# The influence of halophytic compost, farmyard manure and phosphobacteria on soil microflora and enzyme activities

**V. Balakrishnan, K. Venkatesan, K.C. Ravindran**

*Department of Botany, Faculty of Science, Annamalai University, Annamalainagar, India*

**ABSTRACT**

Biocompost has been identified as an alternative to chemical fertilizers that increased soil microbial population and soil enzyme activities in sustainable farming. The objective of this field study was to evaluate the effect of three halophytic composts in combination with farmyard manure and phosphate solubilising bacteria (*Bacillus megaterium*) on soil microflora and enzyme activities. The results show that among nine treatments given, the application of *Suaeda* compost in combination with farmyard manure and phosphate solubilising bacteria ($T_9$) significantly increased the soil microflora such as bacteria, fungi and actinomycetes and soil enzyme activities such as dehydrogenases, alkaline phosphatase, cellulase and urease in soil cultivated with *Arachis hypogaea*.

**Keywords:** biocompost; halophytic compost; soil microflora; dehydrogenases; urease; alkaline phosphatase; cellulase; *Arachis hypogaea*

In many countries throughout the world, agricultural soils are being degraded at an alarming rate by wind and water erosion, salinization, nutrient depletion and desertification as a consequence of improper and inappropriate farming practices. The impact of organic management systems on soil microbiological diversity, activity, and their implication for soil fertility was reviewed by Shannon et al. (2002). According to Nelidov et al. (1988) organic manures stimulated soil microorganisms and thereby increased its productivity. It is believed that most soil enzymes originate from soil bacteria, fungi and plant roots. Soil enzymes are mediating biochemical transformations involving organic residue decomposition and nutrient cycling in soil (McLatchey and Reddy 1998).

Oil seeds constitute the main agriculture sector in India, which leads to "yellow revolution". India accounts for 9.6% of the world's total output of oilseeds, with more than 25 million hectares of land under oilseeds. Despite such considerable area and production of groundnut in the country, the average yield of groundnut is rather poor – only about 900 kg/ha as against 1416 kg/ha of the Asian average and 1275 kg/ha of the world average (Hedge 2005).

Cuevas (1997) recorded the nutrient values of mangroves and observed that nutrient contents in halophytes were higher when compared to green manures (glycophytes). However, halophytes accumulate NaCl in their tissues. The present work is based on the concept that when halophytes are subjected to composting, it is possible that NaCl content present in the tissues degrades during decomposition. $Na^+$ in NaCl may chelate with the organic acids produced during decomposition and release $Cl^-$, resulting thus in the concentration of NaCl. Decomposition nullifies the presence of NaCl content in plant tissues. Watson (2003) also stated that leaching the compost with water reduces the concentration of soluble salts. The main objective of our field study was to evaluate the effect of three different halophytic composts in combination with farmyard manure and phosphobacteria on soil microflora and enzyme activities in soils cultivated with *Arachis hypogaea*.

Supported by the Department of Ocean Development (DOD), Government of India.

## MATERIAL AND METHODS

**Experimental site.** The experiment was carried out in the Thandavaraya Sozhaganpettai village near Pichavaram mangrove forest, 12 km away from the Annamalai University. The experimental field is situated at 11°21'N latitude and 79°50'E longitude, at the altitude of above +5.25 m mean sea level. The experiment outline was entirely randomized block design with three replications.

**Compost preparation.** Three fast growing and dominant halophytes, namely *Suaeda maritima* (L.) Dumort., *Sesuvium portulacastrum* L. and *Ipomoea pes-caprae* (L.) Sweet, were chosen for making compost after a detailed survey. Well-decomposed farmyard manure was mixed with the halophytic compost. Phosphobacteria (*Bacillus megaterium* var. *phosphaticum*) were obtained from the Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, India. For three months, healthy halophytes were harvested from nursery and used for a preparation of compost. The plant materials as well as rice straw were well chopped. The fungus *Pleurotus sajor-caju* (Fr.) Singer, was added to the compost heap to improve its decomposition. The amount of activator used was usually 1% of the total weight of the substrate (Cuevas 1997). By the end of the third month, the compost was ready for use. The soil-available nutrients such as nitrogen (Subbaiah and Asija 1959), phosphorus (Olsen et al. 1954) and potassium (Jackson 1973) were analysed before the beginning of the experiment. The physical-chemical characteristics of the experimental soil presented in Tables 1 and 2 show the nutrient contents of the selected halophytic compost.

