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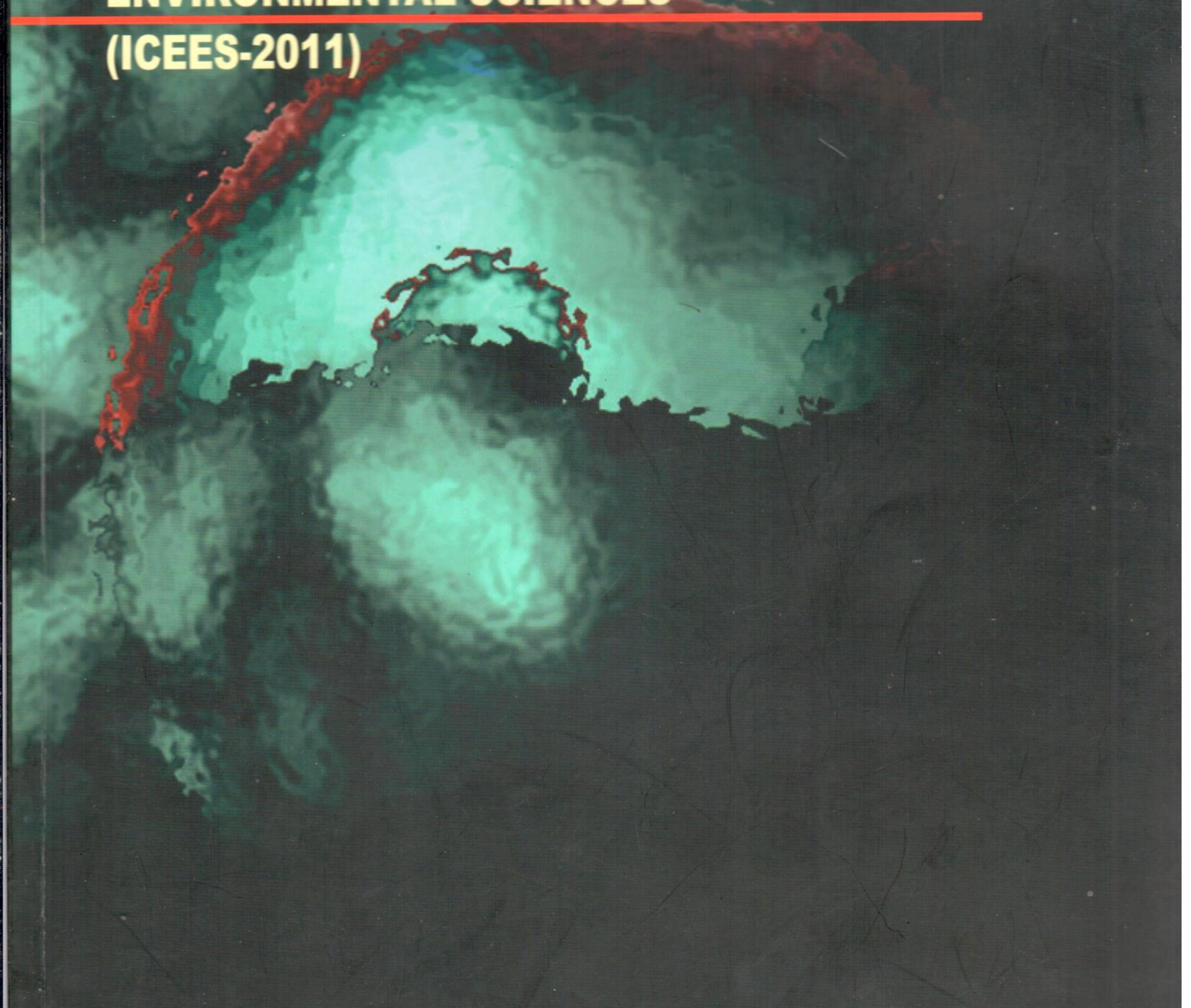

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Solid Waste Management Using Indigenous Microorganisms

