

CHARACTERIZATION OF THE DISSOLVED ORGANIC MATTER PRESENT IN THE WATER OF THE BÍO-BÍO RIVER, VIII REGION OF CHILE. CARACTERIZACIÓN DE LA MATERIA ORGÁNICA DISUELTA PRESENTE EN LAS AGUAS DEL RÍO BÍO BÍO, VIII REGIÓN DE CHILE

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ABSTRACT

The dissolved organic matter in rivers has different origin and its composition can vary both by natural conditions and the anthropic activity that takes place near rivers. This mobile fraction of the organic matter is composed of sub-fractions of different molecular weight and polarity, which confer different complexing capacity for the pollutants and thus a different impact on the environment.

The aim of this study was to isolate and characterize the organic matter dissolved in a representative basin of a temperate zone in South America, such as Bio Bio River located in the VIII region of Chile. The material was characterized chemically and spectroscopically after fractionating by molecular weight and polarity.

It is concluded that the dissolved organic matter of the Rucallhue sector has a terrestrial origin, with a high aromatic carbon content, in contrast with that from Laja and Concepción, which has a greater anthropic impact. The dissolved organic matters from the sectors with high anthropic activity have larger molecular size and higher organic carbon content and conductivity.

**Keywords:** Dissolved organic matter, surface water, organic carbon, molecular weight, fractionation.

INTRODUCTION

Organic matter is a complex and varied mixture of aromatic and aliphatic hydrocarbons with functional groups such as amides, carboxyl, hydroxyl, ketones and others in smaller amounts.<sup>1,2</sup> This organic matter may come from natural sources like the soil or from anthropic sources like organic amendments (compost or biosolids), or spillage from sanitary landfills. Two kinds of organic matter can be identified, usually more abundant, which under normal soil conditions are insoluble (IOM) and another pool of dissolved organic matter (DOM). The latter can migrate to water from different kinds of sources, either natural or anthropic, such as that added to the ecosystem by spillage from sanitary landfills or through the addition of compost or biosolids to the soil (agricultural land, forests, and pastures), among others.<sup>3</sup>

The substances that compose the DOM have the ability to retain and/or mobilize organic and inorganic pollutants in the environment,<sup>1,4</sup> and it is the kind and nature of the existing functional groups present, like carboxyl (-COOH), phenol (benzene-OH), alcohol (-CH<sub>2</sub>-OH) and methoxyl (-OCH<sub>3</sub>) that can influence its interaction ability with the different pollutants.<sup>5,6</sup>

The variety of molecules that constitute the DOM, and their low concentration in water systems make their characterization complex.<sup>7,8</sup> Therefore, to facilitate their study it is often necessary to isolate and concentrate them by methods such as reverse osmosis, evaporation under reduced pressure, freeze-drying, etc.,<sup>7,9</sup> to then fractionate them according to their polarity using fractionating resins like Amberlite XAD, or by molecular size through techniques like ultrafiltration, ultracentrifugation, and high performance size exclusion chromatography (HP-SEC).<sup>10,11</sup> When analyzed by H<sup>1</sup>-NMR, FT-IR, fluorescence, UV-Vis, and GC-MS techniques, these fractions provide information related to the components of the DOM, and they are used as its fingerprints,<sup>7,12,13</sup> characteristics that allow predicting its behavior in the environment and its effect on a variety of pollutants.<sup>14</sup>

In recent years research work related with DOM has increased considerably, because of its effect of retaining and/or transporting organic and inorganic pollutants in natural resources.<sup>15,16</sup>

In Chile several studies aimed at characterizing rivers of high relevance to agriculture, such as the Traiguén River,<sup>17</sup> the Cachapoal and Coya Rivers in the VI Region,<sup>18</sup> and the Laja River in the VIII Region<sup>19</sup> are being carried out with the objective of monitoring the levels of the various pollutants in order to assess the possible effects on the soil and the products generated by this activity.<sup>17,20</sup>

