

Ayten Karaca · Oguz Can Turgay · Nihal Tamer

## Effects of a humic deposit (gyttja) on soil chemical and microbiological properties and heavy metal availability

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**Abstract** The influence of a humic deposit (Gyttja, G) alone (applied at 25 kg ha<sup>-1</sup>) and in combination with mineral fertilizer (G + NP) on soil organic matter content, pH, electrical conductivity, total N content, calcium carbonate content, enzyme activities (urease,  $\beta$ -glucosidase, arylsulphatase, and alkaline phosphatase), microbial biomass C, soil respiration, and availability of Cd, Pb, Ni, and Zn was examined through a 180-day incubation period and compared with the behavior of no treatment (control) and NP treatment. A significant increase in organic matter content was observed in soils treated with G + NP. Compared with G and NP alone, the G + NP-amended soils showed higher values of the selected microbiological properties.

Diethylenetriaminepentaacetic-acid-extractable Cd, Pb, Ni, Cu, and Zn increased significantly with increasing rates of NP, but the addition of G + NP resulted in a considerable decrease in the amount of extractable metals during the incubation period ( $P < 0.05$ ). Based on these results, it can be concluded that the organic matter applied in the gyttja led to an increase in the metal adsorption capacity of the amended soils. This material can be used to reduce the availability and mobility of heavy metals in the soils intensively amended with mineral fertilizers. A combination of G with NP can, therefore, be considered as an alternative approach in the applications of organomineral fertilization.

**Keywords** Gyttja · Enzyme activity · Biomass C · Soil respiration · Soil organic matter

### Introduction

Agricultural soils in Turkey are characterized by low organic matter contents. This is mainly due to the warm and dry climate, the system of cultivation used, and the misuse

of soils with erosion and land degradation. Supplementing soil with organic materials in these agro-ecosystems can support organic matter and nutrient status of soil and also help solve environmental and economic problems. Soils managed with organic inputs generally have larger and more active microbial populations than those managed with mineral fertilizers (Dick 1984). Generally, applying organic materials (poultry manure, sewage sludge, Leonardite, gyttja, etc.) to soil is a common practice to increase organic matter content, improve soil properties (Stevenson 1994), and decrease plant diseases and soil born pathogens (Kheyrodin and Antoun 2002).

Humic substances are probably the most widely distributed natural products on the earth's surface, occurring not only in soils, natural waters, rivers, lakes, sea sediment plants, peat, and other chemically and biologically transformed materials but also in lignite, oxidized bituminous coal, Leonardite, and gyttja (Senesi 1994; Stevenson 1994). The large deposits of humic substances offer possibilities for the commercial production of humate for industrial use, at relatively low cost. Among these deposits, gyttja, which is rich in humic–fulvic acids (Cimen and Ok 2004), is abundant (approximately 1.8 billion tons) between the coal layers of lignite deposits, located in the Afsin–Elbistan region of Turkey. Gyttja is a mixture of both organic and fine mineralogical matters, and its content of organic matter can range from 6 to 40% by weight. Large deposits of gyttja found in the Afsin–Elbistan region are currently being marketed for agricultural uses. Many papers have been published on the influence of the Afsin–Elbistan's gyttja on the soil physical (Akyıldız 1979; Yoruk 1981) and chemical (Barut 1997) properties, but little is known about its effect on microbiological properties of soil. The microbiological properties that are most useful for detecting the deterioration of soil quality are those closely related to nutrient cycles, such as soil respiration, microbial biomass, nitrogen mineralization, and enzyme activities (Visser and Parkinson 1992).

The purpose of this study was to evaluate the effects of gyttja alone (G) and in combination with inorganic fertilizer (G + NP), on urease, alkaline phosphatase,  $\beta$ -glucosidase,

A. Karaca (✉) · O. C. Turgay · N. Tamer  
Soil Science Department, Faculty of Agriculture,  
Ankara University, Ankara, Turkey  
e-mail: akaraca@agri.ankara.edu.tr

and arylsulphatase activities, soil microbial biomass C (SMBc), and soil respiration (SR). In addition to these microbiological properties, changes in soil organic matter (SOM) content, pH, electrical conductivity (EC), total N content, CaO content, and heavy metal (Cd, Pb, Ni, and Zn) availability were also monitored.

