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ORIGINAL RESEARCH

Ecophysiological Responses of Plants After Sewage Sludge Compost Applications

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Abstract Composting is one of the most appropriate methods to recycle sewage sludge. Sewage sludge compost is a suitable solution for improving the quality of barren soil at landfill. Therefore, it is important to investigate the effects of sewage sludge compost on plants. Different compost application methods (mixing and scattering over reclaimed soil) on sawtooth oak (*Quercus acutissima*) and Japanese red pine (*Pinus densiflora*) have been tested. The application of sewage sludge compost markedly increased soil moisture and nitrogen content. Compost treatments resulted in significant increases in both plant height and biomass as compared to controls. Compost treatments led to a significant increase in the N content of plant leaves. Compost treatments resulted in significant increases in the chlorophyll content and photosynthetic rates of the plants. The scattering of compost over reclaimed soil (compost 2) resulted in lower total antioxidant activity and superoxide dismutase activity than mixing the compost with the reclaimed soil (compost 1), or in the control treatment. Since the growth rates, N content, and photosynthetic rates in compost 2 treatment were not markedly different from compost 1 treatment, it (compost 2) would be a better application method from both an ecological and economic perspective.

Keywords Compost · Landfill · Photosynthetic rate · Sewage sludge · Soil characteristics · Antioxidant

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Introduction

Urbanization has led to the generation of large quantities of sewage sludge from wastewater treatment. In Korea, about 1.7 million tons of wastewater sludge is generated every year. In 2005, about 1.1 million tons (64.3%) of sewage sludge was discharged into the ocean, with only 8,000 tons (5.1%) being recycled (Bae 2006). Although sewage sludge contains useful components, such as organic matter, nitrogen (N), and phosphorus, the recycling rate is very low. Ocean disposal of sewage sludge causes environmental problems (O'Sullivan 1971) such as eutrophication. Furthermore, the London Convention 96 Protocol, which prohibits ocean disposal of sewage sludge, will come into effect in Korea in 2011. Hence, the use of sewage sludge as a nutrient source is an interesting research topic. Composting of sewage sludge is a useful method for recycling sewage sludge and involves the degradation of the organic matter (OM) and nutrients within the sewage sludge. Composting is a process that leads to the reduction of many phytotoxic substances in sewage sludge, and it involves the biodegradation of OM and pathogenic microorganisms (Korboulewsky et al. 2002). Therefore, sewage sludge compost is being increasingly considered by many municipalities given its several advantages over other disposal strategies and the potential nutritive benefits to the soil (Wei and Liu 2005).

However, thus far, most studies on sewage sludge were focused on toxic materials, such as heavy metals (Cai et al. 2007; Moreno et al. 1996), or on the composting process (Benitez et al. 1999; Fang et al. 1997) and the effects on soil (Aggelides and Londra 2000; Inubushi et al. 2000). Few studies have reported on ecophysiological reactions of plants after sewage sludge compost application (Han et al. 2004). It has been difficult to locate studies in international journals that report the differences in plant photosynthetic rates and antioxidant activities after compost application. However, decrease of photosynthesis could be the result of stress-induced damage (La Porta et al. 2004) and implying plant conditions (Lee et al. 2004a, b), and antioxidants can be induced by toxic materials of the soil (Patra and Panda 1998) and plant stress (Shim 2006; Zhang et al. 2007; Koricheva et al. 1997). As sewage sludge compost includes heavy metals and induces plant stress (by many phytotoxic substances), antioxidant activity can be an important research item in compost research. Therefore, it is necessary to investigate the ecological and physiological responses of a plant to treatment with sewage sludge compost.

Typically, landfill sites use mined soil for reclamation, and as it has very low levels of OM, a large amount of fertilizer is required to restore soil properties in order to facilitate revegetation. Since the Sudokwon landfill (study site and the source of sewage sludge used) covers a very large area, using fertilizers for revegetation is expensive. Recycling sewage sludge into compost for use in the landfill would be very economical and environmentally friendly. However, since a large amount of soil would be required for the reclamation, mixing compost with the reclaimed soil to create top soil would be very difficult. In addition, since sewage sludge compost can directly affect plant roots, covering a landfill area with a thin layer of compost (typical fertilizer method of scattering over the soil) would reduce the side effects of sewage sludge compost, such as root growth inhibition (Oleszczuk 2008) by indirect contact. The two abovementioned methods were tested by evaluating plant stress. Scattering compost on a small area could have a limited impact because wind and rainfall may spread the compost to the non-composted areas. However, as landfill areas are large, spreading will not reduce the effect of the compost. The best application method can be determined by comparing both methods.

