

A Case Study on Role of Microbial Consortia Assisted Decomposition of Agro-waste for Improvement of Soil Organic Carbon – A Step Towards Sustainable Development

Sonal J. Shinde¹, Arun D. Dixit¹, Ranjeet J. Shanbag¹, Yogesh Kulkarni¹, Mahesh Y. Borde², Vinayak H. Lokhande^{3*}

DOI: 10.18811/ijpen.v9i04.03

ABSTRACT

The availability of optimum soil organic carbon (SOC) in the field is associated with an optimal soil structure, water-holding capacity, nutrient availability, aeration, growth of microflora and thus, crop productivity. In the present investigation, the role of *Azolla*, poultry waste, urea and cattle dung, as a source of nitrogen, was initially investigated for maintaining the optimum carbon: nitrogen (C: N) ratio during the composting of sugarcane agro-waste. The results showed that *Azolla*-fortified agro-waste had a significantly faster rate of composting in comparison to other sources of nitrogen. In the next part of work, *Azolla*-fortified matured compost was used for the isolation of 7 fungal strains, which were combined with 6 bacterial strains for the preparation of microbial consortia. The consortia were used for composting different agro-wastes on open fields of 15 farmers in the presence of cattle dung slurry (nitrogen source) and the compost was then applied on the field for optimizing the level of SOC in the fields of SOC deficient soil. It was found that consortia-induced composting was completed in about 30-45 days as compared to the normal 60 days and the matured compost had achieved the ideal C:N ratio (20:1). The addition of this compost for two cycles significantly increased SOC level (13% and 25% in the first and second cycle, respectively). The study developed the standard operating procedure (SOP) for effective composting of diversified agro-waste (~200 tones) within a short period (~30–45 days) of time in the presence of microbial consortia (200 L ton⁻¹) and cattle dung slurry to retain optimum C:N ratio (40:1–50:1) during composting. The study suggests the application of microbial consortia + cattle dung slurry + diversified agro-waste for on-field rapid composting process and its use as biofertilizer to enhance the SOC of deficient soil in agricultural fields for sustainable development.

Keywords: *Azolla*, Agro-waste, C:N ratio, Composting, Microbial consortia, Soil organic carbon (SOC).

International Journal of Plant and Environment (2023);

ISSN: 2454-1117 (Print), 2455-202X (Online)

INTRODUCTION

The future of agricultural sustainable development resides in the management of optimum soil health in the context of abiotic and biotic stress conditions. The preservation and development of the fertile humus layer of the agricultural soil always remain challenging tasks for the agrarian community to achieve the highest crop yield and productivity to fulfill the requirements of the growing population of the world. It has been correlated with the implementation of improper agricultural management practices, various biotic and abiotic stress factors and lack of awareness among the community for the proper processing of agricultural wastes or crop residues. The estimated amount of agricultural waste produced by the major crops of India was approximately 683 million tons, and a major portion of it was found preferably subjected to burning of the crop residue on open fields (Bhattacharyya *et al.*, 2019, 2021). The burning of agricultural residue has provided short-term gain for the removal of waste from the fields; however, it has drastically impacted the long-term losses in the form of soil health destruction, decreased soil fertility, deficiencies of essential minerals (in terms of C, N, P, K), reduced crop yield, productivity and addition to the non-cultivable lands. Previous studies remarked the adverse effects of agricultural residue burning with reference to air pollution due to the emission of toxic and hazardous noxious gases (Gadde *et al.*, 2009; Sahu *et al.*, 2015; Andini *et al.*, 2018). The burning practices adopted by the farmers

¹Vigyan Ashram, Pabal, Shirur, Pune, Maharashtra, India.

²Department of Botany, Savitribai Phule Pune University, Ganeshkhind, Pune, Maharashtra, India

³Department of Botany, Shri Shiv Chhatrapati College, Bodkenagar, Junnar, Pune, Maharashtra, India.

***Corresponding author:** Vinayak H. Lokhande, Department of Botany, Shri Shiv Chhatrapati College, Bodkenagar, Junnar, Pune, Maharashtra, India., Email: vhl1983@gmail.com

How to cite this article: Shinde, S.J., Dixit, A.D., Shanbag, R.J., Kulkarni, Y., Borde, M.Y., Lokhande, V.H. (2023). A Case Study on Role of Microbial Consortia Assisted Decomposition of Agro-waste for Improvement of Soil Organic Carbon – A Step Towards Sustainable Development. *International Journal of Plant and Environment*. 9(4), 312-322.

Submitted: 16/10/2023 **Accepted:** 28/11/2023 **Published:** 28/12/2023

on a large scale have been recognized and found associated with the incompatibility towards the decomposition of the waste due to the structural and chemical complexity of the agricultural waste such as lignin, cellulose and hemicellulose-rich bio-waste. Further, the mythological thinking of burning waste on open fields for scaling up the productivity of future crops has shown limited benefits to ease of farm operations and pathogen elimination which was drastically responsible for affecting the soil quality of the agricultural field. Besides, the use of toxic concentrations of weedicides to remove the weeds among standing crops, and acidic or alkaline

treatments for the *ex-situ* decomposition of agricultural residue have been found economically and practically non-viable practices of agricultural crop residue management (Zhang *et al.*, 2011; Hosseini and Aziz, 2013).

Therefore, appropriate composting of the agricultural residue with the use of environment-friendly biological sources has been found to be the best solution over the physical or chemical treatments to resolve the problems of agricultural waste management in a sustainable manner. The most viable, economically cheap, and easily available source of waste management is the use of potent strains of lignocellulolytic microbes (bacteria and fungi) to boost up the composting process of agricultural waste (Sahu *et al.*, 2015; Bhattacharyya *et al.*, 2021). The composting of agricultural residue with the use of microorganisms could act as an alternative source of easily and naturally available essential nutrients to future crops and to gain their fruitful effect on soil fertility and crop productivity (Gupta *et al.*, 2004). It could replace the harmful practices of crop residue burning on open field with beneficiary outcomes to the farmers in terms of retaining soil health. The crop residue composting process could be achieved through *in-situ* or *ex-situ* implementation of the composting technology. This has helped in improving soil health due to the enrichment of soil organic carbon (SOC) in the soil, thus directly affecting crop yield and productivity to astonishing levels (Sahu *et al.*, 2015; Zhao *et al.*, 2016; Goswami *et al.*, 2019; Sahu *et al.*, 2019). However, only the use of potent microbial strains for composting of agricultural waste will not solve the purpose of agricultural waste management. It is highly essential to monitor and regulate the optimum concentrations of C:N ratio of the crop residue (Goyal *et al.*, 2005, Goyal and Sindhu, 2011; Sarkar *et al.*, 2011, 2018; Kumari *et al.*, 2018) during the composting process. It could be achieved with the use of naturally available complex organic carbon sources (in terms of agricultural residue – sugarcane trash, paddy straw, etc.), in combination with the addition of suitable nitrogen sources (cattle dung, poultry waste or biological sources such as BGA, *Azolla*, etc.). The optimum C:N ratio of the compost was found responsible for accelerating the composting process of the crop residue or waste with the help of the potent strains of microorganisms. Besides the regulation of the optimum C:N ratio, the enhanced microbial activity during the composting of agricultural residue for the production of matured compost within a short period of time is mainly associated with the optimal levels of temperature and moisture conditions in the composting beds (Dantoliya *et al.*, 2022). Temperature factor during the composting process is solely associated with the diversity and metabolic activities of potent mesophilic and thermophilic microorganisms (Game *et al.*, 2017). In this context, most of the composting processes fails to obtain good quality matured compost by taking longer time for the composting process and production of inferior quality compost with levels of C:N ratio greater than 20:1, low water holding capacity and less availability of organic carbon due to inadequacy of the composting conditions such as use of improper nitrogen source, preferably fresh, partially or over-degraded cattle dung/poultry waste. There are many other alternatives that have not yet been exploited to a greater extent and utilized only at experimental