## Materials and methods

### Soil, gyttja, and fertilizers

The pH of the soil was 7.35, particle ratio was 59:26:15 (clay/silt/sand, respectively), and the SOM content was 1.8% by weight. EC was  $0.16 \text{ dS m}^{-1}$ , total N content 0.11%, available P content 20%, and CEC  $42.16 \text{ meq } 100 \text{ g}^{-1}$ .

Gyttja (G) was obtained from the Afsin–Elbistan Lignite Deposits (Maras, Eastern Turkey). The pH of gyttja was 7.75, EC was  $0.68 \text{ dS m}^{-1}$ , total organic C content was 25.55%, and  $\text{CaCO}_3$  content was 32.50%. Total P content of G was  $17 \text{ mg kg}^{-1}$ , and total N 0.84%. The contents of humic and fulvic acids were 40.78 and 27.49%, respectively.

Urea and triple superphosphate (TSP) were selected as the mineral fertilizers. Total N content of urea was 46.15%, and soluble  $\text{P}_2\text{O}_5$  content of TSP fertilizer was 44.21%. Heavy metal concentrations of G, TSP, and urea were 0.04, 13,  $<0.1 \text{ mg Cd kg}^{-1}$ ; 3.53, 30,  $<0.3 \text{ mg Pb kg}^{-1}$ ; 15.9, 30,  $<1 \text{ mg Ni kg}^{-1}$ ; and 6, 345,  $<0.1 \text{ mg Zn kg}^{-1}$ , respectively. Total heavy metal contents of G were under the organic fertilizer and soil conditioner regulation limits established by Turkish legislation.

### Experimental design

An incubation experiment was conducted in plastic pots, each containing 450 g coarsely sieved soil with various treatments. A constant rate of G was used, while mineral fertilizers were applied to soil in two different doses. Each treatment was replicated three times, and the experiment was carried out in a randomized complete block design. These were the treatments: the control pots with no treatment (treatment C); gyttja was added to soil at a rate of  $25 \text{ kg gyttja ha}^{-1}$  (treatment G); urea and TSP fertilizer were added to soil at a rate of  $1.304$  and  $1.394 \text{ kg ha}^{-1}$ , equivalent to  $0.6 \text{ kg N ha}^{-1}$  and  $0.6 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ , respectively (treatment NP<sub>1</sub>); urea and TSP fertilizer were added to soil at a rate of  $1.956$  and  $2.093 \text{ kg ha}^{-1}$ , equivalent to  $0.9 \text{ kg N ha}^{-1}$  and  $0.9 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ , respectively (treatment NP<sub>2</sub>); G-amended soils were supplemented with the same amount of N and P as for treatment NP<sub>1</sub> (treatment G + NP<sub>1</sub>); G-amended soils were supplemented with the same amount of N and P as for treatment NP<sub>2</sub> (treatment G + NP<sub>2</sub>).

Soil moisture was adjusted to 75% of water holding capacity. The incubation was performed in a growth chamber at  $28^\circ\text{C}$ . Water losses were compensated by the addition of distilled water during incubation.

## Sampling and analysis

SOM, enzyme activities, SMBc, and SR were measured after 1, 30, 60, 90, and 180 days. Soil pH, EC,  $\text{CaCO}_3$ , and total N content were measured after 1 and 180 days, and soil available heavy metal contents were measured after 30, 90, and 180 days of incubation. Extraction of humic and fulvic acids fractions from G was performed according to Schnitzer (1982). Soil pH and EC were measured in a 1:2.5 soil/water mixture (Richards 1954), SOM by a modified Walkley–Black method (Jackson 1962), particle size distribution according to Bouyoucos (1951), and total N by the Kjeldahl method (Bremner 1982). Available Cd, Pb, Ni, and Zn in soils were extracted with a diethylenetriamine-pentaacetic acid (DTPA) solution ( $0.005 \text{ M DTPA} + 0.005 \text{ M CaCl}_2 + 0.1 \text{ M TEA}$ , pH 7.3; Lindsay and Norwell 1978). Total Cd, Pb, Ni, and Zn in G, urea, and TSP were analyzed after digestion with 3 ml  $\text{HNO}_3$ –HF– $\text{HClO}_4$  (1:1:1). Total and DTPA-extractable heavy metals in solution were determined by inductively coupled plasma (ICP-VISTA AX CCD Simultaneous model). Soil enzyme activities were determined according to Naseby and Lynch (1997). SR was measured by the alkaline trap method (Isermayer 1952), and SMBc was determined by the chloroform fumigation–extraction method (Vance et al. 1987). Subsequent statistical analysis was performed using Minitab for Windows (version 2.14).