**Treatment.** The following treatments were used in the present study: $T_0$ – unmanured, $T_1$ – *Ipomoea* compost 6.25 t/ha, $T_2$ – *Sesuvium* compost 6.25 t/ha, $T_3$ – *Suaeda* compost 6.25 t/ha, $T_4$ – *Ipomoea* compost 3.13 t/ha + farmyard manure 3.13 t/ha, $T_5$ – *Sesuvium* compost 3.13 t/ha + farmyard manure 3.13 t/ha, $T_6$ – *Suaeda* compost 3.13 t/ha + farmyard manure 3.13 t/ha, $T_7$ – *Ipomoea* compost 3.13 t/ha + farmyard manure 3.13 t/ha + phosphobacteria $3 \times 10^9$/ha, $T_8$ – *Sesuvium* compost 3.13 t/ha + farmyard manure 3.13 t/ha + phosphobacteria $3 \times 10^9$/ha, $T_9$ – *Suaeda* compost 3.13 t/ha + farmyard manure 3.13 t/ha + phosphobacteria $3 \times 10^9$/ha.

**Soil sampling.** The topsoil samples were taken from the depth of 0–10 cm depth at four equidistant positions in each plot. These samples were bulked to provide one representative sample per plot. At the time of sampling the surface soil in all plots was dry.

**Estimation of the microorganism.** The enumerations of microflora in soil samples amended with the halophytic compost were analysed by soil serial dilution technique, using soil extract agar medium for bacteria, Martin's Rose Bengal agar medium for fungi, and Kenknight's agar medium for actinomycetes (Pramer and Schmidt 1965).

**Estimation of the soil enzymes.** Dehydrogenases and alkaline phosphatase activities were determined by the method described by Tabatabai (1982). Cellulase was estimated by the technique proposed by Galstyan (1965). Urease activity was measured according to the method proposed by Nannipieri et al. (1980).

**Sodium and chloride.** The available sodium was estimated using the flame photometer method (Stanford and English 1949). The available chloride content was estimated with the Mohr's titration method (Jackson 1973).

**Statistics.** The results were analysed by using analysis of variance (ANOVA) and the group means were compared with the Duncan's Multiple Range Test (DMRT) (Duncan 1957). Values were considered statistically significant when $P < 0.05$.

## RESULTS

An experiment was carried out to find out whether the process of decomposition of halophytes

Table 1. Physico-chemical properties of the experimental soil

| Properties | Value |
|---|---|
| **Physical properties** | |
| Coarse sand (%) | 48.86 |
| Fine sand (%) | 34.25 |
| Silt (%) | 5.58 |
| Clay (%) | 10.26 |
| Textural class | Sandy |
| **Chemical analysis** | |
| Available N (kg/ha) | 144.8 |
| Available $P_2O_5$ (kg/ha) | 4.85 |
| Available K (kg/ha) | 156.7 |
| Organic carbon (%) | 0.32 |
| Organic matter (%) | 0.55 |
| Soil reaction (pH) | 7.89 |
| Electrical conductivity (dS/m) | 1.36 |

reduces the NaCl concentration in the compost. From the results (Table 2), it was concluded that NaCl concentrations were drastically reduced when the halophytes were subjected to decomposition. It was also observed that at the end of decomposition, *Suaeda* compost in combination with farmyard manure and phosphobacteria ($T_9$) gained higher nutrient contents when compared to other halophytic composts. Nutrients such as N, P, K, Ca, Mg and micronutrients were found to be higher in $T_9$ compost. However, a reduction in pH, organic carbon and C: N ratio was noticed in $T_9$ compost, as well.

