

RESEARCH PAPER

Effect of organic and mineral amendments upon soil respiration and microbial biomass in a saline-sodic soil

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Abstract

J.E. Celis, M. Sandoval, B. Martínez, and C. Ouezada. 2013. Effect of organic and mineral amendments upon soil respiration and microbial biomass in a saline-sodic soil. Cien. Inv. Agr. 40(3): 571-580. An understanding of soil carbon stocks and fluxes in saline-sodic soils is becoming critical in environmental management because salinity and sodicity are predicted to increase worldwide. The effects of amendment with sewage sludge (SW), mined gypsum (MG) and synthetic gypsum (SG) on the soil respiration rate and soil (S) microbial biomass (SMB) of a saline-sodic soil were assessed *in vitro* over 60 days under controlled conditions. The treatments were: $T1 = S + MG (7 \text{ t } \text{ha}^{-1})$; $T2 = S + MG (7 \text{ t } \text{ha}^{-1}) + SW (90 \text{ t } \text{ha}^{-1})$; T3 = $S + SG (7 \text{ t ha}^{-1}); T4 = S + SG (7 \text{ t ha}^{-1}) + SW (90 \text{ t ha}^{-1}); T5 = S + SW (90 \text{ t ha}^{-1}); T6 = S + SW$ SW (270 t ha⁻¹); T0 = Control (unamended soil). Soil respiration rates were determined by a respirometry method in a closed incubation chamber. SMB was measured by the chloroform fumigation-extraction procedure. The results showed that the highest respiration rate occurred when soil was amended with T6, followed by T5. Microbial respiration was the lowest with T1 and T3. The gypsum amendments did not significantly increase soil respiration, due to low organic matter (OM) intake. All treatments showed a gradual decrease of SMB over the 60-day incubation period. The saline-sodic soil was able to respond positively to large amounts of added sewage sludge, up to 270 t ha-1.

Key words: Arid soil, biological activity, gypsum, organic matter, sewage sludge, soil respiration.

Introduction

Soil erosion, nutrient loss, accumulation of salts and toxicants, waterlogging and desertification are all contributing to soil degradation in most developing countries (Yunlong and Smit, 1994). Soil salinity and sodicity are serious issues worldwide, and unless some strong measures can be applied in order to protect lands, the area of affected soils is predicted to increase dramatically in the future (Wong *et al.*, 2008). Globally, there are approximately 76 million hectares of saline soils (Bell, 1999) and more than 700 million hectares of sodic soils (Fekete and Fehe, 2000). Additionally, approximately 25% of all irrigated land has been affected by salts (Arshad, 2008). Human-induced salinization is the primary contributor to desertification processes in the drylands of the world (Thomas and Middleton, 1993). Desertification has progressed rapidly in

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the northern region of Chile, where approximately 34,000 hectares are affected by salinization (Santibáñez and García. 2000). Salt concentration in the surface or near-surface zones of soils contributes to land degradation and the loss of land for production in a range of environments, leading to falling crop yields (López-Aguirre et al., 2007). Salinity and sodicity especially occur where salts dominated by Na⁺ accumulate in the soil profile (Vukadinovic and Rengel, 2007). Some evidence indicates that is possible to reclaim soil by adding various organic wastes or mineral materials, such as gypsum. However, it is necessary to understand the cycling dynamics of these materials into soil. One way to quantify their applicability is through microbial activity.

High salt content affects the physical, chemical and microbiological properties of soil. Increased Na⁺ concentration in the soil exchange complex leads to deterioration of the soil structure, reducing soil permeability and affecting salt displacement processes (Kochba et al., 2004). There is some evidence showing that increases in salinity clearly decrease soil respiration rates, which negatively affect the decomposition of OM and the release of nutrients required to sustain productivity (Pathak and Rao, 1998; Wong et al., 2008; Shah and Shah, 2011). The reduced activity of microorganisms with increasing salinity is most likely caused by osmotic stress, as a consequence of large concentrations of salts in soil solutions (Oren, 1999). Generally, osmotic stress limits microbial growth and activity in saline soil, whereas microbial growth may be limited by ion toxicities and adverse pH conditions in sodic soils (Rietz and Haynes, 2003). In saline soils, specific ion toxicities from Na and Cl may also inhibit microbial growth (Zahran, 1997).

