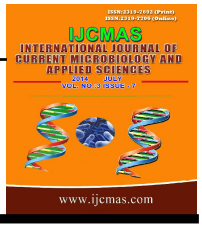
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 7 (2014) pp. 1072-1081**
http://www.ijcmas.com



Original Research Article

Isolation of Plant Growth Promoting Rhizobacteria (PGPR) from Vermicompost and effect on growth of Green Gram (*Vigna radiata L.*)

R.Gopinathan[1] and M.Prakash[2*]

[1]Resarch and Development Centre, Bharathiar University, Coimbatore-641046, Tamil Nadu, India
[2]Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kancheepuram, Tamilnadu, India
**Corresponding author*

Keywords

Plant Growth Promoting Rhizobacteria (PGPR), Vermicompost, *Vigna radiata* L.

A B S T R A C T

Vermicomposting is a low-technology, environmentally-friendly process used totreat organic waste. The resulting vermicompost has been shown to have several positive impacts on plant growth and health. Vermistabilization was carried out with organic substrat (Leaf litter) by using Epigeic species of *Perionyx ceylanensis* and cow dung as the supporting material.The Major bacterial genera (Plant Growth Promoting Rhizobacteria-PGPR) encountered in Leaf litter vermicompost includes *Bacillus, Pseudomonas, Rhizobium, and Azotobacter*. On characterization PGPR tolerant to multiple heavy metals and exhibiting a couple of resistant activities mainly *Pseudomonas sps* were resistant to 400 gml-1 of Hg. The plant growth parameters Shoot length, root length, number of roots hairs, Number of root nodules and number of branches and leaves were determined and also yield of Green gram (*Vigna radiata*) was determined, the results shows that increase level compare with control (without PGPR)

Introduction

Animal manure is a valuable resource as a soil fertilizer because it provides large amounts of macro- and micronutrients for crop growth and is a low-cost, environmentally-friendly alternative to mineral fertilizers. However, the use of manure in agriculture is being abandoned because of increasing transportation costs and environmental problems associated with the indiscriminate and inappropriately-timed application to agricultural fields (Hutchison et al., 2005).

The results of several long-term studies have shown that the addition of compost improves soil physical properties by decreasing bulk density and increasing the soil water holding capacity (Weber et al., 2007). Moreover, in comparison with mineral fertilizers, compost produces significantly greater increases in soil organic carbon and some plant nutrients (Nardi et al., 2004, Weber et al., 2007).

Vermicomposting and Vermicompost Properties

Vermicompost is a nutrient-rich, microbiologically active organic amendment that results from the interactions between earthworms and microorganisms during the breakdown of organic matter. It is a stabilized, finely divided peat-like material with a low C:N ratio, high porosity and high water-holding capacity, in which most nutrients are present in forms that are readily taken up by plants (Domínguez, 2004).

Unlike compost, vermicompost is produced under mesophilic conditions, and although microorganisms degrade the organic matter biochemically, earthworms are the crucial drivers of the process, as they aerate, condition and fragment the substrate, thus drastically altering the microbial activity. Earthworms act as mechanical blenders, and by fragmenting the organic matter they modify its physical and chemical status by gradually reducing the ratio of C:N and increasing the surface area exposed to microorganisms - thus making it much more favourable for microbial activity and further decomposition (Domínguez et al., 2010).

Vermicomposting generally converts organic matter to a more uniform size, which gives the final substrate a characteristic earthy appearance, whereas the material resulting from composting usually has a more heterogeneous appearance (Tognetti et al., 2005).

Karemegam et al. (1999) studied the effect of vermicompost on germination efficiency and growth of green gram and found that germination efficiency in green gram was 93.33% in vermicompost treated plots as compared to 84.17% in untreated plots. The growth and yield performance in vermicompost treated plots was also significantly higher over the control and also reported that number of pods/plant, length of pod, number of seeds per pod and total weight of seeds per plant were significantly higher in experimental plots than control as well as FYM, when vermicompost was used.