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Introduction

Goa, a tiny state on the west coast of India with an area of 3702 sq kms and a population of 13.5 lakh holds a position in UNESCO's world heritage list, as a safe, serene and pleasant tourist destination. However, the influx of visiting tourists is totally disproportionate to the infrastructure development required to receive these tourists. Dealing with waste has become a serious problem and scientific waste management is the immediate need of the hour. Unfortunately, in past years, solid waste management in Goa has consisted mainly of illegal dumping. The Municipal Councils of Panaji and Marmugao do have schemes in operation, designed to bring their garbage processing in line with the MSW Rules 2000 issued under the environment protection act 1986, but are able to process only a small portion of their organic waste. The prerequisite of effective waste management is the availability of reliable information about the quantity and the composition of MSW .This would help in gauging the physical, chemical, and thermal properties of the waste as well as in estimating its material recovery potential and its suitability for composting. Solid waste generated due to domestic and industrial activity is highly variable in composition. The type of waste generated varies with lifestyle, demographics, geographic location and season, due to which its study becomes extremely difficult but all the more essential. If Goa wants to survive on tourism, then a special solid waste management strategy is a must. In the present study an attempt has been made to characterize the physical components of MSW, and a pilot study was conducted to assess the feasibility of composting of source separated organic matter of municipal solid waste (MSW) using indigenous microorganisms.

Materials and Methods

Physical Study

The research study was carried out at Goa University. A survey of MSW generated was carried out in Panaji within an area of 8.2 sq.kms. Sampling for the study was carried out over a period of 2 years, during June to September (Monsoon) and March to May (Summer). Grab samples of MSW were collected from 12 different locations for physical characterization, based on quartering method. For the temporary storage and transport of each sample to the laboratory, water-proof sealed bags were used (USEPA, 2001, 2002). The time interval between collection and analysis was 1–4 h. The representative samples were sorted out into separate fractions, weighted average was calculated and represented as % dry weight. For determination of moisture content about 500 gms of sample was placed in an electric oven at 100° C till dry. The sample was cooled in a dessicator and weighed. Loss in weight of sample (%) was reported as moisture content. Density was determined by packing the MSW in a 1x1x1 m^3 box after chopping to approximately 1/8th inch.

Chemical Study

For chemical analysis an extract of organic waste was prepared as follows.10 gms of sieved air-dried waste was mixed with 200 ml extractant (oxidizing agent i.e. $K_2S_2O_8$ in NaOH ,H_2SO_4, NH_4Cl ,distilled water and a few drops of bromothymol blue) and kept on a shaker for 1 hr. It was then filtered using Whatman filter paper no 44 .The first few drops were rejected and the rest was acidified with acetic acid to pH 4.8. Ca, Mg, Zn, Mn , P , NO_3-N,Fe and K were estimated using the extract. (Iswaran, V., 1980)

pH of the sample was measured potentiometrically using pH meter .The total carbon content of dried and powdered samples was determined by dry combustion method and the total Nitrogen content was determined by Micro-Kjeldahl method (Jackson,1973)

Enzyme Study

Enzyme analysis was carried out on the organic waste samples for cellulase (Pancholy and Rice 1973), Amylase (Miller 1959) , Dehydrogenase (Casida et al 1964),Urease (Modified from Douglas and Bremner 1970 and Zantua and Bremner 1975) and Phosphatase (B. Godden et al 1983)

Microbiological Study

Organic waste samples were pulverized in a blender; suitable dilutions were prepared, plated out on Nutrient agar (Difco), incubated at 37° C for 2 days and enumerated for total viable bacterial counts. Fungi were enumerated on Potato dextrose agar plates after incubating at 28° C for 5 days. Actinomycetes were enumerated on Czapek-Dox agar (Difco). These plates were incubated for 7 days at 37°C. The organisms were screened qualitatively for proteolytic, lipolytic, cellulolytic, amylolytic and phosphate solubilising enzyme activity on milk agar, tributyrin agar, CMC agar, starch agar and hydroxyapatite agar respectively by spot inoculation method. After inoculation the agar plates were incubated for 3-4 days at 37°C. The plates were examined for coloration or clearing zones around the colony. A ratio of zone size to colony diameter indicated the enzyme activity. Enzyme activity was determined so as to select isolates showing highest catabolic activity, for use in composting experiments. Further quantitative screening was carried out in liquid media. Potential isolates were further standardized for inoculum size and incubation time required to obtain optimum enzyme activity. Identification of promising isolates was carried out using standard biochemical media and Bergeys Manual of Determinative Bacteriology.

