

Detection of Copper(II) and zinc(II) binding to humic acids from pig slurry and amended soils by fluorescence spectroscopy

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Pig-slurry application to soil decreased Cu(II) and Zn(II) binding affinities of soil humic acids.

Abstract

The effect of the consecutive annual additions of pig slurry at rates of 0 (control), 90 and 150 m³ ha⁻¹ yr⁻¹ after a 7-year period on the Cu(II) and Zn(II) binding behavior of soil HAs was investigated in a field experiment. A fluorescence titration method and a single site model were used for determining metal ion complexing capacities and stability constants of metal ion complexes of HAs isolated from pig slurry and unamended and amended soils. With respect to control soil HA, pig-slurry HA featured much smaller Cu(II) and Zn(II) binding capacities and stability constants. Pig-slurry application to soil decreased Cu(II) and Zn(II) complexing capacities and binding affinities of soil HA. These effects increased with increasing the rate per year of PS application to soil, and are expected to have a large impact on bioavailability, mobilization, and transport of Cu(II) and Zn(II) ions in pig slurry-amended soils.

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1. Introduction

Recycling in agriculture of organic wastes produced by various animal breeding, such as pig slurry (PS), is a common practice throughout the world, which has recently raised serious environmental concerns. In particular, Cu(II) and Zn(II) ions, which are used abundantly as pig feed additives, may be introduced in relatively large amounts into PS-amended soils, thus representing an actual risk of phytotoxicity and/or leaching downward the soil with potential endangering groundwater quality (L'Herroux et al., 1997; Saviozzi et al., 1997; Giusquiani et al., 1998; Nicholson et al., 1999; Aldrich et al., 2002; Taboada-Castro et al., 2002).

Bioavailability, mobility and transport of metal ions in soils are strongly influenced by binding reactions with soil organic matter, and especially its humified fractions, i.e., humic substances (HS), of which humic acid (HA) is the major component (Senesi, 1992; Stevenson, 1994; Aldrich et al., 2002). For these reasons, the effects of PS application on the compositional, structural and functional properties of native soil HA, and especially on their Cu(II) and Zn(II) binding behavior, need to be accurately evaluated.

Various methods and techniques have been used for studying HS binding to metal ions, among which fluorescence spectroscopy has proven to be one of the most promising and productive (Senesi, 1992; Cabaniss, 1992; Luster et al., 1996; Fukushima et al., 1997; Elkins and Nelson, 2001; Provenzano et al., 2004; Wu et al., 2004a,b; Claret et al., 2005; Glaus et al., 2005; Lamelas et al., 2005; Zhao and Nelson, 2005). In general, HAs exhibit fluorescence that is quenched upon binding to metal ions, especially paramagnetic ones

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(Senesi, 1992). Monitoring the changes of fluorescence spectra of HAs upon titration with Cu(II) and Zn(II) can provide useful qualitative and quantitative information about the solution equilibrium of these ions. In particular, the complexing capacity of HA for Cu(II) and Zn(II) ions and the stability constants of Cu(II) and Zn(II) complexes with HA are of considerable value in predicting the behavior and performances of Cu(II) and Zn(II) in soils. These parameters can be determined using the fluorescence titration technique combined with the mathematical approach introduced by Ryan and Weber (1982). This method is based on the existence of a linear relationship between the fraction of the total ligand bound and fluorescence intensity, and on the assumption of a simple 1:1 stoichiometry for the metal ion–ligand binding.

The objectives of this work were (a) to determine and discuss comparatively the Cu(II) and Zn(II) complexing capacities and the stability constants of Cu(II) and Zn(II) complexes of PS-HA with respect to the unamended soil HA; and (b) to investigate the effect of cumulative PS application rates on Cu(II) and Zn(II) binding behavior of amended soil HAs. To reach these objectives, fluorescence titration and the single site model of Ryan and Weber (1982) were used.

2. Materials and methods

2.1. Pig slurries, soils, field experiments and humic acid samples

The PS samples were collected every year before field application from a pig-breeding farm located in the Toledo province, Spain, which employs a closed-cycle production system. The principal chemical properties of PS samples were determined by conventional methods (Clesceri et al., 1998) as follows: (a) dry matter was measured after heating the sample overnight at 105 °C; (b) pH and electrical conductivity were measured directly on the homogenized sample using a glass electrode and a standard conductivity cell, respectively; (c) total N content was obtained by the Kjeldahl method; and (d) total organic C was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate. The PS samples collected over the years had similar properties whose mean values (\pm standard errors) are as follows: dry matter content, $16 \pm 2 \text{ g L}^{-1}$; pH, 7.5 ± 0.1 ; electrical conductivity, $13.5 \pm 0.9 \text{ dS m}^{-1}$; total N content, $2.2 \pm 0.2 \text{ g L}^{-1}$; and total organic C, $5.8 \pm 1.1 \text{ g L}^{-1}$.

