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Differentiation of some Venezuelan blackwater rivers based upon physico-chemical properties of their humic substances

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Abstract. The dissolved humic substances of seven Venezuelan blackwater rivers, belonging to different tropical biogeographical regions, were analyzed. Fractionation using Sephadex gel together with an analysis of their fluorescence, absorbance and infrared spectral properties, showed differences between their humic substances. These differences were related to their biological sources and humification environment. Statistical analysis of the properties of these humic substances allowed us to separate the rivers into three groups. These groups were directly related to the geological features and vegetation of the terrestrial ecosystems into which they drain:

- The Autana, Atabapo, Chola, Negro which belong to the Amazon basin on the Guayana Shield, each with typical blackwater riverine forest.
- The Caroni, belonging to the Orinoco basin on the Guayana Shield and with tropical humid forest and rainforest.
- The Morichal Largo which draines the Mesa Formation from the eastern Llanos and has gallery forest of *Mauritia flexuosa*.

Introduction

The study of organic matter has increased in importance because of recent interest in elemental behavior in the biogeochemical cycles of the biosphere. It is directly linked to the carbon cycle (Meybeck 1982) and is known to interact with divalent and trivalent metals (Stevenson 1982; Jackson et al. 1978), having a strong influence on their cycles. Aquatic humic substances, the object of our study, make up 40 to 60 percent of dissolved organic carbon. They represent the largest fraction of natural organic matter in water (Thurman 1985), just as humus in soils appears to represent the world's greatest carbon reservoir (Degens 1982). Ertel et al. (1986) found that aquatic humic and fulvic acid represented 60 percent of riverine dissolved organic carbon in the Amazon river system. Precisely how much humus is generated, degraded and oxidized annually, as well as leached into water-bodies, is not fully known. The role of the river in this context is that of transporting organic matter from the different terrestrial ecosystems to the oceans (Meybeck 1982). Tropical rivers in particular seem to drain terrestrial ecosystems where the rate of accumulation of organic matter exceeds that of decomposition, a favorable situation for humic substance formations (Ziechmann 1976; Leenheer 1980). Whilst aquatic humus occurs in dissolved and colloidal forms and in organic detritus, an important portion remains in soluble from and is responsible for the characteristic brown color of water (Leenheer 1980).

Most information already available about blackwater rivers relates to the Amazon river basin, where they are considered as a discrete type of water in Sioli's typology (Sioli 1984). Therefore, the presence of blackwater streams in several Venezuelan regions, which are ecologically and biogeographically different from the Amazon region, lead us to consider how possible it was to generalize in the light of our current knowledge concerning these water types.

Vegas-Vilarrúbia et al. (in press) investigated the physico-chemical characteristics of 18 tropical blackwater rivers to determine if, despite their biogeographical differences, they showed similarities that could be related to their common dark color. One of the main conclusions reached was that, although all were considered as blackwater rivers in Sioli's sense (Sioli 1984), they differed notably in their inorganic water chemistry. This fact probably reflecting the particularities of the different terrestrial environments which they drained.

These results lead us to the hypothesis that their common element, the dissolved humic substances, could also differ in their physico-chemical properties according to the vegetation sources and the environmental conditions associated with each particular river (Tables 1 and 2).

If our supposition was correct, then we should be able to find some physical and/or chemical differences amongst their humic materials. Furthermore, we wondered whether these differences might be used to distinguish natural groups of blackwater rivers.

For this paper we separated the humic substances from 7 rivers draining different biogeographical areas of Venezuela. This separation was carried out by using gel chromatography on Sephadex and the collected fractions were analyzed by spectroscopic techniques.

Determination of the true molecular weight of humic substances was outside the scope of these tests since our aim was basically to separate homogeneous fractions. For this purpose Sephadex filtration is a relatively simple and useful technique, although fractionation of humic substances by this technique has many methodological problems that have not yet been

Table 1. Brief description of rivers and their environmental features. To facilite graphical presentation in many following figures rivers are identified by the assigned numbers in brackets.

Rivers		Geology	Soils	Vegetation
Morichal Largo	(7)	Quaternary (recent and Pleistocene)	Oxisols Entisols Inceptisols	Gallery Forest of *Mauritia Flexuosa* (Morichales)
Caroní	(8)	Guayana Shield Precambrian	Oxisols Entisols Inceptisols	Tropical humid forest, tropical very humid and tropical rainforest
Sipapo	(12)	Guayana Shield Precambrian (Arqueoproterozoic)	Entisols Inceptisols Oxisols	Tropical humid forest, tropical very humid forest, tropical rainforest
Autana	(11)	Guayana Shield Precambrian (Proterozoic)	Entisols Inceptisols Oxisols	Typical blackwater riverine forest
Atabapo	(13)	Guayana Shield Precambrian (Arqueoproterozoic)	Entisols Inceptisols Oxisols	Typical blackwater riverine forest
Chola	(15)	Guayana Shield Precambrian (Arqueoproterozoic)	Spodosols Inceptisols Entisols Oxisols	Typical blackwater riverine forest
Negro	(19)	Guayana Shield Precambrian (Arqueoproterozoic)	Oxisols Spodosols	Typical blackwater riverine forest

Sources: González de Juana et al. 1980; Huber 1982; MARN, 1983.