Furthermore, the experiment proved that soil microflora showed a significant increase in bacteria, fungi and actinomycetes; enzyme activities such as dehydrogenases, alkaline phosphatase, cellulase and urease were increased in soil treated with halophytic compost when compared to unmanured soil during first sixty days of cultivation period. Maximum soil microflora and enzyme activities were identified in *Suaeda* compost treatment in combination with farmyard manure and phosphobacteria ($T_9$). Generally, all the treatments were better than the unmanured soil.

Nearly 70% increase in bacteria, 62% in fungi and 50% in actinomycetes (Figures 1–3) were noticed in the soil treated with *Suaeda* compost along with farmyard manure and phosphobacteria ($T_9$). However, a reverse trend in microflora was observed in soil treated with halophytic compost from 60 days of treatment until the harvest stage, whereas a reduction of about 20% in bacteria, 20% in bacteria, 20% in fungi and 21% in actinomycetes populations was recorded in *Suaeda* compost in combination with farmyard manure and phosphobacteria ($T_9$) when compared to the other treatments and unmanured soil (Figures 1 and 3).

Similarly to soil microflora a significant effect was recorded in enzyme activities and nearly a 70% increase in dehydrogenases, 75% in alkaline phosphatase, 70.1% in cellulase and 70.8% in urease activities was recorded in *Suaeda* compost in combination with farmyard manure and phosphobacteria ($T_9$), as it is shown in Figures 4–7. However, a reverse trend in soil enzyme activity was also observed in soil treated with halophytic compost from 60 days of treatment until the harvest stage and it was noticed that a reduction of about 20% in dehydrogenases, 22.8% in alkaline phosphatase, 20.6% in cellulase and 20.5% in urease activities (Figures 4–7) was observed in *Suaeda* compost in combination with farmyard manure and phosphate solubilising bacteria treated soil ($T_9$).

Table 2. Nutrient contents of different halophytic compost at maturity period (90 days)

| Treatments | pH | C (%) | N (%) | C:N ratio | P (%) | K (%) | Ca (ppm) | Mg (ppm) | Zn (ppm) | Fe (ppm) | Mn (ppm) | Cu (ppm) | Before composting (mg/g fw) | | After composting (mg/g fw) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | Na | Cl | Na | Cl |
| $T_1$ | 7.1 | 54 | 1.80 | 30.0 | 0.68 | 1.46 | 1760 | 1340 | 8.02 | 10 176 | 31.69 | 160 | 24 | 18 | 10 | 9 |
| $T_2$ | 7.3 | 50.20 | 1.84 | 27.28 | 0.71 | 1.52 | 1820 | 1426 | 8.28 | 10 194 | 31.76 | 174 | 22 | 16.5 | 9 | 8 |
| $T_3$ | 7.2 | 47.35 | 1.92 | 24.66 | 0.74 | 1.61 | 1958 | 1554 | 8.59 | 10 296 | 32.62 | 193 | 20 | 15 | 7.5 | 6.6 |
| $T_4$ | 7 | 46.62 | 2.70 | 17.26 | 1.28 | 1.96 | 3260 | 2640 | 10.49 | 10 598 | 34.8 | 370 | – | – | 4.5 | 4.2 |
| $T_5$ | 6.9 | 45.59 | 2.74 | 16.64 | 1.31 | 2.02 | 3320 | 2726 | 10.75 | 10 616 | 34.9 | 403 | – | – | 3.8 | 3.5 |
| $T_6$ | 6.9 | 43.62 | 2.82 | 15.47 | 1.34 | 2.11 | 3458 | 2854 | 11.06 | 10 718 | 35.73 | 580 | – | – | 2.9 | 2.6 |
| $T_7$ | 6.6 | 40.72 | 2.71 | 15.02 | 1.29 | 1.98 | 3274 | 2646 | 11.52 | 10 610 | 35.2 | 384 | – | – | 1.6 | 1.4 |
| $T_8$ | 6.7 | 40.61 | 2.80 | 14.50 | 1.32 | 2.05 | 3336 | 2736 | 11.78 | 10 627 | 35.3 | 426 | – | – | 1.1 | 0.9 |
| $T_9$ | 6.5 | 40.15 | 2.83 | 14.18 | 1.35 | 2.14 | 3474 | 2865 | 12.09 | 10 733 | 35.8 | 595 | – | – | 0.8 | 0.5 |

