracts

Introduction

Hospital is a complex institution frequently visited by people without any dissimilarity of sex, age, religion, and race (Rajakannan et al. 2013). The normal population of hospitals and health care centers are staff and patients. Production of biomedical waste is not only restricted to research activities or hospitals, but biomedical waste can also be generated in rural areas or at home through dialysis or through insulin injection (Deb et al. 2017). Biomedical waste could act as a source of injury and infection to the individual and can also have a serious impact on the environment (Sharma et al. 2013). Around the globe, advancement in health care centers has led to significant improvement for management of biomedical waste in developed countries. In order to protect the environment and to prevent form infections, The Ministry of Environment and Forest has notified and formulated the rules of biomedical waste in 1998 which have to be followed by all the institutions producing biomedical waste (Vasistha et al. 2018).

Without preliminary treatment, direct discharge of hospital effluents into the urban sewage system is one of the major concerns (Ramesh Babu et al. 2009). In hospitals, numerous of chemical substances are used like radionuclides, pharmaceuticals, disinfectants, and solvents for treatment purpose like for disinfection, diagnostics, and for research purpose,

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and from this, many chemical substances resist general wastewater treatment (Chakraborty et al. 2013). Due to inefficient removal in wastewater treatment plant residues of pharmaceuticals are found in normal water (Thakur and Ramesh 2015). The existence of hazardous compounds in surface water or in an aquatic ecosystem may contaminate the entire ecosystem and may be accumulated by the food chain (Capoor and Bhowmik 2017). Hence, concern is rising regarding the safety of the individuals who are utilizing the water after treatment for household purposes, drinking the water, and working at the sewage treatment plant (Rudraswamy et al. 2012).

Cow dung acts as a tremendous bioremediation method. It is cheap, eco-friendly, and economically viable and is locally available in the rural areas of India (Anitha and Jayaaj 2012). The biodegradation process is enhanced by various microorganisms, and it is a natural process (Geetha and Fulekar 2008). Composting is a traditional method for recycling of organic biomedical waste. The organic matter present in waste is broken down to potential fertilizer by bacteria, fungi, and other microorganisms (Wang et al. 2018). Cow dung is a “gold mine” due to its spacious applications in the field of medicine, energy generation, and environmental protection (Randhawa and Kullar 2011). Mismanagement of biomedical waste is a great concern, so there is a need to improve the management of biomedical waste (Patil et al. 2019). Thus, the aim of the present research was to develop an innovative method for the treatment of biomedical waste, to study the rate of degradation of biomedical waste by using organisms and fungus, to study physicochemical parameters of treated waste and soil, and to study phytochemical parameters and heavy metals of plants to substantiate that organic biomedical is converted into potential fertilizer. It is reported that organisms isolated from cow dung are used as a source for degradation of solid waste. Cow dung has mixed organisms culture, and it has different benefits (Gupta et al. 2016). The presence of fungus in cow dung is responsible for the degradation of the organic biomedical waste sample. The fungus is used as an organic approach for biodegradation and for treatment of biomedical waste without existing any trace of dangerous effect on a community (Pandey et al. 2011).

Materials and methods

This study was approved by the institutional ethical committee (ICE), D.Y. Patil University, Kolhapur.

Collection of samples (for each set)

From D. Y. Patil Medical College Hospital and Research Institute Kolhapur, fresh 50 g organic biomedical waste samples (30 g blood swabs, 10 g dressing swabs, and 10 g used cotton) were collected, and then after, samples are doubled autoclaved at 121 °C and prior to use.

Collection of cow dung

Three cow dung samples were collected on three successive days by using the sterile container. A cow was maintained with the same diet pattern for 4 days. After collection, the cow was labelled properly and immediately transported to the laboratory and screened for any gastrointestinal infection using routine microscopy for intestinal parasitic infection, routine bacterial culture, and fecal occult blood for any intestinal bleeding. After confirmation of the above tests, the following analyses were performed for the isolation of bacteria and fungus from cow dung Gupta & Rana 2016.

Preparation of pure culture

One gram of sample was mixed with 10 ml of sterile physiological saline and shakes vigorously in a vortex for 3–5 min and incubated at 37 °C for 20–30 min for microorganism activation. After activation, samples were prepared by using standard dilution method with the help of sterile distilled water. Each test tube contained 9 ml of distilled water and added with 1 ml of activated suspension to the first test tube and serially diluted till the last tube (10th tube) (Patil and Deshmukh 2016).