Table 2. Chemical composition of the blackwater river studied.

River		pH	Cond (μS/cm)	TZ+ meq/l	Color degrees Hazen
Morichal Largo	(7)	5.7	52	2.88	61
Caroní	(8)	5.1	7.5	1.10	67
Autana	(11)	4.5	0.3	0.10	89
Sipapo	(12)	4.0	0.3	0.10	89
Atabapo	(13)	4.0	17.5	0.05	142
Chola	(15)	3.8	17.5	0.28	136
Negro	(19)	3.8	14.5	0.34	150

TZ+ = Ca + Mg + Na + K + Fe (meq/l).

solved. Several factors such as column dimensions, size, pH, concentration and composition of the samples, eluant type and concentration, influence the chromatograms. There is also a general lack of agreement between investigators on the optimal conditions for gel permeation (Posner 1963; Lindquist 1967; Ghassemi & Christman 1968; Gjessing 1971, 1973; Swift & Posner 1971; De Haan 1972a, b; Urano 1980; Sho & Letho 1984; Thurman 1985). Hine & Bursill (1984) have even questioned the usefulness of this method in the analysis of naturally occurring organic materials in raw and treated water. These facts point to the serious analytical problems posed by the complexity of humic substances. Despite these problems, gel filtration has been used to compare humic substances from different sources by several authors (Gjessing & Lee 1967; Ghassemi & Christman 1968; De Haan 1972b; Alderdice et al. 1978; Stabel 1980). Whilst distilled water has been commonly used as eluant, it has been criticised by several investigators for allowing absorption of the solute into the column to take place (Gjessing & Lee 1967; Rashid & King 1971; Swift & Posner 1971; Wershaw & Pinckney 1973; Kemp & Wong 1974; Larson 1978; Stabel 1980). The effects of altering sample or eluant composition can, however, produce changes on chromatograms which are open to ambiguous interpretation (Hine & Bursill 1984) and since we wanted to compare humic substances belonging to different rivers, we decided to maintain their original molecular structure as much as we possibly could. For our purposes it was necessary to ensure that the particular set of conditions chosen would provide, at least, a meaningful fractionation. From the review of available literature it is clear that the gel Sephadex filtration separates humic substances not only according to their molecular weight, but that other physico-chemical properties could also modify elution diagrams. Keeping these facts in mind we have, nevertheless, retained HMW and LMW terminology in this paper because of their widespread use in the literature we reviewed.

The ratio between optical densities at 465 and 665 nm (E_4:E_6) gave some indirect information about free-radical concentration, COOH, O and C contents as well as the total acidity of humic substances (Chen et al. 1977). The fluorescent nature of material per unit absorbance, called f:a ratio, is greater for compounds of lower molecular weight (Stewart & Wetzel 1982). Indeed, all these parameters give us information related to molecular size. The latter is related to the interaction of humic substances with aquatic organisms (Prakash & Rashid 1973; Stewart & Wetzel 1982), and to their mobility and complexing ability (Rashid 1971). Thus its ecological importance may be great and the aim of this project is to provide evidence that our hypothesis has a real base.

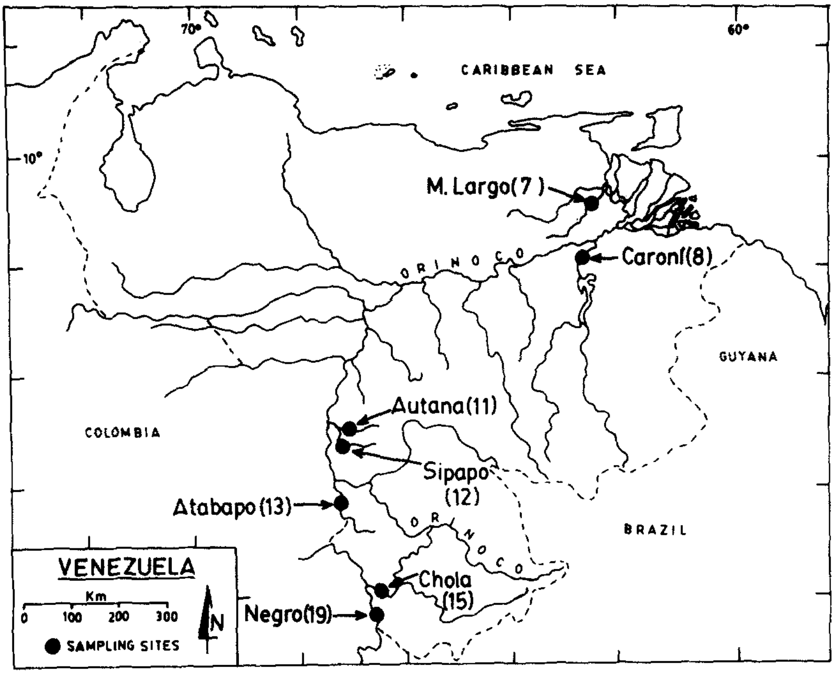


Fig. 1. Location of sampling sites.

Materials and methods

For this analysis, seven rivers were chosen, each representing a different biogeographical region of Venezuela (Fig. 1, Table 1): Morichal Largo, Caroní, Sipapo, Autana, Atabapo, Negro and Chola. Sampling was carried out during the rainy season (July–September 1983). Fifteen to twenty liters of surface water were taken and stored in opaque, plastic containers without preservatives (Gjessing 1976).