The purpose of this study was to investigate the effects of sewage sludge compost on soil and plants. For this purpose, changes in soil properties, plant physiological responses (photosynthetic rate and antioxidant activity), plant ecological responses (growth and biomass increases), and heavy metal accumulations in the soil and plants were investigated. We investigated the validity of sewage sludge compost application to landfills by evaluating reactions in soil and plants.

Materials and Methods

Study Site, Composting, and Experiment Design

Study Site

Reclaimed soil and sewage sludge from the Sudokwon landfill in Incheon, Korea was used in the study. All waste (except toxic materials) from Seoul and Gyeonggi Province (population of ~25 million) was brought to this landfill. The total area of the landfill is about 20 km^2 and the site has established to reclaim 250 million tons of waste from 1992 to 2025. The landfill processes 6,700 tons of leachate and 600 tons of sludge per day, and approximately 25,000 tons of sludge is disposed annually (Lee et al. 2004a, b). After the landfill closes, an eco-park will be constructed on the reclaimed site. The geographic coordinates of the landfill are 37° 34′ 52.14″ N and 126° 37′ 29.06″ E.

Planted pots were placed in an open green field with an irrigation system near the center of the landfill. The annual average temperature in 2006 was 12.7°C, and the annual precipitation was 1,300 mm (KMA 2007).

Composting

Sewage sludge was composted in a warehouse at Sudokwon landfill using 70% landfill sewage sludge carried in from Seoul and Gyeonggi Province (sewage sludge dried until a moisture content of 65% was achieved) and 30% sawdust and bark by volume. An aerobic microorganism solution mixture (0.1%) was added to facilitate composting, and mixing was performed in a 30-m^3 fermenter with continuous air supply of 3.6 $m^3 min^{-1}$. The temperature of the compost increased to 69°C after 5 days. The temperature remained above 65°C for over a week. The water content of the sludge dropped from 64.5% to 52%. After 20 days of composting in the fermenter, temperature-stable compost was piled to a height of 2 m.

Experimental Design

In May 2006, two plant species, sawtooth oak (*Quercus acutissima* Carruth.) and Japanese red pine (*Pinus densiflora* S. et Z.), were grown using four different treatments in the pot experiments (10 replicates each). Each pot had an upper diameter of 19 cm (16 cm lower diameter) and a height of 16 cm. The average height of the sawtooth oak was 32.3 cm before leaves appeared. The average height of the Japanese red pine was 12.7 cm. We used 3-year-old sawtooth oaks and Japanese red pines for the pot experiments. Trees of this age are typically planted in landfills. The plants were irrigated regularly.

In compost 1 treatment, 25% of sewage sludge compost was mixed with 75% (by volume) of reclaimed soil (compost 1). Twenty five percent of the sewage sludge mixture was used because other studies achieved best results with this proportion (25–30%) (Lee et al. 2004a, b; Perez-Murcia et al. 2006). The same volume and proportion of compost and reclaimed soil were used in compost 2 treatment, but the sewage sludge compost was not mixed with reclaimed soil and was scattered over the reclaimed soil after planting. About 3 cm of the soil in the pot was covered with the sewage sludge compost (400 cm^3 by volume and about 250 g by fresh weight; 2.6 g of N per pot). To compare the compost with a fertilizer, we used a controlled-release fertilizer [Osmocote Plus (13(N)+13(P)+13(K)+2MgO) by Scotts International B.V.] treatment. Osmocote treatment was administered (20 g per pot) so that each pot had the same N content. Reclaimed soil (100%) was used in the control treatment. Additional pots were used to measure antioxidant activity.