The Bio Bio basin is part of the VIII Region and it is one of the country's largest basins (24,264 km<sup>2</sup>) and water flows, covering part of the Provinces of Malleco and Cautín in the IX Region. At the national level the basin represents an important economic development center, linked to the forestry sector, the animal raising sector (located mainly in the provinces of Ñuble and Bio Bio), the industrial sector (represented basically by the metallurgical and chemical industries, oil refineries, the textile industry, and the cellulose industries, among others) and the hydroelectric sector, which is the main source of electric power

at the national level. Additionally, the Bio Bio River Basin represents a unique environmental system in South America, characterized by a humid, temperate climate predominant in the South of Chile, with rainfall ranging between 1,200 and 2,000 mm. All this confirms the importance of evaluating the presence of DOM and its physicochemical characteristics, as well as the effect that it can have on the pollutants present in the Bio Bio River.

In general there is little information about the of DOM in South American rivers, except in some rivers in Brazil where several works on the DOM,<sup>21,22,23</sup> have been described, however, such studies consider basins of tropical or warm humid climates, which present flora and fauna very different from that of temperate zones and that would impact notoriously on the composition and dynamics of DOM found in these waters. Currently there is a lack of studies of DOM in rivers of Chile and other South American temperate countries; therefore it is of great importance to characterize these materials because it can change the fate of contaminants in the water, which in turn may be dependent of the environmental conditions in the basin.

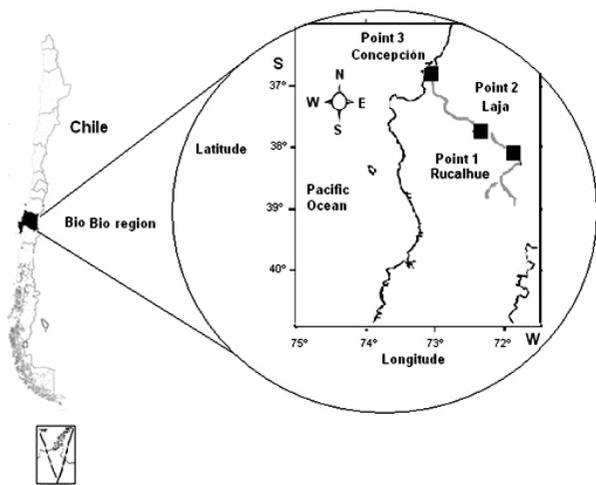
In this context, the aim of the study was to isolate and characterize the DOM present in the Bio Bio River considering three areas where different economic activities take place. Such activities may have an impact on the characteristics of the DOM. The characterization considers the fractionation of DOM in hydrophobic base, hydrophobic acid and hydrophilic fraction present in each area and comparing their chemical and structural characteristics with fractions of other areas of the basin. For this purpose have been used classical techniques for characterization of DOM, which is the first step deeper studies of DOM in temperate rivers of South America.

EXPERIMENTAL

Because of the complexity of the DOM, in a first stage it is necessary to extract it<sup>7</sup> and then fractionate it either according to molecular weight by means of ultrafiltration, or according to polarity by the method described by Leenheer.<sup>24</sup> Then the structural characterization of each fraction is made by techniques like elemental analysis (Fisons-Carlo Erba, model EA 1108), FT-IR (Bruker, model IFS-55 Equinox) UV-Vis analysis (Unicam, model UV2), and H<sup>1</sup>-NMR spectrometry (Bruker, model Advance DRX 300, Digital).

Studied area.

The Bio Bio River receives industrial and household discharges, and its course was followed from its source to its mouth in the Pacific Ocean, considering three sampling zones that differ in their associated anthropic impact. Sample collection was done in June 2011. The first sampling point was the Rucallhue sector, an area with a low water volume characterized by low anthropic impact. The second sampling point was in Laja, between Nacimiento and San Rosendo, with a river width of approximately 2 km, and the samples were taken from a boat; this sector receives wastes from the nearby cellulose plants. The third sampling point was in Concepción, a zone with a large water volume and a high anthropic impact (Figure 1).



**Figure 1:** Geographical location of water sampling points from Bio Bio River, VIII Region, Chile.

#### Water sample collection and characterization.

The samples were collected in High Density Polyethylene (HDPE) bottles that had been decontaminated with 10 %  $\text{HNO}_3$ . In each sector studied ten samples, each containing 2.5 liters of water were collected. Each sample was collected at an interval of 10 minutes, collecting a total of 30 samples for the study. The samples were transported to the laboratory and were stored at 4 °C for their immediate analysis, or they were frozen for later use.