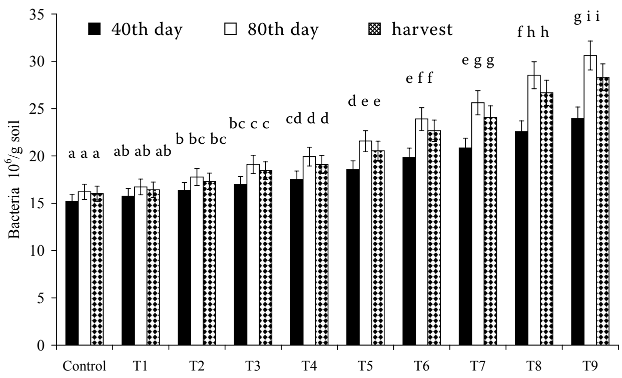


Figure 1. Effect of different halophytic compost application on soil bacteria. Values shown are mean ± SE for three replications (different letters above bars indicate a significant difference at $P < 0.05$ according to the Duncan's multiple range test)

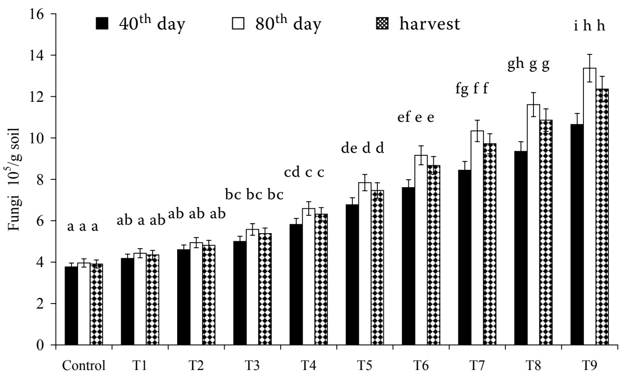


Figure 2. Effect of different halophytic compost application on soil fungi. For further explanations see Figure 1

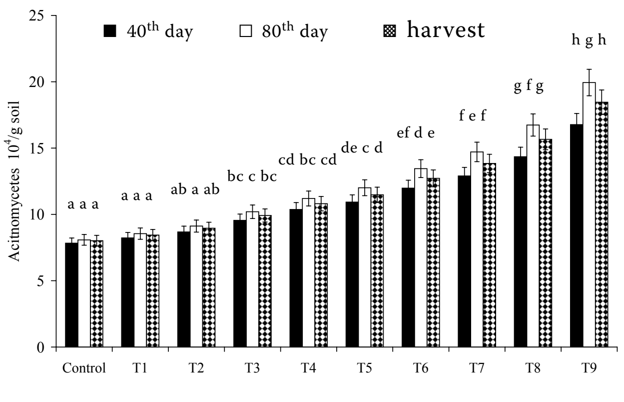


Figure 3. Effect of different halophytic compost application on soil actinomycetes. For further explanations see Figure 1

The results from the present study indicate that the treatment with halophytic compost significantly decreased the pH of the soil. The pH (Figure 8) was reduced to 5.95% in *Suaeda* compost + farmyard manure and phosphobacteria ($T_9$) when compared to other compost treated soil and unmanured soil during harvest stage; in this treatment ($T_9$) EC (Figure 9) was reduced to 24.7%.

The application of halophytic compost significantly decreased the sodium and chloride contents in soil cultivated with *Arachis hypogaea* (Figures 10 and 11). Nearly a 50% reduction in sodium and a 44.4% reduction in chloride content were observed in *Suaeda* compost + farmyard manure and phosphobacteria treatment ($T_9$).