Soil, Compost, and Plant Analyses

Soil Characteristics

The soil in the pots was sampled using 100-ml soil sampling cores (Eijkelkamp BV). Three core-sampled soils from a pot were mixed to obtain one sample for measurement. The soil was then dried at 105 C for 48 h to determine its water content. The OM content was determined by loss on ignition (combustion at 550°C) for over 4 h (Dean 1974). The pH and electrical conductivity (EC) of the soil and compost were determined using a suspension of the soil samples in water (20 g 30 ml^{-1}).

Heavy Metals

One gram of dried and milled soil, compost, and plants was pretreated with 60% HNO_3 for 24 h and heated to 80°C for 2 h. Then, 10 ml of 70% perchloric acid was added and the solution was heated to 200°C until it became clear. The samples were then filtered using Whatman 44 filter paper, and their heavy metal content was analyzed using ICP emission spectrometer (ICPS-1000IV, Shimadzu, Japan). Five heavy metals, all known as major toxic materials in sewage sludge, were investigated.

Analyses of Carbon, Nitrogen, and Hydrogen Content

To determine the C, N, and H contents of the soil, compost, and plants, samples were analyzed using an elemental analyzer (Flash EA 1112; Thermo Electron Co.). NH_4^+–N and NO_3^-–N analyses were performed using Kjeldahl protein/N analyzer (Kjeltec Auto 1035 System; Tecator AB, Denmark).

Photosynthesis and Chlorophyll

Photosynthesis was measured using a portable photosynthesis measurement system (Li-6400; LI-COR Biosciences, USA) in September (30°C; 400 ppm CO_2). The chlorophyll content of leaves was simultaneously measured by extraction with dimethyl sulfoxide extraction method (Hiscox and Israelstam 1979).

Plant Height and Biomass

The height of each potted plant was measured soon after transplantation in May, and the final height and biomass were measured before harvesting in September.

Antioxidant Activity

Sample preparation In late July (nearly 3 months after planting), plants were removed from the pots and replanted for three treatments (control, compost 1, and compost 2). Plants subjected to the control treatments were also replanted to provide the same disturbance during re-planting. Failure to do this may lead to overestimation of plant stress in early stages of compost treatment due to the disturbance of the root during re-planting. Two weeks before the measurement date, 15 pots (three treatments) were established, and the same number of pots was established 1 week before. Using such methods and taking all measurements on the same day helped to minimize the differences in plant stress that resulted from other environmental factors (weather, temperature, etc.).

Crude extracts were prepared by re-suspending the frozen cells in 50 mM phosphate buffer (pH7.2) (Antonyuk et al. 2005). The plant samples (0.1 g) were washed and then the cells were quick frozen in liquid N_2 and milled subsequently. Phosphate buffer (1 ml; Sigma-Aldrich Corp.) was added and the suspension was stirred using a vortex mixer. The waiting time for protein extraction was more than 30 min. After centrifuging at 14,000 rpm at 4°C for 20 min, 400 μl of the supernatant was collected for the experiments (Shim 2006). Absorption was measured using SpectraMax (Molecular Devices, USA).

Protein analysis Protein quantities were determined by using Bradford assay (Bradford 1976) to obtain a standard curve.

Total Antioxidant Activity (TAA) We slightly altered the method established to determine the antioxidant activity of an organic liquid using bathocuproine [US Patent 6613577 (Cruz 2003)]. Our method comprises the following steps (Shim 2006). First, a sample of the liquid (15 μl) was mixed with bathocuproine (200 μM; 585 μl) and stirred using a vortex mixer, and a predetermined quantity (200 μl) of each of the samples was poured into the wells of a multi-well plate. Then, spectrophotometric measurements were performed of the samples at 490 nm (S1). A predetermined quantity (50 μl) of copper sulfate solution was added to each well and incubated at room temperature for 5 min; the reaction was terminated using ethylenediaminetetraacetic acid. Then again, a second round of spectrophotometric measurements was performed at 490 nm (S2). Total antioxidant activity is calculated as TAA=S2−S1 (μM/ml). Finally, units were converted using the Bradford assay results (μM/mg).