The pH, electric conductivity, organic carbon content, and suspended solids of the samples were determined in the laboratory (WTW pH-meter, model PMX 3000, and WTW conductivity meter, model LF 539). The method of Walkley-Black modified for water was used to determine organic carbon in the water samples, prior correlation of the data with the quantification of total organic carbon (TOC) determined with a Vario TOC Select analyzer (Elementar). Reproducible TOC values with an accuracy of  $\pm 1\%$  were always found by injecting 500-  $\mu\text{L}$  aliquots in the analyzer. Suspended solids were determined by the ASTM D5907-09 method.<sup>25</sup>

#### Characterization of the DOM.

An initial volume of 2 L of sample was used, and it was concentrated in a rotavapor reducing its volume to 50 mL, followed by drying in a vacuum oven (WTB Winder), getting a representative solid that was studied by FTIR,  $^1\text{H-NMR}$  and elemental analysis.

#### Collection and fractionation of DOM.

The water samples were filtered through paper (Advantec, 70 mm, N° 2) and then the DOM was separated by filtration through a 0.45 mm cellulose nitrate membrane (Sartorius Stedim Biotech), and this fraction was called DOM.

For the fractionation by molecular weight were used of 3.9 L of DOM solution and a continuous ultrafiltration cell (Amicon), using an ultrafiltration membrane with 1 kDa cutoff. The fractions greater than 1 kDa (F1) and lower than 1 kDa (F2) were concentrated to a volume of 200 mL in a rotavapor, and this volume was then divided into two fractions: one 150 mL fraction was dried in a vacuum drying oven (WTB Winder) and the remaining 50 mL were treated with sodium azide at a concentration of 100 mg  $\text{L}^{-1}$  (100  $\mu\text{L}$  of sodium azide/ 50 mL of solution), to avoid the degradation of the DOM by microorganisms, and it was stored at 4 °C for its later analysis. The organic carbon content of each of the fractions was determined by the method of Walkley-Black modified for water (analysis in triplicate). The dry residue obtained, representative of the DOM fractions, was studied by FT-IR and by the organic carbon analysis.

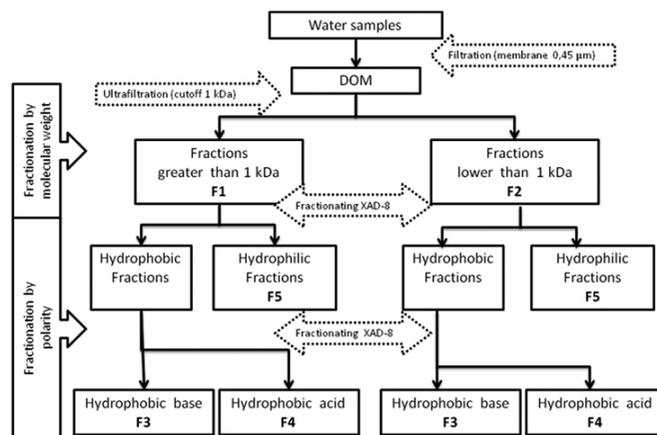
#### Fractionation of the DOM by polarity.

For the fractionation of the DOM according to polarity, use was made of the analytical procedure described by Leenheer,<sup>24</sup> which consists in separating the organic solutes into hydrophobic base (F3), hydrophobic acid (F4); and hydrophilic fraction (F5), depending on their adsorption by an ion exchange resin (XAD-8) (Supelco, Supelite™), an operationally defined separation (Figure 2).

This procedure was applied to the F1 and F2 DOM fractions of the Bio Bio water from the three sampling points (Rucalhue, Concepción and Laja). The organic carbon content of each fraction obtained in the process was determined as it was described above.

#### Analysis of the DOM and its fractions by UV-Vis spectroscopy.

A solution with a carbon concentration of 10 mg  $\text{L}^{-1}$  was prepared at pH 6.5 ( $10^{-3}$  mol  $\text{L}^{-1}$  phosphate buffer) with each of the DOM fractions obtained, and their absorbance was then determined by UV-VIS spectroscopy at wavelengths of 254, 204, 250, 365 and 436 nm. The specific UV-absorbance (SUVA) was determined, defined as the absorbance of a given sample at 254 nm divided by the DOC concentration of the sample.