## Results and discussion

### Soil chemical properties

Changes in selected soil chemical properties (days 1 and 180) are shown in Table 1. With respect to the control soil, the G-amended soils without NP had identical pH values at the beginning and end of the incubation. The highest pH value was found for NP<sub>1</sub> and NP<sub>2</sub> treatment soils at the beginning of the incubation ( $P < 0.05$ ). However, by the end of the incubation, pH had decreased in all NP treatments, with or without G. Total N content of soil increased with increasing rates of NP application ( $P < 0.05$ ). The amount of N was higher in NP treatments than in G + NP treatments. With respect to the control soil, treatment G showed a slight increase in total N at the end of the incubation period. EC increased in all treatments and showed the highest value in the treatment NP<sub>2</sub> at the end of the incubation. The highest  $\text{CaCO}_3$  value was found in treatment G ( $P < 0.05$ ). The increase in soil  $\text{CaCO}_3$  content and EC with the G and NP only treatments, respectively, can be attributed to the large contents of  $\text{CaCO}_3$  in G, and soluble salts in NP.

### Soil microbiological properties

Changes in SMBc and SR during incubation are shown in Fig. 1. For all treatments, SMBc and SR showed a decline throughout. In the soils amended with G, these microbio-

**Table 1** Changes in selected soil chemical properties (1st and 180th days)

	pH		EC (dS m <sup>-1</sup> )		CaCO <sub>3</sub> (%)		Total N (%)	
	1st day	180th day	1st day	180th day	1st day	180th day	1st day	180th day
C	8.05 B	8.02 A	0.15 C	0.19 E	21.70 C	21.18 C	0.195 C	0.167 E
G	8.07 B	8.03 A	0.18 B	0.30 D	23.06 A	23.14 A	0.195 C	0.178 D
G + NP <sub>1</sub>	8.02 C	7.87 C	0.19 B	0.47 B	20.78 D	20.01 F	0.240 B	0.196 C
G + NP <sub>2</sub>	8.00 C	7.98 B	0.20 B	0.39 C	21.86 B	21.52 B	0.246 B	0.205 B
NP <sub>1</sub>	8.11 A	7.97 B	0.24 A	0.47 B	20.80 D	20.78 D	0.268 A	0.217 A
NP <sub>2</sub>	8.10 A	7.98 B	0.27 A	0.62 A	20.80 D	20.20 E	0.279 A	0.218 A
LSD <sub>0.05</sub>	0.025		0.032		0.016		0.019	

Significant differences between treatments at each time point ( $P < 0.05$  level) indicated by different letters

EC electrical conductivity, C Control soil, G soil amended with 25 kg gytija ha<sup>-1</sup>, G + NP<sub>1</sub> soil amended with gytija + 0.6 kg N ha<sup>-1</sup> and 0.6 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, G + NP<sub>2</sub> soil amended with gytija + 0.9 kg N ha<sup>-1</sup> and 0.9 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, NP<sub>1</sub> soil amended with 0.6 kg N ha<sup>-1</sup> and 0.6 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, NP<sub>2</sub> soil amended with 0.9 kg N ha<sup>-1</sup> and 0.9 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>

ological properties were always significantly higher than those of the control soil, indicating that supplementing soil with organic material, i.e., gytija, probably could improve

soil quality since there is a close link between soil quality and microbiological properties of soil (Pascual et al. 1997; Nannipieri et al. 2003). Microbial biomass C and SR of