Superoxide Dismutase (SOD) The superoxide dismutase activity was measured using the methods established by Peskin (Peskin and Winterbourn 2000) and protocols of the WST assay by Dojindo, Japan. Using a WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) solution (Dojindo, Japan) and an enzyme working solution (Dojindo, Japan), SOD activities were calculated under the Dojindo's protocols.

Statistical Analyses

Statistical significance ($P<0.05$) of the differences was determined by one-way ANOVA followed by Duncan's multiple range test for a post hoc comparison.

Results and Discussion

Characteristics of the Soil and Compost

The reclaimed soil was collected from the first, the lowest, layer of the landfill (the landfill has eight layers). The area is newly planted and soil was plowed up before planting, creating usual soil conditions for landfill planting. Soil composition is mostly yellow earth (loess), as usual in Korea. The reclaimed soil showed poorer conditions than expected (Table 1). Because the reclaimed soil was taken from an adjacent land, and came from a depth of up to 100 m, it typically had poor nutrient and low moisture contents. The reclaimed soil had extremely low nutrient levels (about 0.04% of N). Further, the available form of N was very low. As this soil eventually becomes the top soil for the landfill, it would become a limiting factor for plant growth. However, the reclaimed soil contained a relatively high level of heavy metals. As some heavy metals exceeded the national standards (Lee et al. 2005) for hazardous levels (mg kg^{-1}) in soil (Pb=100, Cd=3, As=12, Cr=4, and Cu=70), they could also be hazardous to plants. Given that the soil used for reclamation comes from a depth of up to 100 m, it could have a high heavy metal concentration because of the drilling and breaking of the rock and mineral layers. Furthermore, the leachate and runoff could also accumulate because the soil was collected from the lowest level of the landfill. Although the heavy metal levels did not exceed the national standards, they might impact the physiological activities of plants.

Table 1 Characteristics of the sewage sludge compost and the reclaimed soil of landfill

Items	Reclaimed soil	Compost
pH	8.2	7.8
EC (μS cm^{-1})	182±10	8,500±1,200
Water (%)	10.8±2.1	41.5±8.4
OM (%)	0.7±0.08	35.4±6.80
Pb (mg kg^{-1})	121.3±10.74	119.9±22.01
Cd (mg kg^{-1})	1.08±0.07	0.87±0.18
As (mg kg^{-1})	ND	0.033±0.03
Cr (mg kg^{-1})	7.90±0.55	3.5±0.96
Cu (mg kg^{-1})	68.2±8.97	31.5±11.95
NaCl (%)	0.01±0.00	0.37±0.033
T–N (%)	0.036±0.002	1.7±0.050
NH_4^+ (mg kg^{-1})	0.1±0.0	44.0±2.2
NO_3 (mg kg^{-1})	0.4±0.1	298±8.1
Available P	9±1.7	148±12.9

The data presented are mean±SE of three replicates

ND not detected

The sewage sludge compost had a heavy metal content that was less than 10% of the national standard set for compost (KEI 2003); the salt content was also below the limit. The heavy metal contents were lower than the North American standards set for compost (Hogg et al. 2002), such as those set by the EPA CFR40/503 Sludge Rule. As separate garbage claim and separate reclaim is strictly managed by government, heavy metal sources are not included in sewage and reclaimed garbage. As a result, sewage sludge itself does not contain significant heavy metal content even after composting. The heavy metal content of the sewage sludge compost was lower than that for reclaimed soil. The organic matter, moisture, and N contents in the reclaimed soil were, however, higher than the national standards. Because the N content of the compost was about 50 times that of the reclaimed soil, the application of compost could be very effective and useful for plants.

The soil's N content was examined after 5 months of treatment. The N content of compost 1 treatment was 0.25 ± 0.01^{b}, compost 2 treatment was 0.28 ± 0.01^{a}, Osmocote treatment was 0.25 ± 0.00^{b}, and control treatment was 0.04 ± 0.00^{c} (average±standard error of three replicates, values with same letter are not significantly different from the 0.05 level). The soil moisture content of the compost 1 treatment was 29.9 ± 1.14^{a}, compost 2 treatment was 30.3 ± 0.95^{a}, Osmocote treatment was 22.5 ± 0.75^{b}, and control treatment was 19.7 ± 0.54^{c}. As compost has a water-holding capacity, the difference would be greater if there was no irrigation. Under all conditions, the compost treatments showed a significant increase in the N content of the soil. Osmocote treatments also showed significant increases in N. Increases in the N content and the moisture of the soil could impact the ecological and physiological activities of a plant, such as growth and photosynthesis.