## DISCUSSION

The soil microbial population is related to quantity, type and placement of organic matter addition. In this study it was observed that halophytic compost increased the soil microflora when compared to unmanured soil. Among nine halophytic compost treatments, *Suaeda* compost in combination with farmyard manure and phosphobacteria ($T_9$) increased the soil microbial population when compared to other treatments and control. These results are in agreement with some other findings, in which an application of biofertilizer caused an increase up to 80% in bacteria, 65% in fungi and 53% in actinomycetes populations (Wan and Wong 2004).

Halophytic compost increased dehydrogenases activity and similar results were found, when organic manures with green manure increased dehydrogenases activity up to 75% (Kannaiyan and Kalidurai 1995). Bolton et al. (1985) noticed nearly a 25% reduction in dehydrogenases activity due to green manure incorporation after 50 days of cultivation period. The activity of dehydrogenases basically depends on the metabolic state of the soil biota. It is significantly correlated with soil biomass carbon in organic amended soil (Garcia-Gill et al. 2000). In this experiment halophytic compost also increased the alkaline phosphatase activities and similar observations were recorded in other studies, showing that an application of organic manure with green manure increased the alkaline phosphatase activity up to 82% (Kumar and Kannaiyan 1992). However, Thanikachalam et al. (1984) reported nearly a 25% reduction in alkaline phosphatase activity in the case of field with biofertilizer application after 70 days of cultivation.

In general, alkaline phosphatase activity was higher with the treatment with higher amount of available phosphorus. Spier and Ross (1978)

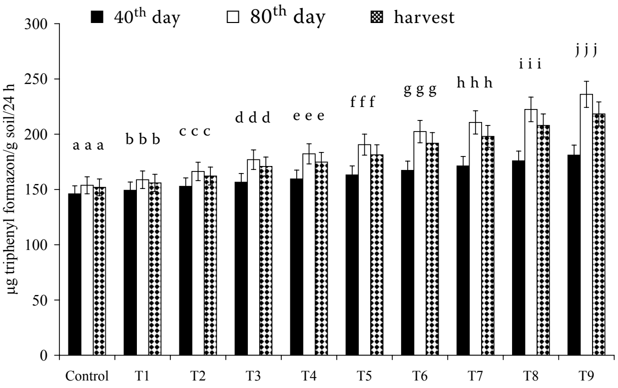


Figure 4. Effect of different halophytic compost application on soil dehydrogenase activity. For further explanations see Figure 1

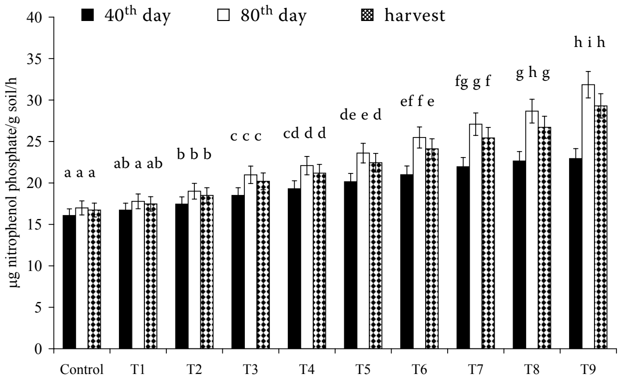


Figure 5. Effect of different halophytic compost application on soil alkaline phosphatase. For further explanations see Figure 1

reported a stimulation of alkaline phosphatase production by organic manure and presence of organic compounds. Higher alkaline phosphatase activity would promote the rate of hydrolysis of organic phosphorous compounds and thus more inorganic phosphorus would be formed.

It was noticed that halophytic compost increased the cellulase activity when compared to unmanured soil. These results are in agreement with the findings of Kumar and Kannaiyan (1992), whose results showed that an application of organic manures increased the cellulase activity nearly to 32% in soil treated with green manure. Bolton et al. (1985) observed nearly a 25% reduction in cellulase activity after 50 days in soil treated with green manure. The increased cellulase activity was significantly correlated with microflora (Kshattriya et al. 1992).