Lab scale Composting Experiment

The composting experiment was performed in large earthenware tubs .A synthetic refuse with characteristics similar to MSW was prepared in the laboratory. The composition was as follows by % wet weight: fruit peels (10%), cooked rice (5%),vegetable trimmings(46%),straw(2%),paper(5%),coconut husk(5%),leaves(5%),oil cake(2%),meat waste (5%), fish waste(10%),egg shells(3%), cotton (2%). 2Kgs of waste prepared by shredding to 1/4th inch was inoculated with 200ml each of a lipolytic, proteolytic, cellulolytic, amylolytic and phosphate solubilising isolates grown in nutrient broth (~8x108 orgs/ml). It was covered with a fine layer of sterile soil weighing approximately 1 Kg. 2 controls were maintained, one with uninoculated organic waste and sterile soil and the other with uninoculated organic waste with normal unsterile soil. Moisture content was adjusted to a suitable level and the composting mass was turned every 4-5 days Temperature, pH and moisture content were monitored throughout the composting run.

Field trials

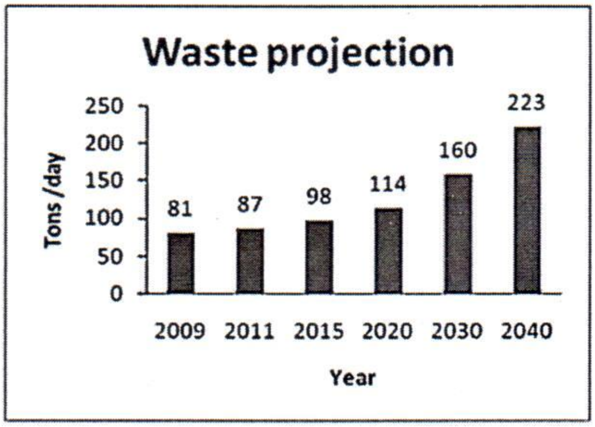
4 pits of 1cu.meter dimension were dug .Approximately 10 Kgs of mixed organic waste with a composition similar to that in the lab experiment was layered in each pit taking care to exclude plastic and other non biodegradables. 5 litres of mixed culture of organisms was used as seed. The experimental setup was as follows. Pit1 had 10 Kgs mixed organic waste + 5 litres inoculum+ 15 litres water; Pit 2 had10 Kgs mixed organic waste + 5 litres inoculum+ 15 litres cow dung slurry; Pit 3 had 10 Kgs mixed organic waste +20 litres cowdung slurry; Pit 4 had 10 Kgs mixed organic waste + 20 litres water .The pits were covered with a fine layer of sterile soil and with palm leaves and sack cloth to prevent moisture loss. The composting mass was turned every week and the moisture level was maintained at 50-60 % with water. Temperature and pH were monitored throughout the run.

Results and Discussion

Physical Study

The most ill fated area in Goa, as far as garbage disposal is concerned is Panaji.CCP is responsible for SWM in Panaji city within its jurisdiction. The resident population as per census 2010 showed a population of approximately 69,470.With the added floating tourist population, the projected population is about 1 lakh.CCP limits encompasses an area of 8.2 sq Kms. The estimated quantity of MSW generated in a day is 87 tonnes. Therefore, on an average approximately 0.77Kg/person/day is generated in Panaji. Waste projections for Panaji city are shown in Figure 1.

Figure 1



It was observed that the MSW was highly heterogeneous but mainly consisted of organic household waste, plastics, straw, paper cartons and construction and demolition waste. (Table 1, 1a, 1b).