Gypsum is calcium sulfate dihydrate $(CaSO_4 \cdot 2H_2O)$, which comes from various sources. Gypsum is a common mineral found around the world in sedimentary rock formations, from which it is mined or quarried (Clark *et al.*, 1999). Flue gas desulphurization gypsum is a synthetic material of identical chemical structure produced as a byproduct from coal-fired electric utilities (DeSutter and Cihacekm, 2009). Both mined and synthetic gypsum can be used as a soil amendment in a range of soils (Fekete and Feher, 2000; Aydemir and Najjar, 2005). Gypsum can be used as mineral amendment to improve soil physical and chemical properties (Clark *et al.*, 1999). Gypsum amendments effectively increase Ca²⁺ concentration in the soil solution, thus decreasing Na⁺ concentration (Vukadinovic and Rengel, 2007).

Adding OM to soil may also help to exchange adsorbed Na⁺ with Ca²⁺ and to displace Na⁺, thus reducing salinity (Avnimelech et al., 1994). Additionally, some evidence suggests that sewage sludge amendments improve the activity of microorganisms in arid soils, implying that adding organic wastes might be a suitable technique to restore soil quality (Pascual et al., 1999). Mixing organic wastes with gypsum increases the solubility of gypsum through the action of chelating ligands present in the organic material or its decomposition products, and amendment with such mixtures may improve soil structure (Kochba et al., 2004). High levels of soil OM are always associated with liberal amounts of calcium, which is a component of gypsum (Aydemir and Najjar, 2005). As a consequence, blends of gypsum and organics increase the value of the organic amendments, especially for soil structure improvement. The role of Ca in clay and soil OM flocculation possibly involves the bridging of clay aggregates to organic materials (Muneer and Oades, 1989).

Some studies using different organic amendments on degraded soils have shown a positive effect on microbial respiration as the dose increased from 15 to 150 t ha⁻¹ (Celis *et al.*, 2009; Celis *et al.*, 2011). According to Banerjee *et al.* (1997), the application of different amounts of sludge significantly increases the amount of SMB. However, some studies performed in saline and sodic soils show contradictory results. Soil respiration and SMB were negatively correlated with salinity as increased substrate C is mineralized for cell maintenance (Muhammad *et al.*, 2008; Tripathi *et al.*, 2006). Another study found that SMB increased positively with salinity, while soil respiration and the metabolic quotient (M_tC) increased as the salinity decreased (Wong *et al.*, 2008). Microbial activity, expressed as CO_2 evolution, depends on the labile C pool, defined as soil organic carbon (SOC) with a turnover time on the order of weeks (Parton *et al.*, 1987). To determine stress in the microbial population, the M_tC is frequently used and measures the ratio of respiration to the SMB (Anderson and Domsch, 1993). The M_bC is the ratio of the SMB to SOC and indicates the substrate availability. Its value increases as available organic C input increases, and its value decreases when the C input decreases (Anderson and Domsch, 1989).

Chilean environmental regulations are becoming stricter to protect soils, especially when large amount of wastes amended to soil need to be completely controlled. Therefore, it is necessary to understand the dynamics of OM decomposition to design sustainable practices. Unfortunately, few studies have examined the effects of sewage sludge mixed with gypsum amended to saline-sodic soils, and fewer have investigated the impact of such amendments on microbial activity (Nelson et al., 1997; Rietz and Haynes, 2003). In particular, little is known about how those amendments influence the respiration rate and SMB of Chilean arid soils. Consequently, the aim of this study was to assess the effect of sewage sludge and gypsums on soil C dynamics as indicated by the microbial activity (respiration rate) and SMB. We examined the effects of different doses of sewage sludge and gypsums in a saline-sodic soil under controlled temperature and moisture conditions.

Materials and methods

Samples

Soil samples (0-20 cm) used in the laboratory were obtained from a sector at Copiapó (27°18'S; 70°25'W), Atacama region, Chile. Municipal sewage sludge was collected from an activated

sludge reactor at the wastewater treatment plant of the city of Chillán (36°36'S, 72°07'W). Mined gypsum was obtained from the local market, and synthetic gypsum from a coal-power plant located in the Atacama region. The physicochemical properties of the soil are given in Table 1, and those for sewage sludge and gypsums are provided in Table 2. The chemical properties of the mined gypsum and synthetic gypsum were very similar.

