International Meeting on Soil Fertility Land Management and Agroclimatology. Turkey, 2008. p: 909-916

Effects of Different Application Doses of Sewage Sludge on Microbial Biomass and CO_2 Production of Soil and Earthworm *Lumbricus terrestris* Cast

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ABSTRACT

This study was carried out in order to determine the effects different application doses of sewage sludge (0, 2, 4, 6, 8 and 10 %) on microbial biomass C, CO_2 production, organic C and total N of soil and earthworm *Lumbricus terrestris* casts. Experimental design was randomized plot design with three replications. The moisture content in soil was maintained around 60 % of maximum water holding capacity by weighing the pots everyday. Changes in the microbiological properties and total C and N were determined in the soil and earthworm casts samples taken in 15, 30, 45, 60, 75 and 90 days after the experiment was conducted.

At the end of the experiment, earthworm casts had higher microbial biomass C, CO_2 production, total organic C, and total N levels than the surrounding soils at all incubation periods and sewage sludge applications significantly (*P*<0,001). Increases in application doses of swage sludge caused increases in microbial biomass C and CO_2 production, significantly (*P*<0,001). It was determined that the microbial parameters of soil and earthworm casts were not significantly changed after the 45^{th} and 60^{th} days of the experiment.

Key words: Sewage sludge, soil, earthworm cast, microbial biomass C, CO_2 production

INTRODUCTION

Organic wastes are added to agricultural soils for increasing plant nutrition and soil fertility and also for soil amendment. It has been added not only plant and animal wastes but also sewage sludge that to provide with refining of urban wastes to agricultural soils for organic matter added. Previous studies have demonstrated favorable plant yield responses to the application of sewage sludge (King and Morris, 1972). In contrast, the effects of sewage sludge on biological process in soil have been questioned by some authors (Knight et al., 1997; Banerjee et al., 1997; Kızılkaya and Bayraklı, 2004). Most papers concerned with the results of sewage sludge studies deal with the influence of sewage sludge on soil biological characteristics. Knight et al. (1997) observed a decrease of soil biological activity such as microbial biomass and enzyme activities, due to sewage sludge application. Conversely, Sastre et al. (1996) and Banerjee et al. (1997) found that the sewage sludge amendment increased soil microbial activity and CO_2 production. These differences might be a result of the some toxic metal content of sewage sludge and of the sewage sludge stability (Tam and Wong, 1990).

In soils, earthworms are known to incorporate organic wastes into soil and casts. Additionally, earthworms enhance the formation of soil structure, incorporation and transformation of soil organic matter, and their importance in improving soil physico-chemical and biological properties are generally accepted (Lee, 1985; Kızılkaya, 2008). In general, earthworms are known to incorporate organic wastes such as plant and animal residues into soil and casts. The nutrient contents of the casts have higher than non-ingested soil (Sharpley and Syers, 1976; Scheu, 1987; Daniel and Anderson, 1992; Parkin and Bery, 1994). Additionally, higher microbial biomass and their activities in casts than the corresponding topsoil were recorded in a range of several studies (Tiunov and Scheu 1999; Kızılkaya and Hepşen, 2004). However, little information is available on the effects of sewage sludge on some biological characteristics in earthworm casts and surrounding soil. Soil biological activity plays an important role in regulating soil fertility and/or soil sustainability. Several biological parameters, such as microbial biomass and CO_2 production have been used to define the status and sustainable development of productivity in soils, and are used as bio-indicators for soil quality and health in environmental soil monitoring (Rogers and Li, 1985). Microbial biomass C is measured to give an indication of the response of soil microbiota to management, environmental change, site disturbance, and soil pollution (Kandeler, 2007). Soil respiration, as measured by the net heterotrophic production of CO_2, is an important measure of aerobic microbial activity and carbon flux through terrestrial ecosystems (Coleman, 1973). The CO_2 produced from the soil results from the mineralization of organic matter, a process in soil microflora play a dominant role (Satchell, 1983).

