

## Research Article

# Soil Application of Tannery Land Plaster: Effects on Nitrogen Mineralization and Soil Biochemical Properties

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Tannery land plaster (TLP) is a byproduct of lime hydrolysis of leather shavings. Its use in agriculture (organic C  $\approx$  17%, N  $\approx$  6% dm) could represent an alternative to landfill or incineration, but the high Cr(III) content ( $\approx$  5% dm) makes it necessary to evaluate the effect on soil biochemical properties. TLP was therefore added at the rates of 220 and 440 kg of N ha<sup>-1</sup> to 2 agricultural soils and incubated for 56 days under controlled conditions. Extractable NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, CO<sub>2</sub>-C evolution, microbial biomass-N, protease activity, and extractable Cr were monitored. The organic N was readily mineralized (>50% in the first week) and a significant increase in microbial activity was measured, regardless of soil type and addition rate. Extractable Cr(III) quickly decreased during the incubation. The absence of a negative impact on soil biochemical properties seems to support the use of TLP in agriculture, although further investigations in long-term field experiments are suggested.

## 1. Introduction

The production of biosolids from municipal solid waste, sewage sludge, and waste of agroindustrial origin is continually increasing [1]. Their potential use in agriculture as an alternative method to landfill or incineration has become an increasingly attractive option, due to current trends in European waste policy [2, 3]. The agricultural use of these byproducts could help in maintaining soil organic matter (OM) content and promoting the recycling of plant nutrients, thus reducing the use of chemical fertilizers [1] and increasing agricultural production sustainability [4]. However, in order to recycle biosolids in soil it is necessary to exclude any hazardous effects for humans, animals, plants, and soil microbial populations. Appropriate control of chemical and physical characteristics of the biosolids, nutrients, and heavy metal dynamics in soils is needed in order to guarantee the agronomical value of the products and environmental safety.

Tannery land plaster (TLP) is a by-product of lime hydrolysis of leather shavings, a residue of the leather production cycle classified as treated industrial sewage sludge [5]. After the alkaline hydrolysis of leather shavings, sulphuric acid is added to neutralize the suspension, and a calcium sulphate precipitate is then separated by filtration, obtaining the TLP. TLP is currently disposed of in landfill or, at best, used to correct soil acidity. However, due to the significant amount of N ( $\approx$  6% dm) its soil application as a source of organic N may represent a more suitable recycling strategy.

TLP originates from Cr tanned animal hides, thus it contains large amounts of Cr(III) ( $\approx$  5% dm), a human, and animal micronutrient but, at the same time, a potentially toxic element [6] that could represent a factor of concern in agriculture and the environment. To date European legislation on sewage sludge [5] does not set any threshold value for Cr, even though the introduction of such a limit is presently under discussion (total Cr in sewage sludge and

TABLE 1: Chemical characteristics of tannery land plaster (TLP).

Parameter	Units	Value (all data are expressed on a dry matter basis)
Water content	(g kg <sup>-1</sup> )	730*
Total solids (TS)	(g kg <sup>-1</sup> )	270*
Ash	(g kg <sup>-1</sup> )	660
Volatile solids (VS)	(g kg <sup>-1</sup> )	90*
pH (in water)	pH	10.6
Electrical conductivity (EC)	(dS m <sup>-1</sup> )	3.6*
Total organic carbon (TOC)	(%)	17
Total nitrogen (TN)	(g kg <sup>-1</sup> )	57
NH <sub>4</sub> <sup>+</sup> -N	(g kg <sup>-1</sup> )	0.7
NO <sub>3</sub> <sup>-</sup> -N	(g kg <sup>-1</sup> )	0.3
C : N ratio	—	3
Total P	(g kg <sup>-1</sup> )	3.1
Total K	(g kg <sup>-1</sup> )	0.24
Total S	(g kg <sup>-1</sup> )	123
Total Ca	(g kg <sup>-1</sup> )	175
Total Mg	(g kg <sup>-1</sup> )	3.9
Total Na	(g kg <sup>-1</sup> )	2.3
Total Fe	(mg kg <sup>-1</sup> )	755
Total Cu	(mg kg <sup>-1</sup> )	3.3
Total Zn	(mg kg <sup>-1</sup> )	8.3
Total Mn	(mg kg <sup>-1</sup> )	36
Total Co	(mg kg <sup>-1</sup> )	9.6
Total Al	(mg kg <sup>-1</sup> )	373
Total Cd	(mg kg <sup>-1</sup> )	<1.0
Total Ni	(mg kg <sup>-1</sup> )	1.6
Total Pb	(mg kg <sup>-1</sup> )	<0.5
Total Cr	(g kg <sup>-1</sup> )	52.0
Cr(VI)	(mg kg <sup>-1</sup> )	<0.5

\* Fresh matter.