As for the urease activity, its increase was observed when halophytic compost was used, compared to unmanured soil. Subramani and Kannaiyan (1989) reported that organic manures increased the urease activity up to 73% in biofertilizer treated soil. However, after 65 days of cultivation period a reverse trend was observed and nearly 26% reduction in urease activity was observed in the same field (Subramani and Kannaiyan 1989). The activity of soil urease was positively correlated to total organic matter and nitrogen content (Reynolds 1985).

In general, a reverse trend in soil microbial population and soil enzyme activity was observed after 60 days to harvest stage. The addition of various organics provided the carbon substrates, which led to the increased soil microflora and enzyme activities during the first 60 days. However, after 60 days these activities subsequently declined because the available carbon content was exhausted by soil microbes (Goyal et al. 1992).

The results also confirm a significant decrease of NaCl in the soil when treated with halophytic compost, which is evidenced by the reduction

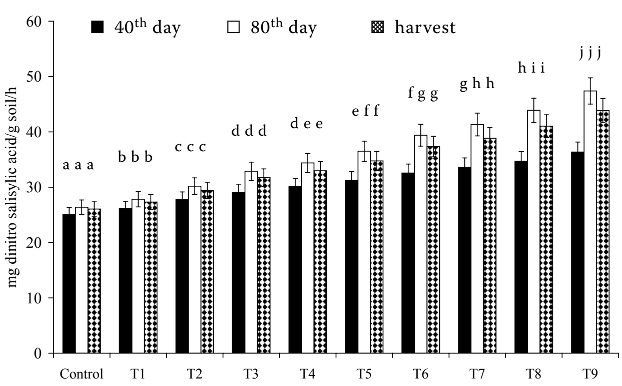


Figure 6. Effect of different halophytic compost application on soil cellulase activity. For further explanations see Figure 1

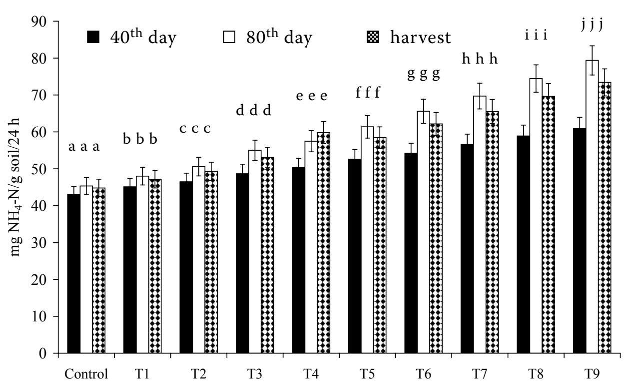


Figure 7. Effect of different halophytic compost application on soil urease activity. For further explanations see Figure 1

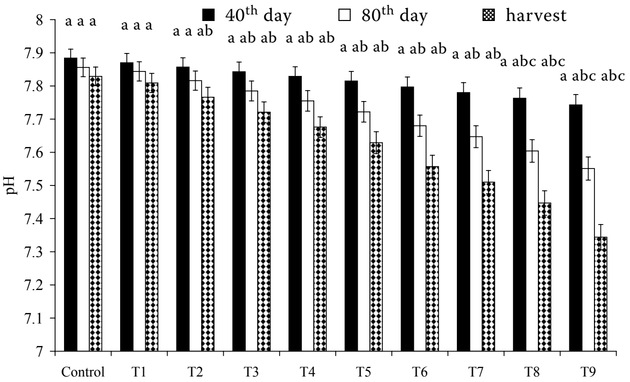


Figure 8. Effect of different halophytic compost application on pH. For further explanations see Figure 1

