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Changes in Chemical and Biological Parameters during Co-Composting of Anaerobically Digested Sewage Sludges with Lignocellulosic Material

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ABSTRACT
This study reports a pilot experiment of composting of anaerobically digested sewage sludges deriving from the production of biogas in a wastewater treatment plant. Two composting piles (about 15 m^3 each) were prepared by mixing 50% and 30% sludges with lignocellulosic material. The composting process was monitored through determination of the main physico-chemical properties. The maturity of the composts was assessed by determination of the respiration index and dehydrogenase activity. The data indicated that, at both sludges concentrations, the process produced a final compost suitable for agricultural applications. The germination and growth indexes of *Lepidium sativum* indicated the lack of phytotoxicity of the final materials. The suitability of the final product as a field improver and horticultural substrate has been attested through bioassays conducted on maize and chrysanthemum.

Keywords: Composting, anaerobic sewage sludges, plant assays

INTRODUCTION
The disposal of sewage sludges from municipal and industrial treatment plants should ensure the protection of public health and environment. The main routes of disposal of sludges are production of biogas, incineration, recycling to agriculture (landspreading), or landfilling. Current trends in European waste policy aim at reducing the deposition of biodegradable wastes in land-fill sites.[1]

Utilization of sewage sludges in soil fertilization can have positive effects on the yield of several crops due to their content of organic matter and macro and micro nutrients.[2,3] However, application of low-quality sludge at high quantity to agricultural soils may adversely affect soil fertility, threaten ground water quality, and lead to food chain contamination.[4] Direct application of sludges also has an unfavourable impact on the environment due to bad odour and the possible presence of pollutants and pathogens. The development of agricultural recycling depends largely on the possibilities for improving the quality of the sludge itself and increasing confidence in sludge quality.[5] The use of sludges in agriculture is regulated by the European Directive 86/278 of 12 June 1986,[6] implemented in Italy by the D.Lgs n° 99 (1992).[7] Both the regulations establish that sludge can have valuable agronomic properties and that encouraging its application in agriculture is therefore justified provided it is used correctly. Before being used in agriculture, sludges must undergo biological, chemical, or heat treatment, long term storage, or any other appropriate process so as to significantly reduce its fermentability and the health hazard resulting from its use.

Composting of sludges ensures quality improvement by biological stabilization and control of hygiene risks. It also implies a reduction in the volume of materials to be transported and distributed, due to the lower water content of the final material.

Sludge characteristics will depend on the type and origin of the waters to be purified, as well as on the type of treatment (aerobic or anaerobic) followed in the wastewater treatment plant. The high moisture content of sludges means that they cannot be composted alone and they need to be mixed with dry materials (such as sawdust, vegetal remains, straw), which act as bulking agents.[8,9] Therefore the nature of sludge and the type and proportion of bulking agent used for composting will influence the composting reaction rate and the final compost quality.

This study reports a pilot experiment on the composting of anaerobically digested sewage sludges deriving from the production of biogas in a wastewater treatment plant. The bulking agents were woodchips of fresh or partially decomposed lignocellulosic material. The composting process was monitored through determination of the main physico-chemical and biological properties. The suitability of the final product as field improver and horticultural substrate has been tested through bioassays conducted on maize and chrysanthemum.

MATERIALS AND METHODS

Starting Materials

An anaerobic sewage sludge from a domestic and industrial wastewater treatment plant in north-western Italy (Cordar spa plant, Biella) was mixed with chopped green wastes in order to prepare two piles (2 m wide, 5 m long, and 1.5 m high) with the following proportions (on a volume basis):

- pile A: 50% sludge, 30% fresh green wastes, and 20% partially decomposed green wastes
- pile B: 30% sludge, 40% fresh green wastes, and 30% partially decomposed green wastes.