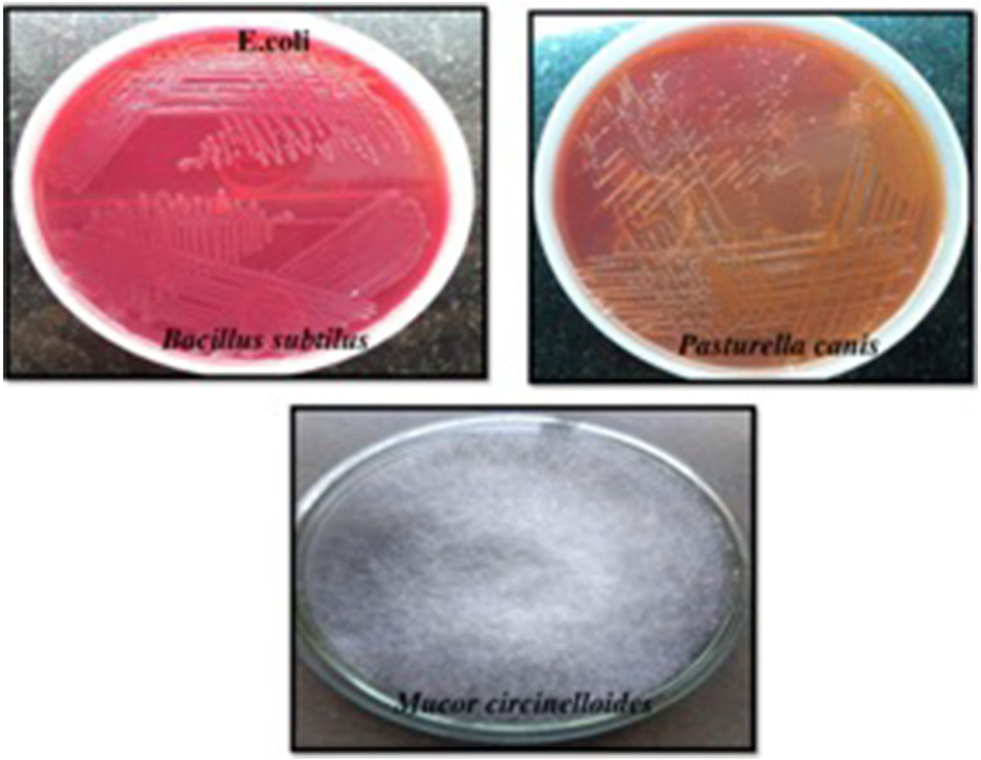
Each test tube contained 0.1 ml of suspension that was transferred to a sterile nutrient agar plate and spread evenly by using a sterile glass rod (spread plate method). Plates were incubated at 37 °C for 24 h. Based on the different colony morphology, bacterial cultures were purified by a streak plate method. Colonies were inoculated into blood agar and MacConkey agar; plates were incubated at 37 °C for 24 h Gupta & Rana 2016.

Isolation of fungus

Pour plate technique was performed to isolate fungus from cow dung sample. One gram of a sample was mixed with 9 ml of sterile normal saline and serially diluted (10^1 to 10^6) (Patil and Deshmukh 2016). One milliliter of sample from each dilution was seeded into Sabouraud dextrose agar (SDA), and plates were incubated at 28 °C for 72 h, and fungal spore forming unit (sfu/g) were calculated. Repeated subculture was performed to obtain the pure culture of the fungi for easy identification.

After getting pure growth of the fungal isolate, macroscopically culture characteristics were studied, and microscopy was performed by Lacto-phenol Cotton Blue method. Slides were observed under × 10 and × 45 magnification. Further confirmation was done by 18 s rRNA sequencing (Accession Number LC413613.1) (Fig. 1).

Fig. 1 Isolation of organisms and fungus (pure culture)



Identification of bacterial isolates

Isolated cultures were identified by using standard microbiology procedures such as catalase test, oxidase test, indole test, methyl red test, Voges Proskauer test, citrate utilization test, urease test, triple sugar iron test, and automated Vitek 2 compact system (Biomeriux) (Table 1).

