Short note - Nota breve

Determination of ergosterol as indicator of fungal biomass in a polluted soil

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1. INTRODUCTION

Soil deterioration is relevant for human, animal and plant health, because mismanaged and contaminated soil can adversely affect air, groundwater and surface water consumed by humans.

The presence and the amount of microorganisms can furnish an integrated measure of soil health. Fungi, in particular, are extremely important contributors to terrestrial ecosystem function. Fungal biomass can be estimated by the quantification of specific membrane molecules such as ergosterol, that is abundant in most filamentous fungi and yeasts (Weete 1989; He et al. 2000). Aim of this work was to determinate by HPLC-UV technique the ergosterol content in a soil historically contaminated by metals. Ergosterol extraction was performed by an alkaline (AE) (Zelles et al. 1987) and a non-alkaline extraction method (NAE) (Gong et al. 2001).

2. STUDY AREA

The samples were collected from the 0–10 cm layer of a loam soil in an exarable area without vegetation and polluted by Cu, Zn and Cd in the province of Alessandria (Piedmont). Sixty samples (30 in summer and 30 in winter) were collected since July 2005 to December 2006. A comparison was also performed with a sample of forest soil collected in the province of Aosta (Aosta Valley).

3. METHODS

3.1. HPLC conditions

The chromatographic system consisted in an Intelligent Pump (D-7100, Merck-Hitachi, Tokio, Japan), a photodiode array detector (L-7450 A, Merck-Hitachi) and a UV-VIS detector (D-7400, Merck-Hitachi). The stationary phase was a RP-18 column (5 μm, 125 mm x 4 mm i.d., Merck, Darmstad, Germany) with precolumn guard RP-18 (5 μm, 15 mm x 4 mm i.d., Merck). The mobile phase was a mixture of methanol/water 95/5 (v/v). Flow rate was 2.0 mL min⁻¹ and UV detection wavelength settled at 280 nm. Injection volume was 100 μl. In these conditions, the retention time of ergosterol was 12.5±0.5 min.

3.2. Alkaline ergosterol extraction (AE): Zelles method

Fresh polluted soil, equivalent to 5.0 g of dry weight, was added with 20.0 ml of methanol, 5.0 ml of ethanol and 2.0 g of KOH. A saponification process was carried out at 70°C for 90 min in a reflux apparatus. After cooling, 5.0 ml of distilled water was added to the solution. In a separatory funnel the apolar species were extracted in two steps by adding first 30.0 ml and then 20.0 ml of petroleum ether. The petroleum ether was evaporated under N₂ flux and the residue collected in 1.0 ml of methanol, filtered (0.2 μm) and injected for the HPLC analysis in the chromatographic system.
3.3. Non-alkaline ergosterol extraction (NAE):

Gong method

Fresh polluted soil, equivalent to 4.0 g of dry weight, was added to a vial containing 4.0 g of acid-washed glass beads (2.0 g of 212-300 μm diameter and 2.0 g of 710-1180 μm diameter) and 6.0 ml of methanol. The vial was vortexed for 10 s and shaken on an orbital shaker for 1 h at 320 rpm, or centrifuged for 15 min at 2000 rpm. After shaking, the soil mixture was allowed to sediment for 15 min. An aliquot of 1.5 ml of supernatant was centrifuged for 10 min at 11000 rpm at 5°C. The supernatant (0.9 ml) was filtered and injected for the HPLC analysis. Using the same protocol, samples of 20.0 g of polluted soil and 8.0 g of forest soil were extracted. Samples of fresh polluted soil equivalent to 4.0 and 20.0 g of dry weight were also treated in three consecutive extractions.

4. RESULTS

A calibration plot was built for ergosterol concentrations ranging between 0.1 and 5.0 mg l⁻¹. A good linearity was observed in the concentration range considered R² being 0.999. The limit of quantification LOQ of the chromatographic system was 0.1 mg l⁻¹.

In the forest soil samples no detectable ergosterol content was observed when extracting the samples by AE and NAE methods. Also when increasing the amount of soil content was observed when extracting the samples by AE tographic system was 0.1 mg l⁻¹ soil (West et al. 2000). The absence of ergosterol in all the soil samples is very likely caused by the negative effects on fungal biomass due to metal contamination. Significant reductions in microbial biomass have been found in metal-contaminated soil when compared to uncontaminated one (Frostegård et al. 1993; Leifa et al. 1995; Barajas-Aceves et al. 2002; Abdel-Azeem et al. 2007). Also the absence of vegetation may have caused a decrease in the number of substrates which can be utilized by heterotrophic communities and thus a reduction in the size of the biomass.

REFERENCES