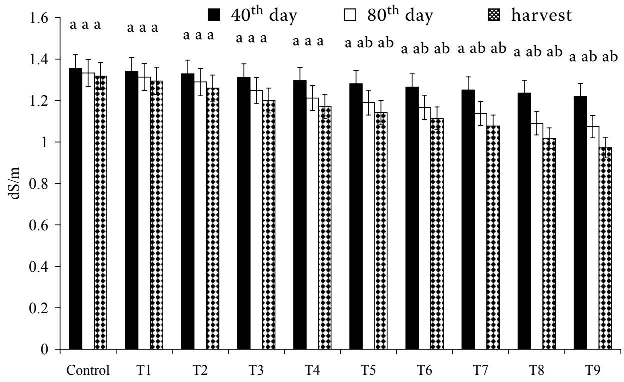


Figure 9. Effect of different halophytic compost application on electrical conductivity. For further explanations see Figure 1

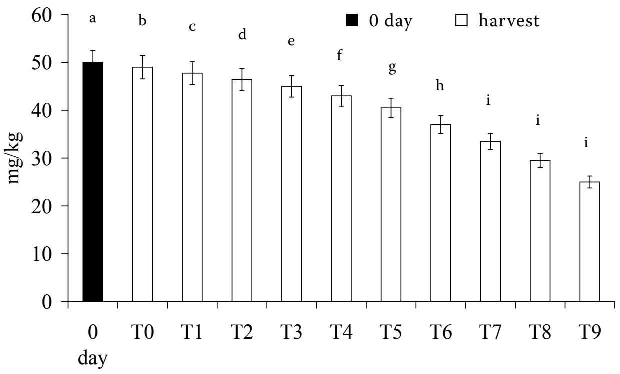


Figure 10. Effect of different halophytic compost application on available sodium in soil. For further explanations see Figure 1

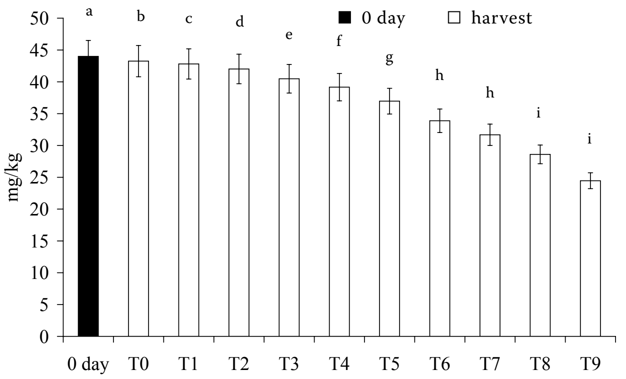


Figure 11. Effect of different halophytic compost application on available chloride in soil. For further explanations see Figure 1

of pH and EC. A similar trend was observed by Wahid et al. (1998). This could be caused by the maturity of finished compost. When fresh organic compost was applied to the soil, the ongoing microbial activity caused the reduction of pH due to a production of organic acids.

To conclude, the addition of various organics provides the substrate with carbon, which leads to an increase of the soil microflora and enzyme activities during the first 60 days; after this period these activities decline since the available carbon content present in the soil is exhausted by soil microbes.

## REFERENCES

Bolton H., Elliott L.F., Papendick R.I., Bezdicek D.F. (1985): Soil microbial biomass and selected soil enzyme activities: Effect of fertilization and cropping practices. Soil Biol. Biochem., *17:* 297–302.

Cuevas V.C. (1997): Rapid composting technology in the Philippines: Its role in producing good-quality organic fertilizers. Food Fert. Techn. Center Extn. Bull., *444*: 1–13.

Duncan B.D. (1957): Multiple range tests for correlated and heteroscedastic means. Biometrics, *13*: 359–364.

Galstyan A.S. (1965): A method determining the activity of hydroloytic enzymes in soil. Počvovedenije, *2:* 68–74.

Garcia-Gill J.C., Plaza C., Soler-Rovira P., Polo A. (2000): Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biol. Biochem., *32*: 1907–1913.

Goyal S.L., Mishra M.M., Hooda I.S., Raghubir B. (1992): Organic matter, microbial biomass relationship in field inorganic fertilization and organic amendments. Soil Biol. Biochem., *24*: 1081–1084.

Hegde D.M. (2005): Striving for self-sufficiency. The Hindu Survey of Indian Agriculture 2005: 58–62.