Preparation of experimental sets

After sample collection, the experimental sets were arranged and kept in anaerobic condition for 288 h. The composition of sets are as follows for control: (50 g of BMW + 30 ml d/w) it is denoted as set 1; for *E.coli* sample (suspension of *E.coli* 15 ml + 50g of BMW + 30 ml d/w), it is denoted as set 2; for *Bacillus subtilus* sample (suspension of *Bacillus subtilus* 15 ml + 50g of BMW + 30 ml d/w), it is denoted as set 3, for *Pasterulla canis* sample (suspension of *Pasterulla canis* 15 ml + 50g of BMW + 30 ml d/w), it is denoted as set 4; for *Mucor circinelloides* sample (suspension of *Mucor circinelloides* 15 ml + 50gm of BMW + 30 ml d/w) it is denoted as set 5, and further, we have taken equivalent quantity of BMW, and it was incinerated by standard method, and it is denoted as set 6.

Table 1 Biochemical reaction of isolated bacteria.

Biochemical test	*E. coli*	*Bacillus subtilus*	*Pasterulla canis*
Catalase	+	+	+
Oxidase	−	−	+
Indole test	+	+	+
Methyl red test	+	−	+
Voges Proskauer test	−	+	−
Citrate utilization test	−	−	−
Urease test	−	−	−
TSI	Acid/acid	Alkaline/alkaline	Acid/acid

Mixing of experimental sets into the soil

After degradation, the equivalent quantities of experimental sets were transferred with soil to confirm their role as a sanitary fertilizer. Set A1 is of control, then further, 50 g set 1 is mixed with 1000 g of soil, and it is denoted as B2; 50 g set 2 is mixed with 1000 g of soil, and it is denoted as C3; 50 g set 3 is mixed with 1000 g of soil, and it is denoted as D4; 50 g set 4 is mixed with 1000 g of soil, and it is denoted as E5; 50 g of incinerated waste set 6 is mixed with 1000 g of soil, and it is denoted as F6.

Plant selection for the experiment

The tomato plants, of (15 days) species *S. lycopersium*, were chosen for plantation, because it is of short duration.

Physicochemical parameters of sample

The analyses of physicochemical parameters were done by measuring the chemical oxygen demand (COD), total dissolved solids, and pH

Physicochemical parameters of soil

The physicochemical parameters of a soil sample that were analyzed are pH, organic carbon content, organic matter, EC, calcium, and magnesium, water-holding capacity, nitrogen, phosphorus, potassium, and heavy metals.

Phytochemical analysis of plants

The phytochemical parameters of tomato plants that were analyzed are chlorophyll content, polyphenol, and protein content.

Results and discussions

Physicochemical parameters of experimental sets

pH

It is reported that superior degradation of the solid waste was obtained at pH values 6–8; 70–90% degradation of total dissolved solids were observed in solid waste at neutral pH value (Dinamarca et al. 2018). Katheem Kiyasudeen S et al also reported that the neutral pH value is good for the degradation of organic waste, and it also supports the microbial activity during the degradation (Kiyasudeen 2016).

The sets of experiments were monitored at different intervals; the pH of control was slightly acidic; in *E. coli*, *Bacillus subtilus*, *Pasterulla canis*, and *Mucor circinelloides*, the pH was neutral, so the degradation value of the experimental sets is far superior, and *P* values are also significant in experimental sets. In Fig. 2b, the pH after incineration is slightly acidic.

Total dissolved solids (TDS) and chemical oxygen demand (COD) of experimental sets

Total dissolved solids can be evaluated by drying the prefiltered sample and then calculating the dry residue mass per liter of the sample. Total dissolved solids are geochemical characterization that links the bulk conductivity to hydrocarbon microbial degradation (Atekwana et al. 2004). S. K. Bansal et al treated a solid waste with cow dung in lab scale batch reactor at a modest temperature within 8 days; there was 68.75% TDS reduction respectively (Bansal et al. 2012). The wastewater of boy's hostel was treated with the vermicomposting technique, and there was an average of 85% reduction in TDS (Lakshmi et al. 2014).