Plant Growth Promoting Bacteria

Plant growth promoting bacteria are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. In last few decades a large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Rhizobium, Flavobacteriaum, Bacillus* and *Serratia* have reported to enhance plant growth (Glick, 1995). Prakash.M and N.Hemalatha, 2013, reported that plant growth promoting bacteria from vermicompost were enhance the performance on black gram (*Vigna munga L.Heeper)*

Even though much work has been done on vermicomposting and plant growth promoting bacteria, very few reports are available related to isolation of Plant growth promoting bacteria from vermicompost and its impact on the plant growth particularly green gram (*Vigna radiata*). In the present study Earthworm, *Perionyx ceylanensis* was used for vermicomposting, followed by isolate the Plant growth promoting bacteria and also study the effect on growth of green gram (*vigna radiata*)

Materials and Methods

Preparation of Vermicompost

Leaf litters of Teak tree (*Tectona grandis*)

(LLT) were collected from Kancheepuram, Tamil Nadu and subjected to initial decomposition in rectangular draining cement tanks of 75cm×60cm×45cm size by sprinkling water, regular mixing and turning of the substrates for 15 days. The cowdung (CD) was collected from nearby cattle sheds in fresh form and allowed to stabilize for one week and used for the study. The stabilization of cowdung was done to make it acceptable by the worms. The earthworm, *P. ceylanensis* for the study, originally collected from culture bank of the Department of Biology, Gandhigram Rural University, Tamilnadu was mass multiplied in cowdung and used for the study.

Leaf litters of Teak tree were mixed with standard bedding material and introduced into standard plastic tubs occupying about 3kg of the materials. The each pre decomposed substrates were mixed with cow-dung in 1:1 ratio on dry weight basis in separate plastic trays of 45cm x 35cm x 15cm size with six replicates for a period of 2 months. Vermicomposting was carried out in an environmentally controlled experimental chamber at a temperature of 27±1°C and the vermibeds were maintained to contain a moisture level of (65-75%) by sprinkling water over the surface daily. Each tray containing vermibed substrate was introduced with 60 adult Epigeic species of Earthworm *P. ceylanensis* were inoculated manually in selected bedding materials in plastic tubs. The culture tubs were placed Indoor in the laboratory. The bedding material upper surface was covered with wire mesh to avoid entry of predators.

Isolation and Identification of Plant Growth Promoting Rhizobacteria

Isolation of PGPR

Leaf litter vermicompost was collected and subjected for the isolation of Plant growth promoting rhizobacteria. All the bacteria were isolated on their respective media; Rhizobium was isolated on yeast extract mannitol agar, Azotobacter on Jensen s medium, Pseudomonas on Cetrimide medium and Bacillus on nutrient agar. Bacterial cultures were maintained on the respective slants.

Biochemical characterization of Rhizobacteria

Selected isolates of *Bacillus, Pseudomonas, Azotobacter* and *Rhizobium* were biochemically characterized by Gram s reaction, carbohydrate fermentation, oxidase test, O-F test, H2S production, IMViC tests, NO2 reduction, starch and gelatin hydrolysis as per the standard methods (Cappuccino and Sherman, 1992)

Characterization of rhizobacteria for PGPR

Production of Indole acetic acid

Indole acetic acid (IAA) production was detected as described by Brick *et al.,* (1991). Bacterial cultures were grown for 72 h (Azotobacter and Rhizobium) and 48 h (Pseudomonas and Bacillus) on their respective media at 36±2 °C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution). Development of pink colour indicates IAA production.

Production of ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in

10 ml peptone water in each tube and incubated for 48 72 h at 36±2 °C. Nessler s reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Production of HCN and Catalase

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lorck (1948). Briefly, nutrient broth was amended with 4.4 g glycine/l and bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 36±2°C for 4 days. Development of orange to red colour indicated HCN production. Bacterial cultures were grown in a nutrient agar medium for 18-24 h at 36±2°C. The cultures were mixed with appropriate amount of H_2O_2 on a glass slide to observe the evolution of oxygen.