Dissolved organic matter was progressively concentrated (Halde 1980) until the total volume of each sample was reduced to 1 to 1.5 liters for each river, after which the samples were freeze-dried. The humic substances were fractioned by chromatography on gel Sephadex G-50 (medium).

Of freeze-dried material, 50 mg was dissolved in 10 m eluant (H_2O) and placed on the top of the column. Fractions of 7.7 ml each were collected in a fraction collector. This was a Fractometer Searle (Buchler Instruments) with a flow rate of 92.4 ml/h (Soechtig 1966; Determann 1967; De Haan a, b, 1972; Fischer 1974; Cameron et al. 1977). The void volume of the column (V_0) was determined using blue dextran 2000 and the total column volume of the gel bed (V_t) was calculated by water calibration of the column. Optical densities on each collected fraction were measured at 360, 425, 400 y 600 nm in a double light beam spectrophotometer (Varian, model DMS 90). Fluorescence spectra of the fractions obtained were determined on a spectrofluorometer (Varian, model SF-330) and the infrared spectra were recorded with a IR spectrophotometer (Shimadzu, model IR-420). KBr was employed to prepare the pellets.

The statistical methods applied to this work were designed to obtain information which would help to differentiate between rivers on the basis of their humic substances. Some methods of bivariate statistics were chosen to allow the grouping of samples based on "n" variables and, as applicable , give hierarchic classification.· Such methods are commonly employed in numerical taxonomy, biometry, morphometry, psychology and other disciplines (Cuadras 1981).

Results and discussion

Sephadex elution curves

Elution diagrams and Kav values for each sample indicated that no adsorption occurred (Fig. 2). Three types of bimodal curves were found with notable differences among their forms. No retention was observed and K_{av} values were always below 1. We agreed with Urano et al. (1980) that the elution volume is strongly affected by the chemical structure of organic compounds, because their different affinities for Sephadex gel and water depend on that property. Thus, if the fractionation is based on characteristics related to the chemical structure of compounds, the elution diagrams obtained indicate inherent chemical differences in humic substances from different provenances with the areas corresponding to LMW generally being larger. In all cases the Negro, the Atabapo, the Autana and the Chola rivers had a large portion of HMW included in the fraction. The Sipapo and the Caroni rivers had a comparatively small HMW fraction, being mainly LMW. However, the Morichal Largo was the only one whose HMW fraction was larger than the LMW fraction. In all cases, at least two distinct fractions were found, coinciding with the data reviewed by Christman and Minear (1971), where significantly different molecular size distribution of humic substances from many surface waters was reported. These results indicated that in the rivers studied with the exception of the Morichal Largo, the fulvic acid fraction prevailed (Prakash & Rashid 1969), indicating its likely responsibility for the dark color of water sampled. LMW fraction also has implications for the ecological role of these aquatic bodies. Some evidence exists to show that smaller size humic substances have a stronger stimulating effect on a number of the physiological processes of aquatic organisms than do the larger fractions (Prakash & Rashid 1968, 1969, 1974; Stewart & Wetzel 1982). They also have a greater complexing capacity for