Ecological and Physiological Responses of Plants

Growth Variations and Chemical Contents of Plants

Table 2 shows that both height and leaf biomass were significantly increased by Osmocote and compost treatment. Compost 1 and compost 2 treatments resulted in a significantly increased leaf biomass in both species. Although compost 2 treatment showed better performance than compost 1, only the height increase in the Japanese red pine showed a statistically significant difference. Plant growths in compost-treated pots were significantly better because compost treatments increased the nutrient status of the soil. Osmocote treatments also showed better performance than the control. As watering was regular and stable, Osmocote decomposed rapidly. So an increase in the release rate of fertilizer would be very effective for the plants. However, in the actual field, and with no constant water supply, the water-holding capacity of the compost would have merits and the compost treatments will result in a relatively improved performance. Overall, the compost treatments showed significantly better performance in growth variations, indicating that an increase in soil nutrients was effective for plant uptake.

Table 3 shows that the N contents of plant leaves were significantly increased by the compost and Osmocote treatments. The compost blend may increase the N concentration of plants (Kahn et al. 2005). The compost 1 treatment showed the most significant increase in N content of the leaves of the sawtooth oak; the compost 2 treatment also showed significant increase in N content compared to Osmocote or the control. The compost 1, 2, and Osmocote treatments showed significant increases in N content of leaves in the Japanese red pine. As reclaimed soil had a very poor N content, the plants' N contents after compost and Osmocote treatments showed dramatic increases. Since the low nutrient contents of reclaimed soil would be a limiting factor for plant growth in landfill (Lee et al. 2005), sewage sludge compost and fertilizer would be an effective solution for landfill revegetation. Further, compost treatments will be more effective in semi-arid areas because compost has a water-holding capacity. The heavy metal contents of the leaves were increased by the compost treatments, but as sewage sludge compost contained less heavy metal than reclaimed soil, the increased heavy metal might be caused not only by the compost but also by the increased physiological activities of the plant resulting from the nutrient supply. The accumulation of heavy metals in the plant leaves was relatively lower than that reported in previous studies (Moreno et al. 1996; Pengcheng et al. 2008; Wei and Liu 2005; Wong et al. 1996), and As was not detected in all the leaf samples. The reason why compost treatments did not show a significant increase in heavy metal accumulation can be explained by the dilution effect. Although the total weight of heavy metal accumulation in compost treatments could be much greater, the per-weight accumulation might be insignificant because of the dilution effect, as fertilized plants grow faster (Korboulewsky et al. 2002). Further, the accumulation of heavy metals in our study was not high, either on its own or in comparison with the controls (Table 3). As the separate reclamation induces separate garbage claim and reduces discharges, and purifying processes of the sanitary landfill reduces the heavy metal concentration of sewage sludge, sewage sludge compost has low heavy metal contents. Therefore, using sewage sludge compost in sanitary landfills presents limited environmental risks.

Although Osmocote treatment showed the best performance in terms of plant growth variations, the N contents in sawtooth oak were lower than in the compost treatments. This result could be explained by differences in the levels of other nutrients. As Osmocote contains P, K, and MgO, these nutrients might encourage plant growth. However, as the nutrient source for sewage sludge compost is OM, other nutrients are usually contained within the compost. Another reason could be the early stress posed to the plants after sewage sludge application. As sewage sludge compost may contain some undetermined (unknown) hazardous chemical

Table 2 Growth variations[a] of trees with assorted compost treatments

Items	Leaf biomass (g)		Height (cm)	
	Sawtooth oak	Japanese red pine	Sawtooth oak	Japanese red pine
Compost 1	11.25±0.36b	2.18±0.20b	8.7±1.62ab	1.18±0.15ab
Compost 2	11.89±0.47b	2.17±0.06b	10.1±1.66ab	1.04±0.15b
Osmocote	13.51±0.33a	2.66±0.05a	12.2±1.40a	1.44±0.13a
Control	9.11±0.80c	1.22±0.09c	6.8±1.20b	0.47±0.07c