The field experiment was conducted in the experimental farm “La Higuera” located in Santa Olalla, Toledo province, Spain, which is very close to the pig farm. The site is characterized by a continental semiarid climate with an average annual rainfall of 487 mm and an average annual temperature of 14 °C. The soil is a Calcic Luvisol (FAO/ISRIC/ISSS, 1998) or Typic Haploxeralf (Soil Survey Staff, 2003). The principal chemical properties of soil samples were determined by standard methods (Sparks et al., 1996) as follows: (a) granulometric composition was obtained by the Bouyoucos method; (b) pH was determined at a ratio soil:water = 1:2.5; (c) electrical conductivity was measured on water extracts obtained at a ratio soil:water = 1:5; (d) total N content was obtained by the Kjeldahl method; and (e) total organic C was determined on 0.5-mm ground sample by dichromate oxidation followed by titration with ferrous ammonium sulphate. The mean values measured on the nine soil surface (0–15 cm) subsamples (see below) (\pm standard errors) are sand, silt and clay contents, 590 ± 2 , 220 ± 2 , $190 \pm 3 \text{ g kg}^{-1}$, respectively (sandy loam texture); pH, 5.8 ± 0.3 ; electrical conductivity, $0.06 \pm 0.01 \text{ dS m}^{-1}$; total N content, $1.2 \pm 0.1 \text{ g kg}^{-1}$; and total organic C content, $13.1 \pm 0.1 \text{ g kg}^{-1}$.

The experimental design included plots ($10 \times 8 \text{ m}^2$) cropped with barley (*Hordeum vulgare* L.), either non-amended or amended yearly with PS at rates of 90 and $150 \text{ m}^3 \text{ ha}^{-1}$ over a 7-year period (PS0, PS90 and PS150, respectively). The PS was applied once per year in mid-September, prior to barley planting (mid-October 1997–2003), and immediately incorporated into

soil at a depth of 0–15 cm. Three replicates were performed for each treatment. Soon after last barley harvest (late June 2004), surface soil subsamples (Ap horizon, 0–15 cm depth) were collected randomly from each plot. Each soil subsample consisted of a mixture of 20 soil cores each of 3-cm diameter. A composite soil sample was then prepared for each treatment by mixing equal amounts (1 kg) of the three corresponding soil subsamples.

The HAs were isolated from the composite PS sample and control and PS-amended soils by a conventional procedure based on alkaline extraction, acidification, separation of HA from FA, purification and freeze-drying, which was described in detail previously (Plaza et al., 2002). The composite PS sample was prepared by mixing equal weights of the seven freeze-dried PS samples collected every year. Prior to HA isolation, the three composite soil samples were air-dried, crushed and passed through a 2-mm sieve after removal of plant residues and stones, and soil carbonates removed by mechanical stirring of each sample for 30 min with 2 M H_3PO_4 . Then soil samples were washed with distilled water until the suspension reached a pH of 7.

2.2. Humic acid analyses

Moisture content of HAs was measured by heating overnight at 105 °C and ash content by burning overnight at 550 °C. The C, H, N, and S contents of HAs were determined in triplicate using a Fisons Instruments (Crawley, UK) elemental analyser model EA 1108. Oxygen content was calculated by difference: $\text{O}\% = 100 - (\text{C} + \text{H} + \text{N} + \text{S})\%$. Carboxyl group content was estimated by direct potentiometric titration as the value of charge at pH 8, phenolic hydroxyl group content was estimated as two times the change in charge between pH 8 and pH 10, and total acidity were calculated by addition (Ritchie and Perdue, 2003).

The absorbances at 465 and 665 nm were measured on solutions of 3.0 mg of each HA in 10 mL of 0.05 M NaHCO_3 , with pH adjusted to 8.3 with 0.02 M NaOH, using a Perkin–Elmer (Norwalk, CT) Lambda 15 UV/Vis spectrophotometer (Chen et al., 1977). The ratio of absorbances at 465 and 665 nm gave the E_4/E_6 ratio.

The Fourier transform infrared (FT IR) spectra of HAs were recorded over the range $4000\text{--}400 \text{ cm}^{-1}$ on pellets obtained by pressing under reduced pressure a mixture of 1 mg of HA and 400 mg of dried KBr, spectrometry grade. A Nicolet (Madison, WI) 5PC FT IR spectrophotometer operating with a peak resolution of 2 cm^{-1} , and Omnic 1.2 software were used to obtain the spectra.

2.3. Fluorescence titrations

A stock solution was prepared for each HA by dissolving 50 mg of freeze-dried HA in 100 mL of 1 M KOH. The mixture was stirred for 30 min under N_2 gas, and then 100 mL of 1 M HNO_3 were added. Subsequently, the solution was brought to a volume of 950 mL with deionized distilled water, the pH was adjusted to a value of 6 by addition of 0.1 M KOH, and the volume was finally brought to 1 L with deionized distilled water. Aliquots of 100 mL of the stock solution of HA were titrated in 150-mL thermostatic vessels with either 0.01 M $\text{Cu}(\text{NO}_3)_2$ or 0.01 M $\text{Zn}(\text{NO}_3)_2$ by using an automatic syringe. To maintain a constant pH, 0.1 M KOH was dispensed using another automatic syringe. Samples were maintained at a constant stirring speed, at a temperature of 298 K, and under N_2 atmosphere throughout the titrations. After each addition of titrant, the solution was circulated for 15 min via a peristaltic pump through a quartz flow-through cell for fluorescence spectral recording and back to the titration vessel.

Three-dimensional fluorescence spectra in the form of excitation–emission matrix (EEM) plots were then recorded using a Perkin–Elmer (Norwalk, CT) LS 55 luminescence spectrometer equipped with the WinLab 4.00.02 software (Perkin–Elmer, Inc., 2001, Norwalk, CT) for data processing. Emission and excitation slits were set at a 5-nm bandwidth, and a scan speed of 500 nm min^{-1} was selected for the emission monochromator. The wavelength emission range was from 350 to 550 nm, while the excitation wavelength increased sequentially by 5-nm steps from 300 to 500 nm. The EEM plots were generated from fluorescence spectral data by using the Surfer 8.01 software (Golden Software, Inc., 2002, Golden, CO).

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