The objective of this study were to determine the effects of different application doses of sewage sludge on microbial biomass C, soil respiration organic C and total N of soil and *Lumbricus terrestris* earthworm cast.

MATERIAL and METHOD

Soil, Sewage Sludge and Earthworms

Surface soil (0-20 cm) was taken from the horticultural soil in Merzifon. The site is located in the Black Sea Region, Northern Turkey (Latitude, 40º 52' N; longitude, 35º 20' W). The climate is semi-arid and the annual average precipitation and temperature are 470.6 mm and 6.6 ^{0}C in February to 23 ^{0}C in August. Sewage sludge obtained from a wastewater facility setup by Ankara Wastewater Treatment Plants, Ankara, Turkey. Sewage sludge used was dried at 65 ^{0}C prior to collection and was sieved (< 2 mm) before analysis. Selected soil and sewage sludge physical-chemical properties were determined by standard methods (Black, 1965; Rowell 1996).

Lumbricus terrestris L. was collected from the site which the soil sample was taken. Earthworms were washed with distiled water and capt for 2 weeks before starting the experiment in containers with soil-sewage sludge combinations at 20 ± 0.5 ^{0}C.

Experimental Procedure

The soil samples (500 g air-dried soil) were placed in 1 L cylindirical plastic containers. The sewage sludges were mixed as homogenous with the soil at a rate equivalent to 0, 20, 40, 60, 80 and 100 g kg^{-1} on an air-dried weight basis. Then, four individuals of *Lumbricus terrestris*, were placed in the sewage sludge-amended soil. The containers were incubated for 90 days in the incubater at 20 ^{0}C and samples were taken from containers per 15 days. The moisture content in soil was maintained at 60% water holding capacity in soil trough out the incubation period. Three replicates per treatment were established. At the end of the each incubation period, samples were collected by hand from earthworm casts deposited on the soil surface and from bulk soil.

Total organic C and Total N Analysis

Total organic C levels of soil and casts were determined by the Walkley-Black method, and total N concentrations were determined by using the Kjeldahl method (Black, 1965; Rowell, 1996).

Biological Analysis

Microbial biomass carbon was determined by the substrate-induced respiration method of by Anderson and Domsch (1978). A moist soil sample equivalent to 100 g oven-dry soil was amended with a powder mixture containing 400 mg glucose. The CO_2 production rate was measured hourly using the method described by Anderson (1982). The pattern of respiratory response was recorded for 4 h. Microbial biomass carbon (C_{mic}) was calculated from the maximum initial respiratory response in terms of mg C g^{-1} soil as 40.04 mg CO_2 g^{-1} + 3,75. Three replicates of each sample were tested. Data are expressed as mg CO_2-C 100 g^{-1} dry soil.

Basal soil respiration at field capacity (CO_2 production at 22 ^{0}C without addition of glucose) was measured, as reported by Isermayer (1952); by alkali ($Ba(OH)_2.8H_2O + BaCI_2$) absorption of the CO_2 produced during the 24h incubation period, followed by titration of the residual OH^- with standardized hydrochloric acid, after adding three drops of phenolphthalein as an indicator. Three replicates of each sample were tested. Data are expressed as μg CO_2 g^{-1} dry soil.

Statistical Analysis

All data were analyzed using SPSS 11.0 statistical software. Analysis of variance (ANOVA) was performed to compare the means of different doses of sewage sludge-amendment; were significant F-values were obtained. Pearson correlation coefficient and P values were calculated for all possible variable pairs. The asterisks, *, **, and ***, indicate significance at $P < 0.05$, 0.01, and 0.001, respectively.