levels as a source of nitrogen for the production of nitrogen-rich biofertilizers such as *Azolla*, BGA, Water hyacinth, etc. (Goyal *et al.*, 2005; Sahu *et al.*, 2018; Trada *et al.*, 2020). Further, after the use of these sources for the composting process at a laboratory scale, their compatibility, performance, and efficiency have not been studied at a large scale, on open fields by many workers. The standard operating procedure (SOP) for mass cultivation of diversified and effective microbial consortia, as well as the implementation of these consortia at large scale on open fields for the rapid composting of complexed agricultural crop residue under the influence of optimum C:N ratio, moisture and temperature conditions for the benefits of the agrarian community have not yet been recognized.

Composting serves not only as an eco-friendly method of waste treatment but also as a recycling process, transforming the end product into agricultural fertilizer. In view of the efficacy of *Azolla*, as a symbiotic pteridophytic nitrogen-fixing organism, in the present investigation, the thought was provoked us with the aim and focus that, the natural microbial diversity of nitrogen fixers associated with the naturally growing *Azolla* could be explored for retaining the optimum C:N ratio and moisture levels during the composting of agricultural residue to a larger extent. Further, the matured compost obtained from *Azolla* supplemented composting process could be used as the source of material for the isolation of potent microbial strains of fungi and its incorporation with well-known composting bacteria strains to develop the *Azolla*-assisted microbial consortia. The development of microbial consortia to a mass scale and its implication for the composting of diversified and complex agricultural crop residue on open field could be able to provide solutions to regain the productivity potential of various crop plants across the SOC deficient fields and further play its role in improving the SOC level to achieve the soil health to optimum levels. With this aim, the current investigation was carried out. The research was planned to provide a fruitful solution and cheaper outcome-based technology to the need-based agrarian community.

MATERIAL AND METHODS

Pilot Scale Experiment for Microbial Consortia Development

Location

The experiment was conducted on the campus of Vigyan Ashram, Pabal, Shirur, Pune, Maharashtra, India (Latitude – 18.8308°N, Longitude – 74.0520°E).

Source of Nitrogen

Pure culture of *Azolla pinnata*, procured from Swami Vivekanand Kendra, Nashik, Maharashtra, India was maintained in the artificially prepared pond (10 x 6 x 3 feet) under controlled conditions (~50% light intensity, 28±2°C temperature, pH 7-8) at Vigyan Ashram. The cultures were regularly sub-cultured in fresh ponds at the interval of one month. The biomass of luxuriantly growing *A. pinnata* was used as natural source of nitrogen and possible microflora rich biomass. The cattle dung and poultry waste were obtained

from Animal and Husbandry Department situated on the campus of Vigyan Ashram, Pabal, Shirur, Pune, Maharashtra, India. The urea (46% N) was purchased from the market with the manufacturer name IFFCO (Indian Farmers Fertilizer Cooperative Limited).

Source of Carbon

Sugarcane trash has high C:N ratio (~100:1) due to complex diversity in chemical composition (lignin, cellulose and hemicellulose), with ease of availability in huge quantity in comparison to other agricultural waste (Goyal *et al.*, 2005). Therefore, in the present investigation, it is used as the source of carbon. The dried, raw sugarcane trash was collected from sugarcane growing field located near study area and chopped into small pieces (~ 2-4 cm) with the help of threshing machine (make- Doodhdhara Agro and Dairy Mart Private Ltd., Model- Chaff Cutter Machine). The chopped trash was directly used for composting process.

Experimental Design

To maintained the C:N ratio in the range of 40:1 to 50 :1 on dry weight basis, the beds (2 x 2 x 2 feet) were prepared on soil surface by mixing the compostable chopped sugarcane trash with the variable nitrogen sources having variable treatments in the following proportions:

- Sugarcane trash (ST) - (Control)
- Sugarcane trash (ST) + Cattle Dung (CD)
- Sugarcane trash (ST) + *Azolla*
- Sugarcane trash (ST) + Urea
- Sugarcane trash (ST) +Poultry Waste (PW)

The bed moisture was maintained to 50% by spraying the water at regular interval throughout the composting process. The moisture of the compost was calculated by following oven dry method (Bremner, 1970). Fresh (100 gm) compost mixture was harvested from the bed and subjected to oven drying at 105°C for 48 hours to record the constant dry weight of the mixture. The actual moisture percent was calculated, then to maintain 50% moisture in all the beds, the water spraying was carried out on the basis of requirement of the bed mixture. The moisture content was assessed regularly at the interval of 5 days.

Estimation of Organic Carbon and Nitrogen

The composting mixture was harvested from all types of treatment beds at the interval of 15 days for the period of 45 days. The sample were subjected for estimation of organic carbon by following the Walkley black method. Besides, total nitrogen content of the composting mixture was recorded by using Kjeldahl's method (Bremner, 1970).

Relative Change in Temperature

The composting process involves breakdown of complex organic substances into simpler form due to the metabolic activities of thermophilic and mesophilic microflora (bacteria and fungi).

The process leads to the temperature shift during the process of decomposition. Therefore, in the present investigation, the change in temperature of the composting mixture from each bed was recorded at the interval of 15 days for the period of 45 days.

Microbial Count

In response to the change in temperature, the diversity of microflora in the composting system at different stages of decomposition were also evaluated during the composting of various organic wastes. The diverse kinds of bacteria and fungi were counted using a conventional sampling approach and appropriate conditions (Bremner, 1970). Sampling (1 gm) of the composting mix from each bed was carried out and subjected to serial dilution, followed by inoculation on culture plates containing potato dextrose agar (PDA) medium for the growth of fungal strains and nutrient agar medium for bacterial growth. The culture plates were incubated for the optimum growth of fungal organism at 27°C for the period of 5-7 days and bacterial strains at 32°C for the period of 48 hours. At the end of the incubation period, the cultures plates were used to record the microbial count by following the colony counter method.

Isolation of Fungal Strains from Compost

For isolation of pure strains of fungal organism from the compost, the composting sample was harvested from the bed (ST + *Azolla*) at the end of composting process and serially diluted in phosphate buffer solution (pH 7.0). About 10 µl of diluted sample was inoculated on PDA medium using streaked plate method under aseptic conditions. The culture plates were incubated at 28°C for the period of 07 days. After the incubation period, the culture plates were observed for the growth of fungal strains. The fungal strains grown on the PDA medium were isolated and sub-cultured for five times on freshly prepared PDA medium for the selection of individual pure strain of fungi. The pure cultures of fungal strains were maintained by sub- culturing on PDA medium at regular interval. Individual distinct colonies were screened, isolated, purified and provided for the sequencing of the 16S rRNA gene for identification.