soil). However, since the form in sewage sludge is Cr(III), characterized by very low mobility and translocation in both soil and plants [7], we believe that its available fraction, rather than its total concentration, is the key parameter determining its potential toxicity. Although Cr(III) is 3 orders of magnitude less toxic than Cr(VI) [8], at high concentrations it can be harmful to the microbial community. Liang and Tabatabai [9] found an inhibitor effect on soil N mineralization processes after the addition of sewage sludge at a concentration of Cr(III) of 550 mg kg<sup>-1</sup>. Soil biological parameters can be used as effective indicators of modification in soil status due to pollution and variation in soil management [9]. Soil respiration, a good indicator of the oxidation of the soil OM to CO<sub>2</sub> by aerobic heterotrophic microorganisms, and enzyme activities related to the cycle of main nutrients could give important indications on the rate of nutrient turnover. These parameters can also be used as a measure of microbial activity and its level of disturbance by inhibiting effects of pollutants and particularly of heavy metals [10, 11]. Plant availability of organic N in a biosolid

TABLE 2: Main physical and chemical characteristics of the two soils (M and P).

Parameter	Units	Soil	
		M	P
Sand	(g kg <sup>-1</sup> )	330	340
Silt	(g kg <sup>-1</sup> )	510	500
Clay	(g kg <sup>-1</sup> )	160	160
Water holding capacity (WHC)	(g kg <sup>-1</sup> )	310	280
pH (in water)	—	8.3	7.9
Total calcium carbonate (CaCO <sub>3</sub> )	(g kg <sup>-1</sup> )	15	20
Cation exchange capacity (CEC)	(cmol <sub>c</sub> kg <sup>-1</sup> )	26	25
Total organic C (TOC)	(g kg <sup>-1</sup> )	10.6	7.7
Total Kjeldahl N (TKN)	(g kg <sup>-1</sup> )	1.1	0.91
C : N ratio	—	10	8.5
Olsen-P	(mg kg <sup>-1</sup> )	18	42
Total Mn	(mg kg <sup>-1</sup> )	42	40
Total Cr	(mg kg <sup>-1</sup> )	25	23
Extractable Cr	(mg kg <sup>-1</sup> )	0.63	0.57
Cr(VI)	(mg kg <sup>-1</sup> )	<0.5*	<0.5*

\* Detection limit.

mainly depends on soil microbial processes of mineralization and immobilization whose turnover is mainly influenced by its C : N ratio, although the soil N turnover cannot be explained by this parameter alone. Soil chemical-physical characteristics and biosolid properties such as soluble C content, N biochemical quality, and phenolic content could play a crucial role in determining N mineralization [12–16]. Specific studies on the mineralization dynamic of the organic N in biosolids are therefore required in order to evaluate N supply to plants and to avoid leaching of N in the environment.

At present little is known about TLP-Cr(III) behaviour in soil and, in particular, on the dynamics of its extractable fraction, likely to be available to soil microorganisms.

The aim of this research was to study the effects of applying agronomical rates of TLP on (i) C and N mineralization, (ii) soil microbial biomass N, (iii) protease activity, and (iv) extractable Cr(III) dynamics in two agricultural soils in a short-term laboratory experiment.

## 2. Materials and Methods

**2.1. Chemical Characterization of Tannery Land Plaster.** A chemical characterization of TLP was carried out (Table 1) to determine water and ash content, total (TS) and volatile solids (VS), pH, electrical conductivity (EC), total organic carbon (TOC), and nitrogen (TN), ammonium (NH<sub>4</sub><sup>+</sup>-N), nitrates (NO<sub>3</sub><sup>-</sup>-N), water extractable hexavalent chromium [Cr(VI)], total nutrients, and the most representative trace heavy metal contents. Water content was determined after oven drying at 105°C for 24 h, pH and EC were measured according to the method reported by Trinchera et al. [17]. TOC was determined by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> oxidation method described by Ciavatta et al. [18], TN using an elemental analyzer (Thermo Fisher Scientific) and mineral N according

to the method reported by Violante [19]. Other nutrients and heavy metals were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Spectro Ciros<sup>CCD</sup>) after digestion of the sample with 65% HNO<sub>3</sub>. The Cr(VI) was determined by 1,5-diphenylcarbazide colorimetric analysis after water extraction [17].

**2.2. Soil Properties and Incubation Conditions.** Two soils (Typic Udifluent, USDA Soil Taxonomy) representative of an important agricultural area located in the southeast of the Po valley (Ravenna, Italy), hereinafter named M and P, were chosen for the experiment because they are involved in a larger study, in which the TLP is used for the N fertilization of maize and tomato under field conditions (Table 2). Soil samples from the top layer (0–20 cm depth) were collected in the early spring of 2007, wet-sieved at 4 mm, and then air-dried. Chemical analyses were carried out using the official Italian methods [19]. Water holding capacity (WHC) was determined as described by Agehara and Warncke [20]. Total Cr was determined by ICP-OES after digestion with 65% HNO<sub>3</sub> while Cr(VI) in the water extract by diphenylcarbazide colorimetric method [21]. Extractable Cr was evaluated using the rhizosphere-based method [22]. Briefly, 2 g of moist soils were mixed with 20 mL of combined solution of acetic, lactic, citric, malic, and formic acids in a 50 mL centrifuge tube (extraction ratio 1:10 w/v). The concentration of the organic acids was 10 mM, and their molar ratio was 4:2:1:1:1, respectively. The soil suspension was shaken by an end-over-end shaker for 16 h (60 rpm), centrifuged at 1000 g for 10 min and filtered with Whatman no. 42 filter paper. Five mL of supernatant were mixed with 5 mL of 2% HNO<sub>3</sub>. The Cr content in soil extracts was determined by ICP-OES.