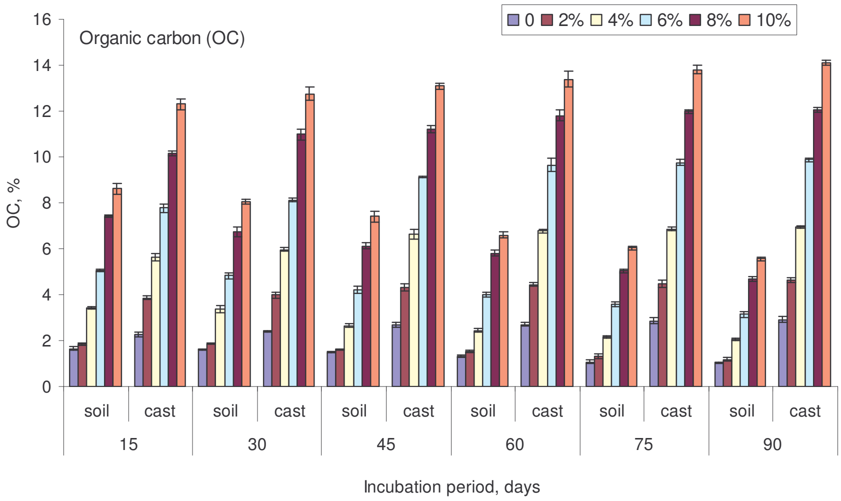
RESULTS

Physical Chemical Properties of Sewage Sludge and the Soil

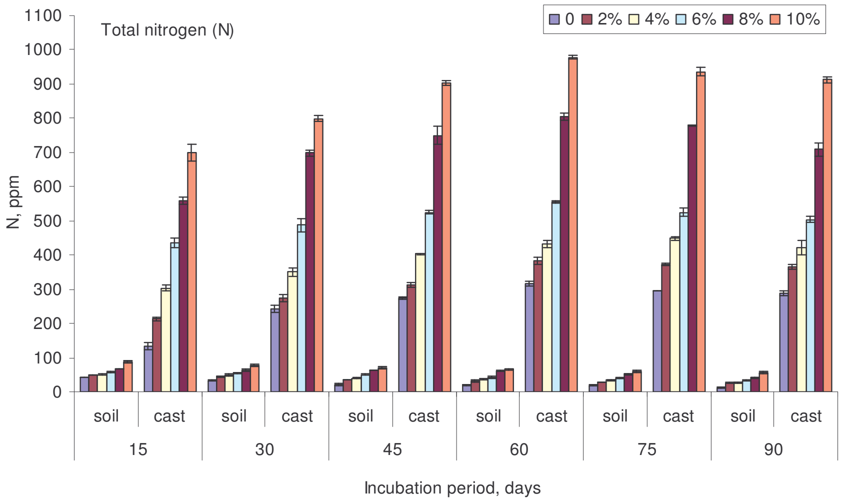
The sewage sludge is composed of approximately 37% weight of oxidable organic matter. The organic fraction comprises 21.2% C and 2.3% N. The pH in water, lime content and C/N ratio were 7.2, 15.8%, and 9.22, respectively. The soil contained 14.1% clay, 10% silt, and 75.9% sand. Soil texture can accordingly be classified as sandy. The pH in water, organic matter content, lime content and C/N ratio were 8.1, 3.29%, 22.5% and 28.1, respectively.

Organic Carbon and Nitrogen Contents in Soil and Casts

The changes of total organic C and total N in earthworm *L.terrestris* casts and surrounding soil during the incubation periods are shown in Figure 1. Organic C and total N in *L.terrestris* casts were generally increased compared to the control treatment at all incubation periods and all sewage sludge doses.