Chemical oxygen demand is a standard method by which amount of pollution which is not oxidized biologically in a water sample is indirectly measured (Singh et al. 2012). The chemical oxygen demand method is based in decomposition of chemicals in inorganic and organic substances dissolved in water (Ali and Yasmin 2014). The solid waste is treated with the cow dung, and there was 35.75% reduction in COD respectively (Bansal et al. 2012). The landfill leachate is treated with the cow dung ash at neutral pH, and 79% removal of COD is achieved, so the cow dung is a good adsorbent, and it should be used for removal of COD and organic pollutant from waste (Kaur et al. 2016). Divya C Das et al. treated the ayurvedic industrial effluent with a microbial consortium and then after with vermifiltration unit, and it was confirmed that there is a significant reduction in COD by 98.03%, and the treated water was disinfected and cleaned enough to be reused for irrigation (Das et al. 2015). For the purpose of the biodegradation, Jianzheng Li et al. treated the sludge waste with cow dung, and within 4 weeks, the superior amount of COD has

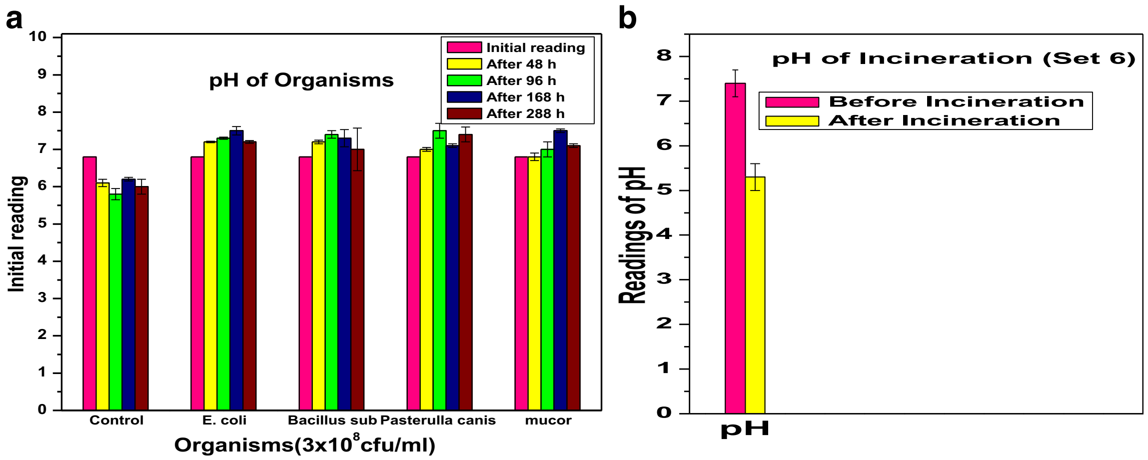


Fig. 2 pH of experimental sets at the different time interval is shown in (**a**), first set is of control, second is of *E. coli*, third is of *Bacillus subtilus*, fourth is of *Pasterulla canis*, and fifth is of *Mucor circinelloides*. The readings of pH before and after incineration are presented in (**b**)