Identification of Fungal Strains

Standard manual was used to identify the fungal isolates (Nagamani *et al.*, 2006). The rDNA gene sequence analysis was used to identify specific fungal isolates. A modified CTAB (Cetyl trimethylammonium bromide) approach was used to extract the DNA (Edward *et al.*, 1991). The internal transcribed regions (ITS) of the rDNA were amplified using ITS 4 and ITS 5 primers (Gardes and Bruns, 1993). 2.5 mM dNTPs, 1.5 mM MgCl₂, 10 p moles of each forward and reverse primer, and 1U of Taq DNA polymerase were used to set up the PCR reaction. The PCR program with initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30s, annealing at 53°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 7 min was used. The PCR products were cleaned using a gel extraction kit procured from Bio-Source Biotechnologies Ltd. in India, and their purity and integrity was assessed using agarose gel electrophoresis (1.5%). Purified PCR products were used for DNA sequencing process. The BLAST search was carried out to examine sequence homology and compared with sequences listed in GenBank. The sequences having the highest identity with the best hit to the query sequence were defined as the sequence-based identities.

Fungal and Bacterial Strains

Pure cultures of phosphate solubilising bacteria (PSB - NCIM No. 5109), Potassium mobilizing bacteria (KMB - NCIM No. 5748), *Rhizobium* species, (NCIM No. 2224) *Azotobacter* species, (NCIM No. 2682), *Bacillus maccrance* (NCIM No. 5055) and *Pseudomonas fluorescence* (NCIM No. 5226) and fungal strains, *Trichoderma viride* (NCIM No. 5060 and 1051), *Aspergillus brasiliense* (NCIM No. 5135) were purchased from the Centre National Collection of Industrial Microorganisms (NCIM) located at National Chemical Laboratory (NCL), Pune, Maharashtra, India. The bacterial cultures were revived on nutrient broth liquid medium (pH 6.5) at 32°C for the period of 48 hrs.; whereas, fungal cultures were grown on PDA nutrient medium. The revived strains of bacteria and fungi were then cultured on sterilized nutrient culture plates and maintained under controlled conditions for further studies.

Preparation and Mass Multiplication of *Azolla* Assisted Microbial Consortia

The standard microbial consortium was prepared using the combination of nitrogen fixing bacterial strains and lignin, cellulose and hemicellulose decomposing fungal strains. The compatibility of the bacterial strains was assessed using gram staining technique. The bacterial strains (PSB, KMB, *Rhizobium*, *Azotobacter*, *B. maccrance*, and *P. fluorescence*) and fungal strains (*T. viride*, and *A. brasiliense*) procured from NCIM, NCL, Pune along with the fungal strains [*A. flavus* (NFCCI 5527), *A. niger* (NFCCI 5528, 5529), *T. harzianum* (NFCCI 5530), *T. atroviride* (NFCCI 5532)] isolated from the *Azolla* assisted matured compost (from the bed of ST + *Azolla*), identified and deposited at NFCCI, Agharkar Research Institute, Pune, Maharashtra, India were grown independently under optimum growth conditions. The liquid broth nutrient cultures were established for all the strains of bacteria and fungi. Aliquots (1 ml) from each liquid broth nutrient cultures were taken and mixed together in the conical flask containing one litre liquid broth nutrient medium. The culture flask was incubated on arbitrary shaker at 150 rpm and 30°C for 3-5 days. This has been used as mother culture for mass multiplication of microbial consortium.

Mass multiplication of *Azolla* assisted microbial consortium was prepared in 200 lit plastic containers by adding 1% jagary and mother culture (1ml lit⁻¹) with microbial count in the range of 10⁻⁸ to 10⁻¹² cfu ml⁻¹. The aeration was maintained by stirring the medium once in a day. The fermentation process was carried out for five days at room temperature. The growth of the fungal and bacterial strains was assessed for their compatibility. The freshly prepared consortia were used to initiate the process of agricultural waste composting at large scale on open field.

Study Area for Open Field Experiment

The experiment was conducted at the village, Wadgaonpir (Lat." 18.8795283 and Long." 74.078536) located in Shirur Tehsil of the Pune District, Maharashtra India (Fig. 1). The village has a total area of 1008.1 hectares; out of which, 735.12 hectares is under agriculture.

Survey

The study area comes under dry and low rainfall regions of Pune district (Fig. 1). Farmers at study area grows several

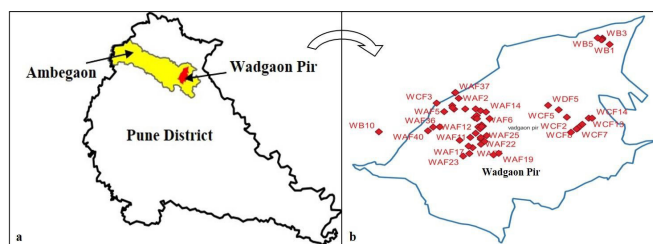


Fig. 1: Map of Pune District showing (a) - site location of Wadgaonpir at Ambegaon Tehsil and (b) - composting units sites of 70 farmers distributed in four blocks (WAF, WBF, WCF, and WDF).

types of crops depending on the type of soil, climate, weather conditions, and availability of water sources. Preferably, onion, bajra, wheat, potato, leafy vegetables, maize, beans, and sugarcane are cultivated by the farmers at study area. The agricultural waste produced after the harvesting of the crops are burnt by the farmers due to inadequate facilities of waste decomposition. The qualitative analysis of the soil for identifying the range of soil organic carbon (SOC) from the different agricultural fields of study area was carried out by following the standard protocol furnished in the soil testing Kit manufactured by Prerna Pvt. Ltd, Hinjewadi, Pune, Maharashtra, India.

Sites Selection

Wadgaonpir village is located in rain-fed area, and have limited sources of irrigation facilities for the agriculture. Total 70 farmers have been identified from study area, depending on their awareness about agriculture waste management through decomposition and their interest towards sustainable agricultural development. These farmers have shown their interest to conduct the waste composting experiment on open area at their field. These farmers have been grouped into four blocks on the basis of their residence and location of agricultural field at Wadgaonpir village. It includes Block 1- Pokharkar Mala-1 (WAF -Farmers from Wadgaonpir Block-A), Block 2 - Pokharkar Mala-2 (WBF -Farmers from Wadgaonpir Block-B), Block 3 - Patil Mala (WAF-Farmers from Wadgaonpir Block-C), and Block 4 - Gavthan (WDF – Farmers from Wadgaonpir Gavthan area Block-D). The block WAF comprise of 40 farmers, block WBF – 11 farmers, block WCF – 14 farmers and block WDF – 05 farmers (Fig. 1).

Preparation of Composting Bed at Study Sites on Open Field

First and Second Cycle

The agricultural waste (bajra husk, wheat straw, dry and waste fodder, sugarcane trash, onion leaves, waste generated from chickpea, mung bean, dried leaves and mixed waste) from the fields of 70 farmers were identified and collected at their own field to establish the composting bed. Approximately 01 tone agricultural waste was taken to develop the composting bed of 10 x 6 x 3 ft (Fig. 2). The waste was thoroughly mixed with cattle dung slurry (as a source of nitrogen) at the ratio in the range of 1:1 to 4:1 on dry weight basis. The moisture level of the bed was maintained to 50% by spraying the water at regular interval with the help of water sprinkler. After 06

hours of bed formation, the composting bed of ~01 tone has been treated uniformly with the 200 litres (10^8 to 10^{10} cfu^{-ml}) of *Azolla* assisted microbial consortia by applying 40 litres of volume at each application, at the interval of 07 days for the period of 45 days. The bed preparation at open field has been illustrated in the Fig. 2. The moisture was maintained by covering the beds with green shed net. The beds were allowed for composting under natural conditions of light and temperature. The observations were recorded for the effective composting process with reference to reduction in bed size, change in colour and texture of the composting mixture, organic carbon level, and C:N ratio. At the end of the experiment, organic carbon level of the compost from each bed was evaluated by following the protocol of Walkley Black method.