**Fig. 1** Changes of soil SMBc (a), SR (b) and urease (c), alkaline phosphatase (d), β-glucosidase (e), and arylsulphatase (f) activities in soils during the incubation. Significant differences between treatments at each time point ( $P < 0.05$  level) indicated by different letters. For abbreviations, see Table 1

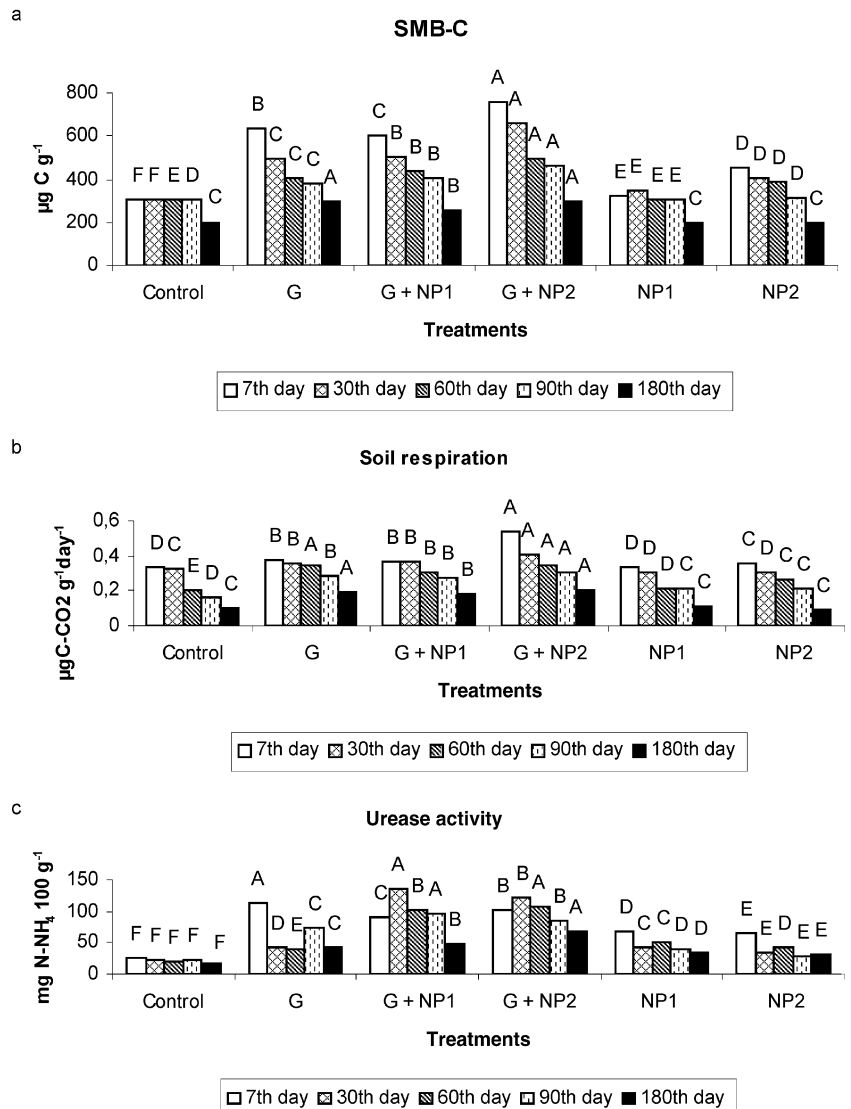
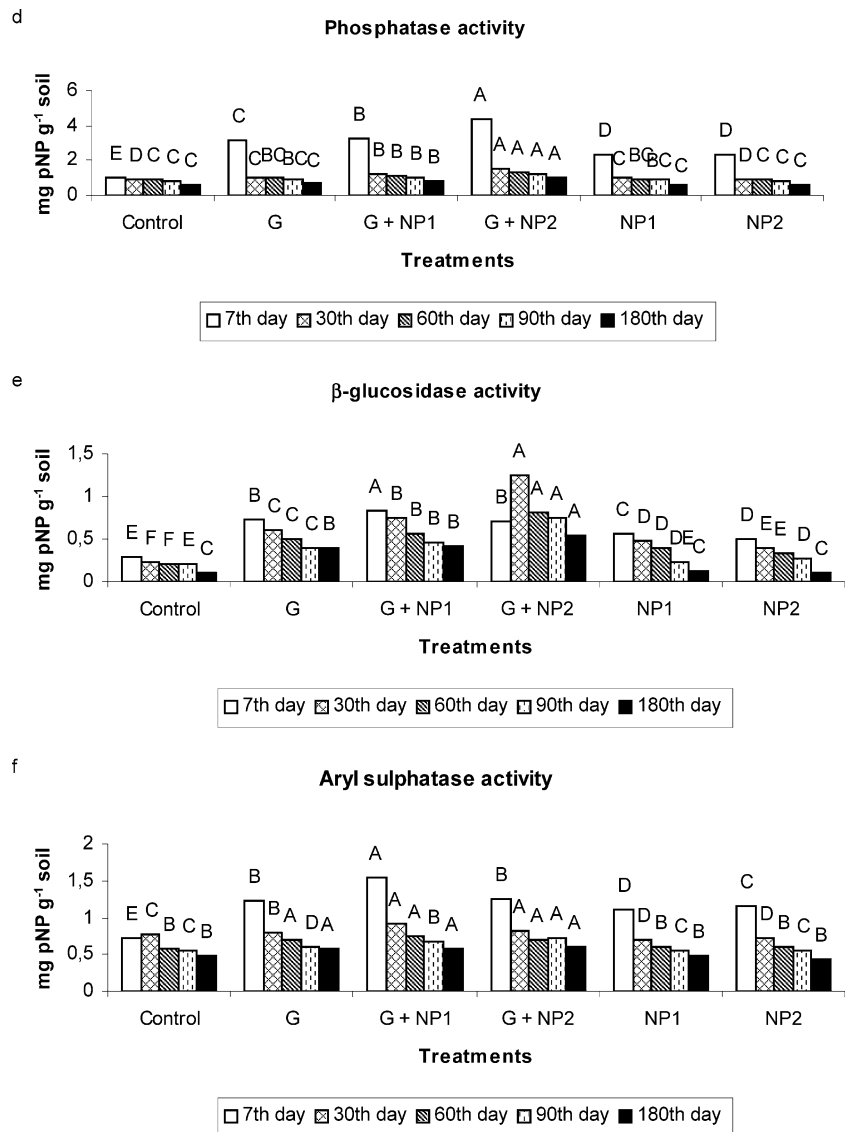