The partially decomposed green wastes were obtained by allowing the material to stand outdoors for four months in static condition. The composting process lasted 90 days; piles were mechanically turned every 7–10 days during the bio-oxidative phase (first 30 days), and then once a month during the maturation phase (2 months). The main characteristics of the starting materials are reported in Table 1. The metal content of the sludges expressed in mg Kg^{-1} were Cd: 0.63, Cr: 650, Hg: 0.92, Ni: 45, Pb: 57, Cu: 234, and Zn: 853. The metal concentrations of the sludges were below the acceptable limits for utilization in composting plants according to European and Italian legislations.[6,7] The relatively low EC (409 µS cm^{-1}) and organic C content (24.5%) and the low C/N ratio (8.4) of the sludges are typical of anaerobically digested sludges, in agreement with the values reported for similar materials.[8,9] The maturation of lignocellulosic wastes leads to a strong decrease in organic C content (from 33.7 to 25.6%) and C/N ratio (from 33.7 to 15.1) attesting to the decomposition of organic matter. The consequence of this transformation is an increase in the electrical conductivity (from 932 to 1599 µS cm^{-1}) due to the release of soluble salts.

Physico-Chemical and Biological Characterization

Temperature was monitored daily in six random locations of each composting pile.

Samples (about 1 Kg) were collected at random locations in each pile at days 0, 7, 19, 28, 58, and 91. The samples were combined and mixed to generate a composite sample. Three subsamples were prepared from the composite sample and analysed independently.

The pH and electrical conductivity were measured on freshly collected samples in 1:5 (w/v) aqueous extract. C and N content were measured by elemental analysis of 0.5 mm sieved samples. The total P content was determined colourimetrically (phosphomolybdic complex), after nitric-perchloric acid digestion. A respiration test was performed on 1 Kg fresh samples brought to 80% maximum moisture capacity with a static respirometer (COMPOSTAB®PC , Athon System srl, Italy). Dehydrogenase activity was determined on the freshly collected samples.[10]

Phytotoxicity was evaluated on cress (*Lepidium sativum*) according to the recommended methods of the Standard UNI 10780: 1998[11] through determination of the germination index and of the growing index. The germination index (I_g) was evaluated on seeds in contact with aqueous extracts of the compost (85% moisture) diluted to 50 and 75% (sample) or with deionized water (control). For each concentration, the germination index was calculated according to the following equation:

$$I_g = \frac{G_s . L_s}{G_c . L_c} . 100$$

G_s = number of germinated seeds – sample
G_c = number of germinated seeds – control
Ls = medium length of the root – sample
Lc = medium length of the root – control

The final result is the average of the I_g values of the two concentrations.

For the determination of the growing index, the substrates were a mixture of siliceous sand and blond peat (1:1, V/V) mixed with 25, 50, and 75 g dry compost/L substrate. A control trial was conducted with the substrate without compost.

At the end of the growing phase (21 days), the aerial parts of the plants were collected and weighed after drying at 60 °C. The growth index was calculated according to the following equation:

$$G_m = \frac{G_s}{G_c} \cdot 100$$

G_s = medium weight of the sample
G_c = medium weight of the control

Agronomic evaluation

Zea mays experiment

The experiment was conducted in the experimental farm of the Department. The growth of a maize hybrid (Pioneer PR35Y65) was tested on substrates prepared as follows: a basal substrate containing 50% sterile quartz sand and 50% sphagnum peat moss was mixed with 20 or 30% (V/V) compost A or B. Five replicates per trial were conducted, each consisting of three maize seeds sown in 20 L plastic pots. The plants were protected by a hail screen and irrigated daily with an automatic apparatus. The experiment lasted two months (3 July – 5 September). At the end of the experiment, the aerial parts of the plants was collected and weighed after drying. The dry weights of the aerial parts of the plants were determined (60 °C × 72 h) and compared with those of control plants grown on the substrate without compost and plants grown in the field.

Chrysanthemum morifolium experiment

Chrysanthemum plants (cv Sepia, by Lazzeri Agricultural Group, Merano, Italy) were grown at the Lanza Nursery (Ronco Biellese, Italy), from June to October. The standard substrate (turf and sand) was mixed with 20%, 30%, and 100% compost; control plants were grown on standard substrate. Each trial consisted of five 2 L pots. Plants were grown under a hail screen and fertilized weekly (18/18/18+3 (N/P/K + Mg) until the 30 August, and 15/15/30+3 (N/P/K + Mg) until the end of the experiment). At the end of October, when the control plants reached commercial size, the quality of the plants was evaluated according to the following criteria: height, diameter, number of flower stalks, and number of flowers on each stalk.