Siderophore production

Siderophore production was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chrom azole S (CAS). Orange halos around the colonies on blue were indicative for siderophore production.

Effect of plant growth using isolated using Plant growth promoting Rhizobacteria bacteria

Plant seeds were obtained from the Govt. District Agriculture institute, Kancheepuram. The bacterial genus, *Rhizobium sps,, Bacillus sps. Pseudomonas sps, Azotobacter sps.* were used for this study. The bacterial strains were obtained from the vermicompost was used in this study. Glycerin-peptone-agar medium used for isolation of bacterial strains the identification of strains relied on standard biochemical and physiological tests according to the classification of Bergey's manual and standard methods. The study of the effect of isolated strains on plant growth was carried out in plot experiments using a vermicompost of Leaf litter and red soil as control.

Experimental work

In this experiment, Vermistabilized leaf litter (Teak) were tested with Green gram (*Vigna radiata*).

T0 – Red Soil (Control - Farmer's practice)
T1 – Vermistabilised Leaf litter (Teak)

The above treatments were replicated thrice in a randomized block design with inoculated plant growth promoting bacteria which was isolated from the vermistabilised organic substrates. The bacteria were grown in glycerine-peptone-medium. Tubes were secured on a rotary shaker (120 rpm; 23°C) and agitated for three days. Seedlings of these plants were inoculated with 1 ml of the bacterial suspension, which resulted in an inoculums' density of 10^6 cfu/ml. The unit plot size was 2.5cm x 2cm.

Plants were grown in plots for 12 weeks. Weeding, irrigation, drainage, crop protection and other intercultural operation were done when necessary. The soil was moistened with water and maintained at 60% of its moisture. The criteria for growth promotion was studied as root, shoot length and other parameters. Data on growth, yield and yield contributing parameters were recorded.

Physiological Observations

Shoot Length

At sampling periods, the seedlings of Green gram (*Vigna radiata*) were plucked out from the plot carefully and washed with tap water to remove the adhering soil. Shoot length was measured from the base to the tip of the lengthiest shoot.

Root Length

At Sampling Periods; their seedlings of Green gram (*Vigna radiata*) were pulled out from the plot carefully without breakage of roots. Root length was measured from the base to the tip of the lengthiest root.

Number of Root hairs

The total numbers of root hairs were counted numerically to find out the influence of plant growth promoting rhizobacteria and organics on the root growth.

Number of nodules

The total numbers of nodules were counted numerically to find out the influence of plant growth promoting bacteria and organics on the root growth.

Number of branches and leaves

The total number of branches and leaves were counted numerically to find out the influence of plant growth promoting bacteria and organics on the plant growth.

Yield of Black gram Plant

The total number of flowers, number of pods and number of seeds present in the each pod were counted numerically to find out the green gram yield for the influence of PGPR and organic substrates. And also determine the weight of fresh and dry weight of the 100 nos of green gram seeds.

Results and Discussion

Table 1, 2 indicates Morphological and characterization for Plant Growth Promoting Rhizobacteria. The results on the effect of inoculation of Plant growth promoting Rhizobacteria along with an uninoculated control in Green gram (*Vigna radiate*) have been presented the data in Table 3 and 4.

Root Length

All the treatments were significantly increasing their efficiency on the growth of roots. Here, this experiment showed the best performance of T1 (23.9) than T0 as control having 22.1 cm in 90^{th} day of plant growth.

Shoot Length

This simple experiment was conducted to find out the effect of PGPR which increased the shoot length over uninoculated control. In this experiment, T1 (39.9 cm) show best performance than T0 as a control (32cm).

Number of Roots Hairs

Plant growth promoting Rhizobacteria increased the number of root hairs growth over the control. In this experiment, the T1 (74) showed the good performance than the T0 control (60)

Number of Root Nodules

All the treatments were significantly increasing their efficiency on the root nodules of Green gram (*Vigna radiata*) plant. Here, this experiment showed the best performance in T1 (23)compare to T0 (16) control.