Table 1 Waste composition	
Bio-degradable	58.34%
Recyclable	20.22%
Non Bio-degradable	21.44%

Table 1a Non Bio-degradable waste	
Construction and demolition	13.39%
Drain silt	5.01%
Street sweeping	3.04%

The relative contribution of recyclables i.e. paper, metals and plastics appeared to be low because of the salvaging of these materials by the rag pickers. 3 basic sources were identified, mainly residential areas, commercial areas and open areas such as beaches and parks. Upto a few years back, the MSW was collected from various bins located at different sites and then dumped at Curca in North Goa and Sonsoddo in South Goa. Later a dumping site was located at Taleigao, but faced strict opposition. An organic waste convertor is supposed to be commissioned for Panaji by November 2011.

Optimum composting conditions involves a balance of 6 factors namely size of particles, moisture content, temperature, oxygen, time and concentration of organic carbon and nitrogen.

Table 1b Bio-degradable waste

Composition	% by wet weight
Fruit peels	10
Rice (cooked)	5
Vegetable trimmings	30
Potato peels	6
Onion peels	10
Straw	2
Paper	5
Coconut husk	5
Leaves	5
Oil cake	2
Meat waste	5
Fish waste	10
Egg shells	3

Table - 2: Particle size, density and moisture content of MSW

Components	Particle size in meter (~)	Density in kg/m^3	Moisture %
Paper	0 - 0.5	20 - 100	1 – 10
Cardboard	0 – 1	30 - 70	1 – 6
Plastics	0 - 0.25	20 - 110	1 – 4
Glass / Ceramics	0 - 0.25	120 - 400	1 – 3
Leather	0 - 0.2	70 - 180	4 – 8
Rubber	0 - 0.5	80 - 180	1 – 2
Textiles / rags	0 – 1	60 - 80	3 – 20
Straw / hay	0 - 0.25	30 - 70	10 – 30
Leaves / Garden trimmings	0 - 1.5	50 - 200	20 – 60
Metals / tin cans	0 - 0.2	40 - 130	2 – 4
Vegetables / putrescibles / compostable matter / kitchen wastes	0 - 0.5	100 - 400	50 – 60
Miscellaneous inerts	0 - 0.25	40 - 100	3 – 10

Analysis of particle size is of importance in the recovery and disposal of materials. Most of the biodegradable matter had a small particle size, whereas non-biodegradables had a larger size and could be segregated easily (Table 2). Size of the particles affects the porosity of the compost. Smaller the particle size, greater is the surface area and faster the decomposition. ¼" to 2" diameter is considered to give best results (Dickerson G.W. 2005). Decomposition within the pile can be aided by increasing the surface area of organic material added to the pile. As microbial activity occurs mostly on the surface of waste added to the pile, increasing the surface area will increase the number of microorganisms working on the material. If the particle size is too small, airflow is inhibited and if it is too large the compost dries out. Density of waste showed a variation depending on the geographic location, season and storage time. Compostable matter had the highest density of upto 400Kg/m^3. It was seen that the organic waste had 50-85% compostable matter and moisture content as high as 50-60%. According to K.Jeevan Rao et al (1993), organic waste having more than 30% compostable matter is preferable for application on agricultural land as biofertilizer. Moisture content is an important factor in deciding whether the organic waste can be composted or not. Moisture is necessary to support the metabolic activity of microorganisms. Ideal moisture content is 50-65%. If the pile is too dry, composting occurs more slowly, while moisture content in excess of 65% develops anaerobic conditions.

Chemical analyses

Among the chemical parameters studied were pH, cellulose and protein content, organic carbon, carbonate carbon, nitrate nitrogen, phosphorus, calcium, magnesium, iron, zinc and manganese content. (Table 3)

Table 3: Chemical parameters

	Cellulose content		Protein content		
Average	90 - 300 mg / g		3 - 70 %		
	Organic C (mg/g)	CO_3 - C (mg/g)	NO_3 –N (mg/g)	Total N (mg/g)	Phosphorus (mg/g)
Run 1	0.31	0.018	0.052	1.8	0.008
Run 2	0.52	0.54	0.012	3.2	0.02
Run 3	1.6	0.248	0.069	2.1	0.8
	Calcium (mg/g)	Magnesium (mg/g)	Iron (mg/g)	Zinc (mg/g)	Manganese (mg/g)
Run 1	0.54	2.32	15.5	0.114	3.76
Run 2	8.42	5.46	4.85	2.8	7.15
Run 3	4.31	8.74	7.62	7.35	1.68