In the second cycle, as per the availability of agricultural waste, water for irrigation and cattle dung resources with the farmers as well as the interest of the farmers and available climatic conditions, the process of agricultural waste decomposition on open field of the farmers have been repeated and analyses were performed as mentioned earlier in the first cycle.

At the end of first and second cycle of composting process, the matured compost was used in the farmer's fields to check its efficiency towards improvement of SOC level and in general the productivity of the standing crop. Therefore, before application of matured compost to the field, the SOC estimation was carried out using the soil testing Kit of Prerna Pvt. Ltd, Hinjewadi, Pune, Maharashtra, India.

RESULTS AND DISCUSSION

In the current scenario, the overgrowth of human population associated with the drastically severe problems of environmental pollution has desperately affected the soil health, worldwide (Elbasiouny *et al.*, 2022). The inadequate water holding capacity of the soil, unavailability of essential nutrients as against the encroachment of toxic heavy metals and salinity problems to severe extent, use of polluted irrigations resources has led to abolish the fertile humus layer of the agricultural soil. Besides, unavailability of useful soil organic carbon at optimum level due to imbalanced ratio of C:N in the agricultural waste has been found negative impact on productivity of standing crops of economic interest. Composting is a natural process, which is hasten with the specific requirement of available composting material in the form of carbonaceous and nitrogenous waste (Waqas *et al.*, 2023). However, the carbonaceous waste used most often includes bajra husk, wheat straw, dry and waste fodder, sugarcane trash, onion leaves, waste generated from chickpea, mung bean, soybean, dried leaves and mixed waste having high lignin content makes the process of decomposition more recalcitrant. It is further made complex, time consuming and incompatible due to absence of proper source of biological nitrogen at an optimum proportion. In this context, present investigation gives emphasis on search for the nitrogen rich biomass like *Azolla* as a source for developing the potent microbial consortia for an effective decomposition of complexed agricultural waste by maintaining the optimum

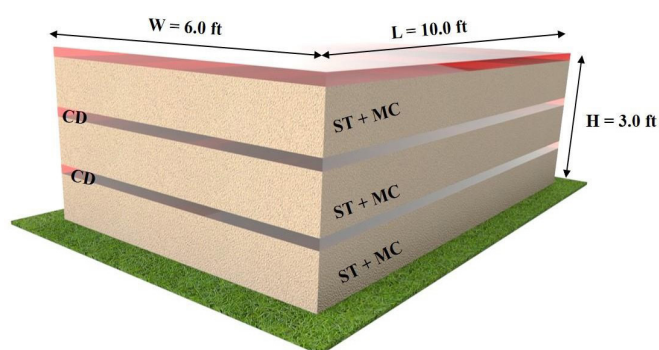


Fig. 2: Composting bed of size 10 x 6 x 3 ft. prepared on open field at study area consist of multiple layers of sugarcane trash (ST) inoculated with spraying of microbial consortia (MC) at the concentration of 40 lit bed⁻¹ at the interval of 07 days for 45 days.

C:N ratio. Further, the application of matured compost produced after the rapid process of composting to the SOC deficient agricultural fields at the doorstep of farmers on open field conditions provides solution for sustainable improvement of soil health by enhancing the SOC level to achievable point.

Effect of Moisture and Temperature on Composting Process

The optimum metabolic activities of the microorganisms during the decomposition process of organic waste have been correlated with the fine tuning of temperature and moisture conditions (Abatenh *et al.*, 2017). It is further correlated with the diversity of the microorganisms, nutrients availability, aeration, source of organic waste and climatic conditions. Moisture content in the range of 50 to 60% with the temperature range between 40 to 60°C have been found optimal for the rapid decomposition of organic waste. The drastic reduction in moisture level below 40% shows significant drop down in the process of composting; however, the rise in moisture level above 60% generates eutrophic condition due to uncontrolled death of microorganisms (Richard *et al.*, 2002). Therefore, in the present investigation, the moisture percent regulated at 50% have shown significant outcome for decomposition of agricultural waste among all the beds (ST + CD; ST + *Azolla*; ST + Urea and ST + PW). The temperature has played major role in composting process (Fig. 3). In the earlier stages, the bed temperature remains constant due to lag phase of microbial activity; however, with increase in incubation period and acclimatization of bacterial and fungal organisms to the available conditions, rate of metabolic processes enhanced due to activity of mesophilic and thermophilic microorganisms. The significant increase in temperature (48°C) was recorded in the bed composed of ST + *Azolla* in comparison to the rest of the treatments (Fig. 3). Significant rise in temperature was recorded at 15 days of composting process and thereafter, drastic reduction was recorded (Fig. 3). In the stationary phase of composting process, the temperature was found in the range of 30 to 34°C (Fig. 3). Rise in temperature of composting bed to the drastic level (48°C) has been correlated with the aerobic activity of mesospheric microorganisms in the compost, which might have accelerated the degradation of proteins and non-

cellulose carbohydrate components of the compost. The lipid and hemicellulose fractions may also be attacked by these bacteria and fungi, although cellulose and lignin seem to be able to feed them off. Our results are in concurrent with the earlier workers (Goyal *et al.*, 2005; Chander *et al.*, 2018) for change in temperature during the composting process in presence of water hyacinth and sugarcane trash. The rapid decomposition of compost during the initial 15 days with significant increase in temperature associated with the higher microbial activity was also reported by Chander *et al.*, (2018). The higher levels of temperature during the composting process helps to destroy the thermos-sensitive pathogens making the composting process effective (Dantoliya *et al.*, 2022).

Effect of Composting Process on C:N Ratio

The composting of sugarcane trash (as a source of carbon) in combination with different sources of nitrogen (CD, *Azolla*, Urea, and PW) have been monitored for 45 days of duration. The crucial stage in rapid composting of agriculture residue was correlated with the optimum C:N ratio, since carbon is utilized as energy source along with the nitrogen for formation of new cells, cellular integrity and multiplication of composting microorganisms (Azis *et al.*, 2023). The imbalance in the C:N ratio results into slowing down the composting process or developing the non-conducive environment for the growth of microorganisms (Palaniveloo *et al.*, 2020). Therefore, in the present investigation, at the beginning of composting process, the C:N ratio of various composting beds was regulated in the range of 40:1 to 50:1 to accelerate the composting process in a superior way. The ST without supplementation of any kind of nitrogen source (control) has not shown any decomposition and thereby resulting into significantly slower rate of organic carbon formation with no change in C:N ratio (Table 1). It has found that, ST alone (control) used for composting has not able

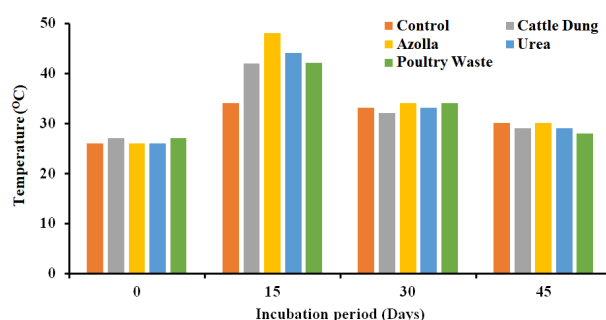


Fig. 3: Changes in temperature during composting of agro-waste maintained with C:N ratio.