(a)



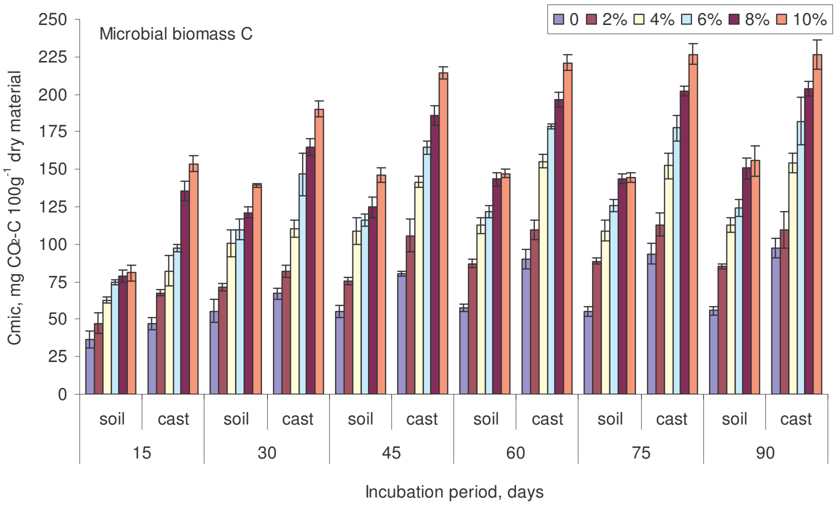
(b)

Figure 1. Changes in C and N of soil amended with sewage sludge during the incubation period. Vertical bars indicate standard error of mean of three replicates at 95 % confidence level
(a) Total organic carbon (b) Total Nitrogen

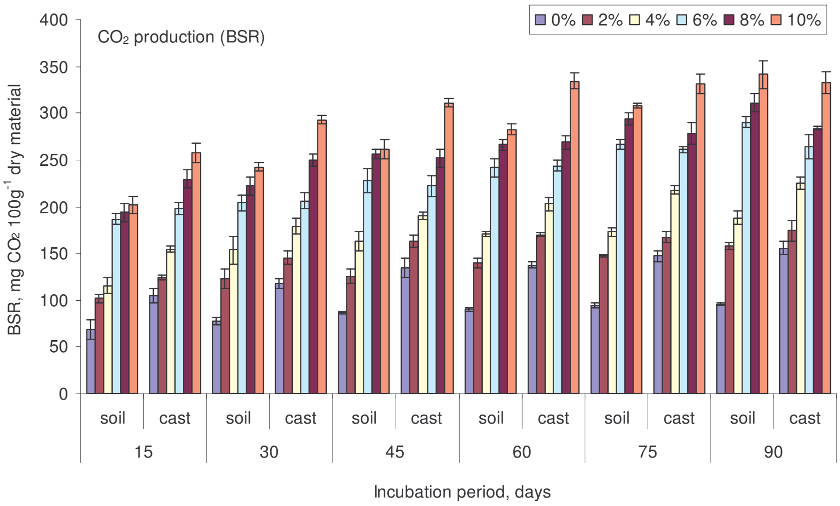
The variations in soil and earthworm casts Organic C and N were statistically significant (Table 1). A marked increase in soil and cast N were found for each increase dose of sewage sludge from 2 to 10%. The highest organic C and total N in soil and casts were obtained with 10% sewage sludge application to soil, and the lowest organic C and total N were found in the control treatment.

Microbial Biomass C and CO_2 Production in Soil and Casts

The changes of microbial biomass C (Cmic) and CO_2 production (BSR) in earthworm *L.terrestris* casts and surrounding soil during the incubation periods are shown in Figure 2.



(a)



(b)

Figure 2. Changes in biomass C and BSR amended with sewage sludge during the incubation period. Vertical bars indicate standard error of mean of three replicates at 95 % confidence level (a) Microbial biomass carbon (b) CO_2 production

Considerable variations in Cmic and BSR were found for the different doses of sewage sludge and different sampling times. Statistically significant variations were found in C mic and BSR at various application rates. Biological properties were also affected by incubation period. The analysis of variance of the results obtained in our experiment on the periodic sampling times with sewage sludge showed that all factors (sewage sludge doses, incubation periods) significantly influenced Cmic and BSR (Table 1). After sewage sludge addition a rapid and significant increase in Cmic and BSR was observed in sludge amended soils followed by a stable in the Cmic and BSR in soils and earthworm casts. At the end of the incubation, the Cmic and BSR measured in treated soils and casts were statistically different from those measured in the control soils.