Table 1. Physicochemical properties of the saline-sodic soil used in the experiment (mean values ± standard deviation).

Property	Unit	Value
pHs	-	8.4 ± 1.1
pH ₂	-	8.1 ± 0.9
ECe	dS m ⁻¹	102.6 ± 8.5
CEC	cmol (+) kg ⁻¹	5.89 ± 1.4
Organic C	%	0.02 ± 0.01
Exchangeable sodium percent (EPS)	%	75.47 ± 2.8
Bulk density	g cm ⁻³	0.80 ± 0.2
NO ₃ -N	μg kg-1	13.4 ± 1.5
Olsen-P	μg kg ⁻¹	15.6 ± 1.7
Total N	%	0.07 ± 0.03

 pH_s and pH_2 refer to the soil pH values of the saturation paste and 1:2 soil-water suspension, respectively.

All samples were slowly air-dried and ground (<2 mm). Soil pH and ECe (electrical conductivity) were measured in saturation paste and extract, respectively, as well as in 1:2 soil-water suspensions. The physical and chemical characterizations of all samples were performed at the laboratories of the Departamento de Suelos y Recursos Naturales of the Universidad de Concepción (certified by the Chilean Soil Science Society since 2000). All samples were maintained at temperatures below 10°C before processing.

Treatments

Different amounts of sewage sludge (SW), mined gypsum (MG) and synthetic gypsum (SG) based on 1 kg of saline-sodic soil (S) each were prepared. Treatments were as follows: T1 = S + MG (7 t ha⁻¹); T2 = S + MG (7 t ha⁻¹) + SW (90 t ha⁻¹);

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Parameter	Unit	SW	MG	SG
pH (water)	-	5.94 ± 1.0	-	-
Organic matter (OM)	%	41.95 ± 5.4	-	-
NO ₃ -N	mg kg-1	17.1 ± 2.1	-	-
NH ₄ -N	mg kg-1	424.0 ± 10.9	-	-
Olsen-P	mg kg ⁻¹	853.2 ± 15.6	-	-
K available	mg kg ⁻¹	$5{,}591.0\pm43.2$	-	-
Water	%	-	0.10 ± 0.07	0.15 ± 0.09
CaSO ₄ x 2H ₂ O	%	-	80.0 ± 4.2	75 ± 8.7
CaCO ₃	%	-	9.6 ± 1.4	8.7 ± 1.1
NaCl	%	-	0.04 ± 0.02	0.03 ± 0.01
MgCO ₃	%	-	0.11 ± 0.06	0.12 ± 0.07
$FeO_3 + Al_2O_3$	%	-	0.20 ± 0.08	0.30 ± 0.06

Table 2. Physicochemical properties of the sewage sludge (SW), mined gypsum (MG) and synthetic gypsum (SG) used in the experiment (mean values \pm standard deviation).

T3 = S + SG (7 t ha⁻¹); T4 = S + SG (7 t ha⁻¹) + SW (90 t ha⁻¹); T5 = S + SW (90 t ha⁻¹); T6 = S + SW (270 t ha⁻¹); T0 = Control (unamended soil). Treatments were set up as totally organic (T5 and T6), totally mineral (T1 and T3), and an organic/ mineral mixture (T2 and T4). All treatments were homogenized, packaged in plastic bags, and then placed in a chamber (with temperature and humidity automatically controlled at 25°C and 60-70%, respectively) for a 10-day pre-incubation period.

Respiration rate

For the incubation process itself, samples consisting of 25 g of the amended and control soils were placed (in triplicate) in glass jars (1 L). Then, a test tube with 15 mL of a 0.05 M NaOH solution and a tube with deionized water were placed in each jar to absorb the CO_2 produced by microorganisms and to keep the humidity constant, respectively. Then, each bottle was sealed hermetically. The bottles were placed in an incubation chamber at 22°C for 60 days. Measurements of non-neutralized NaOH over time were accomplished by titration (0.1 M HCl). Then, 1 mL of NaOH was extracted (in duplicate), and 2 mL of BaCl₂ (0.5 M) was added to precipitate the inorganic C as insoluble BaCO₃. Drops of phenolphthalein were added as an acid-base indicator, so that the non-neutralized NaOH was titrated directly with HCl (Ianotti *et al.*, 1994). The amount of CO_2 -C generated from the incubated mixtures was calculated according to the formula suggested by Anderson (1982) as modified below:

$$R_{rat} = \frac{45 \times (B - V) \times N \times 1000}{S_{g} \times (1 - h)}$$

where R_{rat} is the respiration rate (µg CO₂-C g⁻¹), B is the volume of HCl used in the titration of NaOH in the blank (mL), V is the volume of HCl used in the titration of NaOH from the amended soil (mL), N is the HCl normality, S_g is the amount of soil incubated (g), and h is the humidity (as decimal) of the incubated soil.