**Figure 2:** DOM fractionation scheme by molecular weight and polarity.

## RESULTS AND DISCUSSION

#### Characterization of the water.

The organic carbon content (Table 1) of sampling point 3 was greater by one unit than that of points 1 and 2, whose values were 2.4 and 2.9 mg  $\text{L}^{-1}$ , respectively. The conductivity was 89.4, 114 and 125  $\text{mS cm}^{-1}$  for points 1, 2 and 3, respectively. The results of the determination of the pH of the water were similar, with an average of 7.6 for the three sampling points, values which involve the predominance of acidic functional groups such as carboxylic, in dissociated form and basic functional groups such as amines, in protonated form. With respect to suspended solids, sampling points 1 and 2 had similar values of 12.9 mg  $\text{L}^{-1}$ , in contrast with sampling point 3, whose value was almost three units lower, in agreement with the flow volume differences between the sampling points, with the flow at point 3 (3443  $\text{m}^3\text{s}^{-1}$ ) greater than in points 1 and 2, with 1439 and 2073  $\text{m}^3\text{seg}^{-1}$ , respectively.<sup>26</sup>

DOM concentrations measured as organic carbon are reported as 1 - 10 mg  $\text{L}^{-1}$  in rivers; 1 - 50 mg  $\text{L}^{-1}$  in lakes, 8.6 mg  $\text{L}^{-1}$  in waste water, and 0.5 - 52 mg  $\text{L}^{-1}$  in soil.<sup>27</sup> This organic matter can interact with the different pollutants present in the soil, and in the specific case of heavy metals the organic matter can favor their stabilization in the soil solution,<sup>28</sup> while in the specific case of DOM from residues such as compost, it can mobilize heavy metals through the soil profile.<sup>29</sup>

**Table 1:** Point coordinates altitude and main physicochemical characteristics of the surface waters in the sites under study.

Sector	Rucalhue	Laja	Concepción
Point	1	2	3
Elevation / (m)	846	139	144
S	37°43' 16,9''	37°17' 02,5''	36°49' 37,9''
W	72°14' 39,7''	72°42' 59,9''	73°04' 17,8''
$C_{\text{org}}$ / (mg $\text{L}^{-1}$ )	2.38 $\pm$ 0.07*	2.88 $\pm$ 0.00	4.30 $\pm$ 0,71
Electric Conductivity / ( $\mu\text{S cm}^{-1}$ )	89.4 $\pm$ 0.4	114.2 $\pm$ 0.1	125.4 $\pm$ 0.3
pH	7.63 $\pm$ 0.01	7.55 $\pm$ 0.01	7.56 $\pm$ 0.00
Suspended Solids / (mg $\text{L}^{-1}$ )	12.87 $\pm$ 0.19	12.88 $\pm$ 0.46	9.97 $\pm$ 0.33

$C_{\text{org}}$ : Organic carbon content  
\* Standard deviation.

Characterization of the DOM.

The mass of DOM from river water at sampling points 1, 2 and 3 was 70.7, 63.4 and 56.4 mg L<sup>-1</sup>, respectively. With respect to elemental microanalysis of the solid fraction of the DOM, the percentages for sampling point 1 were N: 0.1, C: 4.9, H: 1.02; for sampling point 2: N: 0.5, C: 4.6, H: 1.04; and for sampling point 3: N: 0.7, C: 5.3, H: 1.00.

Because of the complexity of the structure of the DOM, the H<sup>1</sup>-NMR spectra were considered as a pattern of signals rather than as a means of identifying specific compounds (Figure 3). It must be noted that the distribution found resembles the spectra reported by Ma et al.,<sup>30</sup> for fulvic acid, and that corresponding to the hydrophilic fraction with a signal at 0- 4 ppm for the aliphatic region and 6-8.6 ppm for the aromatic region.