Fig. 1 (continued)



NP<sub>1</sub>- and NP<sub>2</sub>-treated soils were significantly higher than in the control soil during the incubation, but at the end of the incubation period, there was no significant difference between the control soil and the treated soils. The highest SMBC and SR values were found in the G + NP<sub>2</sub> treatment and compared with the control and NP-treated soils; G-amended soils with and without NP had higher SMBC and SR values. In light of these results, it seems that the organic matter added in the form of gytja stimulated both microbial growth and activity in soil.

Previous studies have shown that organic materials added to soil supply energy through their decomposition and stimulate microbial activity and microbial growth (Azmal et al. 1996; Fließbach and Mader 1999). One of the assumptions for the augmenting effects of organic materials on the SMB is the rapid assimilation of nutrients, which may be directly absorbed by soil microflora. Our results confirm what has already been reported in bibliography: The addition of mineral nutrients alone may not have marked effects on microbial biomass, whereas in-

corporation of degradable organic materials may cause changes by providing readily available energy sources and substrates for metabolism (Lovell and Jarvis 1996). In G-amended soils, there was a considerable increase in SMBC and also SR, indicating that the G was being used as a substrate by the microorganisms for both metabolism and growth. This is consistent with the general view that the metabolic efficiency of a microbial community is supposed to be reflected by its specific respiration rate, and the specific respiratory activity has been proposed as a measure of substrate availability in soil (Anderson and Domsch 1990).