RESULTS AND DISCUSSION

Temperature

The temperature of pile B (30% sludges) reached 55 °C, an optimal value for aerobic fermentation of organic matter, on the 21st day. In contrast, the maximum temperature of pile A (50% sludges) was 43 °C on the 26th day. A further decrease was then observed in both piles as usually expected in such a process. At the end of the experiment, the composts were in equilibrium with the ambient temperature. The fact that the temperature of pile A remained relatively low even during the exothermic phase of the process suggests that a high content of sludges (50%) does not promote the best conditions for biooxidation. This result is in agreement with those of Banegas et al.[9], who found that in composting of anaerobic sludges mixed with sawdust, the thermophilic temperatures were lower in the pile at a sludge:sawdust ratio of 1:1 than in that at 1:3. Banegas et al.[9] explained such behaviour by the toxic effect on microorganisms of Zn at high concentration and by the presence of other toxic substances derived from the anaerobic treatment. Such a toxic effect was not observed at low sludge concentration because of the dilution by the higher proportion of the bulking agent. The toxic effect of Zn cannot be excluded in our experiment, although the Zn content of the sludges (853 mg Kg^{-1}) was much lower than that reported by Banegas et al.[9]: (2195 mg kg^{-1}). A minor increase in temperature has been also related to a too low C/N ratio (15) of the starting material by Huang et al.[12] during the co-composting of pig manure and sawdust at two different ratios. These authors suggest that the scarcity of available carbon source at the beginning of composting does not provide a favourable condition for the growth and biological activity of microorganisms. In our experiment, although the formation of toxic compounds cannot be excluded, the low temperature observed in pile A is more likely related to an insufficient aeration of the material due to the relatively low amount of bulking agent, which did not ensure an adequate microbial activity.

Physical-Chemical Properties

The main characteristics of piles A and B during composting are reported in Tables 2 and 3 respectively.

pH

The pH values increased during the first 19 days to values close to 9, and then decreased to final values of 6.9 and 7.3 in composts A and B respectively. The pH trend reflects the steps of composting: usually easily degradable carbon sources, such as monosaccharides, starch, and lipids, are utilized by microorganisms in the early stage of composting. The pH decreases because organic acids are formed from these compounds during degradation. In the next stage microorganisms start to degrade proteins, resulting in the liberation of ammonia and an increase in the pH.[13] The successive pH decrease is induced by the rapid dissipation of NH_3, mainly by volatilization and nitrification.

Electrical conductivity

The electrical conductivity of the two composting materials increased during the process from about 800 $\mu S\ cm^{-1}$ to final values close to 1500 $\mu S\ cm^{-1}$. An increase in EC usually takes place during composting as a result of the release of salts due to the mineralization of organic material. The range of variation of this parameter is dependent on the nature of the initial feedstocks: increases from about 600 to 800 $\mu S\ cm^{-1}$ during composting of sheep manure and straw,[14] from 1166 to 2230 $\mu S\ cm^{-1}$ during composting of sewage sludges mixed with wood shavings,[15] from 900 to 1600 $\mu S\ cm^{-1}$ during composting of pig manure and sawdust, and from about 2000 to 3000 $\mu S\ cm^{-1}$ during composting of food waste[16] were reported. In contrast, Li et al.[17] observed a decrease from 4360 to 2750 $\mu S\ cm^{-1}$ during composting of a mixture of sewage sludges, pig manure, and sawdust.

Total carbon content

The total organic carbon content was higher in mixture B at the beginning and during the first 30 days of the process, probably due to the major presence of C-rich lignocellulosic material. After about one month, both the piles exhibited an organic carbon content close to 25%. These results are very close to those reported by Albrecht et al.[18] during the composting of digested municipal sewage sludges and green wastes. The relatively low variation in the organic carbon content could be attributed to the more stable nature of the organic matter contained in anaerobic sludges as a consequence of the stabilization it had undergone in the treatment plant. Such a statement has been expressed by Banegas et al.[9] by comparing the basal respiration of aerobic and anaerobic sludges during the composting with sawdust. In contrast, Zubillaga and Lavado[19] observed a strong decrease in C content during composting of digested sewage sludges with different proportions of sawdust to abnormally low final values (close to 4 g Kg^{-1}).

