Nitrification of pig slurry added to high heavy metal-content soils

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Introduction

For the effective development of phytoremediation strategies, agronomic management practices need to be optimised for commercial usefulness. Therefore, some organic materials used in agriculture, like animal manures, compost and peat, have been employed recently in different bioremediation experiments for soils contaminated with heavy metals (Bernal et al., 2007).

Pig slurry has been used traditionally in agriculture, especially in semiarid Mediterranean areas, where soils are often characterised by low organic matter contents. However, this waste is currently one of the most abundant in Europe, especially in Spain, where the number of pig breeding farms has increased greatly during the last three decades, producing a significant amount of manures (25.2×10^6 Tm; MAPA, 2004). Thus, the efficient use of these wastes without damage to the environment is now considered to be of high priority. Pig slurry is characterised by high levels of nitrogen (especially as ammonium), slightly basic pH, high electrical conductivity and some undesirable compounds, such as xenobiotics and potentially harmful trace metals (e.g. copper and zinc), and pathogens. Thus, rational use of pig slurry as a soil organic amendment represents a valuable way to provide plant micro and macronutrients, especially nitrogen, and also soluble organic matter which contributes to the acid-base buffering capacity of soils, plays an important role in metal speciation, affects microbiological activity and improves aeration and moisture retention (Bernal, 1990).

Soil fertility depends strongly on the turnover rate of soil organic matter, driven by the activities of different microbial communities. Nitrogen is one of the main factors for plant growth. Its cycle is very important for the agricultural economy and nitrogen losses, as nitrate to groundwater or as greenhouse gas emissions to atmosphere pose environmental risks (Martínez, 1997; Martínez and Peu, 2000). The oxidation of NH_4^+ to nitrate (NO_3^-) is a critical part of the nitrogen cycle which contributes to the availability of soil nitrogen to plants and microbiota. Because of the high specificity of the implicated bacteria, soil nitrification potential depends on many factors: availability and chemical form of N-sources, CO_2 status, redox potential, salinity, pH, organic matter, moisture, soil texture and the presence of heavy metals (Gilmour 1984). Soil pH and organic matter are the most influential parameters for the nitrogen cycle (Sauvé et al., 1999). Although low soil pH does not seem to exclude nitrification in vegetated soils, there are also many acid soils (pH<5) from which nitrification appears to be absent (Robertson, 1982).
Furthermore, pH is one of the main factors controlling bioavailability of toxic compounds for the nitrogen cycle, such as heavy metals, which can affect the N-cycle by inhibiting soil organisms development and damaging the soil-plant ecosystem (Lee et al., 1997). However microbiota can take up metals from the soil solution and it seems that nitrogen cycle inhibition is more related to easy-bioavailable metal concentration rather than total concentration (Semerci y Çeçen, 2007).

Pig slurry properties may indicate a potential use as an organic amendment for

phytoremediation of metal-contaminated soils. However, knowledge of the influence of heavy metals on nitrogen dynamic in metal-contaminated soils is needed to estimate the nitrogen availability to plants used for phytoremediation and thus to optimise the efficiency of pig slurry as fertiliser.

The aim of this work was to determine the nitrification processes in metal-contaminated soils after treatment with pig slurry, using an incubation experiment with soils having different properties and heavy metal concentrations.

Materials and methods

Two soils affected by the pyrite toxic spill of the Aznalcóllar mine were selected: an acid (6A), pH 4.97, and a neutral (3A), pH 7.06. These soils are non-calcareous loam with low levels of OM (1.9%), 46% sand, 34% silt and 20% clay. Both soils have similar total heavy metal concentrations, however their 0.1 M $CaCl_2$-extractable metals (equivalent to soluble and exchangeable forms) are very different, 3A soil: Mn 1.4; Zn 3.8 (mg kg^{-1}) and 6A soil: Mn 120; Zn 180 (mg kg^{-1}). An uncontaminated (Az) control soil (pH 8.24) was collected from a nearby area unaffected by the toxic spill. The texture of this soil was very similar with 40% sand, 34% silt and 26% clay. All the soils were classified as Typic Xerofluvent according to the American Soil Taxonomy, the main characteristics are shown in Table 1. Soils were collected from the top 20 cm, air-dried, and sieved to <2 mm for analysis.

The organic amendment was pig slurry (PS) (Table 1), collected from the installations of CEMAGREF (Rennes, France). This waste is slightly basic (pH 8.23), with high electrical conductivity (32.1 dS m^{-1}), and is rich in organic carbon (34.7 g l^{-1}) and nitrogen (6.6 g l^{-1}), especially as $N\text{-}NH_4^+$ (3.7 g l^{-1}) equivalent to 56.1% of total-N. PS was kept refrigerated at 3ºC before addition to the soil in order to prevent its degradation.

