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Isolation and seasonal effects on characteristics of fulvic acid isolated from an Australian floodplain river and billabong

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Abstract

Fulvic acids from an Australian floodplain river and billabong were isolated using DEAE and DAX-8 resins, and characterised with the use of size exclusion chromatography and solid-state CP-MAS ^{13}C NMR spectroscopy. Differences between the two resin isolates were evident. Fulvic acids isolated using DEAE-cellulose had higher apparent M_n and M_w values, while the DAX-8 resin showed a slight preference for aliphatic components. Fulvic acids from the river and billabong had the same functional groups present, however, the river fulvic acids had higher apparent M_n (number average molecular weight) and M_w values (weight average molecular weight), and were more polydisperse than the billabong fulvic acid. There were no significant changes in the characteristics of the fulvic acid isolated from the river at four sampling times: summer, autumn, winter and spring. In contrast, fulvic acids isolated from a billabong displayed seasonal variation in molecular weights. This work emphasizes the importance in ecological studies of isolation procedure for the operationally defined fulvic acids.

Keywords: Fulvic acid; Resin; Sample preparation; River; Billabong

1. Introduction

Dissolved organic matter (DOM) is the major form of organic carbon in almost all aquatic ecosystems [1,2]. DOM in aquatic environments represents one of the largest active organic carbon reservoirs in the biosphere [3] with the amount of DOM in the oceans alone being comparable to the amount of CO_2 carbon in the atmosphere [4]. It is composed of a complex mixture of substances, including non-humic solutes such as amino acids, hydrocarbons, carbohydrates, fats, waxes, low molecular weight acids and resins and humic substances (HS). The latter are the main constituents of DOM [5], typically comprising 40–70% [6,7], and as high as 90% [8]. HS can be classified as either fulvic acids, humic acids or humin based on solubility characteristics and it is the fulvic acids that predominate in aquatic ecosystems [9].

HS have been the subject of intense scientific interest [6] due to their importance in the global ecosystem [10–13]. However, HS are defined operationally according to their isolation procedure [14] with fulvic acids representing the fraction of HS that is soluble at all pH values. An understanding of the impact of sample isolation on their chemical makeup is therefore critical [15–17]. Despite this importance few papers have examined the impact of isolation procedure on structural characteristics.

Traditionally, the isolation procedure for aquatic HS has been based on the use of the non-ionic macroreticular resins (typically the XAD-8 resin) that adsorb and release ionic species through hydrophobic and polar interactions. This resin, however, is no longer manufactured, and the substitute resin Supelite DAX-8 has been developed. The question arises as to whether or not the HS isolated using different resins, are in fact the same. Studies by Peuravuori et al. [14] found that slight variations between the two resins occurred. While the recoveries of the bulk humic material were similar, the DAX-8 resin exhibited hydrophobic–hydrophilic sorption-desorption properties that isolated slightly different humic solutes. However, characterization was restricted to pyrolysis–gas chromatography–mass spectrometry, with offline tetramethylammonium hydroxide (TMAH) derivatisation of different aquatic samples [14].

An alternative isolation procedure exploits the weakly acidic character of humic substances by employing anion exchange materials such as diethylaminoethyl cellulose (DEAE cellu-

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lose) for their isolation and concentration from water [18,19]. An advantage of using this resin is that the natural complex organic acids can be obtained at neutral pH values and with high recoveries, whereas the XAD technique does not concentrate the colourless low molecular weight organic acids in water [18]. In general, the main XAD fractions and DEAE isolates have been thought to consist of almost identical organic compounds [20] as analysed by various techniques [8,21–23], with the structural composition of the humic solutes obtained by DEAE being a combination of the hydrophobic and hydrophilic acidic solutes obtained by the XAD technique [21].

There are no additional studies of this critical topic and further, there have been few investigations that characterise dissolved organic matter in Australian aquatic systems [24], let alone the humic fraction. The main structural characteristics of HS stem from their different organic matter origins [5]. Furthermore, the terrestrial environment influences the dynamics of organic matter inputs into streams [25]. Australian forests have significantly different vegetation to northern hemisphere rivers [24] being uniquely dominated by Eucalyptus species [26]. An examination of the characteristics of HS in an Australian aquatic system is therefore warranted.