The soils were preconditioned for 14 days at 70% of their WHC at 25°C, to enable acclimatization to incubation conditions. At the end of this period, 3.16 and 6.32 mg g<sup>-1</sup> dry soil of TLP (previously lyophilised and grounded) corresponding to 0.18 and 0.36 mg N g<sup>-1</sup> of dry soil, respectively, were added to 150 g soil samples. The application rates were calculated on an agronomic basis and corresponded to 220 and 440 kg of N ha<sup>-1</sup>, respectively. Unamended soil was taken as the control. The experiment was carried out in triplicate and soils were sampled after 0, 3, 7, 14, 28, and 56 days of incubation that was performed in the dark at the same conditions adopted in the pre-conditioning phase.

**2.3. Extractable Mineral N.** Aliquots of 5 g of soil samples were extracted with 50 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> for 1 hour. Extractable NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were determined by the Bran Luebbe AACE 5.46 Auto Analyzer method. The NO<sub>2</sub><sup>-</sup>-N content was assumed to be negligible in comparison with NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N.

The cumulative amount of mineral N released from TLP at time  $t$  ( $N_{TLP}$ ) was calculated using the following equation:

$$N_{TLP} = N_m(\text{TLP-treated soil})_t - N_m(\text{control})_t - N_m\text{TLP}, \quad (1)$$

where  $N_m$  is the mineral N content (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N).

The percentage of organic N released from TLP at time  $t$  (%N<sub>TLP</sub>) was calculated using the following equation:

$$\%N_{TLP} = \left( \frac{N_{TLP}}{N_{0TLP}} \right) * 100. \quad (2)$$

$N_{0TLP}$  is the amount of organic N in TLP sample calculated as follows:  $N_{0TLP} = TN_{TLP} - MN_{TLP}$ , where  $TN_{TLP}$  is the total N in TLP samples and  $MN_{TLP}$  is the mineral N in TLP sample.

The percentage of mineral N released was fitted to a first-order model [21] using a nonlinear curve-fitting procedure:

$$\%N_{TLP} = \%N_0 * [1 - \exp(-k_0 * t)], \quad (3)$$

where %N<sub>0</sub> is the % of potentially mineralizable organic N added;  $k_0$  is the first-order rate constant (day<sup>-1</sup>).

The  $N_0$  and  $k_0$  values were deemed significantly different ( $\alpha = 0.05$ ) if the 95% confidence intervals did not overlap.

**2.4. Biochemical Analysis.** Ninhydrin (2,2-dihydroxyindane-1,3-dione) reactive N content of the microbial biomass was determined on soil extracts obtained using the fumigation extraction method [23]. Moist soil portions, equivalent to 10 g of oven dried soil each, were fumigated with ethanol-free chloroform for 24 h, then extracted with 40 mL 0.5 M K<sub>2</sub>SO<sub>4</sub> for 30 minutes. Unfumigated soil samples were similarly extracted. Microbial biomass ninhydrin reactive N was calculated by the difference between the ninhydrin N value extracted by K<sub>2</sub>SO<sub>4</sub> from fumigated samples subtracted with the ninhydrin N value extracted by K<sub>2</sub>SO<sub>4</sub> from unfumigated samples [24].

Protease activity was determined according to Ladd and Butler [25]. Moist soil (1 g oven dry basis) was mixed with 5 mL TRIS (2-Amino-2-hydroxymethyl-propane-1,3-diol) buffer (pH 8.1), and 5 mL of 2% Na-casein (suspended in the TRIS buffer). The soil mixture was incubated in a shaking water bath at 50°C for 2 h. Controls were performed by adding the substrate suspension after the incubation. The reaction was stopped with 5 mL of 15% trichloroacetic acid solution (TCA) and the suspension was centrifuged for 10 min at 5000 rpm. The clear supernatant (5 mL) was placed in tubes, treated with 7.5 mL of a 50:1:1 mixture of 0.06 M NaOH, 5% Na<sub>2</sub>CO<sub>3</sub> 0.5%, CuSO<sub>4</sub>·5H<sub>2</sub>O, and 1% potassium sodium tartrate and incubated for 15 minutes. After the incubation, 5 mL of 33% Folin-Ciocalteu reagent (FCR) were added and after 1 h, the absorbance was determined at  $\lambda$  700 nm.