to produce the matured compost due to recalcitrant nature of complex carbon content in the form of lignin, cellulose and hemicellulose. It has been correlated with significantly the highest content of the C, as against the unavailability of N in the compost as well as making the microbial decomposition unfavorable and slower in presence of naturally occurring non-composting microorganisms. However, the carbon source (ST) combined with the nitrogen source (*Azolla*) have shown significantly the higher rate of composting process of complexed carbon material of ST due to the highest metabolic activities of favorable composting microorganism in comparison to incorporation of other nitrogen sources (CD, Urea, and PW) in the beds (Table 1 and 2). It has been correlated with the significant reduction in total organic carbon level along with the drastic increase in nitrogen content of the compost, thus reduction in the C:N ratio with time to optimal level which was stabilized in the range of 9 to 10 (Table 1). This was further justified by the rise in temperature during the composting of ST + *Azolla* mixture (Fig. 3). The C:N ratio 10:1 to 15:1 has been found a significant sign of mature compost which is also found in vermicompost (Yadav and Garg, 2011). The rapid reduction in C:N ratio was found

Table 1: Change in carbon, nitrogen and C:N ratio at different days (0 to 45) during composting of sugarcane trash (carbon source) in combination with different nitrogen sources (Cattle Dung, *Azolla*, Urea, and Poultry Waste).

Treatments (Source of Carbon : Nitrogen)	Temporal change in											
	Organic carbon content (%)				Nitrogen content (%)				C:N Ratio			
Duration (days)	0	15	30	45	0	15	30	45	0	15	30	45
Sugarcane trash (ST; Control)	51.5	47.0	45.0	44.0	0.00	0.00	0.00	0.00	51.5	47.00	45.00	44.00
Sugarcane trash (ST) + Cattle dung (CD)	48.5	46.0	42.0	41.3	0.90	1.22	1.42	1.46	53.89	37.70	29.58	28.29
Sugarcane trash (ST) + <i>Azolla</i>	41.7	38.0	31.0	28.1	0.90	0.90	2.20	3.00	46.33	42.22	14.09	9.37
Sugarcane trash (ST) + Urea	43.2	39.5	37.0	36.0	1.00	0.92	1.60	1.80	43.20	42.93	23.13	20.00
Sugarcane trash (ST) + Poultry waste (PW)	43.9	38.5	32	34.2	1.00	0.8	1.40	2.40	43.90	48.13	22.86	14.25

Table 2: Growth pattern of bacterial and fungal population with respect to time (days) during composting of waste maintained with C:N ratio.

Treatments	Bacterial population (*10 ⁷ g ⁻¹ material)				Fungal population (*10 ⁸ g ⁻¹ material)			
	0	15	30	45	0	15	30	45
Sugarcane trash (ST; Control)	1 ^d	8 ^e	20 ^d	24 ^e	0.4 ^d	10 ^d	12 ^c	10 ^d
Sugarcane trash (ST) + Cattle dung (CD)	10 ^b	28 ^b	49 ^c	36 ^b	8 ^b	17 ^b	25 ^c	15 ^c
Sugarcane trash (ST) + <i>Azolla</i>	15 ^a	45 ^a	66 ^a	54 ^a	10 ^a	32 ^a	34 ^a	23 ^a
Sugarcane trash (ST) + Urea	5 ^c	18 ^c	52 ^b	30 ^c	4 ^c	18 ^b	34 ^a	21 ^b
Sugarcane trash (ST) + Poultry waste (PW)	5.5 ^c	14 ^d	54 ^b	27 ^d	5 ^c	12 ^c	30 ^b	21 ^b

associated with significantly the highest activity of carbon consumption by the favorable composting microorganisms from the available organic matter and its release in the form of CO₂ during the process of respiration (Goyal *et al.*, 2005). Symbiotically associated nitrogen fixing bacteria available on the root surface as well as the highest amount of moisture content (more than 90%) of the *Azolla* made it suitable for retaining the conducive environment for microbial decomposition of ST, and therefore, found responsible for the highest rate of composting with the significant reduction in C:N ratio to the optimal level (9:1) as shown in Table 1. The decreased C and increased N content along with significant reduction of C:N ratio has been reported by many workers as an indicator of successful formation of mature compost while working on composting of different types of agricultural waste in combination with the variety of nitrogen sources (Goyal *et al.*, 2005; Goyal and Sindhu, 2011; Yadav and Garg, 2011; Game *et al.*, 2017; Patil *et al.*, 2021).

The C:N ratio of the matured compost, after 45 days composting of various organic wastes (ST + CD, ST + *Azolla*, ST + Urea, and ST + PW) was ranged between 9.36 to 28.28 in comparison to control (ST), which was found significantly lowest in the case of *Azolla* and the highest in ST + CD (Table 1). From the commercial point of view, minimum time (in days) required for composting process and formation of matured compost with the optimum C:N ratio (10:1 to 15:1) and suitable texture has found greater significance in taking the multiple cycles or number of batches of composting per annum, which could add the benefit to the farmer for effective productivity with higher income. In the present investigation, surprisingly, we could able to reduce the duration of composting process (up to 30 days) for the production of matured compost (with C:N ratio ~ 14:1) with the help of combination of ST and *Azolla* in comparison to rest of the treatments – ST, ST + CD, ST + Urea, and ST + PW (Table 1). Further continuation of composting process up to 45 days yielded formation of high-quality product (matured compost) with the ideal properties (C:N ratio – 10:1, optimum water holding capacity, and superior texture – crumbly black colour) in comparison to other treatments. It has been observed that, the incorporation of Urea in the composting process leads to the partial loss of N in the gaseous form and partially flush out due to its solubility in the water. Similarly, the sources of nitrogen in the form of PW and CD have shown reduced levels of C:N ratio but inferior to *Azolla*, which might be due to absence of naturally growing nitrogen fixing bacteria, as well as use of improperly composted CD and PW. Therefore, the efficacy of *Azolla*, as a source of nitrogen has been found superior as over the other sources such as CD, Urea and PW, suggesting its direct implications for rapid composting of complexed agricultural waste on large scale. To the best of our knowledge, for the first time *Azolla* has been utilized as a direct source of nitrogen by incorporating it with the agro-waste for accelerating the composting process as over the common use of CD.