The results of this study are also consistent with that described by Lam et al.,<sup>31</sup> for the H<sup>1</sup>-NMR spectra of DOM present in natural waters, where the signals in the vicinity of 1 ppm would account for the presence of aliphatic groups, signals around 2 ppm of acetate groups, signals between 3 and 4 ppm corresponding to carbohydrates; signals between 5.5 to 6 ppm due to the presence of double bonds, and signals over 7 ppm corresponding to aromatic rings or amides.

In a study of the DOM, but conducted in wastewater Dignac et al.<sup>32</sup> described similar results, identifying signals for aliphatic chains as key signals in the zone between 0.8 to 1.5 ppm, and other signals corresponding to the resonance of carbohydrates in the zone between 3.2 to 4.2 ppm

The results of the FT-IR analyses of the DOM are presented in Figure 4 for the three sampling points, showing agreement between the spectroscopic profiles of the different DOM, indicating that the structures and functional groups are similar. The main signals are seen at 3422.3 cm<sup>-1</sup>, corresponding to the H-bonded OH stretching of carboxyl, phenol and alcohol; a lower intensity signal at 1637 cm<sup>-1</sup> corresponding to the C=O stretching of COO<sup>-</sup>, ketonic C=O, and aromatic C=C conjugated with the COO<sup>-</sup> of pyridine to amino acid; a signal at 1425.1 cm<sup>-1</sup> corresponding to -C-H of carbohydrates, and a strong signal at 1096.6 cm<sup>-1</sup> corresponding to the C-O group of polysaccharides; only for the DOM from points 2 and 3 a signal is seen at 1384.6 cm<sup>-1</sup> that corresponds to polyhydroxylated phenols. The spectroscopic profiles obtained for the DOM from the three sampling points agree largely with the FT-IR spectrum of a humic acid, with the difference that the DOM does not present the signal at 2900 cm<sup>-1</sup> corresponding to aliphatic C-H stretching.<sup>33</sup>

The fractionating of DOM by molecular weight was done using a 1 kDa cutoff, because it was determined that the amount of organic carbon present in the fractions obtained with molecular weights greater than 30 kDa was ten times smaller than that obtained using the 1 kDa cutoff size. Table 2 shows the results of the determination of the organic carbon content in fractions F1 and F2. Upon carrying out the mass balance it is seen that part of the DOM is lost during the ultrafiltration process, which in general takes several days for its performance (120 continuous hours), so it is possible that DOM degradation processes as well as adsorption of it on the ultrafiltration membrane used may continue taking place.<sup>7</sup> The results showed that the predominant fraction in the DOM for the three sampling points is the one greater than 1 kDa, with a higher organic carbon content.

In relation to the FT-IR of the F1 of the DOM (Figure 5), a stronger signal is seen for sampling point 2 at 3500 cm<sup>-1</sup> corresponding to a H-bonded OH stretching of carboxyl, phenol and alcohol; at 1630 cm<sup>-1</sup> corresponding to C=O stretching of COO<sup>-</sup>, ketonic C=O and aromatic C=C conjugated with COO<sup>-</sup> of pyridine to amino acid; at 1400 cm<sup>-1</sup> corresponding to -C-H of carbohydrates, and at 1225 cm<sup>-1</sup> corresponding to C-O stretching, and O-H bending mainly of carboxyl groups. The signal at 1512 cm<sup>-1</sup>, corresponding to aromatic C=C, N-H bending, and C=N stretching is seen only in fraction F1 from sampling point 1.

In relation to the FT-IR of F2 (Figure 6), it is noted that the signal at 2920 cm<sup>-1</sup>, corresponding to -OH groups is not seen, and the signals at 1630 and 1400 cm<sup>-1</sup> are weaker.

The spectroscopic profiles of the DOM from the three sampling points agree among fractions with the same molecular weight.