Microbial respiration was measured using the method described by Isermeyer [26]. CO<sub>2</sub> evolution was measured after 1, 2, 3, 5, 7, 9, 14, and 21 days on aliquots of moist soils (10 g oven dry basis; 70% WHC) incubated at 25°C in glass jars by means of 10 mL 1 M NaOH traps. Three replicates were carried out as well as blank CO<sub>2</sub> traps without soil samples. CO<sub>2</sub> evolution was determined by adding 2 mL of 0.5 M BaCl<sub>2</sub> to CO<sub>2</sub> traps and titrating to 8.8 pH with 0.025 M HCl. The respiration rate ( $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil h}^{-1}$ ) and the cumulative evolved CO<sub>2</sub> ( $\mu\text{g CO}_2\text{-C g}^{-1}\text{ soil}$ ) were calculated.

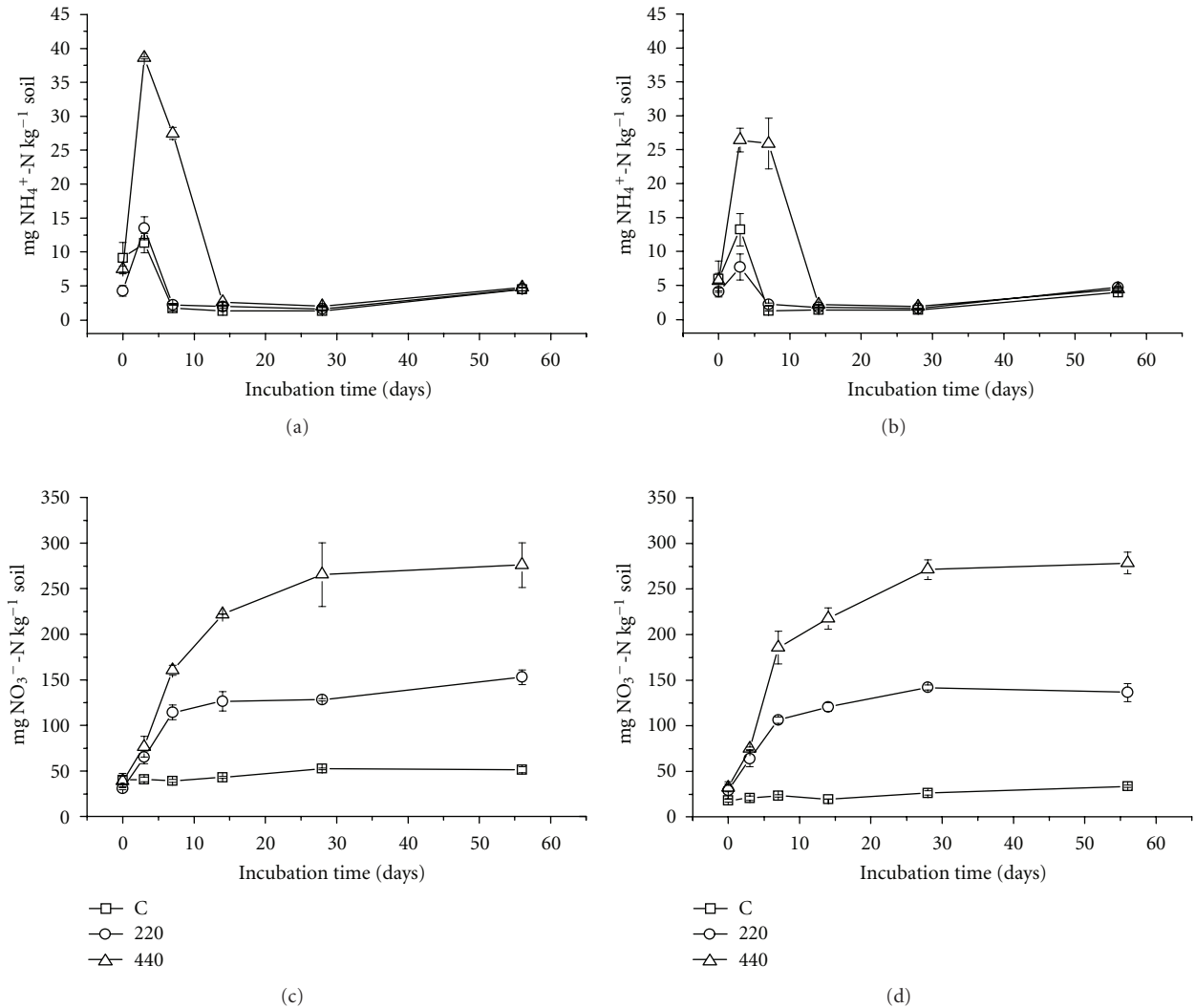


FIGURE 1: Concentrations of K<sub>2</sub>SO<sub>4</sub> extractable NH<sub>4</sub><sup>+</sup>-N in soil M (a) and soil P (b) and K<sub>2</sub>SO<sub>4</sub> extractable NO<sub>3</sub><sup>-</sup>-N in soil M (c) and soil P (d) at different sampling times. C: controls; 220: TLP addition rate at 220 kg N ha<sup>-1</sup>; 440: TLP addition rate at 440 kg N ha<sup>-1</sup>; bars represent standard deviation ( $n = 3$ ).