The data presented are the mean±SE of ten replicates in the pot experiment. The means within a column followed by the same letter are not significantly different at the 0.05 level. Every weight is a dry weight

[a] The height values indicate the increase for each tree

Table 3 Chemical contents of leaves with different compost treatments

	Sawtooth oak				Japanese red pine			
	Com 1	Com 2	Osmo	Con	Com 1	Com 2	Osmo	Con
C (%)	48.0±0.2	47.8±0.2	47.6±0.2	48.1±0.3	43.5±0.7	44.2±0.1	44.1±1.0	44.9±0.3
N (%)	1.80±0.03a	1.45±0.01b	1.27±0.02c	0.98±0.04d	1.24±0.06a	1.29±0.02a	1.34±0.05a	0.82±0.03b
Cr (mg/kg)	0.16±0.01a	0.12±0.00b	NM	0.09±0.01c	0.17±0.02	0.17±0.03	NM	0.12±0.03
Cu (mg/kg)	0.30±0.01a	0.29±0.01ab	NM	0.26±0.01b	0.23±0.03	0.27±0.02	NM	0.28±0.01
Cd (mg/kg)	0.01±0.00	0.01±0.00	NM	0.01±0.00	0.02±0.00	0.01±0.00	NM	0.02±0.00
Pb (mg/kg)	1.33±0.05a	1.20±0.06ab	NM	1.12±0.05b	1.39±0.03a	1.25±0.02b	NM	1.16±0.04b

The data presented are the mean±SE of three replicates. Means within a row followed by the same letter are not significantly different at the 0.05 level

Com 1 compost 1, *Com 2* compost 2, *Osmo* Osmocote, *Con* control, *NM* not measured

compounds (Oleszczuk 2008), it could be phytotoxic to plants before adapting to the new conditions. Hence, the initial growth of plants with Osmocote treatments could be better than that with compost treatments. For this reason, plant stress surveys after compost treatments could be important because early or continuous stress of the plants by sewage sludge compost can affect ecological and physiological reactions of plants. And less plant-stressed methods can be better treatment methods to improve soil conditions.

Physiological Activities of Plants

Table 4 shows that the chlorophyll content of plant leaves are significantly increased by compost and Osmocote treatments. Sawtooth oak showed the highest total chlorophyll content in the compost 2 treatment. Japanese red pine also showed higher chlorophyll contents in the compost treatments, but the increased value was not significant. Overall, the Osmocote treatment showed the highest content. The sawtooth oak showed the best performance in terms of the N and chlorophyll contents, but Japanese red pine showed the best performance under Osmocote treatment. Japanese red pine has a relatively high drought tolerance and is typically found in drier habitats (Lee and Lee 2003); hence, an increased level of soil moisture following the compost treatment was not as effective as it was for the oak species. Osmocote together with no initial stress and other nutrients works better on the pine species. Overall, the increased N content in the soil impacts a plant's physiological activities and chlorophyll content, and this difference will become more apparent upon analyzing the photosynthetic rates.

The photosynthetic rate of the sawtooth oak is significantly increased by the compost treatments (Fig. 1). The control treatments showed very low light saturation points (about 700 μmol $m^{-2}s^{-1}$) because low nutrient and moisture levels in the soil would limit the plants' ability to process high intensity light (Kim et al. 2002). Compost treatments showed almost double the photosynthetic activity, thereby suggesting a much better status of the plants. As compost treatments have a positive impact on a plant's physiological activities, the application of sewage sludge compost to a landfill is likely to be very effective.