In all treatments, urease activity decreased with time (Fig. 1c). At the beginning of the incubation, the addition of G significantly increased the urease activity with respect to all other treatments ( $P < 0.05$ ). By the end of the incubation period, all treatments amended with G and NP had significantly higher urease activity than the control ( $P < 0.05$ ). However, this increase was much greater in the treatment G + NP<sub>2</sub> than in the treatments G and G + NP<sub>1</sub>. Urease

activity decreased with increasing NP rate in the NP-amended soils. The effect of organic residues on soil urease enzyme activity has been reported extensively (Burns 1978; Azam and Malik 1985; Nannipieri 1994; Moreno et al. 1999). Dick (1994) showed that by increasing rates of ammonia-based N fertilizer, urease activity decreased. Mc Carty et al. (1992) showed that N products derived by microbial uptake of inorganic N depressed urease synthesis. Madejon et al. (2001) found that the application of organic materials to soil stimulated urease activity. They indicated that the highest value of urease activity was observed in soil amended with municipal waste compost (MWC), probably due to the higher amount of organic N incorporated with MWC promoting urease synthesis.

Phosphatase activity (EC 3.1.3.1) is important agronomically because it catalyses the hydrolysis of organic P to inorganic P, which can be assimilated by plants. Alkaline phosphatase activity decreased with time until the last

sampling and was significantly increased by all treatments in the study (Fig. 1d). There was no significant difference between control soil and NP<sub>1</sub>- and NP<sub>2</sub>-treated soils. Phosphatase activity decreased by increasing the NP rate. However, G + NP<sub>2</sub> amendment resulted in significantly greater alkaline phosphatase activity compared to all other treatments ( $P < 0.05$ ). Different studies have shown that phosphatase activity increases as a consequence of organic fertilization (Pascual et al. 1999; Chakrabarti et al. 2000). Generally, P fertilization under field conditions may depress phosphatase activity in arable soils (Clarholm 1993; Nannipieri 1994; Olander and Vitousek 2000). However, contradictory results have been reported since phosphatase activity in a low organic matter soil was increased by P fertilization, whereas a soil with a higher organic matter content amended with P fertilizer showed no change in phosphatase activity (Dick 1997). Our results showed that phosphatase activity was negatively correlated

**Fig. 2** Changes in the SOM content (a) and DTPA-extractable Cd (b) Pb, (c), Ni (d), and Zn (e) in soils during the incubation. Significant differences between treatments at each time point ( $P < 0.05$  level) indicated by different letters. For abbreviations, see Table 1