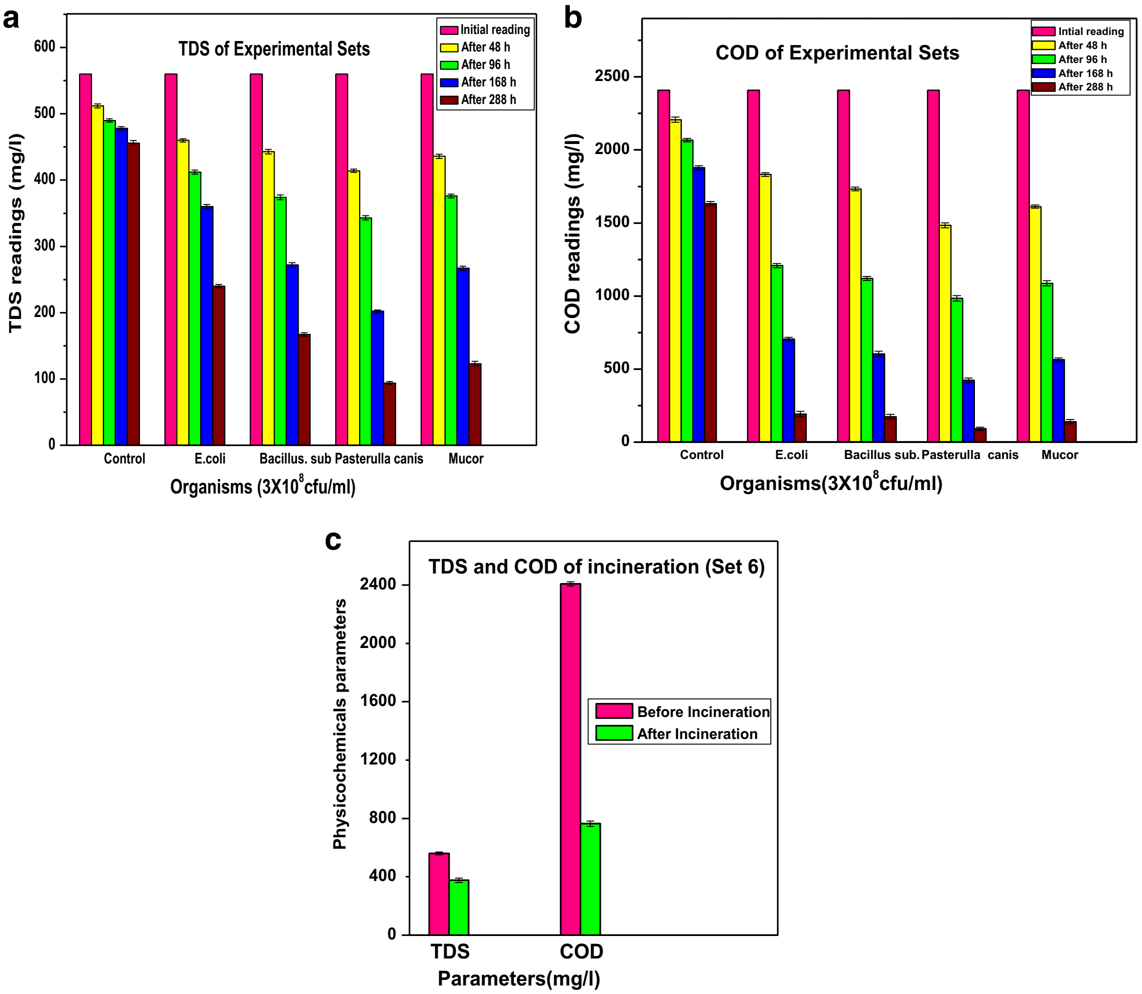


Fig. 3 TDS and COD of experimental sets at the different time interval are shown in (**a**), first set is of control, second is of *E. coli*, third is of *Bacillus subtilus*, fourth is of *Pasterulla canis*, and fifth is of *Mucor circinelloides* (**b**). The readings of TDS and COD before and after incineration are presented in (**c**)

been degraded (Li et al. 2011). So looking toward the ecofriendlly approach, we have designed that the experimental sets and parameters are analyzed to reveal the biodegradation rate of the waste. Figure 3 shows the COD values of experimental sets at various concentrations (mg/l).

The minute amount of organic matter and inorganic salts in solution are expressed as total dissolved solids (TDS). The TDS in experimental sets should be less than 700 mg/l, to make it eligible for the application of fertilizer. The TDS of different sets is shown in Fig.3a for a different time interval. It was confirmed that in over 288 h of the operating period there was progressive decrease in TDS in the case of *Pasterulla canis* (560 to 094 mg/l) than that of control and of incineration. The *P* value of the experimental sets is also extremely significant.

From Fig. 3b, we can conclude that there is a high degradation of biomedical waste within 288 h when it was treated with organisms and fungus except from control and of incineration. *P* value is also significant than that of required ($P > 0.05$). In experimental sets such as control, *E. coli*, *Bacillus subtilus*, *Pasterulla canis*, and *Mucor circinelloides*, incineration COD has decreased from 2408 to 1632 mg/l, 2408 to 192, 2408 to 174, 2408 to 91, 2408 to 140, and 2408 to 764 respectively This remarkable reduction in COD and in all other parameters suggests that organisms and fungus can effectively destroy pathogens which are present in biomedical waste with respect to time. As we know, cow dung is gold mine; it contains various microorganisms, and these organisms help to degrade the biomedical waste. Olga et al. (2015) has used common fungal strain of *Mucor circinelloides* and degraded diesel oil hydrocarbons. The readings of Fig. 3c shows TDS and COD before and after incineration. The TDS and COD decreased from 560 to 376 and 2408 to 764 respectively. It was far higher than that of the required value for fertilizer.