Table.1 Morphological and biochemical characterization for Plant Growth Promoting Rhizobacteria

Morphological and biochemical characterization	***Bacillus* sp**	***Pseudomonas* sp**	***Azotobacter* sp**	***Rhizobium* sp**
Grams reaction	Gram positive	Gram negative	Gram negative	Gram negative
Shape	rods	rods	rods	rods
Pigments	-	+	+	+
Lactose	+	-	+	+
Dextrose	+	+	+	-
Sucrose	+	+	+	-
Mannitol	+	-	+	+
Oxidase	-	+	+	+
OF test	-	+	+	-
H2S production	-	+	-	+
Indole	-	-	+	+
Methyl red	-	-	+	+
Vogues Proskauer	+	-	+	+
Citrate utilization	+	+	+	-
Nitrate reduction	+	+	+	+
Starch hydrolysis	+	+	+	+
Gelatin hydrolysis	+	-	-	-

Table.2 PGPR Characterization

PGPR Characterization	***Bacillus* sp**	***Pseudomonas* sp**	***Azotobacter* sp**	***Rhizobium* sp**
IAA Production	+	+	+	+
Ammonia Production	+	+	-	+
Catalase Production	+	+	+	+
Siderophore Production	-	+	-	-

Table.3 Effect of vermicompost on plant growth parameters* of Green gram *(Vigna radiate)*

Treatment	No. of days	Shoot length (cm)	Root length (cm)	No. of Root nodules	No. of Root hairs	No. of branches	No. of leaves
T0	15	7.1	3.4	0	6	2	3
T1		9.0	4.7	0	9	3	4
T0	30	14.2	8.1	13	13	5	8
T1		14.7	9.5	16	17	6	9
T0	45	18.3	10.9	15	31	7	12
T1		22.1	13.7	21	45	8	14
T0	60	21.0	15.8	16	43	9	14
T1		24.3	18.3	23	52	10	19
T0	75	25.0	18.6	17	55	10	23
T1		31.8	21.2	25	69	12	29
T0	90	32	22.1	16	60	11	27
T1		39.9	23.9	23	74	13	36

T0 = Red Soil; T1 = PGPR from vermicompost *Mean of Three replications,

Table.4 Effect of vermicompost on the yield of Green gram *(Vigna radiate)*

Treatment	No. of days	No of Flowers	No of pods Per plant	No of seeds Per pod	Weight of 100 seeds (fresh wt., g)	Weight of 100 seeds (dry wt., g)
T0	15	-	-	-	-	-
T1		-	-	-	-	-
T0	30	-	-	-	-	-
T1		-	-	-	-	-
T0	45	0	0	0	-	-
T1		2	0	0	-	-
T0	60	6	2	5	6	4
T1		9	6	6	6.7	4.6
T0	75	7	6	6	6.9	4.7
T1		8	12	7	7.7	5
T0	90	7	16	8	7.8	5
T1		9	23	8	8.1	5.1

Number of branches and leaves

Plant growth promoting Rhizobacteria increased the number of branches and leaves on plant growth over the control.Here, this experiment showed the best performance of T1 than T0 control.

Effect of vermicompost on the yield of Green gram (*Vigna radiata*)

The number of main parameters on the yield of green gram was significantly influenced by application of PGPR treatments (Table.5). The number of flower per plant ranged form 2 to 9. The highest number of pods (23) produced per plant by the application of PGPR on the 90th day. The highest number of seeds present per pod of plant by the application of PGPR is 8 than T0 control.The fresh weight and dry weight of 100 seeds were higher in the treatments T1 than in T0 (control) (Table. 5).

Leaf litter is the major pollutant of the water resource. Disposal of these by physical and chemical process is highly expensive (Gohil, 1985). Therefore biological treatment methods have received much attention and considered as efficient, low cost treatment for Leaf litter.

