Studi Trent. Sci. Nat., 86 (2009): 149-150 ISSN 2035-7699

Atti XVIII Convegno Gadio 2008: *Un mondo che cambia: successioni ecologiche, invasioni biologiche ed alterazioni antropiche*
Sessione 8 - Poster

Short note - Nota breve

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

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RIASSUNTO - *Determinazione dell'ergosterolo come indicatore della biomassa fungina in un suolo contaminato* - L'ergosterolo, presente nella membrana plasmatica della maggior parte dei funghi filamentosi, è un utile indicatore della biomassa fungina. Scopo del lavoro è determinare mediante tecnica HPLC –UV l'ergosterolo in un suolo contaminato da metalli e privo di copertura vegetale. L'assenza di ergosterolo, al limite di determinazione del metodo cromatografico (LOQ= 0,2 μg g^{-1} suolo secco), nei campioni analizzati può presumibilmente essere correlata all'effetto inibente dei parametri ambientali sulla biomassa fungina e al limitato apporto di sostanza organica disponibile per le comunità eterotrofe.

Key words: soil health, fungi, ergosterol, Alessandria (Italy)
Parole chiave: salute del suolo, funghi, ergosterolo, Alessandria (Italia)

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 photodidode 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 N_2 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 R^2 being 0.999. The limit of quantification LOQ of the chromatographic system was 0.1 mg l^{-1}.

In the polluted soil samples no detectable ergosterol content was observed when extracting the samples by AE and NAE methods. Also when increasing the amount of soil to 20.0 g no peak was observed. In the forest soil samples, ergosterol contents, determined by NAE, were 24.5 and 27.6 μg g^{-1} dry soil (deviation: 11.9%) with single centrifuge and Gallenkamp extraction, respectively.

5. DISCUSSION

In this study we selected an area without vegetation and historically contaminated by metals, in particular Cu, Zn and Cd. In every polluted soil sample collected since summer 2005 to winter 2006, the quantitative analysis, performed by HPLC-UV after the alkaline extraction method (AE) and non-alkaline extraction method (NAE), revealed the absence of ergosterol at least at the limit of quantification LOQ of the chromatographic system (LOQ= 0.1 mg l^{-1} which correspond to 0.2 μg of ergosterol for 1 g of dry soil). On forest soil samples, NAE method performed by single centrifuge and Gallenkamp extraction, allowed to obtain ergosterol contents corresponding to 24.5 and 27.6 μg g^{-1} dry soil, respectively. These results lie within the range of ergosterol concentrations reputed between 0.08 and 230 μg g^{-1} soil (West *et al*. 1987; Hill *et al*. 1993; Ruzicka *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; Leita *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.

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