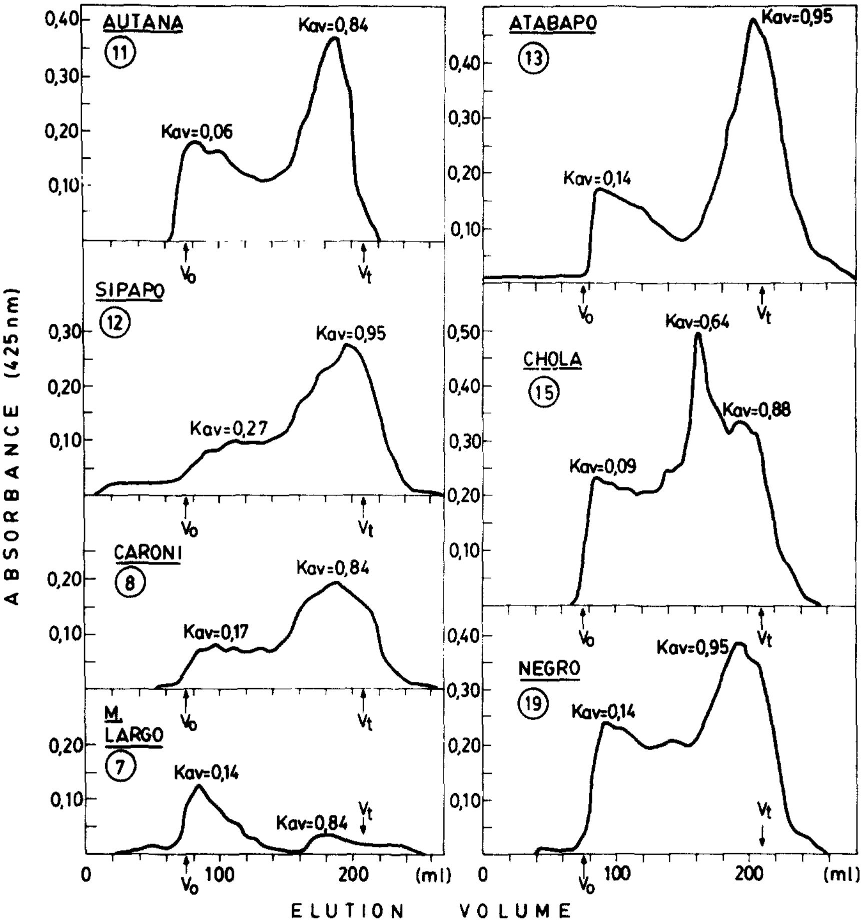
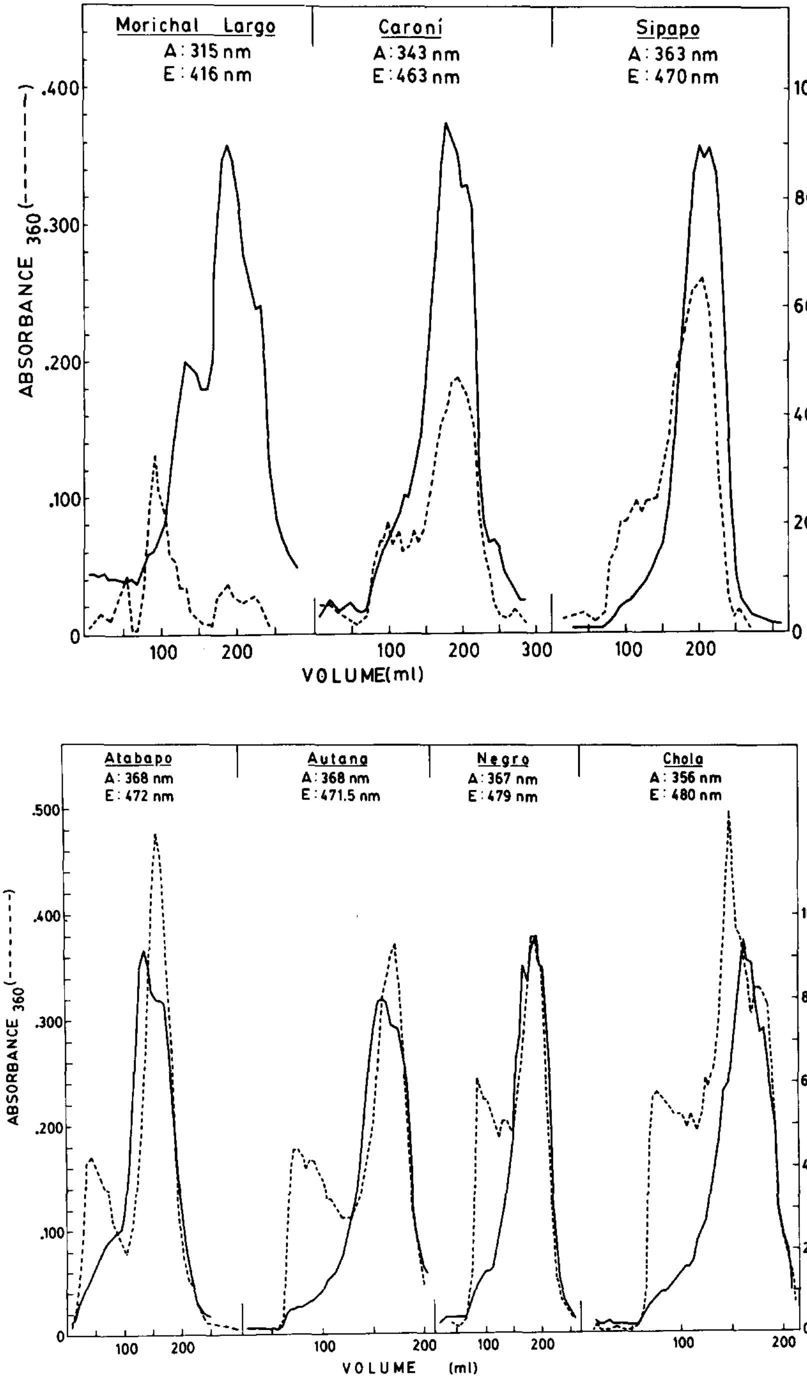


Fig. 2. Elution curves on Sephadex G-50. Pointed lines indicate the separation volume between HMW and LMW fractions. Ve = partial elution volume.

cations (Rashid 1971; Stevenson 1982). However, we do not know if the cations found in low concentrations in Venezuelan blackwater rivers (Vegas-Vilarrúbia et al. in press), are present as free aqueous ions or complexed by this fraction. This might also be of ecological interest, because Ca, Fe, Mg and K are indispensable for aquatic organisms. Whether or not metal-humic complexes in such waters have a positive or a negative effect on aquatic biota still remains unknown.

Absorbance and fluorescence analysis

Figures 3 and 4 represent the elution diagrams obtained through measurement of the optical density and fluorescence at the wavelengths indicated. Most HMW fractions showed no fluorescence, except in the case of the Morichal Largo, so that the fluorescence peaks belonged to the smaller



Figs. 3, 4. Elution curves by measurement of the absorbance at 360 nm and the fluorescence at the indicated wavelength. A: excitation wavelength, E: emission wavelength.

fractions and tended to coincide with the absorbance peaks or slightly higher volumes. This weak fluorescence of the HWM fractions may be due to the reduction of fluorescence (quenching) as result of binding of metal ions (Lévêsque 1972; Underdown et al. 1981; Ryan & Weber 1982) or to a decrease in the carboxylic hydroxyl content (Hall & Lee 1974).