Table 1. Analysis of Variance (ANOVA)

	Organic C	Total N	Cmic	BSR
Material (cast or soil)	48381.081***	174410.363***	2440.156***	627.643***
Incubation period (IP)	14.738***	495.510***	458.993***	537.360***
M x IP	736.838***	786.759***	19.288***	13.789***
Sewage sludge doses (D)	20434.538***	8751.207***	1487.319***	3649.125***
M x D	1646.809***	6695.104***	78.806***	74.232***
IP x D	6.326***	17.184***	6.430***	12.011***
M x IP x D	30.927***	16.673***	1.986**	6.948***

DISCUSSION

At the end of this study, It was determined that organic C, N, Cmic and BSR levels increased depending on increasing doses of sewage sludge treatment compared to the control. This situation may be related to the organic matter and nutrient concentrations in sewage sludge.

In some researches it was determined that the sewage sludge application to soil has significantly increased the biological characteristics, nevertheless at the other researches that the sewage sludge application to soil has destructed the biological characteristics (Sastre et al, 1996; Knight et al, 1997; Kızılkaya and Bayraklı, 2004).The differences among the data that to carry in conclusion of studies completely depend on sewage sludge characteristics (organic matter content, C/N ratio, nutrient content etc.) and soil physico-chemical characteristics (Hinesly et al. 1972; Kızılkaya and Bayraklı, 2004). It was determined that organic C, N, Cmic and BSR levels of earthworms casts into both sewage sludge treated and no treated pots is higher than the surrounding soil. Similarly most of the experiments reported that the earthworm casts contain higher level of organic matter and nutrient than soil and thus contain biological characteristics like biomass and BSR at higher levels (Lee and Foster, 1991; Scheu and Parkinson, 1994; Haynes and Fraser, 1998; Kızılkaya and Hepşen, 2004, 2007). Nevertheless, Zhang et al. (2000) determined some earthworm species' casts contain lower level microbial activity. This is due to that some earthworm species feed some microorganisms such as fungal spores and bacteria. In this incubation experiment, organic C, N, Cmic and BSR in cast and soil increased until 30th and 45th days depend on increasing doses of sewage sludge, but there is not a significantly increase after 45th day. This situation probably interests in lack of nutrient sources in order to earthworm feeding (Lee, 1985) and stabilization of biological characteristics in earhworm growing media.

REFERENCES

Anderson, J.P.E. and K.H. Domsch. 1978. A physiological method for the quantative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10: 215 – 221.

Anderson, J.P.E. 1982. Soil respiration. In. Page, A.L. (Ed.), Methods of soil analysis. Part 2, Chemical and microbiological properties. Vol. 9, 2nd edition. ASA-SSAA, Madison, Wisconsin, USA pp.831-871.

Banerjee, M.R., D.L. Burton and S. Depoe 1997. Impact of sewage sludge application on soil biological characteristics. Agriculture, Ecosystem and Environment 66: 241-249.

Black C.A., 1965. Methods of soil analysis, Part 2, Chemical and microbiological properties, Agronomy 9, American Society of Agronomy Inc. Madison, Wisconsin, USA.

Coleman, D.C. 1973. Soil carbon balance in a successional grassland. Oikos 24: 195-199.

Daniel, O. and J.M. Anderson. 1992. Microbial biomass and activity in contrasting soil material after passage through the gut of earthworm *Lumbricus rubellus* Hoffmeister. Soil Biology and Biochemistry 24, 465-470.

Haynes, R. and P. Fraser. 1998. A comparison of aggregate stability and biological activity of the earthworms *Lumbricus terrestris* and *Aporrectodea giardi* and consequences on C transfer in soil. European Journal of Soil Biology 36: 27-34.

Hinesly, T. D., R.L. Jones and E.L. Ziegler. 1972. Effects on corn applications of heated anaerobically digested sludge. Compost Science 13: 26-30.

