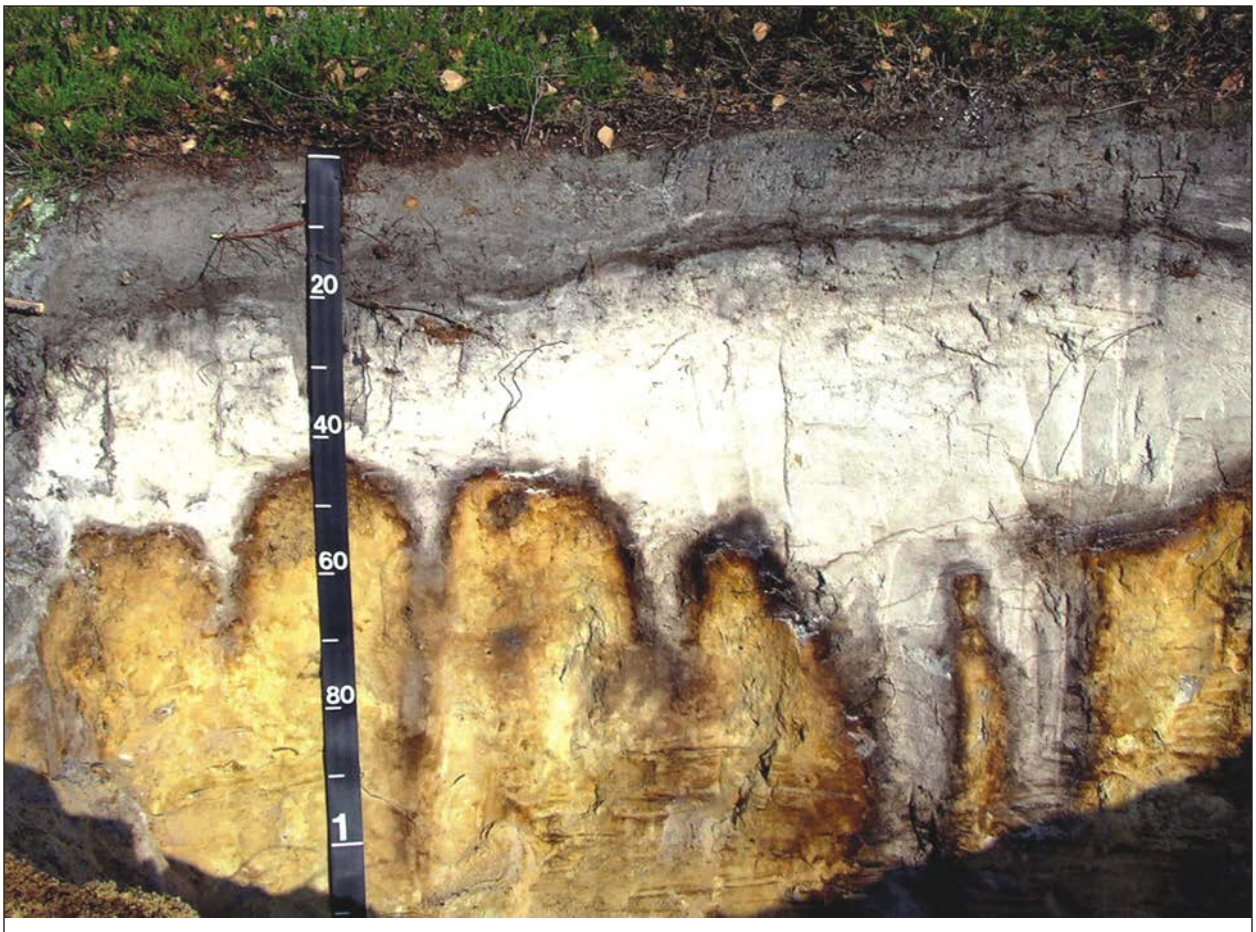


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# A Polyacrylamide Gel Electrophoretic Approach of Fingerprinting Soil Polyphenols

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**Abstract:** Present study reports a novel method to fractionate and analyse a mixture of polyphenols without prior purification. It is based on compound separation of the extracts using one dimensional polyacrylamide gel electrophoresis (1D-PAGE) and subsequent determinations of approximate molecular masses and concentrations on the gel. This is a simple and rapid technique, which has important applications in complex interactions of soil-plant systems and also in different areas of plant sciences. These initial results justify the importance of its further improvements, to be used as a cost-effective tool for polyphenol research, compared to conventional analytical methods.