Soil microbial biomass

SMB was measured by the chloroform fumigation–extraction procedure described in Vance *et al.* (1987). There was a negligible effect on dichromate oxidation by Cl⁻ interference following calculation of the Cl⁻ correction factor. All analyses were performed in triplicate. The metabolic quotient, M_tC, and microbial quotient, M_bC , were determined at the end of the 60-day incubation period as follows:

$$M_t C = \frac{R_{rat}}{SMB}$$

$$M_b C = \frac{SMB}{SOC}$$

where R_{rat} is the respiration rate (µg CO₂-C g⁻¹), SMB is the soil microbial biomass (µg C g⁻¹), and SOC is the soil organic carbon (µg C g⁻¹).

Statistical analysis

Results obtained from *in vitro* respirometry trials were expressed as μ g CO₂ - C g⁻¹ soil (dry matter basis, oven at 105°C x 24 h). Mean values for each sampling time were used. Data were normalized using the expression (x+0.5)^{0.5} and then processed by means of ANOVA, and the comparison among means was performed using Tukey's tests. In all cases, the level of significance used was 95% (P \leq 0.05). The values were processed using SAS statistical software (SAS Institute Inc, Cary, NC, USA).

Results and discussion

Figure 1 shows the CO_2 -C evolution in the salinesodic soil when amended with different doses of sewage sludge and gypsums. In general, a non-linear increasing trend of microbial activity with time was observed, similar to other studies performed in a degraded arid soil amended with urban wastes (Ros *et al.*, 2003), a Patagonian soil amended with salmon sludge (Celis *et al.*, 2009), and a degraded granitic soil amended with salmon sludge (Celis *et al.*, 2011). The control followed a similar trend until the first 20 days of incubation and then decreased. This pattern may be explained as a consequence of microorganisms

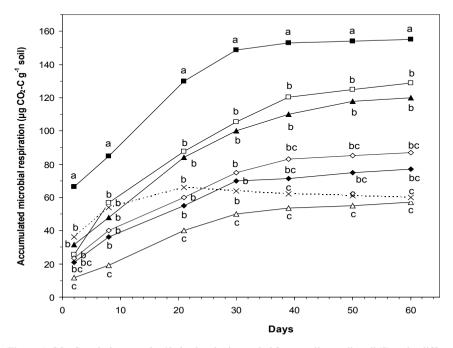


Figure 1. CO₂-C evolution over the 60-day incubation period from a saline-sodic soil (S) under different amendments with sewage sludge (SW), mined gypsum (MG) and synthetic gypsum (SG). T1 (Δ) = S + MG (7 t ha⁻¹); T2 (\Box) = S + MG (7 t ha⁻¹) + SW (90 t ha⁻¹); T3 (\blacklozenge) = S + SG (7 t ha⁻¹); T4 (\diamondsuit) = S + SG (7 t ha⁻¹); T4 (\diamondsuit) = S + SG (7 t ha⁻¹); T5 (\blacksquare) = S + SW (90 t ha⁻¹); T6 (\blacksquare) = S + SW (270 t ha⁻¹); T0 (\times) = Control (unamended soil). Different letters by the same day indicates a significant difference (P≤0.05).

having rapidly depleted the low level of organic C in the saline-sodic soil (0.02%, Table 1).

There was more CO₂ evolution as the amount of OM added to soil increased. Sewage sludge amendments at 270 t ha-1 (T6) notably produced more CO₂-C than other treatments ($P \le 0.05$) during the whole incubation period. It is possible that sewage sludge became available in treatments T5 and T6 due to the controlled soil moisture content during the experiment. This could result in increased respiratory activity of the microbial population, with the highest rates of respiration occurring in the high-sewage-sludge treatment. The rest of the treatments showed levels of CO₂-C production not significantly higher than those of the control (T0), with the exception of mined gypsum amendments (T1), which showed the lowest ($P \le 0.05$) microbial respiration rate. Water-soluble C can provide a large proportion of the substrate, which is immediately available and has the potential to be

replenished rapidly by the continued dissolution of OM (Jandl and Sollins, 1997).