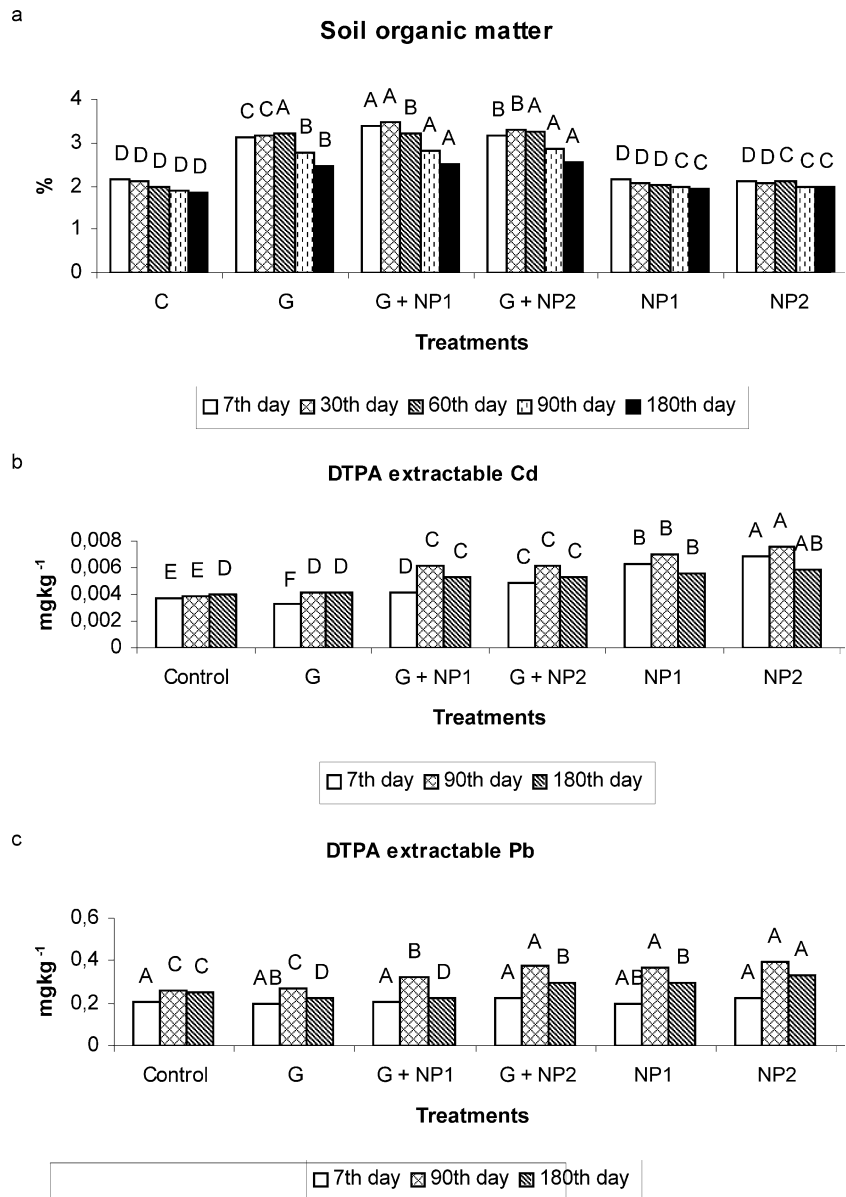
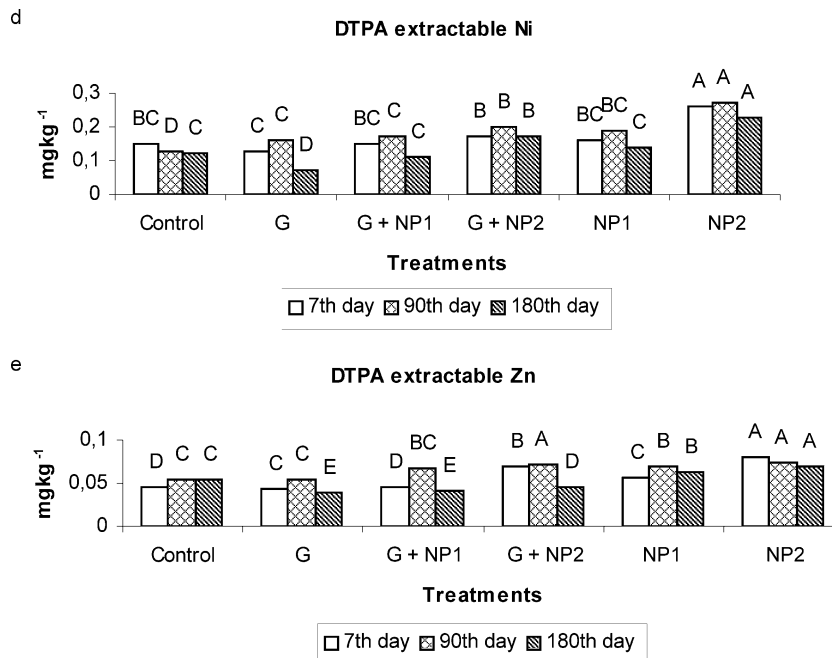




Fig. 2 (continued)



with available Pb ( $r = -0.553$ ,  $P < 0.05$ ). However, it must be noted that it is not possible to distinguish inhibition by heavy metals and stimulation by organic matter addition with the present enzyme assays (Nannipieri 1994).