The antioxidant activities of plants can be induced by environmental stress. The role of a plant's antioxidant enzymes that act as tolerance mechanisms in different environmental extremes has been widely researched (Bor et

Table 4 Chlorophyll contents (mg/L) of leaves

Species	Treatment	Chl-*a*	Chl-*b*	Total Chl
Sawtooth oak	Compost 1	17.95±2.68a	11.56±1.91	29.50±4.58ab
	Compost 2	18.19±0.56a	11.35±0.65	30.40±1.94a
	Osmocote	18.12±0.85a	10.66±0.27	28.77±1.06ab
	Control	12.75±2.34b	8.17±1.38	20.90±3.71b
Japanese red pine	Compost 1	5.73±0.51b	2.66±0.23b	8.39±0.74b
	Compost 2	5.97±0.65b	2.75±0.24b	8.71±0.90b
	Osmocote	11.10±1.02a	4.77±0.42a	15.87±1.44a
	Control	4.90±0.34b	2.31±0.11b	7.20±0.44b

The data presented are the mean±SE of three replicates. Means within a column followed by the same letter are not significantly different at the 0.05 level

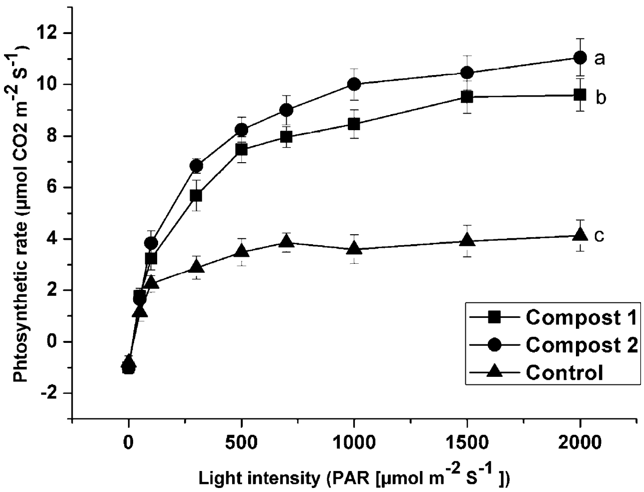

Fig. 1 Photosynthetic rates of sawtooth oak 4 months after treatment. The data presented are mean±SE of five replicates. The *means followed by the same letter* are not significantly different at the 0.05 level

al. 2003; Patra and Panda 1998). Additionally, low levels of soil nutrients can induce higher antioxidant activity (Agrawal and Rathore 2007). Increased heavy metal content in the soil can also induce antioxidant activity (Patra and Panda 1998), and heavy metals in sludge also increase the antioxidant activity (Singh et al. 2004). Since sewage sludge compost contains toxic materials including heavy metals and organic pollutants (Oleszczuk 2008), researching plant stress in terms of antioxidant activity after compost application is very important.

Total antioxidant activity of the old (pre-established) pots was significantly lower than that for newly planted pots, thereby indicating stresses associated with re-planting (Table 5). The compost 1 treatment did not show a difference compared to the control. However, the compost 2 treatments showed significantly lower TAA, implying that direct contact with the compost in the compost 1 treatment induced certain stress in the plants. Maybe the undetermined toxic substances could cause differences. And as sewage sludge compost is highly concentrated (Table 1), reverse osmosis could cause plant stress and increased TAA.

As the control treatment was significantly higher, the low nutrient content of the reclaimed soil also would be a stressful condition to the plants. The TAA of plants 7 days after treatment was not significantly different between treatments, implying that disturbance during re-planting is the major cause of plant antioxidant activity. However, after 2 weeks, plants in the control treatment became more stable, but compost treatments showed more antioxidant activities. The higher antioxidant activity after 2 weeks is explained by an increase in the leaching of the heavy metals in compost and by the active uptake of metals by the stabilized plants. As compost 2 treatment showed significantly lower activity than the compost 1 treatment, non-direct contact with the compost would be a better application method for plants.

The activities of SOD are induced in plant species by heavy metals, and SOD is the major enzymatic action in H_2O_2 and O_2 formation (Zhang et al. 2007). As SOD is frequently used as an indicator of pollution stress and is increased by heavy metals (Koricheva et al. 1997), SOD activities between treatments were tested to determine the precise effect of heavy metals. As the compost 1 treatment showed the highest SOD activity in every period, direct contact with sewage sludge seems to cause stress to plants. However, compost 2 treatments showed significantly lower SOD activities, indicating the merits of non-direct application. As reclaimed soil has high heavy metal contents, the SOD activities of plants would increase (Landberg and Greger 2002; Zhang et al. 2007). As a result, the SOD activities of plants in the control treatment were also high. Since Cr was higher than other heavy metals (in reclaimed soil), compared to national standards for hazardous levels, Cr may be the major reason behind the increase in SOD activity (Panda 2007). However, as the control soil is loess, one of the most heavy metal absorbing soil, the leaching of heavy metal can be weak (Seo and Ahn 2004; Lee et al.