Total nitrogen content

The trend of the total nitrogen content included an initial decrease due to the degradation of the proteic material, followed by a stabilization at values close to 2%. Similar results have been reported in composting of sewage sludges with green wastes[18] and maize straw.[20]

C/N ratio

The two mixtures exhibited a low C/N ratio (close to 11) at the beginning of the experiment. This is due to the low C/N values of the starting materials (especially sludges and partially decomposed green wastes; Table 1). Only a minor variation in this parameter was observed during composting, leading to final values of 12.0 and 13.6 for piles A and B respectively. Usually, the C/N ratio is much higher (25 to 30) at the beginning of composting and decreases during the thermophilic phase in relation to mineralization of the organic matter.[14,16,21,22] The optimum C/N ratio has been reported to be 25 ± 4.[13] However, in the case of sewage sludges based mixtures several studies report a low initial C/N ratio and a reduced change in this parameter during the process in agreement with our results.[15,18,20,23] Several studies have been conducted in order to verify the effect of the initial C/N ratio on the composting process by using different compositions of starting materials. Eiland et al.[24] observed that low initial C/N ratios caused a fast degradation of fibres during the first three months of composting of straw and pig manure (hemicellulose: 50–80%, cellulose: 40–60%). Huang et al.[12] concluded that co-composting of pig manure with sawdust at a low initial C/N ratio would require a longer composting period to reach maturity. In a similar study conducted on sewage sludges and rice straw, Iranzo et al.[23] observed that a low C/N ratio induces a higher oxygen consumption than other larger ratios. Such behaviour was attributed to the fact that a low C/N ratio increased the microorganisms' growth and provided a better homogeneity of mixture and consequently a greater microbiological activity.

Phosphorous content

Phosphorous content was measured at the beginning of the process and then in the mature composts. It increased from 1.2% to 2.3% for mixture A and from 1.8% to 2.3% for mixture B. Such a trend due to organic matter mineralization is reported by several authors.[12,15,25,26]

Potassium content

Both the starting mixtures exhibited a high total K content (5.6 and 4.4 g Kg^{-1} in mixtures A and B respectively) which increased to 7.1 and 8.2 g Kg^{-1}. Although K is an important nutrient, its concentration in compost is seldom reported. A compost obtained from sewage sludge mixed with woodchips exhibited a K concentration of 2.3 g Kg^{-1} [15] while about 10 mg Kg^{-1} K was found in a vermicompost of organic wastes.[25] K concentration is largely dependent on the starting materials. According to the data of Solano et al.,[14] it is much higher in manures than in sewage sludges and green wastes.

Respiration Index and Dehydrogenase Activity

Dehydrogenase activity is commonly used as a valid bioindicator of the composting process especially if correlated to other parameters such as oxygen consumption during the composting process.[27]

Dehydrogenase activity decreased dramatically during composting from 1.53 µMTPF g^{-1} (pile A) and 1.07 µMTPF g^{-1} (pile B) to 0.02 µMTPF g^{-1} in both composts at the end of the experiment. Such a trend is usually associated with an adequate maturation of the compost.[28–32]

The respiration indexes measured at the end of the composting process were respectively 51 and 37 mg O_2 $Kg^{-1}VS$ h^{-1} in composts A and B. An insufficiently mature compost has a strong demand for O_2 and high CO_2 production rates due the intense development of microorganisms as a consequence of the abundance of easily biodegradable compounds in the raw material,[20] while a low respiration rate is likely due to the stability of the anaerobic sludges as observed by Banegas et al.[9] The method we used measures the maximum rate of O_2 required to degrade the volatile solid unit (VS) in static conditions. It is generally considered that, in these conditions, a respiration index < 200 mg O_2 $Kg^{-1}VS$ h^{-1} attests to a sufficient maturity of the compost, confirming the indication given by the decrease in dehydrogenase activity.

Several authors report respiration data in compost [9,23,29,31,33–35] but the variety of methods used for the determination and for the expression of the results does not allow us to compare the results.

Plant Assays

Lepidium sativum

The germination indexes of the composting materials of piles A and B are reported in Tables 4 and 5 respectively. According to the Italian standard, a non phytotoxic material exhibits a medium germination index > 70%. In both piles, the germination index at the end of the experiment was > 75%, attesting to the lack of phytotoxicity of the composts. Some values < 70% were found during composting, probably due to the production of toxic compounds. The determination of the germination index of different seeds has been used to follow the phytotoxicity of composting mixtures of sludges and green wastes[9,15] and of manures and ligno-cellulosic bulking agents of different origins[12,14]. The results reported in such experiments confirm that the final material exhibited a high germination index, although some lower values were found during the transformation process. However the germination index determination, the only test performed to assess the phytotoxicity of composts in most of the studies dealing with composting processes, was not reliable enough. Oleszczuk[36] observed that seed germination proved to be the relatively weakest indicator of sewage sludge toxicity and pointed out that criteria for the evaluation of sewage sludge and composts with respect to their ecotoxicity are strongly needed. Aslam and VanderGheynst[37] proposed an improvement of the germination index determination consisting of direct seeding into compost-amended soil mixtures.