Growth and survival of microorganisms is greatly influenced by the pH of the environment. Each species grows within a specific pH range. The pH of the organic waste was found to vary from 5.8 to 8.3, a range where most organisms will grow or survive and therefore ideal for composting. Chemical analysis of the organic waste revealed that there was good potential in the organic waste for converting it microbiologically into soil conditioner, as it contained many important trace elements such as Ca, Mg, Fe, Zn & Mn which are essential for plant growth, though not in excess.

Enzyme analyses

Enzyme analyses revealed the presence of enzymes such as cellulase, amylase, dehydrogenase, phosphatase & urease in the degrading organic waste (Table 4).

Table 4: Enzyme analysis

Enzymes	Average activity
Cellulase µg/g	2.5 - 35
Amylase µg/g	7.2-10
Dehydrogenase µg/g	1 - 170
Phosphatase nM/g	0.2 - 16.5
Urease (µg of N/ml)	75 – 145

The presence of enzymes in organic waste indicated inherent microbial activity. Microbes in the compost pile cannot directly metabolize the insoluble particles of organic matter (Chanyasak V et al 1982, Godden B et al 1983 and Garcia C et al 1991). Rather, they produce hydrolytic extracellular enzymes to depolymerize the larger compounds to smaller fragments that are water-soluble. The water-soluble components dissolve in water and are assimilated by microorganisms in the compost. The insoluble components on the other hand, are decomposed by secreted microbial enzymes (extracellular enzymes) into water-soluble matter and subsequently absorbed into the microbial cells (Hankin LH 1976).

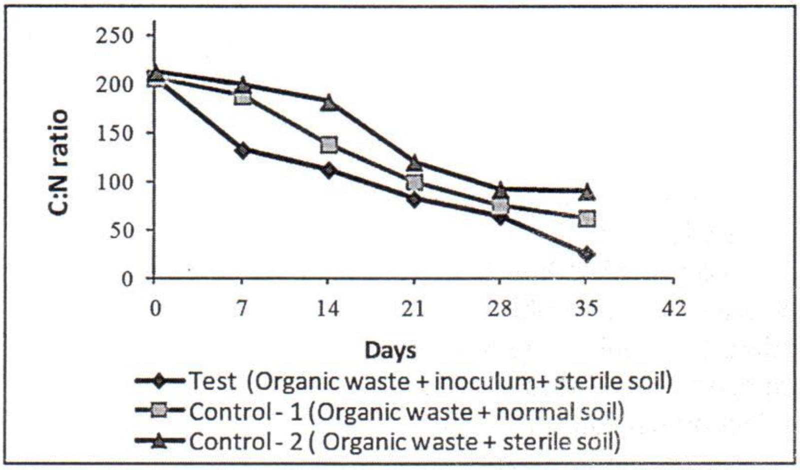
Microbiological Analyses

Indigenous organisms were isolated from the soil at the waste dump site using milk agar, tributyrin agar, CMC agar, starch agar, hydroxyapatite agar, PDA and Wickerhams agar. Proteolytic, lipolytic, cellulolytic, amylolytic, phosphate solubilizing organisms, fungi and actinomycetes were isolated. Organisms were identified upto the genus level using biochemical media as per Bergey's Manual of Determinative Bacteriology. The organisms were screened quantitatively for enzyme production at pH 4.5, 7.0 and 9.2. Of the 138 isolates obtained, 23 were phosphate solubilizers, 29 were cellulose degraders, 30 were protein degraders, 14 were starch degraders and 42 were lipid degraders. Among the organisms showing major enzyme activities were *Aspergillus, Bacillus* and *Cellulomonas* for Cellulase; *Vibrio* and *Aerobacter* for Amylase; *Pseudomonas* and *Bacillus* for Protease; *Micrococcus* for Lipase; *Pseudomonas and Micrococcus* for Acid and Alkaline Phosphatase. The isolates were tested for multiple enzyme activity as well as antagonistic action against each other. Optimum inoculum size was determined by taking 25, 50 and 125 ml culture per 250 gms organic waste and testing utilization of substrate. The optimum inoculum size was found to be between 50- 75%.