Jackson M.L. (1973): Soil chemical analysis. Asia Publ. House, Bombay, India.

Kannaiyan S., Kalidurai M. (1995): Nitrogen fixing potential of stem nodulating *Sesbania rostrata* and its effect on rice yield. In: Kannaiyan S. (ed.): Rice Management Biotechnology. Assoc. Publ. Co., New Delhi, India: 173–195.

Kshattriya S., Sharma G.D., Sharma R.R. (1992): Enzyme activities related to litter decomposition in forests of different age and altitude in northeast India. Soil Biol. Biochem., *24*: 265–270.

Kumar K., Kannaiyan S. (1992): Changes in the activity of soil enzymes during decomposition of N fixing green manures. 33$^{rd}$ Ann. Conf. Assoc. Microbiol. Goa Univ., India.

McLatchey G.P., Reddy K.R. (1998): Regulation of organic matter decomposition and nutrient release in a wetland soil. J. Environ. Qual., *27*: 1268–1274.

Nannipieri P., Ceccanti B., Cervelli S., Matarese E. (1980): Extraction of phosphatase, urease, protease, organic carbon and nitrogen from soil. Soil Sci. Soc. Am. J., *44*: 1011–1016.

Nelidov S.N., Vasilevo L.V., Mishustin E.N. (1988): Use of crop residues to increase productivity of rice on ameliorated alkaline soils. Biol. Bull. Acad. Sci., USSR, *1*: 31–43.

Olsen S.R., Cole C.V., Watanable F.S., Dean D.A. (1954): Estimation of available phosphorus in soil by the extraction with sodium bicarbonates. USDA, Circular No. 939.

Pramer D., Schmidt E.L. (1965): Experimental soil microbiology. Burgess Publ. Co., Minneapolis.

Reynolds C.M., Wolf D.C., Armbruster J.A. (1985). Factors related to urea hydrolysis in soil. Soil Sci. Soc. Am. J., *49*: 104–108.

Shannon D., Sen A.M., Johnson D.B. (2002): A comparative study of the microbiology of soils managed under organic and conventional regimes. Soil Use Manag., *18*: 274–283.

Spier T.W., Ross D.J. (1978): Soil phosphatase and sulphates. In: Burrens R.G. (ed.): Soil Enzymes. Acad. Press.

Stanford S., English L. (1949): Use of flame photometer for analysis of Na, K and Ca. Agron. J., *41*: 446–447.

Subbaiah B.V., Asija G.L. (1959): A rapid procedure for the determination of available nitrogen in soils. Curr. Sci., *25:* 259–260.

Subramani S., Kannaiyan S. (1989): Effect of the water fern *Azolla* on enzyme activity in soil under rice cultivation. Mikrobiologia, *58*: 118–121.

Tabatabai M.A. (1982): Soil enzymes. In: Page, Miller R.H., Keeney D.R. (eds.): Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Ann. Soc. Agron., Madison, WI: 903–947.

Thanikachalam A., Rajakannu K., Kannaiyan S. (1984): Effect of neem cake, carbofuran and *Azolla* application on phosphatase activity in soil. 25$^{th}$ Ann. Conf. Assoc. Microbiol. Pant Nagar, India.

Wahid A., Akhtar S., Ali I., Rasul E. (1998): Amelioration of saline-sodic soils with organic matter and their use of wheat growth. Commun. Soil. Sci. Plant Anal., *29*: 2307–2318.

Wan J.H.C., Wong M.H. (2004): Effects of earthworm activity and P-solubilising bacteria on P availability in soil. J. Plant Nutr. Soil Sci., *167:* 209–213.

Watson M.E. (2003): Testing compost. In: Extension Fact Sheet, School of Natural Resources, Columbus, OH: 1–4.

Received on August 16, 2006

*Corresponding author:*

Dr. Konganapuram Chellappan Ravindran, Annamalai University, Department of Botany, Annamalainagar, 608 002 Tamil Nadu, India
phone: + 91 4144 238 248, fax: + 91 4144 238 145, e-mail: drkc_ravi@rediffmail.com