The result of the growth test on substrates containing compost at different concentrations added to a mixture of peat and sand is reported in Table 6. The plant growth index of both compost (about 85%) was not significantly different from that of the control without compost, confirming the lack of phytotoxicity. Although plant assays are largely used for the evaluation of the maturity of the compost, the information they give depends greatly on the plant species and the methodology. In performing bioassays to evaluate the maturity of composts with different species *(Lepidium sativum, Raphanus sativus,* and *Brassica chinensis*), Warman[38] concluded that the commonly used compost extract test and the compost-soil germination and growth tests were not sensitive enough to detect differences between mature and immature composts. The nature of the plants has been demonstrated to be a major factor affecting the results of the assays. In a study evaluating the potential toxic effect of sewage sludges using *Brassica rapa, Lolium perenne*, and *Trifolium pratense*, Ramirez et al.[39] observed that this last plant was more sensitive than the other species. Aslam and VanderGheynst[37] performed experiments to evaluate the phytotoxicities of different composts by determination of the germination index of seeds of *Lepidium sativum, Raphanus sativus, Brassica rapa,* and *Lactuca sativa* on compost-amended soil mixtures. They observed that *Lepidium sativum* was more susceptible than the other tested species. Aslam and VanderGheynst[37] develop a sigmoidal model to relate phytotoxicity (measured as seed germination) with compost stability (measured as carbon mineralization rate and potential mineralizable carbon).

Taking into consideration the variability of the bioassays, it is useful to check the suitability of the composts in relation to their potential destination. For example, Ostos et al.[40] checked the suitability of substitution of peats with composts in a nursery, conducting a growth assay with *Pistacia lentiscus* L. (Anacardiaceae).

Taking into account the possible applications of the composts further assays have been conducted with maize and chrysanthemum, selected as representative species.

Growth of chrysanthemum

The results of the growth of chrysanthemum on substrates containing 20, 30, and 100% compost are illustrated in Table 7. In the trials with 20 and 30% compost, the height of the plants was slightly lower than that of the control but the diameter was not significantly different and the number of flower stalks and buds was higher. Since these last parameters are considered the most important to evaluate the commercial value of the plants, we can conclude that substitution of more than half of the amount of peat with compost has led to an improvement in the quality of the plants. In the trial in which all the peat was substituted by compost, some symptoms of phytotoxicity took place, as demonstrated by the minor development of the plants and the low number of stalks. This was due to plants flowering before the expected time, probably because of an excess of nutrients.

Growth of maize

The results expressed as the dry weight of the epigeous part of the plants are reported in Table 8. Plants grown on the mixtures containing compost did not show any sign of phytotoxicity but did not reach the dimensions of the control plants grown in the field. These results suggest that the release of nutrients from the composts was too slow to ensure the development of maize plants and that additional mineral fertilization, as normally used in experiments with maize,[35,41,42] was needed. The growth of the control cultivated on a mixture of peat and sand was dramatically low because of the inadequacy of the substrate. The addition of compost, especially at the highest percentage, provoked a significant improvement in the growth of the plants, suggesting that the composts are able to partially compensate the lack of nutrients of the control substrate. In a previous experiment, performed in the same conditions but with a non-composted municipal sludge, the plant growth was not different from that of the field-grown plants (data not shown). This suggests that the increase in the stability of the sludges by composting induces a slow release of nutrients that is too slow to sustain a fast and exigent crop such as maize.

CONCLUSION

The composts obtained from anaerobic sewage sludges and green wastes exhibited physico-chemical properties and a maturity level suitable for agricultural use. The different proportions of sewage sludge in the starting materials did not influence the characteristics of the composts, notwithstanding the minor temperature reached in the pile with 50% sludges.