Table 3 represents the maximum excitation and emission value for the LMW fractions. These appeared to be very similar for the Sipapo, Autana. Atabapo, Negro and Chola rivers, whilst the Caroni and the Morichal Largo showed lower values. Such similarities may be related to their biological sources. Table 1 and 2 show a considerable variety of riverine vegetation, edaphic, geological and physico-chemical conditions, doubtless implying a variety of sources and environmental situations. Since fluorescence is linked to the molecular structure of humic substances (Gjessing 1976; Lévêsque 1972; Underdown et al. 1981) such differences could be evidence to support the theory that their origin and humification conditions have an effect on their structure. Similarly Larson & Rockwell (1980) demonstrated significant differences between the fluorescence properties of humic substances of diverse origins and suceeded in relating them to known compounds of their humic structure. Brooks et al. (1976, 1977) also showed that certain simple organic products, derived from organisms living in aquatic environments, were the source for the neosynthesis of more complicated humic substances.

Infrared analysis

Infrared spectra provided some information on general similarities and differences among riverine humic compounds. Considerable overlapping of the absorption bands hindered our interpretation regarding the presence and distribution of functional groups. It also made it difficult to obtain information about differences in the nature and chemical structure of carbon

Table 3. Maximum excitation and maximum emission wavelengths for LMW fractions.

No	River	Maximum excitation wavelength	Maximum emission wavelength
7	Morichal Largo	315	416
8	Caroni	343	463
11	Autana	368	471.5
12	Sipapo	363	470
13	Atabapo	368	472
15	Chola	386	480
19	Negro	367	474

skeletons. All infrared spectra show strong absorption bands for hydroxyl, carbonyl, free carboxyl and carboxylate functional groups. We were thus able to verify that the acid character of humic materials coincides in general outlines with infrared spectra found by Stevenson & Goh (1971) for soil humic substances and by Leenheer (1980) and Brinkman (1981) for some Amazonian blackwaters.

Infrared spectra (Fig. 5) of both fractions looked very similar, although some differences, due to the presence or absence of certain bands, were observed. In all cases, infrared spectra indicate strong absorption towards 3400 cm^{-1} (stretch of hydrogen-bonded OH), 1600 cm^{-1} (stretch of aromatic

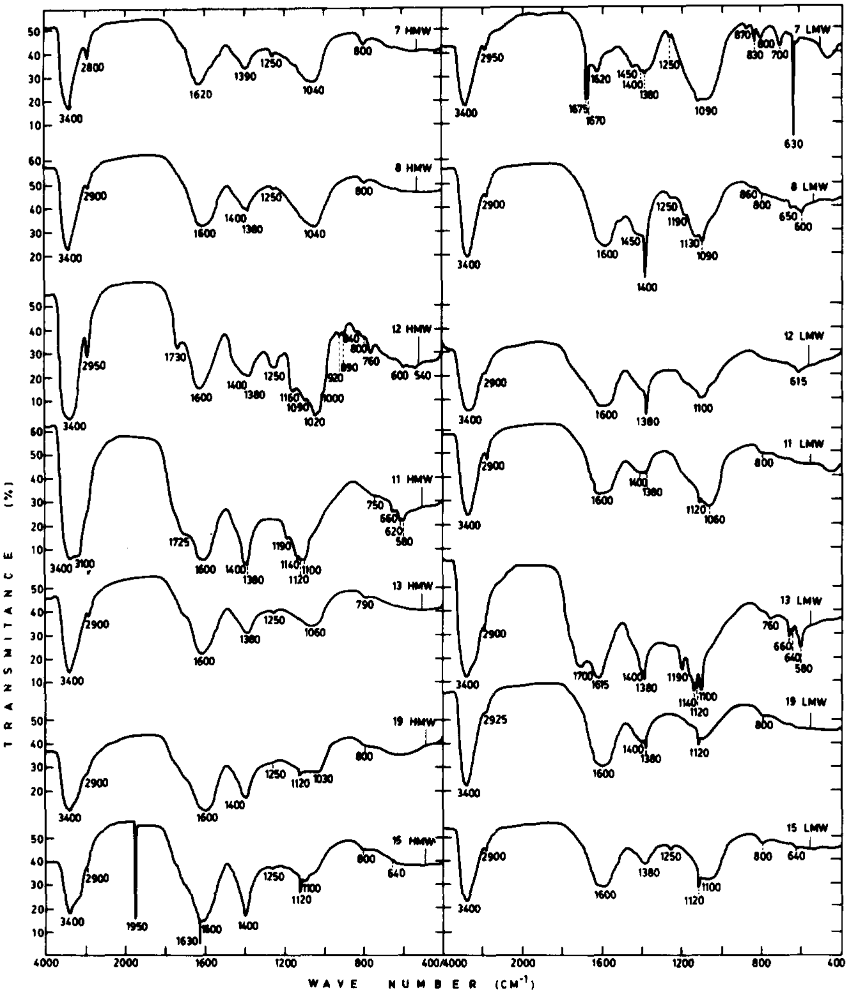


Fig. 5. Infrared spectra of HMW fraction (left) and LMW fraction (right).