**2.5. Data Analysis.** An overall analysis of variance (ANOVA) was used for each dependent variable; the model included the main effects of soil type and amendment and their two-way interaction.

Data obtained at each sampling time and from each soil type were subjected to one-way analysis of variance and to mean separation by *Bonferroni* test at  $*P \leq 0.05$  significant levels.

### 3. Results and Discussion

**3.1. Pattern of Mineral-N Release.** The mineralization process started immediately after TLP addition and a significant accumulation of NH<sub>4</sub><sup>+</sup>-N was observed in both soils treated with 440 kg N ha<sup>-1</sup> during the first week of incubation (Figure 1). The concentration of NH<sub>4</sub><sup>+</sup>-N then rapidly decreased and from the 2nd week of incubation was very similar to the other treatments. On the contrary, both soils

treated with 220 kg N ha<sup>-1</sup> showed values of extractable NH<sub>4</sub><sup>+</sup>-N similar to the controls.

Amendment with TLP caused a significant increase of NO<sub>3</sub><sup>-</sup>-N in both soils (Figure 1), detectable from the 3rd day of incubation. After a week, a significant effect of the application rate was detected in both soils and at the end of incubation the NO<sub>3</sub><sup>-</sup>-N concentration a stabilization was shown.

The percentages of net cumulative mineral N (NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N), fitted to a first kinetic order model, are reported in Figure 2. After 14 days of incubation, from 45 to 55% of the N added with TLP was mineralized and the values reached 57 to 68% at the end of the experiment.

The organic N added with TLP was readily mineralized to N-NH<sub>4</sub><sup>+</sup> and further converted into N-NO<sub>3</sub><sup>-</sup>, regardless of the soil type and the addition rate. The extent and dynamics of N mineralization observed in this study are in agreement with other results obtained with different organic fertilizers

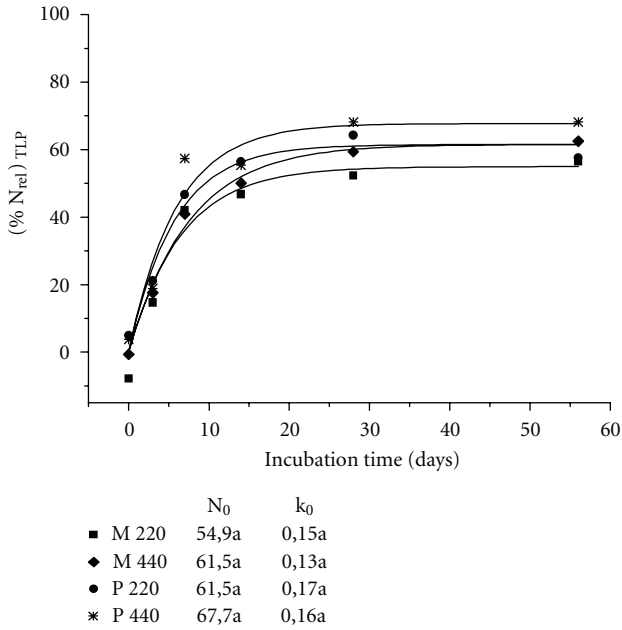


FIGURE 2: Percentage of the net cumulative N released by TLP into M and P soils. The  $N_0$  or  $k_0$  values followed by the same letter are not significantly different at  $\alpha = 0.05$ .

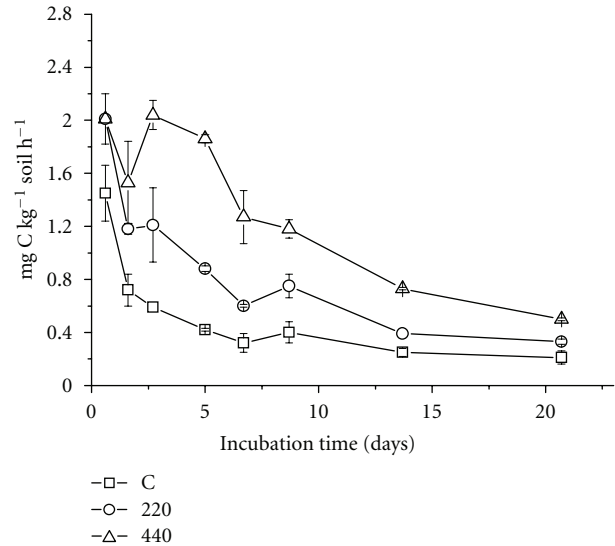
such as meat and bone meal [27], and with blood meal [21]. It is generally accepted that the main factors affecting N mineralization in soil treated with organic materials are the C:N ratio and the biochemical quality and N content [15, 28].