Lab scale Composting Experiment

Composting was selected as the ideal method of organic waste disposal, based on the physical and chemical analysis of organic waste. Composting involves the decomposition of organic matter by a mixed microbial population in a warm, moist and mostly aerobic environment. More precisely, composting is a breaking down as well as building up process where microbial enzymes play the major role. In fact inoculation or seeding at the onset of composting has been reported to be effective in accelerating the degradation reaction. The quantity and nature of the microbial population and the substrate used determines the progress of the reaction. The composting process involves the generation of heat, production of CO_2, loss of water vapour, loss of mass and production of relatively stable humus that is free of offensive odors. Laboratory trials were carried out using a mixture of the locally isolated indigenous microorganisms such as *Pseudomonas, Bacillus, Micrococcus, Vibrio and Aspergillus*. In the laboratory experiments using locally isolated microorganisms, when 2.5 kgs of organic waste was seeded with 1.5 liters of test cultures, it showed better and faster decomposition than the uninoculated organic waste controls. Compost should contain at least 5% or more oxygen for optimum aerobic composting. Turning of a compost pile provides only limited aeration. Therefore good porosity is important for natural convection and diffusion of oxygen. This in turn is related to the particle size. Insufficient oxygen results in anaerobic conditions and the production of objectionable odors from chemicals such as Hydrogen sulphide, methane and organic acids. A succession of microbial populations was observed during the composting process. The bacteria increased in number before the temperature of the composting mass rose and then declined, whereas cellulolytic organisms and actinomycetes in general increased in the thermophilic stage. Presumably, the mesophilic bacteria rapidly attack the more readily available organic constituents, resulting in a temperature increase. The increased temperature favors the cellulolytic organisms, and the mesophilic bacteria largely disappear. The actinomycetes appeared in the final stage to such an extent that the surfaces of the compost piles were white or gray. These organisms are known to play a role in the humification of organic matter, which results in a stabilized product (Golueke, C. G.et al 1953). The carbonate carbon showed an increase from 0.018 mg/g to 0.286 mg/g. Nitrate nitrogen increased from 0.011 mg/g to 0.123 mg/g. Phosphorus increased from 0.008mg/g to 1.256mg/g. The C/ N ratio decreased from 203 to 20. (Figure 3)

Fig 2: Temperature and pH during composting



Field trials

Field composting experiments were also performed using locally isolated microorganisms and organic waste. Best results were obtained when earthworms were also added towards the end of composting. pH dropped initially during the 5th-10th day, then increased upto the 40th day. Typical time courses of temperature and pH during composting of the waste biomass are shown in Figure 2.

The composted mass cooled to ambient temperature (20 to 30° C) by the 40th day. A pH of 8.0 was obtained within 30 days. The temperature was highest at 59.1°C on the 8th day. Mesophilic organisms are generally active only at temperatures between 20 to 40° C. High temperature favors cellulose degradation and growth of cellulolytic bacteria (Stutzenberger al.,1970; Gazi A.V et al.,2007). A temp of 55°C for 48-96 hrs is required to make compost pathogen free. Temperatures between 55 to 65° C are considered ideal, as it kills most weed seeds and pathogens. This temperature should be maintained for as long as possible. At temperatures above 70°C microbial activity ceases. Therefore turning of the organic waste is essential to bring the temperature down. When the composting temperature drops to 37° C or less and fails to reheat after turning, it is considered ready and may be left to cure for 1-6 months under natural aerobic conditions .The time taken for degradation in our experimental setup was approximately 40 days. Active thermophilic composting, indicated by pH and temperature rise, began only after 8 days. From a public health standpoint, it is desirable that a temperature above 48° C be maintained long enough to destroy pathogens like Salmonella. In our experimental setup the thermophilic stage of composting reached within 10 days and continued till almost 30 days. During composting, a lot of heat is generated. In the initial stage of composting, intense mineralization processes take place, which were manifested by considerable decrease in total organic carbon and increase in nitrate nitrogen respectively in all treatments. The carbonate carbon increased from 0.239 mg/g to 0.48 mg/g, as against the control, which showed only 0.38 mg/g. Similar results were recorded by A.K. Inoko et al