Finally, it is not known if the fractionated components of DOC, including HS alter at different times of the year, particularly in lotic systems [24]. It was therefore the aim of this study to investigate the characteristics of fulvic acids isolated from an Australian floodplain river and adjacent billabong[1] using DAX-8 and DEAE resin procedures. Seasonal comparisons between the isolates were also examined. Solid-state CP-MAS ^{13}C NMR spectroscopy, percent carbon, and number and weight-averaged molecular weights (M_n and M_w, respectively) were used to characterize the isolated fulvic acids, and Suwannee River fulvic acid as a commercial standard. These characteristics were selected as being important in terms of the bioreactivity of HS. The accepted model of bioavailability is that the degree of recalcitrance to bacterial breakdown is positively correlated with the size of the organic moiety. Although this concept is still widely accepted, recent studies indicate that some high molecular weight compounds are rapidly utilized by bacteria [3]. This suggests that molecular size may not be the sole factor controlling bioavailability and Sun et al. [27] found evidence of an association between elemental ratios and bioavailability of DOM to bacteria. Interestingly, they emphasized that bioavailability was positively correlated with the percentage of aliphatic carbon in a sample.

2. Experimental

2.1. Study sites

The test site was within the Murray–Darling Basin which covers an area of 1,061,469 km^2 over south-eastern Australia and is the largest river system on the Australian continent. The floodplains of this system are distinguished by low gradients, meandering rivers, and a characteristic profusion of cut-off meanders (ox-bows or billabongs), abandoned channels and swales and ephemerally filled depressions. The lateral exchange of particulate and dissolved organic matter is an important component for the physical and biological components of large river systems [28], linking the floodplain and rivers into integrated ecosystems [29] during high flow events via surface flow and groundwater pathways.

The Murrumbidgee River is approximately 1600 km in length and drains an area of approximately 82,000 km^2. It begins in the Snowy Mountains, and flows west across the semi-arid Riverine Plain before joining the Murray River near Balranald.

Berry Jerry Lagoon (billabong) is a 6.6 ha (117 ML) meander cut-off lagoon located approximately 30 km west of Wagga Wagga, Australia (Fig. 1). It is connected to the Murrumbidgee River by overbank flows, and when the channel flow reaches 16,700 ML day^{-1} (as recorded at the Wagga Wagga gauge). This lagoon was chosen as a study site due to it being one of the lower lying lagoons on the floodplain that is regularly connected to the river during summer irrigation flows, however, due to drought conditions, there was no connectivity during this study.

2.2. Isolation of fulvic acids

2.2.1. Preparation of water samples

Water samples were collected in 10 L and 15 L carboys. Prior to sampling, each carboy was acid washed and rinsed several times with Milli-Q water. They were then filled with Milli-Q water and allowed to stand overnight to ensure the adequate removal of contaminants. The water was emptied, and the carboys rinsed twice with sample water before filling. During the course of this study, 344 L of Berry Jerry Lagoon water and 1068 L of Murrumbidgee River water were collected.

The filtration of water samples was conducted via a two-step process. Water was pre-filtered using a Buchner filtration apparatus fitted with a 90 mm GF/C filter. The water was then filtered using a SolvCycle Nalgene® filtration apparatus fitted with Advantec MFS 0.45 μm mixed cellulose ester or cellulose acetate membrane filter to obtain dissolved organic carbon. Fulvic acids were isolated from the dissolved organic carbon.

2.2.2. Isolation of fulvic acids using DEAE cellulose

2.2.2.1. Pre-treatment and regeneration of DEAE cellulose. HCl (0.5 M, 1.0 L) was added to DEAE cellulose (microgranular, 50 g) in a 5.0 L glass beaker and stirred for 1 h. The cellulose was then rinsed with Milli-Q water in a Buchner filtration apparatus fitted with a 90 mm Whatman GF/C filter until the pH was neutral. DEAE cellulose was re-suspended in NaOH (0.5 M, 1.0 L), stirred for 1 h, and again filtered and rinsed with Milli-Q water until the pH was neutral.

Fine particles were removed by re-suspending the cellulose in Milli-Q water, allowing it to settle for 1 h and decanting the supernatant. Pre-treated cellulose was stored in a glass Schott bottle in the dark at 4 °C.

[1] A billabong is a shallow lentic water-body that is formed on the middle and lower reaches of floodplain rivers when the main channel of a river changes its course.