**3.2. Biochemical Properties.** Dynamics of C mineralization, measured as  $CO_2$ -C evolution rate, were characterized by a peak occurring after 3 days of incubation, followed by a progressive decrease (Figure 3). The amount of evolved  $CO_2$ -C was clearly affected by the application rate; soils treated with larger amounts of TLP were characterized by a significantly higher rate of  $CO_2$ -C evolution versus the soils treated with the lowest amount of TLP. On the contrary, the soil type did not affect the  $CO_2$ -C evolution rate (Table 3).

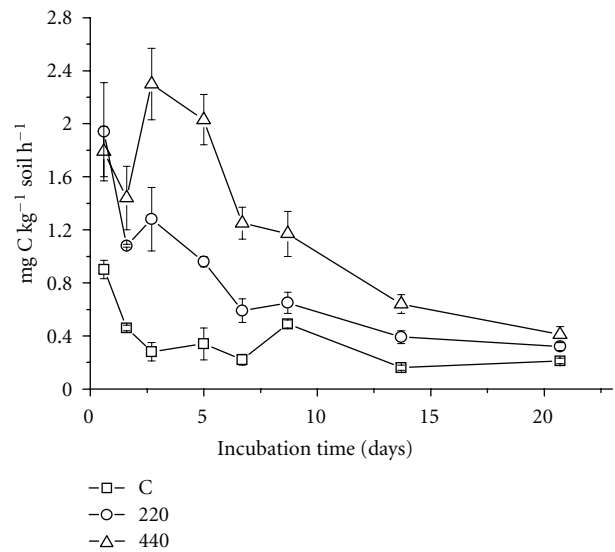
In both soils and for both rates of application, TLP induced a significant increase in  $B_{NIN}$  versus the control soils, 3–7 days after the amendment (Figure 4). However, the turnover of the microbial biomass N in soils treated with TLP was quite rapid with a tendency to decrease towards values approaching the controls by the end of the incubation. The ANOVA showed a significant influence of both the soil type and the amendment, but did not detect a significant effect of the TLP application rate (Table 3).

Protease activity (Figure 4) showed a marked increase with a maximum occurring 7–14 days after the TLP addition; in soil P the increase was faster than in soil M. The protease activity of treated soils remained significantly higher than the controls until the end of the incubation period, and the ANOVA revealed a significant effect of both the soil type and the TLP application rates (Table 3).

Dilly and Nannipieri [29] reported that the increase in soil respiration, enzyme activities, and microbial biomass



(a)



(b)

FIGURE 3: Dynamics of  $CO_2$ -C evolution rate in soil M (a) and soil P (b) at different sampling time. C: controls; 220: TLP addition rate at  $220 \text{ kg N ha}^{-1}$ ; 440: TLP addition rate at  $440 \text{ kg N ha}^{-1}$ ; bars represent standard deviation ( $n = 3$ ).

after the addition of easily decomposable substrates to the soil are clear indicators of an increased microbial activity. In our experiment, these parameters were positively influenced by the TLP addition, pointing out to the absence of a significant action of potentially toxic or detrimental substances that could hamper microbial growth or activities. Dynamics of  $CO_2$ -C evolution showed that TLP addition caused an increase in soil respiration rate, indicating the presence of readily available substances that soil microorganisms can use as sources of C and energy. The extra cumulative  $CO_2$ -C that evolved after 21 days of incubation with TLP (23–32% of the added C) was higher with respect to the values recorded with other substrates of animal origin, such as meat and



TABLE 3: Results of the *F*-test from an overall ANOVA for extractable  $\text{NH}_4^+$ -N, extractable  $\text{NO}_3^-$ -N, protease activity, respiration rate, microbial biomass, and extractable Cr.

Parameter	Soil	Fertilization rate			Soil type fertilization rate
		Control versus 220	Control versus 440	220 versus 440	
Extractable $\text{NH}_4^+$ -N	ns	ns	<0.05	<0.05	ns*
Extractable $\text{NO}_3^-$ -N	ns	<0.05	<0.05	<0.05	ns
Protease activity	<0.05	<0.05	<0.05	<0.05	ns
Respiration rate	ns	<0.05	<0.05	<0.05	ns
Microbial biomass	<0.05	<0.05	<0.05	ns	ns
Extractable Cr	ns	<0.05	<0.05	<0.05	ns

\* ns: not significant at  $P > 0.05$ .