Table 1. Characteristics of the soils and pig slurry (PS) used in the experiment

Parameter	Az soil	3A soil	6A soil	PS†
pH	8.24	7.06	4.97	8.23
EC (ds m^{-1})	0.21	2.27	2.15	32.1
CEC (cmolc kg^{-1})	27.9	15.1	16.3	ND
TOC (g kg^{-1})	7.3	10.5	10.5	34.7
OM (%)	1.3	1.9	1.9	3.1
Total-N (g kg^{-1})	0.9	1.3	1.4	6.6
NH4+-N (mg kg^{-1})	2.3	3.5	3.1	3700
Total-P (g kg^{-1})	ND	ND	ND	1.6
Mn (mg kg^{-1})	446	762	692	37.3
Cu (mg kg^{-1})	50	200	196	10.7
Pb (mg kg^{-1})	24	736	395	0.3
Zn (mg kg^{-1})	124	796	567	5.5

† Values expressed on a fresh weight basis. ND: not determined

An aerobic incubation experiment was carried out using two treatments in each soil: PS-treated soil (5 g of dry soil + 0.2 ml of PS) equivalent to 150 mg $N\text{-}NH_4^+$ kg^{-1} of soil (dry weight) and unamended soil (5 g) as a control. The incubations were placed in individual open plastic vessels (40-ml capacity) without drainage holes. The soils were wet in order to bring their moisture content to 60% of the water-holding capacity. The vessels were closed with parafilm to keep soil moisture and maintain adequate O_2 levels to avoid anaerobic conditions. Each treatment for each sampling-day was repeated four times.

The incubation was carried out in darkness in a temperature-controlled incubator at 26º C. Soils were sampled after 1, 3, 7, 14, 21, 28, 42, 56 and 175 days of incubation, and were analysed for pH and for different nitrogen forms (three samples).

Total-N was measured by Kjeldahl digestion and pig slurry-N-NH_4^+ was determined by distillation in alkaline medium (MgO). Evolution of the different nitrogen forms in the soil was followed after sequential extraction with two steps: ultrapure water for 2h (1:5 w/v), for water-soluble ammonium, nitrates and nitrites and 2M KCl for 2h (1:5 w/v) for exchangeable-ammonium forms. Nitrates and nitrites in soils were analysed by ionic chromatography. Water-soluble and exchangeable ammonium were analysed by the salicylate method (Kempers and Zweers, 1986) using dichloroisocyanurate sodium as chlorine source. Soil pH was determined for 1:2.5 soil:water aqueous extracts after 2h shaking. Soil metal concentrations were determined in water and KCl extracts by AAS. All analyses were performed at least in duplicate.

Results and discussion

The NH_4^+-N and NO_3^--N dynamics in the PS-treated soils showed a similar pattern in the non-polluted (Az) and neutral (3A) soils. Both soils exhibited greater NO_3^--N concentrations than the acid soil (6A), showing the highest nitrification of the nitrogen from pig slurry.

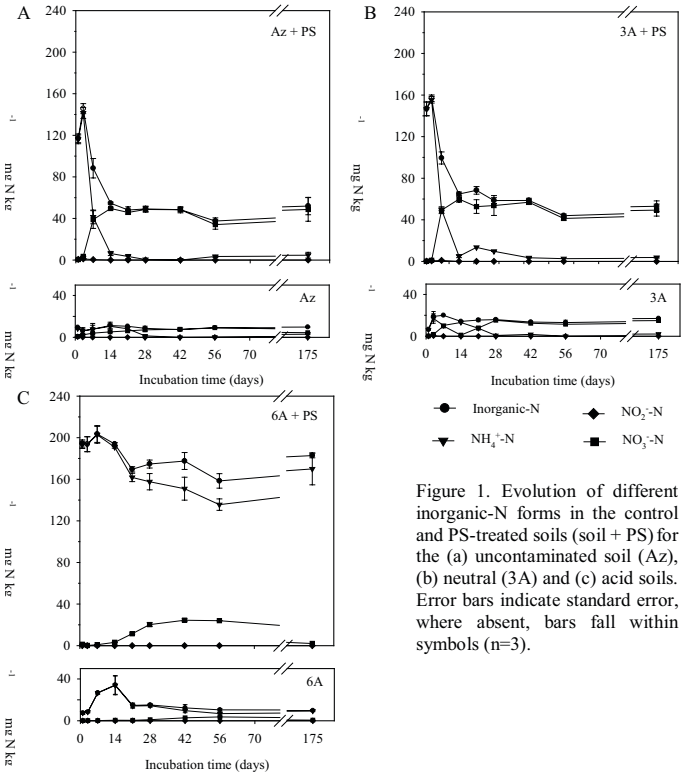
NH_4^+-N and NO_3^--N evolution in these two soils (Az and 3A) showed two different phases. An initial increase, after 1 day of incubation, of NH_4^+-N was shown, associated to the presence of easy decomposable compounds in the pig slurry (such as urea). After this, during two weeks and important decrease (>90%) of NH_4^+-N in PS-treated soils occurred, reaching very low values at the end of the experiment (6.3 and 4.9 mg NH_4^+-N for Az and 3A soils, respectively). NH_4^+-N in the soil remains in an equilibrium between exchangeable and soluble forms (data do not shown). Soluble-ammonium forms are the most easily-available fraction for soil microorganisms which can take it up as nitrogen source for growth (immobilisation) or oxidize to nitrate (nitrification). Both processes produce decreases in the ammonium present in the soil solution, and NH_4^+-N is displaced from the soil exchange complex to the soil solution.