**Key words:** 1D/PAGE, gel electrophoresis, polyphenols, soil

## Introduction

Polyphenols are polymeric phenolic substances that occur in various organs of higher plants as secondary metabolites, especially in dicotyledons (Kogel-Knabner, 2002). They are released to the soil during litter decomposition. The compounds of polyphenols include tannins, large molecules of quinones like hypericin, etc., which have molecular weights ranging from 500 to 3000 Da (Haslam, 1996). Phenolic constituents have a variety of roles in the life of the plant. They contribute to the plant colour, act as signal molecules in legume-*rhizobium* symbiosis and protect plant from herbivory or microbial infections. They are important economically as medicines, flavors and colours for foods, drinks etc. and as a tanning agent of leather. Purified materials of these have been analyzed using spectroscopic, chromatographic (paper-chromatography, thin-layer chromatography, column chromatography, gas chromatography, high performance liquid chromatography) and capillary zone electrophoretic methods (Cowan, 1999; Waterman and Mole, 1994). As they are variable and highly heterogeneous structures showing spatial and temporal variabilities in nature, their composition in plant tissues, soils or sediments is not amenable to direct chemical analysis without prior isolation (Martens, 2002). Present study attempts to analyse a mixture of extracted polyphenols using gel electrophoresis.

## Materials and Methods

This study was conducted in December 2003. Soils used in the study represented major land use patterns of the dry, intermediate and wet zones of Sri Lanka (Fig. 1). They had pH ($H_2O$) 5.5-7.2, 0.9-1.9% organic carbon, 0.07-0.1% total N and 0.31-0.63% total polyphenols. The soils were air dried and sieved (<2 mm). In addition, a dry zone paddy soil was incubated at 30°C for 10 days with dried

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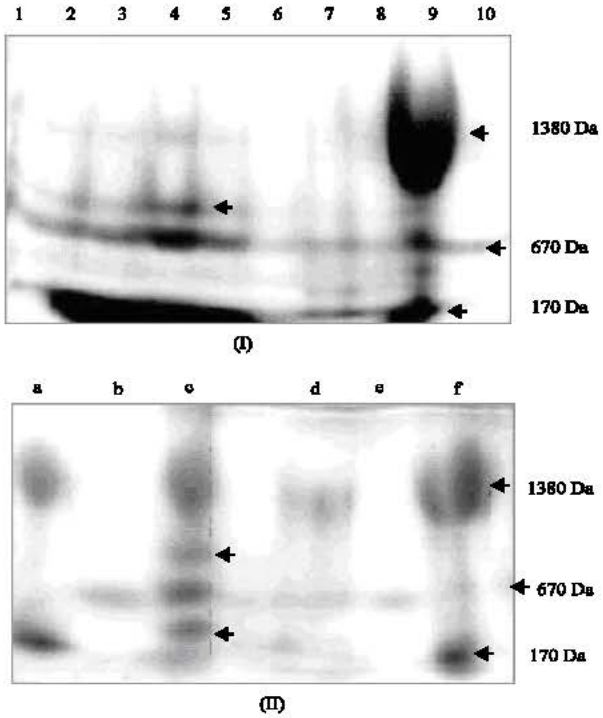


Fig. 1: Polyphenol profiles on 1D-PAGE gels of (I) soil extracts and (II) extracts of a soil incubated with plant materials. (I) lanes 1, 6, 7 and 8 are soils from a forest, shifting cultivation and two paddy fields, respectively of the dry zone; lane 2 is a soil from a vegetable cropland of the intermediate zone; lanes 3, 4 and 5 are soils from a tea estate, forest and a rubber plantation, respectively of the wet zone of Sri Lanka. Lanes 9 and 10 are of standards of commercial tannic acid + bromophenol blue and bromophenol blue alone, respectively. (II) lanes a and f are standards of commercial tannic acid and lanes b and e are of bromophenol blue. Lanes c and d are of a dry zone paddy soil incubated separately with rice (*Oryza sativa*) straw or fresh gliricidia (*Gliricidia sepium*) leaves, respectively for 10 days at 30°C. Molecular weights given with the gels are of standard compounds. The diffused bands/spots on the gel are due to low molecular weights of the compounds, because molecules with low masses show higher self-diffusion (Qiu and Bousmina, 2002)

and ground gliricidia (*Gliricidia sepium*) leaves and rice (*Oryza sativa*) straw (48 mg per 20 g of air dried soil, i.e., at a rate of 3 t $ha^{-1}$), in order to compare plant polyphenols released during the decomposition of fresh leaves and crop residues, respectively. All soils were extracted using 70% acetone (Hagerman and Klutcher, 1986).