The lack of phytotoxicity pointed out by the assay with *lepidium sativum* was confirmed in the case of chrysanthemum. The growth of maize in pots has also given promising results although they should be confirmed by field experiments.

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Table 1. Main characteristics of the starting materials.

	Sludges	Fresh lignocellulosic material	Mature lignocellulosic material
pH (H_2O)	8.4	7.0	7.9
EC (μS cm^{-1})	409	932	1599
C (%)	24.5 ± 0.30	33.7 ± 0.28	25.6 ± 0.25
N (%)	2.9 ± 0.04	1.0 ± 0.05	1.7 ± 0.07
C/N	8.4	33.7	15.1

Table 2. Changes in chemical parameters during composting of pile A.

Time, days	pH	EC, uS cm^{-1}	C (%)	N (%)	C/N
0	8.3	800	26.5 ± 0.20	2.4 ± 0.03	11.0
7	8.5	671	26.2 ± 0.16	2.4 ± 0.02	10.8
19	8.8	1027	26.2 ± 0.05	2.1 ± 0.03	12.5
28	8.1	982	25.7 ± 0.26	2.2 ± 0.13	11.9
58	7.2	1111	24.7 ± 0.13	2.0 ± 0.06	12.3
91	6.9	1561	24.2 ± 0.20	2.0 ± 0.11	12.0

Table 3. Changes in chemical parameters during composting of pile B.

Time, days	pH	EC, uS/cm	C (%)	N (%)	C/N
0	8.4	747	28.9 ± 0.25	2.6 ± 0.09	11.1
7	8.7	857	30.1 ± 0.15	2.2 ± 0.06	13.4
19	8.8	1178	27.9 ± 0.21	1.9 ± 0.04	14.6
28	8.3	1224	25.6 ± 0.24	1.9 ± 0.07	13.2
58	7.4	1143	25.3 ± 0.19	2.0 ± 0.06	12.6
91	7.3	1489	25.4 ± 0.23	1.9 ± 0.08	13.6

Table 4. Germination index of *L. sativum* at two concentrations of the compost extract and different composting times of pile A (mean of 5 replicates ± SE)

Days	0	7	18	28	91
50%	80.6 ± 5.0	97.3 ± 9.3	57.2 ± 6.0	107.3 ± 9.7	86.1 ± 7.2
70%	67.8 ± 6.2	66.6 ± 10.5	40.3 ± 6.0	84.2 ± 9.3	86.5 ± 11.2

Table 5. Germination index of *L. sativum* at two concentrations of the compost extract and different composting times of pile B (mean of 5 replicates ± SE).

Days	0	7	18	28	91
50%	87.6 ± 10.9	72.6 ± 5.7	111.9 ± 9.9	92.0 ± 5.4	78.3 ± 4.7
70%	78.8 ± 11.5	48.9 ± 7.6	98.1 ± 10.4	105.2 ± 11.6	83.8 ± 9.3

Table 6. Growth index of *L. sativum* at different application rates of compost at the end of the experiment (mean of 5 replicates ± SE).

	Pile A	Pile B
25 g L^{-1}	85.4 ± 11.6	84.2 ± 7.0
50 g L^{-1}	86.0 ± 9.2	102.5 ± 9.5
75 g L^{-1}	86.0 ± 7.6	70.7 ± 6.2

Table 7. Effect of addition of different amounts of compost on the growth of chrysanthemum plants (mean of 5 replicates ± SE).

	Control	Compost A			Compost B		
% compost	0	20	30	100	20	30	100
Height	35.4±1.03	29.2±0.53	31.4±0.93	28.7±0.88	34.3±1.00	28.6±0.75	28.0±0.58
Diameter	52.8±1.66	51.7±0.95	50.4±1.72	41.7±0.89	51.0±1.71	53.4±1.78	43.3±0.88
Number of stems	52.4±5.34	76.2±1.35	80.8±1.89	38.3±1.76	71.3±2.16	63.2±3.31	46.0±5.13
Number of flower buds	308±38	477±17	454±32	261±26	388±27	331±35	155±21

Table 8. Effect of addition of different amounts of compost on the growth of maize (mean of 5 replicates ± SE).

	Pot control	Field control	Compost A		Compost B	
% compost	0	0	20	30	20	30
Dry weight,g	11.8±3.42	169.8±4.36	63.8±2.7	82.6±5.3	60.8±2.03	79.3±5.00