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В материалах конференции представлены работы, освещающие наиболее актуальные проблемы, характеризующих современное состояние черноземов. В сборнике представлены результаты работ по вопросам генезиса, географии и классификации черноземов, их физических, химических, биологических, агрохимических, экологических и других свойств, вопросам диагностики и оптимизации экологического состояния.

Публикуемые материалы представляют интерес для широкого круга специалистов в области почвоведения, биологии, экологии, географии, охраны окружающей среды, сельского хозяйства, для преподавателей, аспирантов и студентов вузов.


The materials of the conference contains papers covering the most urgent problems of the current state of chernozems. This volume presents the results of work on the genesis, geography and classification of chernozems, its physical, chemical, biological, agrochemical, environmental, and other properties, the diagnosis and optimization of ecological status. Published material is of interest to a wide range of experts in the field of soil science, biology, ecology, geography, environment, agriculture, for educators, students and postgraduates.

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EFFECTS OF SEWAGE SLUDGE ON MICROBIAL BIOMASS AND BASAL RESPIRATION OF SOIL AND EARTHWORM LUMBRICUS TERRESTRIS CAST

Ridvan Kızılkaya, Coşkun Güler
Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 55139 - Samsun TURKEY, ridvank@omu.edu.tr cgulser@omu.edu.tr

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, basal respiration, 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, basal respiration, 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 sewage sludge caused increases in microbial biomass C and basal respiration, significantly ($P<0.001$). It was determined that the microbial parameters of soil and earthworm casts were not significantly changed after the 45th and 60th days of the experiment.

Key words: Sewage sludge, soil, earthworm cast, microbial biomass C, basal respiration

1. 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 Bayrakh, 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 (Sharples 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 (Tsimov 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 basal respiration (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, basal respiration, organic C and total N of soil and Lumbricus terrestris earthworm cast.

2. MATERIAL AND METHOD
2.1. 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, 40° 52' N; longitude, 35° 20' W). The climate is semi-arid and the annual average precipitation and temperature are 470.6 mm and 6.6 °C in February to 23 °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 °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 distilled water and capp for 2 weeks before starting the experiment in containers with soil-sewage sludge combinations at $20 \pm 0.5$ °C.
2.2. Experimental Procedure
The soil samples (500 g air-dried soil) were placed in 1 L cylindrical 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 incubator at 20 °C and samples were taken from containers per 15 days. The moisture content in soil was maintained at 60% water holding capacity in soil through 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.

2.3. 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).

2.4. 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 CO₂ 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 (Cmic) was calculated from the maximum initial respiratory response in terms of mg C g⁻¹ soil as 40.04 mg CO₂ g⁻¹ + 3.75. Three replicates of each sample were tested. Data are expressed as mg CO₂-C 100 g⁻¹ dry soil.

Basal respiration at field capacity (CO₂ production at 22 °C without addition of glucose) was measured, as reported by Isermayer (1952); by alkali (Ba(OH)₂·8H₂O + BaCl₂) absorption of the CO₂ 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 CO₂ g⁻¹ dry soil.

2.5. 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.

3. RESULTS
3.1. Physical chemical properties of sewage sludge and the soil
The sewage sludge is composed of approximately 37% weight of oxidizable 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.

3.2. 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.

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(a)
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.

3.3. Microbial biomass C and CO$_2$ production in soil and casts
The changes of microbial biomass C (Cmic) and basal respiration (BSR) in earthworm *L. terrestris* casts and surrounding soil during the incubation periods are shown in Figure 2.
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 Cmic 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)

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

### 4. 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; Kizilkaya and Bayralp, 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; Kizilkaya and Bayralp, 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; Kizilkaya 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 earthworm growing media.