Physicochemical parameters of the soil sample

Numerous chemical processes are affected by soil pH, so the pH of the soil was acknowledged as a master variable. In most

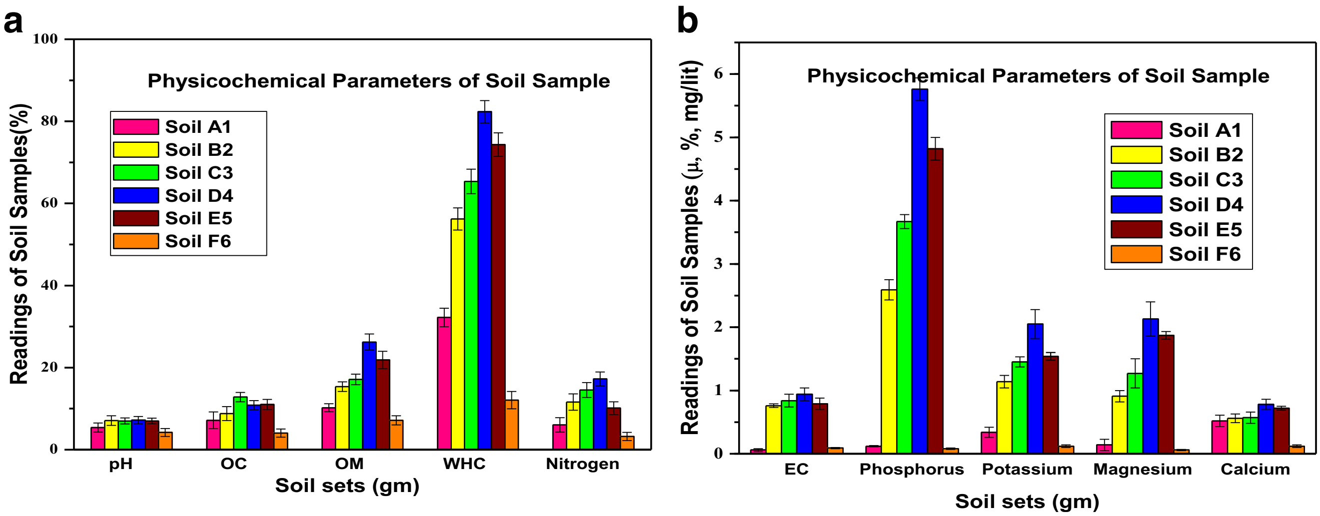


Fig. 4 Physicochemical parameters of all soil samples were pH, EC, organic carbon, organic matter, water holding capacity, nitrogen, phosphorus, potassium, magnesium, and calcium

of the plants, the optimum pH value is between 5.5 and 7.5 (Randhawa and Kullar 2011). Soils electrical conductivity varies depending on the moisture content held by particles in the soil. The change in electric conductivity will influence crop yield (Corwin and Lesch 2005). Organic matter which is present in the soil is known as soil organic matter; at various stages of decomposition, it consists of animal and plant residues, tissues and cells of organisms, and synthesized substances by organisms (Campion et al. 2015). To provide regulatory ecosystem, organic matter exerts various positive effects on the soil. In the soil, phosphorus is found in minerals and organic compounds. As compared with the total amount of phosphorus, readily obtainable phosphorus is very low (Bennett et al. 2001). Potassium is involved in the activation of an enzyme within the plant, which can affect the production of starch, protein, and adenosine triphosphate (ATP) (Van Raij et al. 2008). The amount of water that the given soil can hold is referred to as water holding capacity. In the process of photosynthesis, the opening and closing of stomata are regulated by potassium. (Ashley et al. 2006). Nitrogen is the most important building blocks of proteins, nucleic acid, and other constituents. Careful management of plants is warranted by the nitrogen (Bodelier and Laanbroek 2004). Each and every plant needs calcium for its growth and development as calcium helps to maintain the soil chemical balances; calcium reduced soil salinity that improves water penetration. Magnesium has a wide range of key roles in numerous plants functions. In the process of photosynthesis, magnesium plays its well-known role the building block of chlorophyll; the leaves appear green due to magnesium (Karhu et al. 2011). Different physiochemical parameters of the soil before and after the addition of the respective experimental sets are shown in Fig. 4.