The worms along with organic manure's can be utilized as an alternative to costly inorganic fertilizers (Senappathi, 1980). Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals. This study observed few rhizobacteria tolerant to multiple heavy metals and exhibiting a couple of PGP activities Pseudomonas sps were resistant to 400 gml-1. It was also apparent that more cultures of PGPR isolated from vermicompost were tolerant to elevated levels heavy metals. The selection of microorganisms both metal tolerant and efficient in producing PGP compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils.

All the bacterial isolates in the present study were able to produce catalase. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical, and chemical stress. Some of the above-tested isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. Similar to our findings of multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored (Gupta *et al*., 1998).

There is a paucity of information on effect of Plant growth promoting bacteria from leaf litter vermicompost.on the growth of plants. Hence, the present investigation carried in this direction. The seedlings of Green gram (*Vigna radiata),* with plant growth promoting bacteria from vermicompost using earthworm, *perionyx ceylanensis*, resulted in increase of growth parameters like the root length, shoot length, number of twigs and leaves and total biomass of the plant. Similar observations were madein the black gram *(vigna munga L.Heeper)* by Prakash and Hemalatha (2013) clearly indicated that to obtain healthy seedlings

Reference

Allen, G.N. 1953. Experiment in soil bacteriology. Burgers Publ. Co., Minneopolis, Minn, USA 127.

Bhattacharya, P, Nath, D.S. and Banik, S.1990. Organic manures in relation to rhizohere effect, effect of organic manures on the quantitative and qualitative distribution of bacterium,

Fungi and actinomycetes in the rhizosphere soils of rice. Ind. Agri., 34:157-162

Bouche A., 1992. Earthworm species and ecotoxicological studies, Intercepts, Andover, 20-35

Callahan C.A., 1988. Earthworms as ecotoxicological tools, SPB Academic Publishing, The Hague, 295-301.

Cappuccino, J. C., Sherman, N., 1992. In: Microbiology: A Laboratory Manual, New York, pp. 125 179.

Domínguez, J. 2004. *State of the art and new perspectives on vermicomposting research*. In:

C.A. Edwards (Ed.). Earthworm Ecology (2nd edition). CRC Press LLC. Pp. 401-424

Domínguez, J., Aira, M. and Gómez Brandón, M. 2010. *Vermicomposting: earthworms enhance the work of microbes*. In: H. Insam, I. Franke-Whittle and M. Goberna, (Eds.),

Microbes at Work: From Wastes to Resources (pp. 93-114). Springer, Berlin Heidelberg.

Edwards, C.A., 1998. The use of earthworms in the breakdown and management of organic wastes. In: Earthworm Ecology, CRC Press LLC, Boca Raton, FI, pp 327-354.

Edwards C.A. and Bohlen P.J., 1996.Biology and Ecology of Earthworms, 3rd ed. Chapman and Hall, London, 426.

Gajalakshmi, S., Ramasamy, E.V. and Abbasi, S.A. 2005 'Composting-vermicomposting of leaf litter ensuing from the trees of mango (*Mangifera indica*)', *Bioresour. Technol*., Vol. 96,pp.1057–1061.

Glick, B.R., 1995. The enhancement of plant growth by free living bacteria.Can. J. Microbiol. 41, 109 114.

Gohil, M.B. 1985. Treatment of pulp and mill waste case studies in pollution management in Industries Environmental Publication. Karad, 16-20.

Hutchison, M.L., Walters, L.D., Avery, S.M., Munro, F. and Moore, A. (2005). Analyses of livestock production, waste storage, and pathogen levels and prevalence in farm manures. *Applied and Environmental Microbiology* 71, 1231–1236

Kale R., Vermiculture. 1991. Scope for new Biotechnology. Calcutta: Zoological Survey of India, (6) 122-176.

Kloepper, J.W., R. Lifshitz and Zablotowicz, R.M., 1989. Free-living bacterial inocula for enhancing crop productivity. Trends. Biotechnol. 7: 39 43.

Ismail, S.A. (1997) Vermicology. The biology of earthworms. Orient LonMan pub Ltd, Chennai. 1-73.