C=C conjugated with other double bonds, stretch of C=O in carbonyl or quinone, stretch of C=O in carboxylate, and stretch of C=O in diketones) and 1400 cm^{-1} (stretch of C=O carboxylate and bend of O—H in alcohols and phenols). According to Jones (1959) absorption between 1000 cm^{-1} and 1200 cm^{-1} is due to C—OH stretch in alcohols. Thus, two kinds of fractions can be distingushed, those absorbing between 1000 cm^{-1} and 1100 cm^{-1} (cyclohexanol type) and those absorbing between 1100 cm^{-1} and 1120 cm^{-1} (secondary alcohols). It is remarkable that, except for the HMW fractions of the Atabapo, the Sipapo and the Autana rivers and the LMW fraction of the Atabapo, no fractions show absorption bands in the region of 1725 cm^{-1} (stretching of C=O in CO_2H and ketonic C=O) (Wagner & Stevenson 1965; Stevenson & Goh 1971; Leenheer 1980). The decrease in the carboxylic absorption or its total suppression occurs as a consequence of electrovalent linkages with cations. This produces an increase of absortion, or its total suppression, at 1600 cm^{-1} due to the resulting carboxylate anions (Schnitzer & Skinner 1963). Coincidentally relatively lower cation concentrations were found in the Sipapo, the Autana and the Atabapo rivers (Vegas-Vilarrúbia et al. in press). To some extent a proportion of the different absorption levels in the spectrum regions could be due to linkages of phosphorus and nitrogen organic compounds (Jones 1959). Therefore, since phosphorus and nitrogen are limiting elements for many aquatic organisms, the interation of aquatic humus with these elements ust be considered of importance. Phosphorus is present in the biota of a wide variety of organic compounds, characterized by either weak P—O—C ester bonds or stable P—C bonds. Its role as a mobile entity of cell metabolism and as a basic structural element of cell materials is critical (Richey 1983). Some evidence exists of the formation of humic-metal-phosphate complexes in lacustrine environments (Jackson & Schindler 1975). Nitrogen is also needed for biological processes as fundamental as protein and nucleic acid synthesis. In addition, a large number of organisms demand specific chemical species of nitrogen, whilst the nitrogen potentially available is commonly limiting. The low concentrations of phosphorus and nitrogen found in the dissolved phase of our tests (Vegas-Vilarrúbia et al. in press) may be explained by applying this principle.

Statistical analysis

The distinct relationship between the fluorescent and absorbant properties (E_4:E_6, f:a) of both fractions, could indicate differences in their molecular structures since optical properties, although related to molecular size, depend mainly on the chemical structure of the humic material. In order to discover some relationship between these two parameters (that is, the proportion of fluorescence to be found in the absorbing groups of humic matter analyzed) we studied their linear relationship in the case of each river (Table 4, Fig. 6). Use of the F_{max} test (Snedecor & Cochran 1971) showed their residual mean square of regression deviations to be homogenous, with the exception of the Caroni river, which showed a significantly different level at $\alpha \leqslant 0.05$. These results allowed us to compare the regression lines of all the rivers, except the Caroni, using a covariance analysis (Snedecor & Cochran 1981). The fluorescence versus absorbance plot (Fig. 6) shows that the Negro, Atabapo, Autana and Chola rivers seem to have a similar relationship, whilst the Sipapo appears as a transition between these rivers and the Caroni. The Morichal Largo, however, differed markedly from the rest of the rivers, its regression coefficient being negative. Since its correlation was also markedly lower (Table 4), the linear relationship between the two parameters seems to be less strong than in the others. Finally, the broken lines indicate the distance between the average values of fluorescence and absorbance for each fraction and their corresponding regression line. This distance could be taken as an indicator of just how strongly each fraction influences the relationship between fluorescence and absorption: so LMW have a stronger incidence on it. The dispersion of average values around regression lines may be responsible for the variation of slopes. Once again, LMW fractions seem to be the major determinant of this variation, with HMW fractions showing a weaker dispersion. Therefore, the fluorescent nature of humic substances per unit absorbance appears to be more regular in the LMW fraction. This conclusion gives us additional capacity when analyzing the blackwater river's humic matter.

In order to discover further contrasts among both type of fractions, we examined the relationship between their E_4:E_6 and f:a quotient values (Figs. 7 and 8). Firstly we took only the values corresponding to the two absorption peaks and 30 ml before and after them, for each river. These were used to calculate their averages and give what we considered to be the most

Table 4. Linear correlation coefficients among fluorescence and absorbance values significant at a le $\alpha \leqslant 0.01$.