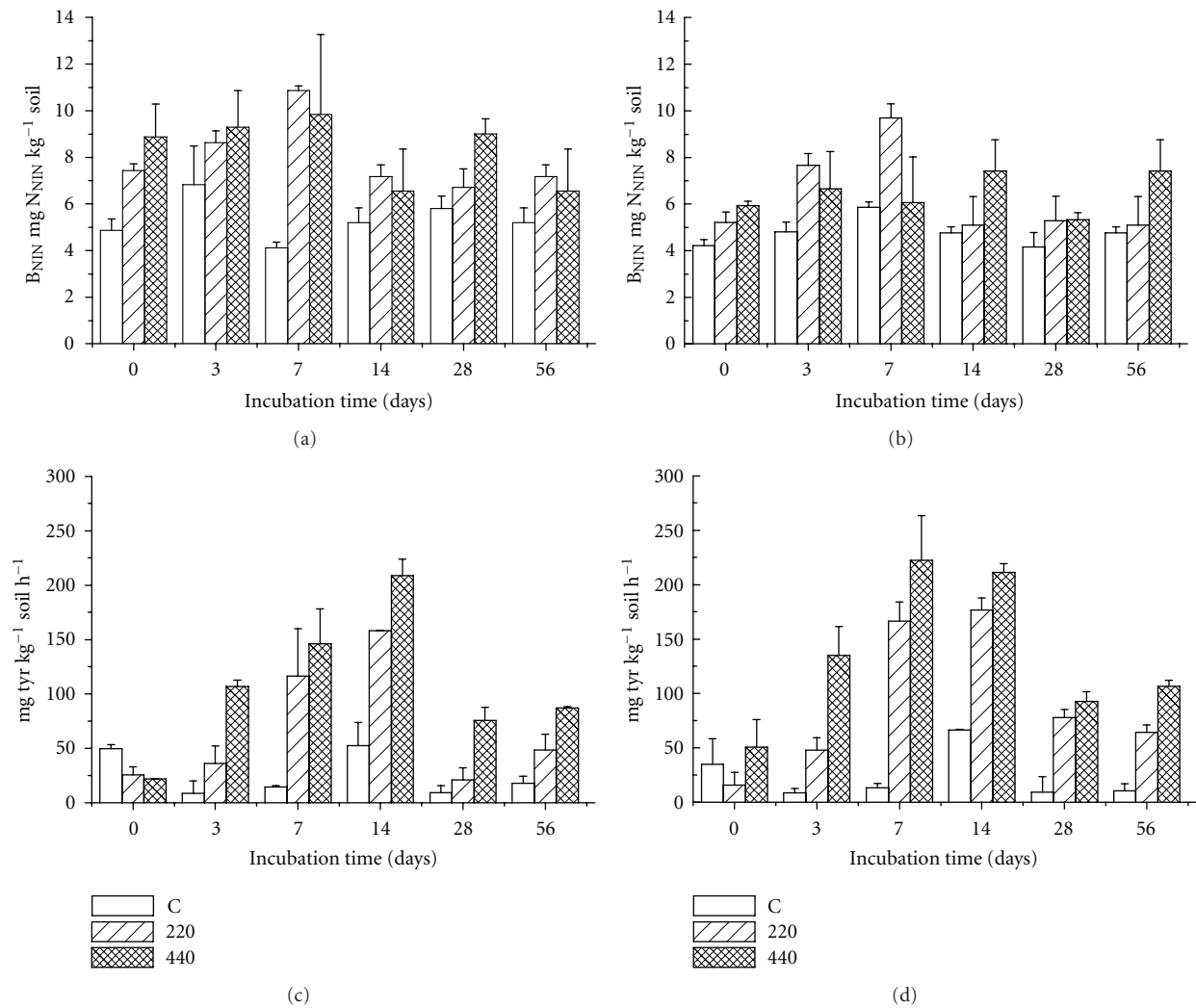


FIGURE 4: Microbial biomass ninhydrin-reactive N ( $\text{B}_{\text{NIN}}$ ), in soil M (a) and soil P (b) and protease activity in soil M (c) and soil P (d) at different sampling time. C: controls; P. 220: TLP addition rate at 220 kg N ha<sup>-1</sup>; 440: TLP addition rate at 440 kg N ha<sup>-1</sup>; bars represent standard deviation ( $n = 3$ ).

bone meal (10–16%) [27], poultry manure, and pig slurry (16 and 19% resp.) [30]. This could be due to differences in the microbial use efficiency of the added C and could have caused the limited and transitory increase of microbial biomass shown by the  $\text{B}_{\text{NIN}}$  parameter.