Microbial Growth During the Composting Process

The maintenance of optimum C:N ratio in the composting bed determines the growth of beneficial microorganisms (bacteria and fungi) for the rapid destruction of complex organic compounds of agricultural organic waste. The

consortium of mesophilic and thermophilic bacteria as well as fungal organisms plays an important role in the process of composting which also affects the quality of mature compost (Ishii *et al.*, 2000; Ryckeboer *et al.*, 2003; Goyal *et al.*, 2005). In the present investigation, the emphasis was given on the determination of overall growth of bacterial and fungal population in response to the combination of carbon source (ST) with the variety of nitrogen sources (CD, *Azolla*, Urea, and PW) in the composting beds. The population of the composting organisms (bacteria and fungi) was found significantly increased with increase in the incubation period up to 45 days (Table 2). The microbial count was significantly higher in the logarithmic phase as observed on 30th day of incubation and found declined with increase in incubation period up to 45 days, which has been recognized as stationary phase (Table 2). The significant increase in microbial count during the process of composting has also been reported during the composting of different types of organic carbon rich compost with the addition of variable sources of nitrogen (Goyal *et al.*, 2005; Chander *et al.*, 2018; Patil *et al.*, 2021; Dash *et al.*, 2022). Among the variable sources of nitrogen (CD, *Azolla*, Urea, and PW) incorporated in the composting beds, in combination with the carbon source (ST), the bed comprised of ST + *Azolla* has shown significantly the highest count of bacteria and fungi (66×10^7 and 34×10^8 g⁻¹ material, respectively) in comparison to other sources of nitrogen (CD, Urea, and PW) (Table 2). However, due to availability of recalcitrant form of carbon source in the ST without presence of nitrogen source (control) has revealed significantly the lower count of microorganisms (Table 2). CD, which has been widely utilized as a richest source of natural nitrogen has found higher count of microorganisms next to the *Azolla*, which could be utilized in the composting process under the circumstances of *Azolla* unavailability. Our study claims the superiority of *Azolla* incorporation over the use of CD as a nitrogen source along with the varied sources of carbon during the composting process. Most of the times, it has been observed that, the use of CD as a nitrogen source has generated difficulties in the process of composting due to incorporation of poorly composted or sometimes use of fresh or over-degraded CD. However, *Azolla* has found to be the cheapest, beneficial microflora rich and easily available source of nitrogen with the efficiency of rapid multiplication within a short period of time (3-5 days of doubling rate in ideal conditions), significantly the highest amount of moisture (90-95%) which favours the moisture retaining capacity of the degrading agro-waste during composting process and provides the higher water holding capacity to the processed or matured compost. To the best of our knowledge, *Azolla* has widely explored as a source of biofertilizer and nitrogen in the open paddy fields as substitute to the urea (Thapa and Poudel, 2021). The present investigation recommends the effective multiplication of *Azolla* and its incorporation in the composting bed to accelerate the composting process by providing the natural microbial consortium, optimum moisture content and production of matured compost with significantly the lowest C:N ratio and having optimum water holding capacity. The application of such compost to the crop plants will help to enhance the productivity to the significant level.

Effect of Azolla Assisted Microbial Consortia on Composting of Agriculture Waste on Open Field

On the basis of compatibility of bacterial strains (PSB, KMB, *Rhizobium*, *Azotobacter*, *B. maccrance*, and *P. fluorescence*) with the fungal strains (*T. viride*, *A. brasiliense*, *A. flavus*, *A. niger*, *T. harzianum*, *T. atroviride*), the mass multiplication of the *Azolla* assisted microbial consortia was carried out which revealed optimum growth when grown under optimum conditions by providing the jaggery as a source of carbon and energy. Since the large-scale composting of agricultural waste on open field requires huge quantity of *Azolla* biomass with its continuous supply, it was recognized as limiting factor for large scale composting process on open field. Therefore, in the current investigation, instead of direct incorporation of *Azolla* biomass, as a source of nitrogen in the composting bed, the microbial strains (fungi) isolated from matured compost of *Azolla* fortified sugarcane trash bed and combined with the inoculum of different bacterial cultures were used to develop the *Azolla* assisted microbial consortia. It has resulted into rapid mass multiplication of consortia within short period of time and its use as biological source for rapid composting of agricultural waste. The growth of the *Azolla* assisted microbial consortia as well as composting process has found accelerated with the incorporation of cattle dung as a base for the inoculum of the consortia. It has been observed that, the combination of *Azolla* assisted microbial consortia with the cattle dung slurry was found responsible to boost up the growth of the microorganisms in the consortium, which has been found beneficial for the acceleration of composting process.

In the first cycle, 70 farmers from the four blocks (WFA, WFB, WFC, and WFD) of study area have responded to investigate the effect of *Azolla* assisted microbial consortia on composting process of agricultural waste. Out of the 70 farmers from these four blocks, 40 farmers have effectively completed the process of composting for their agricultural waste in presence of *Azolla* assisted microbial consortia. Further the matured compost obtained at the end of composting was incorporated in the fields of farmer. However, in the second cycle, as per the availability of agricultural waste, water for irrigation and cattle dung resources with the farmers and climatic conditions, out of the 40 farmers, 15 farmers from the 4 blocks have responded successfully for performing the process of agricultural waste decomposition at their field. At the end of first and second cycle of decomposition, the significant reduction in bed size from 10 x 6 x 3 ft. to 10 x 4 x 0.5 ft., crumbly texture, dark brown or black appearance of the compost with moisture level 30-35%, temperature in the range of 28-30°C and pH of the compost 6.5 to 7.5 have been recorded and considered as the criteria for complete decomposition of agricultural waste. The C:N ratio of the matured compost was recorded to be 20:1, which was in concurrent with the optimum range (between 15:1 to 20:1) of the matured compost of other organic wastes as reported earlier (Patil *et al.*, 2021).

In the present investigation, to explore the efficacy of *Azolla* assisted microbial consortia fortification with the complexed agricultural waste for its rapid decomposition, the foremost criterion of change in soil organic carbon (SOC) level has been monitored. For this, the matured compost obtained from the composting process was applied by the farmers to the standing

Table 3: Effect of microbial consortia fortified agro-waste compost on percent increase in soil organic carbon level at different composting sites.

Composting treatment Sites	Level of Soil Organic Carbon (%)			Percent increase in Soil Organic Carbon after composting treatment	
	Composting treatment				
	Before	After		1 st cycle	11 nd cycle
		1 st cycle	11 nd cycle	1 st cycle	11 nd cycle
WAF20	0.29	0.4	0.6	11	31
WAF21	0.71	0.78	0.91	7	20
WAF35	0.2	0.26	0.42	6	22
WAF36	0.7	0.8	0.82	10	12
WAF37	0.72	0.82	0.82	10	10
WAF39	0.4	0.6	0.72	20	32
WAF40	0.4	0.82	0.89	42	49
WCF3	0.4	0.59	0.72	19	32
WAF10	0.81	0.9	0.92	9	11
WAF6	0.67	0.76	0.85	9	18
WAF7	0.39	0.42	0.63	3	24
WBF10	0.92	0.94	1.2	2	28
WAF9	0.84	0.9	0.98	6	14
WBF11	0.51	0.82	0.92	31	41
WBF9	0.62	0.82	0.95	20	33
Average				13.67	25.13

crops (wheat, bajra, corn, onion, brinjal, tomato, cabbage, cauliflower, etc.) on open field, which have shown significant improvement in SOC level after the first and second cycle of matured compost applications.

Initially, before the application of matured compost to the field, the average SOC level of the study area was recorded as 0.57% (in the range of 0.29 to 0.92%). This has been correlated with the diversity observed in the type and texture of the soil, water holding capacity, pH of the soil, naturally available microbial flora, soil nutritional status and cropping pattern in the fields of the study area. The recorded levels of SOC in the study area have been found significantly lower in comparison to the optimum level, which should be greater than 1.0% (Liu *et al.*, 2006). It has been proved that, optimum levels of SOC in the agricultural field plays a crucial role in maintaining the soil health parameters as well as growth and productivity of the field crops. In the present investigation, application of the *Azolla* assisted microbial consortia fortified matured compost for two successive cycles has significantly improved the level of SOC of the fields at study area (Table 3). At the end of the first cycle, the average increase in SOC was observed from 0.57% to 0.70% which was in the range of 9 to 42% with the drastic increase in organic carbon level, after the treatment of matured compost application (Table 3). Interestingly, application of matured compost successively in the second cycle has astonishingly improved the average level of SOC from 0.57% to 0.83%. Overall, the significant increase in the level of SOC in the first and second cycle of matured compost application was 13.67% and 25.13%, respectively. Since, the *Azolla* assisted microbial consortia fortified matured compost application to the field have not been studied before by any other workers, the outcome of present investigation suggests the effective use of *Azolla* assisted microbial consortia for the improvement of SOC level of deficient agricultural soils. The improvement in the crop growth, productivity, soil health parameters could be achieved with the use of such kind of microbial consortia for sustainable development in a holistic manner.