Karmegam, N., Alagumalai, K. and Daniel, T. 1999. Effect of vermicompost on the growth and yield of green gram (*Phaseolus aureus* Roxb.). *Tropical Agriculture* 76, 143-146.

Karmegam, N. and Daniel, T. (2009a) 'Investigating efficiency of *Lampito mauritii* (Kinberg) and *Perionyx ceylanensis* Michaelsen for vermicomposting of different types of organic substrates', *Environmentalist*, Vol. 29, pp.287–300.

Karmegam, N. and Daniel, T. (2009b) 'Growth, reproductive biology and life cycle of the vermicomposting earthworm, *Perionyx ceylanensis* Mich. (Oligochaeta: Megascolecidae)', *Bioresour. Technol*., Vol. 100, pp.4790–4796.

Karsten, G.R., and Drake, H.L. 1995. Comparative assessment of the aerobic and anaerobic microfloras of earthworm and forest soils *Appl. Environ. Microbiol*. 61: (3) 1039-1044.

Manna MC, Singh M, Kundu S, Tripathi AK and Takkar PN. 1997. Growth and reproduction of the vermicomposting

earthworm *Perionyx excavatus* as influenced by food materials. Biology and Fertility of Soils 24(1):129–132

Nardi, S., Morari, F., Berti, A., Tosoni, M. and Giardini, L. (2004). Soil organic matter properties after 40 years of different use of organic and mineral fertilisers. *European Journal of Agronomy* 21, 357-367.

Nogales R, Melgar R, Guerrero A, Lozada G, Beniteze E, Thompson R, Gomez M and Garvin MH. 1999. Growth and reproduction of *Eisenia andrei* in dry olive cake mixed with other organic wastes. Pedobiologia. 43(6):744–752

Prakash, M., M.Jaikumar and Karmegam,N. 2008a. Physico-chemical Characteristics and Fungal Flora in the Casts of the Earthworm, Perionyx ceylanensis Mich. Reared in Polyalthea longifolia Leaf Litter. J.Appl. Sci. Res. 4(1): 53-57.

Prakash, M., and Karmegam, N. 2010. Shift of total microflora in the course of vermicomposting and their association with moisture content during incubation of vermicompost In: Microbial Biotechnology (Eds.) P. Ponmurugan and V. Balakrishnan.Excel India Publishers, New Delhi. Pp.258-264.

Prakash, M., and Hemalatha,N. 2013. Dynamics of Microorganisms during Vermistabilization of organic substrates and enhances performance of Plant Growth Promoting Rhizobacteria on Black Gram. *Int.J.Curr.Microbiol.App. Sc.i* 2(7): 171-187.

Senappathi, K, M.C. Dash, A.K. Pana and Panda, B.K. 1980. Observation on the effect of earthworm in the decomposition process in soil under laboratory Conditions. Comp.physio.Ecol.5:140-142.

Sreenivas C, Muralidhar S and Rao MS. 2000. Vermicompost, a viable component of IPNSS in nitrogen nutrition of ridge gourd. Annals of Agricultural Research 21(1):108–113.

Suthar, S. (2006) Potential utilization of guar gum industrial waste in vermicompost production. *Biores. Technol*., 97: 2474-2477.

Tognetti, C., Laos F., Mazzarino, M.J. and Hernández, M.T. (2005). Composting vs vermicomposting: A comparison of end product quality. *Compost Science and Utilization*, 13, 6-13.

Thopate, A.M., P.R. Hapase and Jadhau, S.B.1997. Sugarcane products An important source for vermicompost production. Seminar on Recycling of Biomass and Industrial By-products of sugar, TNAU.Cuddalore. India. pp.49-53.

Vermi Co. 2001. Vermicomposting technology for waste management and agriculture: an executive summary. (http://www.vermico.com/summary.htm) PO Box 2334, Grants Pass, OR 97528, USA: Vermi Co.

Weber, J., Karczewska, A., Drozd, J., Licznar, M., Licznar, S., Jamroz, E. and Kocowicz, A. 2007. Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. *Soil Biology and Biochemistry*, 39, 1294-1302.