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Quantitative determination of sterols and other alcohols in overland flow from grazing land and possible source materials

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Abstract

Organic marker compounds (biomarkers) can be used to identify the sources of waterborne pollutants. This paper examines sterols and other alcohols in overland flow from pasture-based grazing systems, possible agricultural source materials and water extracts of these source materials as a preliminary step to developing chemical profiles that can be used for tracing pollutants. The biomarkers were quantified using gas chromatography and gas chromatography-mass spectrometry techniques.

Analyses of plant material show that some pasture species contain unique compounds, enabling their identification. For example, *Arctotheca calendula* (capeweed) contains an as yet unidentified compound (*Arctotheca m/z* 163). Other pasture species that do not contain unique compounds do contain unique ratios of phytol, hexacosanol, octacosanol and 24-ethylcholesterol, enabling their identification.

Analyses of faecal samples show that the ratios of sterols to stanols enable faeces to be distinguished from the pasture species, e.g. the ratio of 24-ethylcholesterol to 24-ethylcoprostanol was <1, generally <0.25 for faeces, while for most pasture species this ratio was >4. Using this ratio, qualitative apportioning of the sources of pollutants in overland flow to vegetation or faeces could be performed, but only in extreme cases (i.e. when the ratio <1 or >4). Decaying organic matter and surface soil appear to contain a composite of plant and faecal sterols.

Sterols, being sparingly soluble in water and surface active, were not expected to be present in overland flow samples. Surprisingly, cholesterol and 24-ethylcoprostanol were found in both the particulate and filtrate fractions of most overland flow and water extracts of most source materials. Using the ratios of sterols to stanols, particulate organic material in water could be traced back to its broader source, i.e. vegetation or faeces.

Keywords: Biomarkers; Sterols; Lipids; Overland flow; Runoff

Abbreviations: GC, gas chromatography; GC-FID, gas chromatography-flame ionisation detector; GC-MS, gas chromatography-mass spectrometry; DAP, diammonium phosphate; SSP, single-superphosphate; MRF, Macalister Research Farm; ND, not detected; SD, standard deviation

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1. Introduction

Water contaminants, such as excessive nutrients, sediments and organic matter in streams and their receiving waters, are a worldwide problem (Cooke et al., 1993; European Environment Agency, 1998; USEPA, 1996). Increased nutrients and organic matter (eutrophication) can lead to algal blooms (CSIRO Australia, 1996) that reduce the amenity of water. For these pollutants to be a problem, there needs to be (1) a source of the pollutant, (2) mobilisation of the pollutant into water, (3) transportation of the pollutant to a location and (4) adverse effects expressed, often through a vector such as a blue-green algae.

Agricultural sources of water pollutants include plants, animal wastes, soils and fertilisers and have been discussed in detail elsewhere (Nash and Halliwell, 2000). Pollutants from these sources can be mobilised by either erosion or dissolution, and while erosion is a physical process resulting in transport of material $>0.45\,\mu m$, dissolution results in materials being either truly dissolved or associated with colloids $<0.45\,\mu m$. The initial transportation of pollutants at the farm scale can often occur in overland flow. Identifying the source of pollutants in the overland flow, where sources are limited to the farm environment, is an important step in the development of effective remedial action. Organic marker compounds (biomarkers), such as lipids, have useful attributes for tracing water pollutants to their source in these environments (Nash and Halliwell, 2000).

Lipids are a heterogeneous class of organic compounds that includes fats and oils. The predominance of carbon-to-carbon and carbon-to-hydrogen bonds in lipids results in an essentially non-polar structure with low solubility in water but high solubility in non-polar organic solvents, such as *n*-hexane. Lipids contain variable length, branched, hydrocarbon chains, including cyclic hydrocarbons, and a range of functional groups, and as a result are often species specific (Jones et al., 1994).

The species specificity of lipids, especially the neutral lipids from the sterol and, to a lesser extent, hopanol groups, has facilitated their use in tracing studies (Nash and Halliwell, 2000). Sterols are formed by the cyclisation of the C_{30} isoprenoid hydrocarbon squalene in both plants and animals and have important biological functions, e.g. in cell membranes (O'Leary et al., 1994). The feature regarded as distinguishing plant-derived sterols from those of animal origin is an additional methyl or ethyl unit in the C_{24} position of the side chain (Goad, 1977). Hopanoids are characterised by an interlocking pentacyclic (five-ring) structure consisting of one cyclopentane (five carbon) and four cyclohexane (six carbon) rings and are found in all bacteria and higher plants but are absent in animals (Jones, 1993). Hydroxyl and amino group substitution in the side chain of hopanoids distinguishes chemical species within the groups.

Coprostanol is an example of a sterol used to study faecal pollution (Leeming and Nichols, 1996; Nichols and Leeming, 1991; Nichols et al., 1996). It is formed by the hydrogenation of the double bond between C_5 and C_6 in the second hexane ring of cholesterol. While only trace amounts of cholesterol are found in plant tissues, it is an important membrane component of animal cells (Christie, 1989). Consequently, cholesterol consumed by meat-eating animals is converted to coprostanol on passage through the gut and is found in the faeces (Martin et al., 1973; Rosenfeld and Gallagher, 1964, 1971; Rosenfeld et al., 1967). Similarly, 24-ethylcoprostanol and 24-ethylepicoprostanol can be used to distinguish between faeces from herbivores and carnivores (Leeming et al., 1994). In both cases, the sterol profiles of the faeces reflect the diet of the source animal and conversions in the digestive tract (Leeming et al., 1995).

This study investigates the concentrations of neutral lipids of the sterol and other alcohol groups in overland flow exported from pasture-based grazing systems and source materials. Lipids were extracted directly from source materials, the particulate ($>0.45\,\mu m$) and filtrate fractions ($<0.45\,\mu m$) of the overland flow, and from the particulate and filtrate fractions of water extracts of source materials. These experiments are part of an initial investigation that aims to use biomarkers to trace the sources of pollutants in river systems (Nash and Halliwell, 2000).

2. Materials and methods

2.1. Sample collection and storage

Details of the source materials and overland flow used in these studies are presented in Table 1. 'Replicates' in Table 1 refer to physically separate samples of the same source type. Source materials were collected in November 2000 from dairy farms at Ellinbank (38°16′S, 145°55′E) and Darnum (38°10′S, 146°3′E) in the Gippsland Region of southern Australia. Additional samples were collected during January 2001 from the Macalister Research Farm (MRF) at Maffra in the Macalister Irrigation District (38°00′S, 146°54′E).

All equipment (i.e. spatulas, scissors, soil corer, glassware, bottles, sieves) used in the study were washed thoroughly with a dichloromethane/methanol (both Mallinkrodt Laboratory Chemicals, USA; nanograde) mixture prior to use and between samples to minimise contamination. Aluminium foil and glass fibre (GF/F) filters (Schleicher and Schuell, Germany; Ref. No. 10370008) were muffle furnaced (450 °C for 24 h) to