CONCLUSION

Present investigation provided *Azolla*, as an operative source of nitrogen for development of effective microbial consortia and its use in rapid composting process of diverse agricultural waste within a short period of time (30-45 days). *Azolla* has shown its efficacy over the CD, Urea, PW as a source of nitrogen for maintaining the optimum ratio of C:N during the composting process. Further, the matured compost obtained from *Azolla* fortified agricultural waste could act as a source of beneficial microorganisms to prepare modified consortia with the supplementation of choice based bacterial strains, on the basis of their compatibility with the fungal organisms. Besides, the study provides the easy way of mass multiplication of *Azolla* assisted consortia with the use of jagarri solution, as a carbon and energy source. This actively growing specially designed microbial inoculum has rapidly hastened the large-scale composting process of diversified agro-waste with the addition of CD, on open field. The success of the investigation laid into the significant SOC improvement of SOC deficient agricultural soil through the successive

application of matured compost in the standing crops on the field of the farmers from study area. The improvement in SOC of the soil almost by 25% has found as the big achievement towards sustainable development and gives solution to the management of agricultural waste in an effective manner.

ACKNOWLEDGMENTS

The senior author is thankful to Vigyan Ashram, Pabal, Shirur, Pune, Maharashtra, India and Department of Botany, Savitribai Phule Pune, University, Pune for their infrastructural support during the project tenure.

AUTHOR CONTRIBUTIONS

S.J.S., M.Y.B., V.H.L., A.D.D., R.B.S., and Y.K. designed and supervised the experiments.

S.J.S. performed the laboratory and field work. S.J.S. and V.H.L. analysed the results and drafted the manuscript.

FUNDING

The senior author is grateful to Department of Science and Technology (DST), Ministry of Science and Technology and Earth Science, New Delhi for providing the funding in the form of Women Scientist -B (WOS-B) fellowship under the scheme of KIRAN (Knowledge Involvement in Research Advancement through Nurturing) for the project (Project File No - DST/WOS-B/2018/1311) entitled 'Recycling of agricultural residue using microbial consortia for organic farming'.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

LIST OF ABBREVIATIONS WITH FULL FORM

SOC- Soil Organic Carbon
 TOC - Total Organic Carbon
 SOP- Standard Operating Procedure
 WHC - Water Holding Capacity
 N- nitrogen
 P- Phosphorus
 K - Potassium
 WF - Wadgaonpir farmer
 WAF- Wadgaonpir - Pokharkar Mala 1
 WBF- Wadgaonpir - Pokharkar Mala 2
 WCF- Wadgaonpir -Patil Mala
 WDF- Wadgaonpir- Gavthan
 CD-Cattle Dung
 ST- Sugarcane Trash
 PW- Poultry Waste
 C: N- Carbon and Nitrogen ratio
 SOP- Standard Operating Procedure
 BGA- Blue Green Algae
 PSB-Phosphate Solubilizing Bacteria
 KMB- Potassium Solubilising Bacteria
 PDA- Potato Dextrose Agar
 NA- Nutrient Agar
 CFU- Colony Forming Unit
 NCIM - National Collection of Industrial Microorganisms
 NCL -National Chemical Laboratory