$\beta$ -Glucosidase (EC 3.2.1.21) activity catalyses the hydrolysis of  $\beta$ -glucosidase bonds and thus the breakdown of some carbohydrates (Martinez and Tabatabai 1997). The NP<sub>1</sub> and NP<sub>2</sub> amendments, in the absence of G, significantly decreased  $\beta$ -glucosidase activities with respect to the G treatments ( $P < 0.05$ ). However, the NP<sub>1</sub> and NP<sub>2</sub> treatments also resulted in significantly greater  $\beta$ -glucosidase activities than the control soil until the 90th day of incubation (Fig. 1e), whereas such differences were not observed at the 90th day of incubation. The addition of G + NP<sub>2</sub> resulted in significantly greater  $\beta$ -glucosidase activity than any other amendments at the end of the incubation period ( $P < 0.05$ ). Fauci and Dick (1994) noted that repeated inorganic N applications over a period 305 days did not significantly affect  $\beta$ -glucosidase activity of soil, whereas stimulation occurred with organic amendments. Pascual et al. (1999) indicated that the increase in  $\beta$ -glucosidase activity in soil amended with municipal solid waste compost was probably due to the increase in microbial activity. Madejon et al. (2001) showed that the increase in  $\beta$ -glucosidase activity of soils treated with organic materials can be attributed to the high  $\beta$ -glucosidase activity of the material itself, as well as to the stimulation of microbial activity.

Arylsulphatase (EC 3.1.6.1) activity is believed to be involved in the mineralization of ester sulphate in soils (Tabatabai 1994). In our study, the G amendment substantially increased the arylsulphatase activity, both with and without NP addition ( $P < 0.05$ ), and this effect persisted at the end of the incubation (Fig. 1f). In the treatment G + NP<sub>1</sub>, arylsulphatase activity was significantly greater than

that in the treatments G and G + NP<sub>2</sub> at the beginning of the incubation, whereas such differences were not observed at the end of the incubation. The NP<sub>1</sub> and NP<sub>2</sub> amendments, in the absence of G, significantly decreased arylsulphatase activities with respect to the G treatments ( $P < 0.05$ ). There were no significant differences between treatments NP<sub>1</sub>, NP<sub>2</sub>, and control. Dick (1994) showed that arylsulphatase and  $\beta$ -glucosidase activities, which are not directly involved in the N cycle, did not correlate with N fertilizer input levels.

Higher enzyme activities were associated with higher microbial biomass C and basal respiration values of soil treated with G and G + NP; this indicates that the increase in enzyme activities of soil is associated with higher microbial activity and microbial growth. Pascual et al. (1999) found that the addition of organic amendment had a positive effect on some soil enzyme activity, probably due to the higher microbial biomass. Kheyrodin and Antoun (2002) stated that the high soil enzyme activity in organic amended soils is not only the result of a greater microbial biomass but also of a higher rate of enzyme production by the microflora. Soils treated with organic inputs generally have higher enzyme activity than those treated with mineral fertilizers (Burns 1978; Dick 1984; Nannipieri 1994).

#### SOM and heavy metal availability

A significant increase in the SOM content was observed in response to the application of G, and this increase persisted up to the end of the experiment (Fig. 2a). The changes in the amount of DTPA-extractable Cd, Pb, Ni, and Zn during the incubation time are shown in Fig. 2. The application of NP alone increased the concentration of heavy metals in soil. Higher NP input resulted in con-

sistently higher metal concentration than did lower NP input ( $P < 0.05$ ). This is most probably related to the presence of metal in P fertilizers widely used in rural areas (Alloway 1993).

We found that the concentrations of DTPA-extractable metals increased by increasing NP fertilizer rate, whereas the presence of G generally decreased the concentration of DTPA-extractable metals. The G material used is rich in humic acids, and probably the humic substances form chelate complexes. According to Alloway (1993), carboxyl and phenoxyl groups of humic acids are responsible for the binding of metals.

## Conclusion

According to our findings, increasing values of microbial biomass C, SR, and enzyme activities in all gytija-treated soil reflect the inputs of organic matter by gytija. Therefore, gytija can improve fertility of agricultural soils by increasing the SOM content and improving microbiological properties of soil. Gytija also decreased the availability of Cd, Pb, Ni, and Zn in soil. Applying such organic fertilizers to soil contaminated with heavy metals along with mineral fertilizer can decrease the availability of heavy metals. However, the beneficial effects of gytija need to be also proved in long-term field experiments.

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