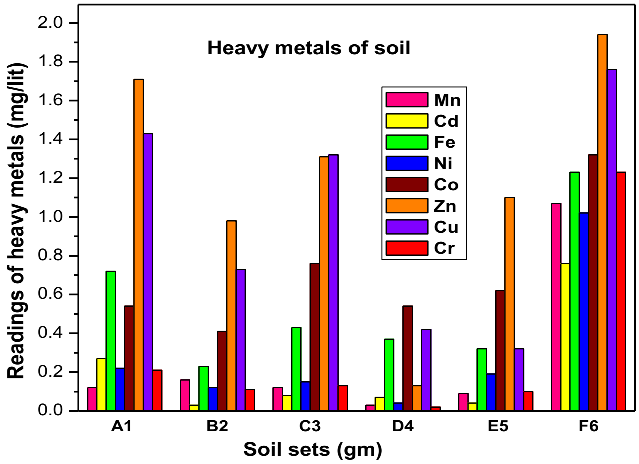


Fig. 5 Represents heavy metals of A1, B2, C3, D4, E5, and F5 soil samples

Slightly acidic pH is observed in A1 soil, whereas neutral pH is seen in B2, C3, D4, E5 soils which increases the soil microbial activity, but in F_6 soil, acidic pH is observed. EC content of F6 soil is lower than that of control, and soil D_4 has more EC content. EC strongly correlates to soil size, soil particles, and soil textures, depending upon the moisture held by soil particle EC varies (Xu et al. 2006). After addition of treated organic biomedical waste to the soil, the organic matter and organic carbon content of D4 soil were increased as compared with others. The water holding capacity of treated soils has also enhanced as compared with F_6 soil control soil. Disposal of biomedical waste has become a well-known problem, so when we used this waste as a potential fertilizer, it contributes to minimize waste as well as enhance the parameters of soil. Phosphorus, potassium, nitrogen, calcium, and magnesium of the treated sets have enhanced as shown in the evaluation of the control A1 and F6 soil.

Table 2 Phytochemical parameters of (tomato plant)

Sr. no.	Name of set	Chlorophyll (mg/100 g)	Polyphenol (mg/1000 g)	Protein (mg/g)
1	A1	098.72 ± 1.01	18400 ± 5.02	0.34 ± 0.01
2	B2	173.42 ± 1.04	45700 ± 6.23	1.24 ± 0.02
3	C3	184.31 ± 1.45	53400 ± 5.56	2.01 ± 0.24
4	D4	236.24 ± 1.34	86300 ± 4.27	2.89 ± 0.13
5	E5	194.81 ± 1.32	63800 ± 4.12	2.12 ± 0.08

Heavy metals of the soil sample

The heavy metal in solid waste mainly enters through the use and disposal of products such as batteries, pigmented plastics, glasses, ceramics, electronics products, paints, etc. There are several methods and mechanisms by which the heavy metals are removed; they are chemical precipitation, particulate settling and trapping, and plant uptake; they are also removed through binding to organic substances, accumulation into plant tissues, sedimentation of suspended particles, adsorption of soil components, filtration, etc (Wuana and Okieimen 2011). The heavy metals of soil are analysed, and the results of heavy metals are mentioned in Fig. 5. From these results, the heavy metals are within the permissible limit. As there are no toxic metals in the soils, there is no risk to the environment. So with respect to time, organisms and fungus can effectively destroy pathogens which are present in the biomedical waste.

Phytochemical parameters of (tomato plant)

The tomato plants were sown, and phytochemical analysis of the treated tomato plant was studied. Chlorophyll plays a crucial role in the process of photosynthesis, and plants take energy from light. The substance which is soluble enhances the chlorophyll content and improves the growth of the plant (Baglieria et al. 2014). The condensed tannins are known as the most abundant polyphenol, which is found in all plant family. Larger polyphenols are often present in the epidermis, leaf tissue, fruits, and flowers which also play a crucial role in forest decomposition and in the nutrient cycle in forest ecology. Proteins which are present in plants are highly complex compounds. Enormous nutritional values are present in protein and they are involved directly in the chemical processes which are necessary for life.

In Table 2, the results of phytochemical parameters of tomato plants are shown and from the table, we can conclude that D4 show higher chlorophyll contents than that of other plants. There was maximum polyphenol content in plant D4 followed that of others.

Conclusion

From the study, it can be found that the organisms, and fungus show excellent degradation of organic biomedical waste. It was found that physicochemical parameters of experimental sets, treated soils, phytochemical, and morphological parameters of a plant were high in the set D4. So with respect to time organisms can effectively destroy pathogens which were present in the biomedical waste. As it is one kind of biological waste treatments, and it is an alternative method to recycle the organic matter and produce a stable. So using organism's cultures, organic biomedical is converted into potential fertilizer for a cleaner environment.

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Compliance with ethical standards

This study was approved by the institutional ethical committee (ICE), D.Y. Patil University, Kolhapur.

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