Isermayer, H. 1952. Eine einpache Methode zur Bestimmung der Pflanzenatmung und der Karbonate in Boden. Zeitschrift für Pflanzenernährung und Bodenkunde 56: 26 - 28.

Kandeler, E., 2007. Physiological and Biochemical Methods for Studying soil Biota and Their Function. In: Soil Microbiology, Ecology, and Biochemistry. Paul E.A. (Ed.). Academic Press. pp. 53-83.

Kızılkaya, R., 2008. Dehydrogenase activity in *Lumbricus terrestris* casts and surrounding soil affected by addition of different organic wastes and Zn. Bioresource Technology 99, 946 – 953.

Kızılkaya, R. and Ş. Hepşen. 2004. Effect of biosolid amendment on enzyme activities in earthworm (*Lumbricus terrestris*) casts. Journal of Plant Nutrition and Soil Science 167, 202-208.

Kızılkaya, R. and Ş. Hepşen. 2007. Microbiological properties in earthworm *Lumbricus terrestris* L. Cast and surrounding soil amended with various organic wastes. Communication in Soil Science and Plant Analysis 38, 2861-2876.

Kızılkaya, R. and B. Bayraklı. 2005. Effects of N-enriched sewage sludge on soil enzyme activities. Applied Soil Ecology 30: 192-202.

King, L.D. and H.D. Morris. 1972. Land disposal of liquid sewage sludge: 2. The effects on soil pH, manganese, zinc, and growth and chemical composition of rye. Journal of Environmental Quality 1: 325-329.

Knight, B.P., M.J. McGrath, J.W. Doran, R.G. Cline, R.F. Haris and G.E. Schuman, 1997. Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, or zinc. Applied Environmental Microbiology 63: 39-43.

Lee, K. E. 1985. Earthworms. Their ecology and relationships with soils and land use. Academic Press, Sydney.

Lee, K.E. and R.C. Foster. 1991. Soil fauna and soil structure. Australian Journal of Soil Research 29: 745-775

Parkin, T.B. and E.C. Berry. 1994. Nitrogen transformations associ ated with earthworm casts. Soil Biology and Biochemistry 26: 1233–1238.

Rogers, J.E. and S.W. Li. 1985. Effect of heavy metal and other inorganic ions on soil microbial activity: Soil dehydrogenase assay as a simple toxicity test, Bulletin of Environmental Contamination and Toxicology 34: 858 – 865

Rowell, D.L. 1996. Soil Science: Methods and Applications. 3rd Edition Longman. London, UK.

Sastre, I., M.A. Vicente and M.C. Lobo. 1996. Influence of the application of sewage sludges on soil microbial activity. Bioresource Technology 57: 19-23.

Satchell, J.E., 1983. Earthworm Microbiology. In: J.E. Satchell (Ed.). Earthworm Ecology from Darwin to Vermiculture. Chapman and Hall, London. pp. 351-364.

Sharpley, A.N. and J.K. Syers. 1976. Potential role of earthworm casts for the phosphorus enrichment of run-off waters. Soil Biology and Biochemistry 8: 341-346.

Scheu, S., 1987. Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). Biology and Fertility of Soils 3: 230-234.

Scheu, S. and D. Parkinson, 1994. Effects of earthworms on nutrient dynamics, carbon turnover and microoganisms in soils from cool temperate forests of t he Canadian Rocky Mountains: Laboratory studies. Applied Soil Ecology 1: 113-125.

Tiunov, A.V. and S. Scheu, 2000. Microbial biomass, biovolume and respiration in *Lumbricus terrestris* L. cast material of different age. Soil Biology and Biochemistry 32: 265-275.

Zhang, B., G. Li, T. Shen, J. Wang and Z. Sun. 2000. Changes in microbial biomass C, N and P and enzyme activities in soil incubated with the earthworms *Metaphire guilelmi* or *Eisenia fetida*. Soil Biology and Biochemistry 32: 2055-2062