REFERENCES

- Abatenh, E., Gizaw, B., Tsegaye, Z., Wassie, M. (2017). The Role of Microorganisms in Bioremediation- A Review. *Open Journal Environmental Biology* 2(1), 38–46. <https://doi.org/10.17352/ojeb>.
- Andini, A., Bonnet, S., Rousset, P., Hasanudin, U. (2018). Impact of open burning of crop residues on air pollution and climate change in Indonesia. *Current Science* 115, 2259–2266. <https://www.jstor.org/stable/26978589>
- Azis, F.A., Rijal, M., Suhaimi, H., Abas, P.E. (2022). Patent Landscape of Composting Technology: A Review. *Inventions*, 7, 38. <https://doi.org/10.3390/inventions7020038>
- Bhattacharyya, S., Sahu, A., Manna, M.C., Patra, A.K. (2019). Potential of surplus crop residues, horticultural waste and animal excreta as a nutrient source in the central and western regions of India. *Current Science* 116, 1314–1323. <https://www.jstor.org/stable/27138035>
- Bhattacharyya, S., Sahu, A., Phalke, D. H., Manna, M. C., Thakur, J. K., Mandal, A., Tripathi, A. K., Sheoran, P., Choudhary, M., Bhowmick, A., Rahman, M. M., Naidu, R., Patra, A.K. (2021). In situ decomposition of crop residues using lignocellulolytic microbial consortia: a viable alternative to residue burning. *Environmental Science and Pollution Research* 28(25), 32416–32433. <https://doi.org/10.1007/s11356-021-12611-8>
- Bremner, J.M. (1970). Total organic carbon in methods of soil analysis Part-2. Chemical and microbiological properties. Page, A.L (ed). II Edn. Amer. Soc. Agron. Inc. and Soil. Sci. Amer. Inc. Madison, Wisconsin, USA, pp. 475–594.
- Chander, G., Wani, S.P., Gopalakrishnan, S., Mahapatra, A., Chaudhury, S., Pawar, C.S., Kaushal, M., Rao, A.V.R.K. (2018). Microbial consortium culture and vermi-composting technologies for recycling on-farm wastes and food production. *International Journal of Recycling of Organic Waste in Agriculture* 7(2), 99–108. <https://doi.org/10.1007/s40093-018-0195-9>.
- Dantoliya, S., Joshi, C., Mohapatra, A., Shah, D., Bhargava, P., Bhanushali, S., Pandit, R., Joshi, C., Joshi, M. (2022). Creating wealth from waste: An approach for converting organic waste in to value-added products using microbial consortia. *Environmental Technology and Innovation*, 25. <https://doi.org/10.1016/j.eti.2021.102092>.
- Dash, P.K., Padhy, S.R., Bhattacharyya, P., Pattanayak, A., Routray, S., Panneerselvam, P., Nayak, A.K., Pathak, H. (2022). Efficient Lignin Decomposing Microbial Consortium to Hasten Rice-Straw Composting with Moderate GHGs Fluxes. *Waste and Biomass Valorisation* 13(1), 481–496. <https://doi.org/10.1007/s12649-021-01508-9>
- Edward, K., Johnstone, C., Thompson, C. (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acid Research* 19(6), 1349. <https://doi.org/10.1093/nar/19.6.1349>
- Elbasiouny, H., El-Ramady, H., Elbehiry, F., Rajput, V.D., Minkina, T., Mandzhieva, S. (2022). Plant Nutrition under Climate Change and Soil Carbon Sequestration. In *Sustainability (Switzerland)* (Vol.14, Issue 2). MDPI. <https://doi.org/10.3390/su14020914>.
- Gadde, B., Bonnet, S., Menke, C., Garivait, S. (2009). Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. *Environmental Pollution* 157(5), 1554–1558. <https://doi.org/10.1016/j.envpol.2009.01.004>.
- Game, B.C., Deokar, C.D., More, P.E. (2017). Efficacy of Newly Developed Microbial Consortium for Composting of Rural and Urban Wastes. *International Journal of Current Microbiology and Applied Sciences* 6(6), 626–633. <https://doi.org/10.20546/ijcmas.2017.606.074>.
- Gardes, M., Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes- application to the identification of mycorrhizae and rusts. In *Molecular Ecology*, (Vol. 2). <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Goswami, S.B., Mondal, R., Mandi, S.K. (2019). Crop residue management options in rice- rice system: a review. *Archive of agronomy and Soil Science* 66, 1218–1234. <https://doi.org/10.1080/03650340.2019.1661994>
- Goyal, S. Sindhu, S.S. (2011). Composting of rice straw using different inocula and analysis of compost quality. *Microbiology Journal* 1, 126–138. <https://doi.org/10.3923/mj.2011.126.138>
- Goyal, S., Dhull, S. K., Kapoor, K. K. (2005). Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. *Bioresource Technology* 96(14), 1584–1591. <https://doi.org/10.1016/j.biortech.2004.12.012>
- Gupta, P.K., Sahai, S., Singh, N., Dixit, C.K., Singh, D.P., Sharma, C. (2004). Residue burning in rice-wheat cropping system: causes and implications. *Current Science* 87, 1713–1715. <https://www.jstor.org/stable/24109770>
- Hosseini, S.M., Aziz, H.A. (2013). Evaluation of thermochemical pretreatment and continuous thermophilic condition in rice straw composting process enhancement. *Bioresources Technology* 133, 240–247. <https://doi.org/10.1016/j.biortech.2013.01.098>
- Ishii, K., Fukui, M., Takii, S. (2000). Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. *Journal of Applied Microbiology* 89, 768–777. <https://doi.org/10.1046/j.1365-2672.2000.01177.x>
- Kumari, P., Chaudhary, S., Dhanker, R., Verma, N., Goyal, S. (2018). Assessment of quality of compost prepared from paddy straw and distillery effluent. *Chemical Science Review and Letters* 7(25), 222–227. CS042049031
- Liu, X., Herbert, S.J., Hashemi, A.M., Zhang, X., Ding, G. (2006) Effects of agricultural management on soil organic matter and carbon transformation – a review. *Plant soil environ.*, 52, 2006 (12): 531–543. <https://doi.org/10.17221/3544-PSE>
- Nagamani, A., Kunwar, I.K., Manoharachary, C. (2006). *Hand book of Soil Fungi*. I.K. International New Delhi, pp. 477.
- Nelson, D.W., Sommers, L.E. (1982). Carbon and organic carbon and organic matter. In: Page, Miller, A.L., Keeney, R.H., D.R. (Eds.), *Method of Soil Analysis*. American Society of Agronomy, Madison, pp. 539–574. <https://doi.org/10.2136/sssabookser5.3.c34>
- Palaniveloo, K., Amran, M.A., Norhashim, N.A., Mohamad-Fauzi, N., Peng-Hui, F., Hui-Wen, L., Kai-Lin, Y., Jiale, L., Chian-Yee, M.G., Jing-Yi, L., Gunasekaran, B., Razak, S.A. (2020). Food waste composting and microbial community structure profiling. In *Processes* (Vol. 8, Issue 6, pp. 1–30). MDPI AG. <https://doi.org/10.3390/pr8060723>.
- Patil, S.A., Navale, M., Deokar, D., Patil, A. (2021). Development and assessment of microbial consortium for composting of organic waste. *Journal of Pharmacognosy and Phytochemistry* 10(2), 241–245.
- Richard, T., L. Hamelers., H., M, Veeken, A., Silva, T. (2002). Moisture relationships in composting processes. *Compost Science and Utilization* 10(4), 286–302. <https://doi.org/10.1080/1065657X.2002.10702093>.
- Ryckeboer, J., Mergaert, J., Coosemans, J., Deprijs, K., Swings, J. (2003). Microbiological aspects of biowaste during composting in a monitored compost bin. *Journal of Applied Microbiology* 94(1), 127–37. <https://doi.org/10.1046/j.1365-2672.2003.01800.x>
- Sahu, A., Bhattacharjya, S., Atoliya, N., Manna, M.C., Patra, A.K. (2018). Rapid and effective method for exploring cellulase-producing potential of bacterial strains. *Environmental Ecology* 36, 828–834.
- Sahu, A., Bhattacharjya, S., Manna, M.C., Patra, A.K. (2015). Crop residue management: a potential source for plant nutrients. *JNKVV Research Journal* 49, 301–311.
- Sahu, A., Manna, M.C., Bhattacharjya, S., Thakur, J.K., Mandal, A., Rahman, M.M., Singh, U.B., Bhargav, V.K., Srivastava, S., Patra, A.K., Chaudhary, S.K., Khanna, S.S. (2019). Thermophilic ligno-cellulolytic fungi: the future of efficient and rapid bio-waste management. *Journal of Environmental Management* 244, 144–153. <https://doi.org/10.1016/j.jenvman.2019.04.015>
- Sarkar, P., Meghvanshi, M., Singh, R. (2011). Microbial Consortium: A new approach in effective degradation of organic kitchen wastes. *International Journal of Environmental Science and Development* 2(3), 67–71.
- Sarkar, S., Singh, R.P., Chauhan, A. (2018). Crop residue burning in northern India: increasing threat to Greater India. *Journal of Geophysical Research: Atmospheres*, 6922. <https://doi.org/10.1029/2018JD028428>
- Thapa, P., Poudel K., (2021). *Azolla*: Potential biofertilizer for increasing rice productivity, and government policy for implementation. *Journal of Wastes and Biomass Management* 3(2), 62–68. <http://doi.org/10.26480/jwbm.02.2021.62.68>
- Trada, N.N., Malam, K.V. (2020). *Azolla* (Aquatic Fern) as Bio Fertilizer (Eco-Friendly Agriculture). *Agriculture and Environment*, 008.

- Waqas, M., Hashim, S., Humphries, U.W., Ahmad, S., Noor, R., Shoaib, M., Naseem, A., Hlaing, P.T., Lin, H.A. (2023). Composting processes for agricultural waste management: A comprehensive review. In Processes (Vol. 11, Issue 3). MDPI. <https://doi.org/10.3390/pr11030731>.
- Yadav, A., Garg, V.K. (2011). Recycling of organic wastes by employing *Eisenia foetida*. Bioresource Technology 102(3), 2874–2880. <https://doi.org/10.1016/j.biortech.2010.10.083>
- Zhang, J., Zeng, G., Chen, Y., Yu, M., Yu, Z., Li, H., Yu, Y., Huang, H. (2011). Effects of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting. Bioresource Technology 102, 2950–2956. <https://doi.org/10.1016/j.biortech.2010.11.089>
- Zhao, B., Zhang, J., Yueyue, Y., Douglas K., Xiyin, H., (2016). Crop residue management and fertilization effects on soil organic matter and associated biological properties. Environmental Science and Pollution Research 23, 17581–17591. <https://doi.org/10.1007/s11356-016-6927-3>