A series of tests was conducted to select suitable gel concentrations for the stacking and separating gels and to find suitable buffer strength for obtaining maximum resolution in 1D PAGE (Hames, 1996; Acquaah, 1992). Commercial tannic acid was used as the standard. Optimal monomer concentrations were found to be 43% acrylamide and 3% bis-acrylamide. Two molar tris-borate was found to be suitable for the tank buffer. Seven molar urea was added in preparing

separation gel. Mini gel (Sigma, model E-5889) system was used and electrophoresis was carried out for 3 h at a constant voltage of 65 V for stacking gel and 85 V for separating gel. Folin-Denis reagent (Anderson and Ingram, 1993) was used to stain polyphenols in the gel, which appeared as blue colour bands. After specifying the gel conditions for the characterization, extracted polyphenols and commercial tannic acid were loaded on the gel. Bromophenol blue was used as an additional molecular weight standard. Gels were run and analysed using a computer software (Gel Compar, 1993).

## Results and Discussion

Seventy percent acetone extracts low molecular weight phenolics and polyphenols (Hagerman and Klutcher, 1986). For commercial tannic acid, two bands/diffused spots appeared on the gel (Fig. 1), because, it is hydrolysed in acidic media into a gallate group (molecular weight ca. 170 Da) and a core of galloyl glucose with a variable structure (Hagerman and Klutcher, 1986). Its molecular weight reported by the manufacturer is usually a theoretical value based on a presumed composition. This value cannot be reliably used to determine the composition of the commercial preparation. Therefore, the molecular weight of the gallate group and bromophenol blue (670 Da) and their relative front (Rf) values were used to calibrate the molecular weight of the core galloyl glucose, which was ca. 1380 Da. Molecular weights of other bands on the gels were calculated using the relationship between Rf values and molecular weights of tannic acid components and bromophenol blue. Concentrations of the two components of tannic acid were calculated using their optical densities (Jayasinghearachchi, 2004). In this calculation, a simple equation was derived to account mass fractionation of tannic acid to the two components. It was:

$$m_1 = m[od_1w_1/(od_1w_1 + od_2w_2)];$$

where, $m_1$ is the mass of component 1,

m (= $m_1 + m_2$; $m_2$ being the mass of component 2) is the mass of tannic acid initially used in the gel, $od_1$ and $od_2$ are optical densities of components 1 and 2, respectively, $w_1$ and $w_2$ are molecular weights of components 1 and 2, respectively.

With these masses and their molecular weights, concentrations of the two components of tannic acid were calculated and then the relationship between optical densities and concentrations was established. Thereby, concentrations of the other bands on the gels were calculated. The diffused bands/spots on the gel are due to low molecular weights of the compounds, because molecules with low masses show higher self-diffusion (Qiu and Bousmina, 2002).

Three major compounds of polyphenols with molecular weights of ca. 1380, 850 (shown with an arrow on the lane) and 670 Da appeared in the gel lane of the wet zone forest soil (Fig. 1). Their concentrations ranged from 0.7-2 mM. The two compounds with 850 and 670 Da were also common in soils of the vegetable cropland in the intermediate zone and the tea estate and rubber plantation in the wet zone. One compound with a molecular weight of ca. 610 Da and at a concentration of ca. 1 mM was predominantly present in soils of the forest, shifting cultivation and paddy fields of the dry zone. Of all soil samples, monomer forms of phenolic compounds retained as a diffused band at the bottom of the gel, with a molecular weight of ca. 110 Da (possibly catechol) and concentrations from ca. 1-2 mM. Both gliricidia and rice straw treated soils had two compounds in common, which corresponded to the two components of the commercial tannic acid. This indicates that tannic acid could be used as a reference compound for plant polyphenol studies on the gel. In addition, two other

compounds with molecular weights of ca. 365 Da and ca. 910 Da were detected for rice-straw treated soil (shown with arrows on the lane). Another compound with a molecular weight of ca. 670 Da was observed in glyricidia and rice straw. Gliricidia had lower concentrations of the compounds (i.e. ≤ 0.1 mM), compared to rice straw that contained those with concentrations ranging from 0.6 to 3 mM. This indicates that crop residues release polyphenols to the soil faster than fresh leaf materials.

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenolics or their oxygen substituted derivatives (Cowan, 1999). Different plants produce different sets of phenolics. Soil conditions (i.e., stress etc.) also affect the plants to determine, which compounds are to be produced. These compounds are released to the soil during stem flow, litter decomposition etc. Hence these compounds reflect soil-plant interactions of the habitat and their patterns on the gels can be used as fingerprints of the interactions in terrestrial ecosystems.

In conclusion, present polyphenol fingerprinting facilitates for a simultaneous comparison of variable and complex interactions of soil-plant systems of different habitats and is a rapid and convenient method for such evaluations. Small quantities of polyphenols can be assayed from this technique at relatively little outlay and analytical cost. Compounds separated on the gel can be eluted and subject to structural elucidation. This fingerprinting also has important implications and applications in chemotaxonomy and natural product chemistry. Different solvents (e.g., 70% methanol, 1 M NaOH etc.) extract different compounds of polyphenols (Cowan, 1999). Therefore, further studies should be conducted to expand and improve and also to investigate other potentials of this technique.

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