Soil extracellular proteases are produced by a wide range of bacteria, especially actinomycetes, and fungi, are stimulated by the addition of organic residues containing N in proteinaceous forms through mechanisms of substrate induction and are positively correlated with the amount

of added substrates [31]. On the other hand, when high levels of end products such as amino acids,  $\text{NH}_4^+\text{-N}$  and easily available C sources are abundant, the allocation of resources to protease production may be repressed. However, a fraction of soil proteases can be stabilized by interaction with the soil matrix and may be insensitive to environmental conditions that affect microbes. TLP addition caused a marked increase in protease activity and this increase was positively influenced by the TLP addition rate. The presence of end products apparently did not repress proteases. During the rapid phase of organic N mineralization, the lack of evident protease repression could be due to the fact that substrate induction was the dominant mechanism regulating enzyme synthesis and release and dwarfed the repression mechanisms caused by directly available C and N sources [31]. After 8 weeks of incubation, the levels of protease activity in the amended samples remained relatively constant and significantly higher than the controls. We hypothesize that stabilization processes of extracellular protease had occurred [27, 29], preventing the enzymatic activity from falling below basal levels [32].

The dynamics of  $\text{CO}_2\text{-C}$  evolution,  $B_{\text{NIN}}$  and protease activity suggest a tendency of soils microorganisms to use the substrate to produce energy, with a reduced ability to promote a stable microbial growth. The energy obtained by the intense processes of C and N mineralization could have been used to sustain the intense enzymatic synthesis observed in the treated soils.

**3.3. Chromium Oxidation and Extractable Fraction.** The high concentration of Cr(III) in TLP is a matter of great concern for its agronomical use. As known, Cr(III) is characterized by a scarce mobility in both soil and plants, and generally only a small fraction of the Cr in soil is available to plants and microorganisms [6, 33]. On the contrary, Cr(VI) shows high mobility in soils and toxicity to plants and animals. In order to exclude the oxidation of Cr(III) to Cr(VI) in soil, the determination of water extractable Cr(VI) was carried out at each sampling time: Cr(VI) was never detected throughout the incubation experiment (data not shown).

Since the extent of the extractable fraction is the main element determining the effect of Cr on microorganisms and plants, we monitored its dynamics during the TLP mineralization. Figure 5 shows the dynamics of the extractable Cr fraction, using the extraction method described by Feng et al. [22]. At the beginning of the incubation period from 12.6 to 16% of the total Cr added to the soil with TLP was extractable by the organic acid solution. After 3 days of incubation, the percentage of extractable Cr decreased to 50%, regardless of the soil type and the TLP addition rate. The extractable Cr fraction then steadily decreased and at the end of the incubation period from 3.9 to 4.3% of the total Cr added was still extractable, although it was not influenced either by the soil type or TLP application rate (Table 3). The values of the controls were significantly lower (Table 2) and stable during the incubation period.

The extractable Cr fraction was clearly reduced during the period of incubation. This could be due to the precipita-

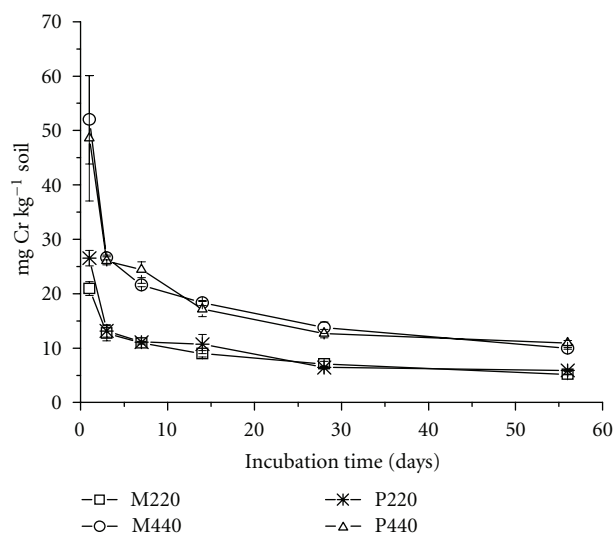


FIGURE 5: Dynamics of extractable Cr in soils M and P. 220: TLP addition rate at 220 kg N ha<sup>-1</sup>; 440: TLP addition rate at 440 kg N ha<sup>-1</sup>; bars represent standard deviation ( $n = 3$ ).

tion of Cr(III) in insoluble forms (e.g., oxide and hydroxide Cr forms) in these alkaline soils relatively rich in carbonates [34].

## 4. Conclusions

TLP added to the soil was characterized by a fast mineralization. The net potential mineralization accounted for more than 50% of the added TLP-N during the first two weeks of incubation, indicating that TLP is a good source of readily available N. The addition of TLP caused a significant increase in the size and activity of microbial biomass, showed by soil respiration, N microbial biomass and protease. The increase of these parameters indicates no toxic or detrimental substance that could hamper microbial growth or inhibit microbial activity like protease at least in the short-term period. The extractable Cr(III) fraction was quickly reduced during the incubation and was probably involved in an intense precipitation process. Assuming that the rate of N mineralization and the effect on biological and microbiological soil properties represent some of the most important factors that we have to consider in the evaluation of the agronomical value of biosolids of industrial origin, we can conclude that TLP should be recycled in agriculture. However, the effect of repeated applications of TLP should be tested in order to evaluate the possibility of long-term accumulation of Cr in soil and its effect on biochemical parameters, in order to confirm the results obtained here.

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