(1979) with a decrease of total carbon including hemicelluloses and cellulose, and an increase in carbonate carbon, similarly an increase in total nitrogen, crude ash and lignin during maturation of city refuse compost has been reported. Phosphorus increased from 0.025 mg/g to 1.34 mg/g whereas control showed 0.18 mg/g. Nitrate nitrogen increased from 0.022 mg/g to 0.138 mg/g whereas the control had 0.125 mg/g. B.Chefetz et al (1998) reported the total amount of soluble nitrogen decreases during composting and it represents mineralization. During maturation the ammonium nitrogen level continues to decrease while the nitrate level increases. The increased ratio of $NH_4^+ : NO_3^-$ is an indicator of compost maturity (R. J. Riffaldi et al 1986). N. Morisaki et al (1989) also reported that the greatest decrease of ammonia nitrogen occurred after the thermophilic stage leading to an increase of nitrate concentration through nitrification. In aerobic process the percentage conversion of ammonia to nitrate is known to be high due to continuous aeration of waste. The changes in the C/N ratio reflect organic matter decomposition and stabilization during composting process because microorganisms use carbon as a source of energy, and nitrogen for building cell structure. C/N ratio reduced from 261 to 24 whereas control showed a ratio of 90 (Figure 3).

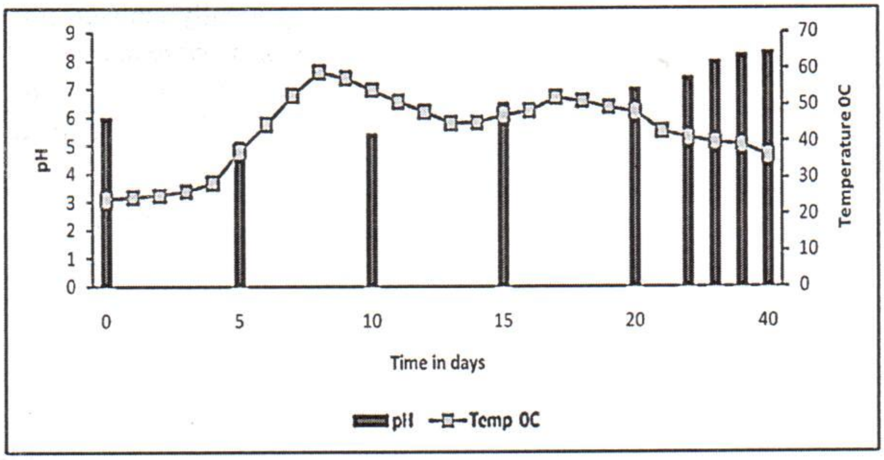


Fig 3: C/ N ratio during composting in laboratory trials

The C/N ratio of 24 lies within the acceptable limits but is inclined towards the higher values, meaning the compost may need supplements to increase its nitrogen value. C/N ratio of the waste could have been adjusted to an optimum level by adding cow manure, poultry manure, garden waste etc. Several researchers have drawn attention to the fact that successful preparation of MSW composts depended upon the nature of the organic materials, the proportion of nitrogenous compound to carbohydrates, the temperature of decomposition and the microbial population involved in the process (Babyranidevi & Bhoyar, 2003; Xi et al., 2003) If the C/ N ratio had been set to an optimum of about 30:1, the rate of organic matter decomposition would have been much faster and the quality of compost would also be better.

Conclusion: MSW from Panaji has a high percentage of biodegradable organic matter, acceptable moisture content and many important trace elements such as Ca, Mg, Fe, Zn &

Mn , indicating a good potential in the organic waste for converting it microbiologically into soil conditioner. On composting, not only the volume of the waste reduced but it also resulted in a crumbly, earthy smelling, soil-like, compost. Analysis of the MSW has shown that it is ideal for composting using indigenous microorganisms, provided proper segregation is carried out.

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