After 60 days of incubation, soil treated with sewage sludge at 270 t ha⁻¹ (T6) accumulated significantly more CO₂-C than all of the other treatments. These highest CO₂-C values reflected greater C and energy availability for soil heterotrophic microorganisms as a consequence of an abundant provision of OM and N, thus stimulating microbial activity (Zagal et al., 2002; Ros et al., 2003). According to Jünemann (1969), as cited by Esparza et al. (2004), soils with low OM content, such as the saline-sodic soil investigated. respond positively to sludge addition. The addition of organic wastes stimulates microbial growth. increasing OM decomposition (Barrena et al., 2006). For that reason, chemical amendments with gypsums showed lower CO₂-C values than organic amendments with sewage sludge. Evolution of CO₂-C due to large sewage sludge addition to saline-sodic soil suggests a positive effect on

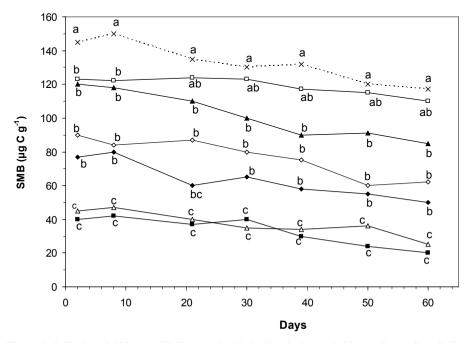


Figure 2. Soil microbial biomass (SMB) over the 60-day incubation period in a saline-sodic soil (S) under different amendments with sewage sludge (SW), mined gypsum (MG) and synthetic gypsum (SG). T1 (Δ) = S + MG (7 t ha⁻¹); T2 (\Box) = S + MG (7 t ha⁻¹) + SW (90 t ha⁻¹); T3 (\blacklozenge) = S + SG (7 t ha⁻¹); T4 (\diamondsuit) = S + SG (7 t ha⁻¹) + SW (90 t ha⁻¹); T5 (\blacktriangle) = S + SW (270 t ha⁻¹); T0 (\times) = Control (unamended soil). Different letters by the same day indicates a significant difference (P≤0.05).

microbial activity, as has been similarly reported by Varnero *et al.* (2004). Moreover, wastes rich in potassium, such as the sewage sludge used in this study (Table 2), may improve the soil chemical properties by reducing Na⁺ through electrostatic repulsion, in which K⁺ replaces Na⁺ to form potash humate (Tchouaffe, 2007).

Figure 2 shows the effect of the different amendments on SMB over the incubation period. There were significant interactions between treatments and time over the 60-day incubation period ($P \le 0.05$). The control (T0) displayed the highest levels of SMB with time, while the sewage sludge at the highest rate (T6) and mined gypsum (T1) displayed the lowest levels of SMB. In all treatments, there was a gradual decrease of SMB over the 60-day incubation period. In our study, SMB decreased over the experimental incubation period because SMB most likely decreased the amount of available C, as also noted by Wong *et al.* (2008).

Table 3 shows the effects of the organic and mineral amendments on M_tC and M_bC at the end of the 60-day incubation period. M_tC was highest in the control (T0), whereas M_bC was lower in treatment T5. The control also showed the highest M_bC . Clearly, the organic and mineral amendments decreased soil salinity, while the control (T0) most likely reflected a shift in population structure to one dominated by less active microorganisms, mainly fungi, with lower respiration rates compared to a population dominated by more active microorganisms, mainly bacteria (Adu and Oades, 1978; Sadinha et al., 2003), Our results are similar to those found by Wong et al. (2008). The respiration rate was higher in the treatments with sewage sludge, indicating that OM provision decreased the levels of Na⁺ in the saline-sodic soil, thus decreasing salinity after a 60-day incubation period. A large proportion of the OM comes from water-soluble C, which is immediately available for microorganisms (Jandl and Sollins, 1997). In saline soils it is possible that the additional OM is also readily available and easily decomposable (Wong et al., 2008). Therefore, the microbial population increased due to increased nutrient supply, but as the food supply decreased, the respiration rates and SMB decreased

Although more research is needed to evaluate the physical, chemical and biological properties of the soil, this study revealed that the saline-sodic soil was able to respond positively to large applications of sewage sludge, up to 270 t ha⁻¹, which allows the possibility of recycling municipal wastes into salinity-affected soils in the northern part of Chile as part of future sustainability programs.