The greatest decrease in NH_4^+-N in 3A and Az soils appeared during the first two weeks of incubation. The slightly basic pH of these soils could favour nitrogen losses by ammonia volatilisation, especially in the Az soil, because the equilibrium $NH_4^+ \Leftrightarrow NH_3 + H^+$ is favoured to NH_3 at pH>8. However, these losses were found to be negligible in a separate set of incubation because the organic material was homogeneously mixed with the soil. Not all the slurry-NH_4^+-N was recovered as NO_3^--N, and only 17.9% in Az and 14.3% in 3A of total-N added was found as NO_3^--N after 175 days of incubation. This was probably due to immobilisation by microbial biomass, which is reported to be a fast process in the soil after pig slurry application (Bernal and Roig, 1993). The addition of carbon and nitrogen, as in the case of pig slurry, to these poor soils (Table 1) can enhance microbial activity and produce a decrease in nitrogen availability by immobilisation. Bertrand et al. (2007) did not find nitrification after 35 days of aerobic incubation in limed (pH 8.1) and unlimed (pH 5.1) soils. These authors assumed a strong immobilisation period of nitrogen. Overall, pig slurry addition induces a reactivation of soil microorganisms growth and activity, as indicated that PS-treated soils exhibited a higher nitrification rate than control soils.

After 14 days, NH_4^+-N decrease was concomitant with NO_3^--N increase, reaching 50 and 60 mg NO_3^--N kg^{-1} for non-polluted and neutral soils respectively (Figure 1). Small amounts of NO_2^--N were detected but only for the first week (0.4 and 0.9 mg kg^{-1} for Az and 3A soils, respectively). After the greatest increase, the NO_3^--N concentration was almost stable in both soils until 42 days and then slight decreases in Az and 3A were shown after 56 days. Inorganic-N decreased in all the soils at the end of the incubation (reaching 93.8, 65.2 and

11.6 mg kg^{-1} for 3A, Az and 6A respectively). However, the greatest decreases were found during the first two weeks of incubation for 3A and Az (82 and 64 mg N kg^{-1} respectively), equivalent to a net decrease of 34 and 24% of the total-N added by the pig slurry for each soil. From 14 to 175 days of incubation, slight decreases in inorganic-N were found in 3A and Az (14.5 and 1.3 mg N kg^{-1}) and only 6A soil showed net mineralisation in this phase (13 mg N kg^{-1}). Although preferential microbial immobilisation of NH_4^+ over NO_3^- is generally accepted (Azam et al. 1992) these decreases could be due to microbial assimilation of NO_3^- as nitrogen source (Myrold and Posavatz, 2007), but also denitrification could occur (Bergstrom et al., 1994).

Figure 1. Evolution of different inorganic-N forms in the control and PS-treated soils (soil + PS) for the (a) uncontaminated soil (Az), (b) neutral (3A) and (c) acid soils. Error bars indicate standard error, where absent, bars fall within symbols (n=3)



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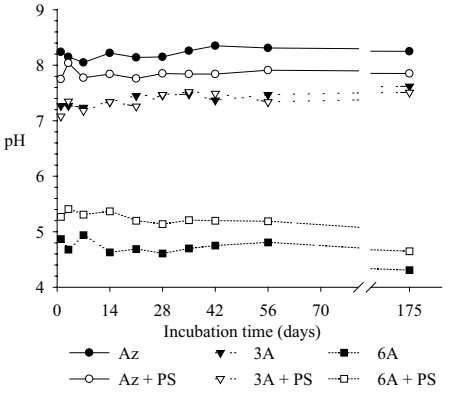
The addition of pig slurry to the non-polluted soil (Az) decreased the pH (Figure 2), reaching values close to 7.8 during the incubation due to the nitrification process. However, the values for soil 3A soil with or without pig slurry were very similar during incubation (Figure 2).