Number	River	n	r
7	Morichal Largo	24	0.590
8	Caroní	24	0.941
11	Autana	39	0.743
12	Sipapo	27	0.878
13	Atabapo	30	0.799
15	Chola	33	0.725
19	Negro	36	0.804

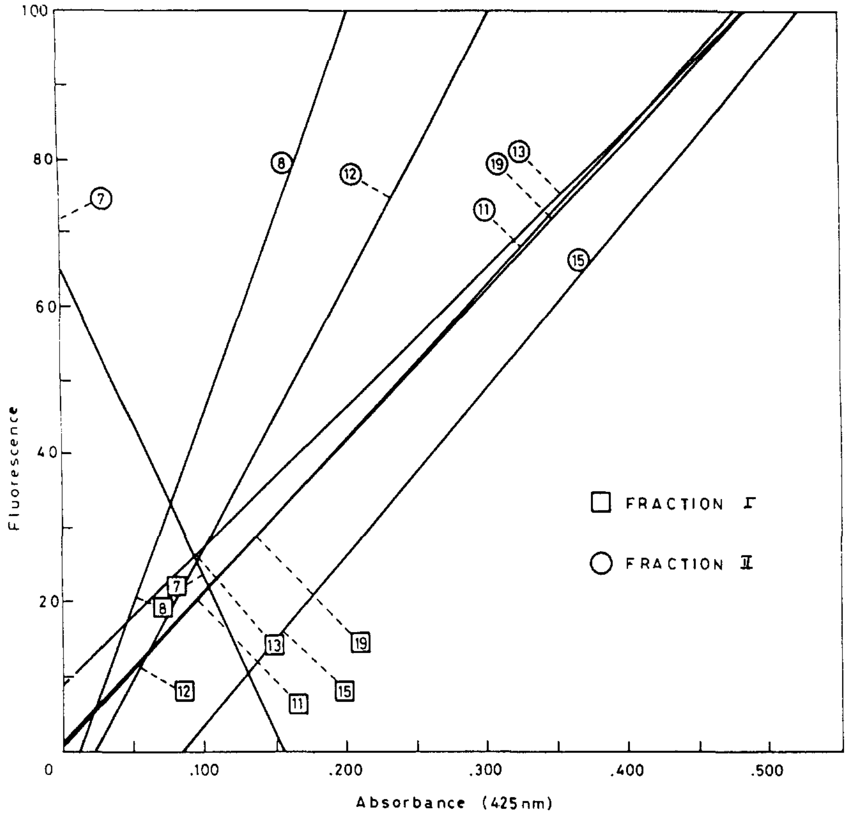


Fig. 6. Regression lines between fluorescence and absorbance; average values of HMW and LMW fractions of each river in their corresponding position to their respective regression lines. Equations from regression lines and correlation coefficient values are significant at a level $\alpha \leqslant 0.01$.

representative f:a values. Secondly, dispersion values were calculated by adding or subtracting standard deviations. Finally, these averages were represented (Fig. 7) by boxes on a symbolic dispersion diagram (Hideux 1967). Results indicated the tendency of river fractions to either form into groups or conversely to scatter. Thus, the Autana, Negro, Atabapo and Chola rivers come together as a well defined group. Within this grouping, the Chola and Autana appeared very similar, whilst the Negro is almost identical to the Atabapo. Once again, the Sipapo river seemed to represent a transition between this group and the Caroni. The results for the fall outside the scale indicated by Morichal Largo the LMW fractions from the rest of the rivers, which would indicate important optical differences in the humic molecules.

In the second symbolic dispersion diagram (Fig. 8) E_4:E_6 values are related to f:a values for each fraction separately. Data was calculated as before and the plotted points for each fraction were joined to give an

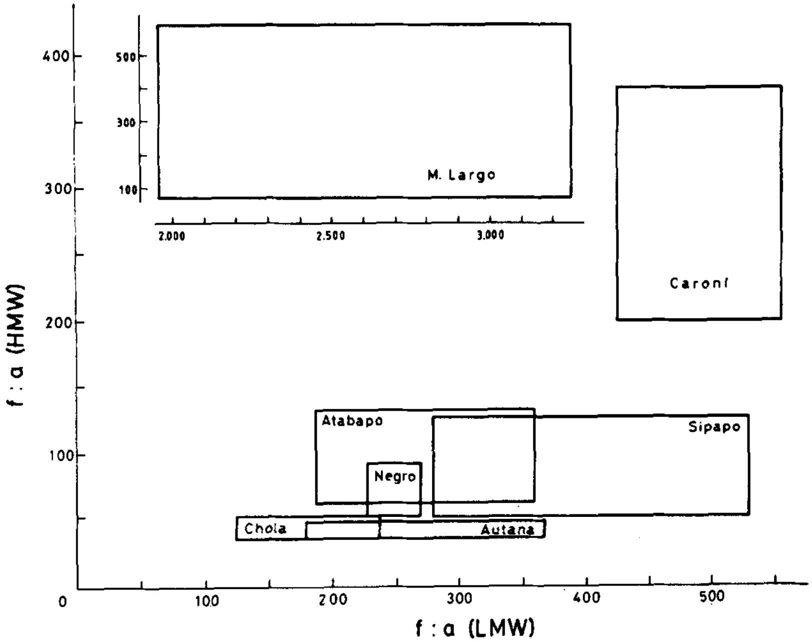


Fig. 7. Symbolic dispersion diagram of fluorescence: absorbance relationship for each fraction of the seven rivers.