In conclusion, the application of sewage sludge stimulated soil microbial CO_2 evolution. The highest respiration rate occurred when soil was amended with sewage sludge at the highest rate. Microbial respiration was lower when sewage sludge was mixed with gypsum before application to soil. Adding either only mined or synthetic gypsum to soil did not significantly increase

Table 3. Effects on the metabolic quotient (M_iC) and microbial quotient (M_bC) after 60-day incubation period in a saline-sodic soil amended with different doses of sewage sludge and gypsums.

Treatments	M_t^C (µg CO ₂ -C µg ⁻¹ C)	M _b C (%)	ECe (dS m ⁻¹)	ESP (%)
T6 (S + 270 t ha ⁻¹ SW)	1.32	1.03	7.51	15.73
T5 (S + 90 t ha ⁻¹ SW)	1.41	1.01	8.69	15.08
T4 (S + 7 t ha ⁻¹ SG)	1.40	1.86	10.13	17.04
T3 (S + 7 t ha-1 SG)	1.54	1.45	20.14	18.03
T2 (S + 7 t ha-1 MG + 90 t ha-1 SW)	1.17	1.07	26.07	17.09
T1 (S + 7 t ha ⁻¹ MG)	2.28	1.24	27.43	19.75
T0 (unamended soil)	3.00	3.56	100.47	73.68

S = saline-sodic soil; SW = sewage sludge; SG = synthetic gypsum; MG = mined gypsum.

the production of respired CO_2 -C, most likely due to the low OM intake. The highest levels of soil microbial biomass were reached with the unamended soil over the 60-day incubation period, while the highest levels of microbial activity, measured by soil respiration, were found with high-sewage sludge amendment. This is attributed to additional substrate becoming available for the microbial population in the soil with increasing salt concentration, with the effects due to salinity more evident than those due to sodicity. This study showed that salinesodic soil responds positively when amended with large amounts of sewage sludge.

Resumen

J. E. Celis, M. Sandoval, B. Martínez, v C. Ouezada. 2013. Efecto de enmiendas orgánicas y minerales sobre la respiración y biomasa microbianas en un suelo salino-sódico. Cien. Inv. Agr. 40(3):571-580. El entendimiento del flujo de carbono en suelos salino-sódicos se está tornando crítico en el manejo ambiental, a medida que la salinidad y sodicidad están aumentando a nivel global. Se analizaron in vitro los efectos de lodos urbanos (SW), yeso mineral (MG) y yeso sintético (SG) en la respiración y biomasa microbiana del suelo (SMB) sobre un suelo salino-sódico (S) por un periodo de 60 días bajo condiciones controladas. Los tratamientos fueron: $T1 = S + MG (7 \text{ t } \text{ha}^{-1})$; $T2 = S + MG (7 \text{ t } \text{ha}^{-1}) + SW (90 \text{ t } \text{ha}^{-1})$; T3 = S+ SG (7 t ha⁻¹); T4 = S + SG (7 t ha⁻¹) + SW (90 t ha⁻¹); T5 = S + SW (90 t ha⁻¹); T6 = S + SW (270 t ha^{-1}) ; T0 = Control (suelo sin enmendar). El CO₂ producido por los microorganismos se determinó mediante respirometría en una cámara de incubación cerrada. SMB se midió a través del método de fumigación-extracción con cloroformo. La mayor respiración ocurrió cuando el suelo fue enmendado con T6, seguido por T5. La respiración microbiana fue la más baja cuando se aplicaron al suelo T1 y T3. Las enmiendas con veso no aumentaron significativamente la producción de CO₂ respirado debido al bajo aporte de materia orgánica (OM). Todas las enmiendas mostraron un descenso gradual de SMB en los 60 días de incubación. El suelo salino-sódico fue capaz de responder positivamente a grandes cantidades de lodo urbano enmendado a dosis de hasta 270 t ha-1.

Palabras clave: Actividad biológica, lodo urbano, materia orgánica, respiración del suelo, suelo árido, yeso.

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