In the acid soil (6A), the transformation of NH_4^+-N to NO_3^--N was very slow and at the end of the experiment a high NH_4^+-N concentration was still found in the soil (170 mg NH_4^+-N kg^{-1}). An important decrease (>90%) in NO_3^--N was shown in 6A soil from 56 to 175 days of incubation (Figure 1). Pig slurry promoted an increase of soil pH with respect to the values without pig slurry, reaching values greater than 5 during the incubation experiment (Figure 2). It has been shown that nitrification is limited at low pH (<5) but the values reached in soil 6A were not low enough to avoid nitrification.

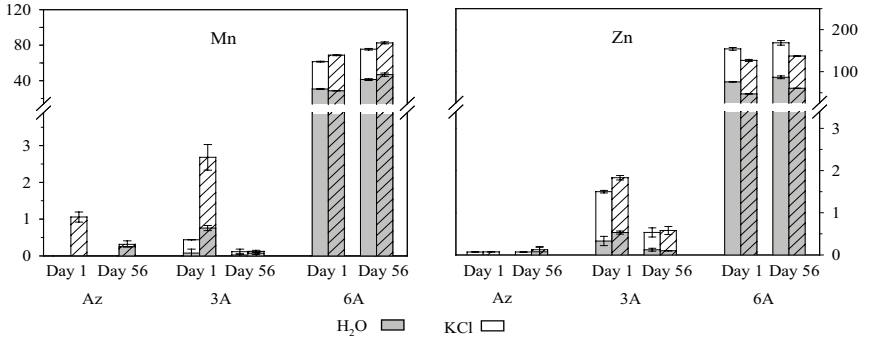
So, the differences in the nitrogen dynamic between these soils, with respect to neutral contaminated and non-polluted soils, could be due to the presence of easily-available metals in this soil, which exert a significant impact on the performance of nitrifying bacteria, affecting the nitrogen balance in the soil (Giller et al., 1998, Lee et al., 1997).

Figure 2. Soil pH of the non-contaminated (Az; solid line), neutral (3A; medium dash line) and acid (6A; dotted line) contaminated soils incubated with (open symbols) and without (close symbols) pig slurry



High concentrations of soluble and exchangeable Mn and Zn were found in 6A soil after 56 days of incubation (Figure 3). Pig slurry slightly decreased soluble and exchangeable Zn related to the soil-pH increase after pig slurry application (Figure 2); however the values did not decrease with time. This could affect both the population and activity of soil microorganisms, limiting immobilisation and nitrification. Hinojosa et al. (2004) found that enzyme activities were negatively correlated with bioavailable Cd, Cu and Zn, but positively with pH in soils affected by the Aznalcollar toxic spill. Furthermore, they showed significant differences in N-mineralisation and nitrification between non-contaminated soils and others affected by the pyrite mud spill. However, the concentrations of water soluble and exchangeable Cu, Zn, Pb and Mn in the soil (KCl extractable) in Az and 3A were very low during the experiment (Figure 3), especially Cu and Pb (data not shown). Both fractions of Zn and Mn decreased with the incubation time in 3A control and PS-treated soils. Only slight increases between PS-amended and non-amended soils were found for Mn after 1 day of incubation (1.1 mg kg^{-1} and 2.3 mg kg^{-1} for Az and 3A soil respectively). Bhuiya and Cornfield (1974) in soils treated with 1000 ppm of Pb and Zn addition reported similar values of nitrate production between two soils (one neutral and another basic).

Figure 3. Soil soluble and exchangeable heavy metals in the non-amended control soil (without fill) and in the PS-treated soils (soil+PS; with fill) after 1 and 56 days of incubation



Overall, the similar behaviour between non-polluted and neutral-polluted soils shows that total heavy metals were not limiting the nitrification process in the soil, nor was the soil pH. However, the soluble and exchangeable metal fractions were more related to nitrification inhibition. Thus, the presence of high levels of easily-available heavy metals in the acid soil affected adversely the soil microorganism activity involved in the nitrification process.

Acknowledgements

The authors wish to thank the CSIC for the grant awarded to Carlos de la Fuente (I3P pre-doctoral). The work was financed by the Spanish Ministry of Education and Science through the project REN2004-06715-C02-02.

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