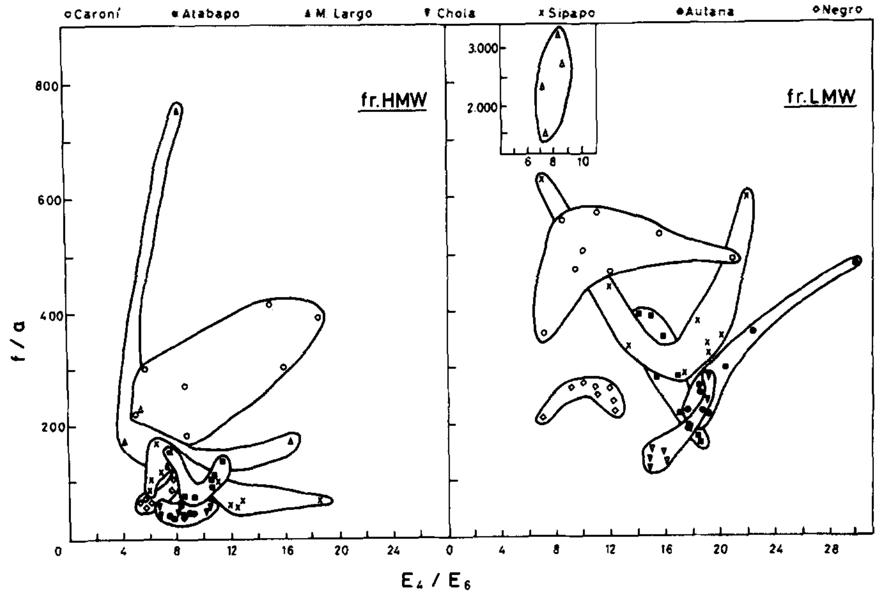


Fig. 8. Groups based on the relationship among E_4:E_6 and f:a ratios for HMW and LMW fractions of each river.

individual profile. The degree of intersection, union or disjunction amongst them was taken to represent their relationship to one and other. HMW fractions did not allow a good separation or classification of the rivers. LMW, however, indicated that the Atabapo, Autana and Chola fell into one group. The Sipapo again showed an intermediate position, lying closest to the Caroní river, which did not resemble any of the others. The Morichal Largo once more showed a profile which was quite different from that of the other rivers.

This statistical comparison of the rivers, based on molecular weight and optical characteristics due to chemical structure, indicated a notable similarity between the Negro, Chola, Atabapo and Autana rivers. The Caroní and Morichal Largo resembled none of the others and were also quite different from each other, whilst the Sipapo fell into an intermediate position between the latter two and the Amazonian group.

Conclusions

Indirect evidence indicated divergences in the chemical structure of isolated humic fractions, which could be due to the distinct vegetation sources and environmental conditions under which they were formed. Based on their humic material content, three groups of rivers could be distinguished:
— Those of southern Venezuela (Autana, Atabapo, Chola, Negro) situated in the Cuchivero geological province, where volcanic (mainly acid) and plutonic (mainly alkaline granite) rocks predominate, having typical blackwater riverine forest (Huber 1982) and highly colored waters with very low pH and conductivity values (Tables 1 and 2).

— The Caroní, representing the eastern Guayana Shield and draining four geological provinces: the Cuchivero, the Imataca with silicic rocks (granitic gneisses with intrusions of calcareous-alkaline composition), the Roraima, which consists of a volcanic basal complex below a stratified formation with several layers of sandstone, lutites and siltstones and the Pastora, formed by a sequence of acid and basic volcanic and metasedimentary rocks (amphibolite facies). This river is surrounded by tropical humid rainforest, its water is less colored and shows low pH and conductivity values (Tables 1 and 2).

— The Morichal Largo, which drains the Mesa formation, an unconsolidated quaternary alluvial deposit of sands and gravels. Surrounded by savanna vegetation with a gallery forest of *Mauritia flexuosa*, it has the least colored water with the highest conductivity and pH values (Table 1 and 2).

The Sipapo falls into an intermediate position between the first group and the Caroní. In outline this grouping is similar to that based on inorganic chemistry and physical characteristics found by Vegas-Vilarrúbia et al. (in press) for the same rivers. We would, however, emphasize that this grouping should not be interpreted as a classification, due to the limited number of rivers examined. It is, nonetheless, a good indicator that physico-chemical characteristics do give rise to some differentiation.

Thus, in comparing these groups we were able to observe several relationships. The presence of typical blackwater riverine forest seemed to determine the difference between the rivers of the first group and the Sipapo. This river enjoys the same geological and soil conditions, but differs markedly in its surrounding vegetation, which more resembles that of the Caroní river. This fact would partly explain its intermediate position between the rivers of southern Venezuela and the Caroní and also shows the possible effect of vegetation on humic material. The Morichal Largo differs from the others in that it drains a savanna region, which in turn implies that its environmental conditions are also completely different.

It must be pointed out that the river bank soils described in Table 1 are all sandy soils. Oxisols are typical of the Llanos region. On the banks of the Morichal Largo we observed sandy Entisols and Inceptisols (MARN, 1983). Spodosols, sandy inceptisols and Entisols belong to different soil orders, and due to their light texture have a high permeability. This characteristic allows water to percolate and restricts the adsorption of humic materials, thus leaching humic substances into the rivers. This feature seemed to be a common characteristic of the blackwater river banks we examined. Nevertheless, detailed information about these particular edaphic conditions is still very scarce.

It should be remembered that humic substances are a dynamic group of compounds which are extremely complex in their structural makeup and change in space and time. Therefore further characterization of organic matter in tropical blackwater rivers and their environmental and ecological implications requires additional investigation, taking into account temporal and spatial variations.

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