Table 5 Antioxidant enzyme activities in sawtooth oak after treatment

Day	Treatment	TAC (μM/mg protein)	SOD (U/mg protein)
7 days after treatment	Control	557.5±28.9	90.7±5.3b
	Compost 1	584.8±110.7	99.7±5.4a
	Compost 2	510.4±83.8	83.5±9.7b
14 days after treatment	Control	489.2±22.8b	71.1±1.0b
	Compost 1	722.8±109.8a	97.8±4.1a
	Compost 2	532.3±70.2ab	65.1±4.3b
3 months after treatment	Control	146.7±7.6a	78.7±0.8b
	Compost 1	158.4±21.8a	99.6±6.5a
	Compost 2	92.0±23.2b	80.5±1.1b

The data presented are the mean±SE of five replicates. Means within a column followed by the same letter are not significantly different at the 0.05 level

2004a, b). Thus, absorption of heavy metals by plants can be smaller than compost and show less SOD activity than compost treatments though the reclaimed soil has much more Cr contents (Table 1). Although there was a significant difference between the control and the compost 1 treatment, the difference was not large. The reason why SOD activity does not differ over time (like TAA) is that the leaching of heavy metal and the plant uptake occurs constantly. TAA increases can be promoted by many environmental stresses, such as disturbance of roots and soil (non-heavy) metal organic pollutants, and soil conditions; these sources can be stabilized later. However, leaching of heavy metals and their uptake by plants can occur at anytime during the growing season, often resulting from rainfall and the active transpiration of plants. Therefore, unlike TAC, which includes many environmental stress reactions, SOD activities are not reduced over time. As the compost 2 treatment showed significantly lower activity than the compost 1 treatment in terms of both TAA and SOD, the compost 2 treatment (non-direct contact of compost) would be a better application method for plants. Although the difference was not large, early-stage plants will respond to this difference because it is not stabilized. This difference could alter the survival rate and early growth rate of the plants.

Conclusions

Compost made from the sewage sludge from sanitary landfill contains significant amounts of OM and N. The heavy metal contents were lower than those specified by the EPA CFR40/503 Sludge Rule. Application of sewage sludge compost significantly increased the soil moisture and N contents—both in mixing with the reclaimed soil method (compost 1) and scattering over reclaimed soil method (compost 2). Osmocote treatment showed increased soil N but did not significantly change the soil moisture content. Osmocote treatment showed the best plant growth and compost treatments showed a significant increase in the height and biomass compared to the control. Osmocote and compost treatments showed dramatic increases in the N content of plant leaves. The heavy metal contents of leaves in the compost treatments were lower than those found in previous studies because the heavy metal content of the sewage sludge compost itself was very low. Plants exposed to compost treatments showed significantly increased chlorophyll contents and photosynthetic rates. Since compost treatments showed better performance in plants, and heavy metal accumulation was not appreciable, application of sewage sludge compost to landfill soil is a good way to condition the soil for plants.

Compost 2 treatments showed increased TAA and SOD activity compared to the control, but compost 2 treatments showed less activity than the control treatments. As compost 2 treatment showed significantly lower activity than the compost 1 treatment in both the TAA and SOD, non-direct contact with the compost would be a better application method for plants. Since the growth rate, N contents, and photosynthetic rates of the compost 2 treatment were not significantly different from the compost 1 treatment, covering the reclaimed soil would be a better application method. As the scattering method requires less effort and can be done at less cost, it would be an ecologically and economically advantageous option for landfill sites. And application for restoration of degraded (disturbed, contaminated) areas such as closed mine fields, closed non-sanitary landfills, and military shooting range could be also ecologically and economically advantageous.

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