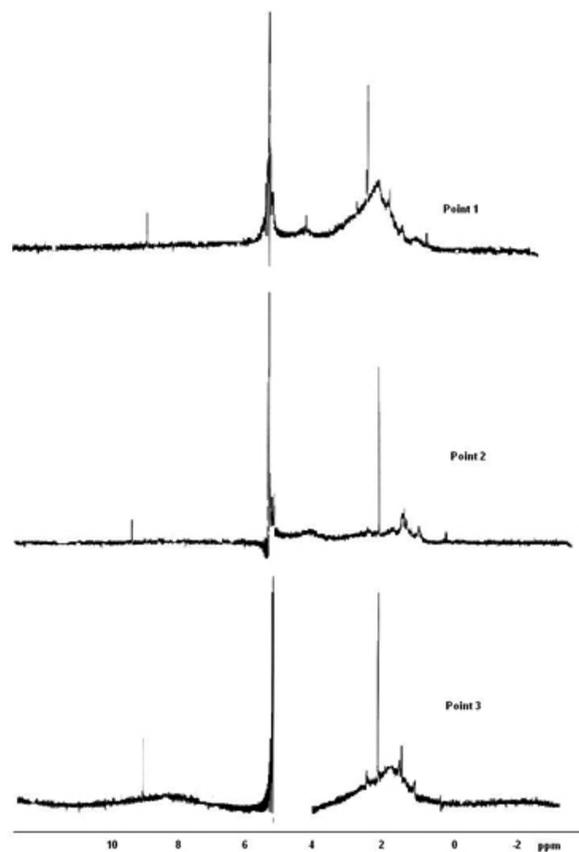


Figure 3: H<sup>1</sup>-NMR spectra of DOM obtained from points 1, 2 and 3.

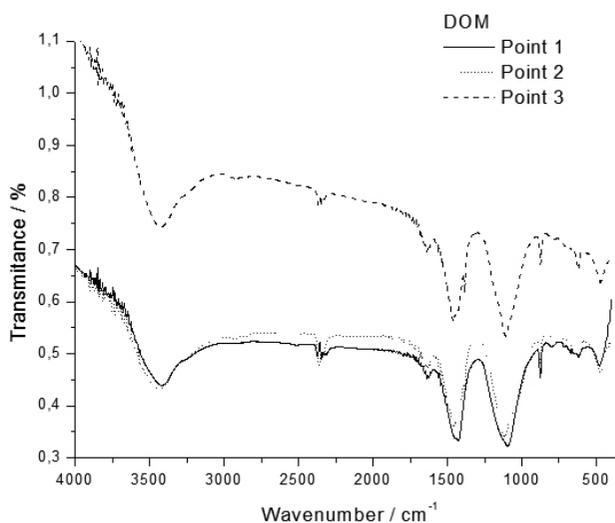


Figure 4: FT-IR spectra of DOM fraction lower than 0.45 μm.

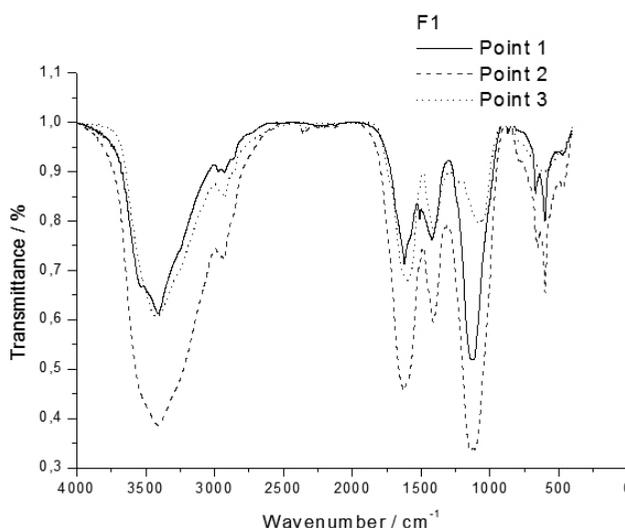


Figure 5: FT-IR spectra of DOM fraction (F1) greater than 1 kDa.

Table 2: Characterization of DOM fractions by UV-Vis spectroscopy and organic carbon content.

Point	1	2	3	1	2	3	1	2	3
Fraction	Lower than 0.45 mM			Greater than 1 kDa (F1)			Lower than 1 kDa (F2)		
$C_{org} / (mg L^{-1})$	2.38± 0.07	2.88± 0.00	4.30± 0.71	0.88± 0.34	2.05± 0.44	2.38± 0.47	0.6 ± 0.09	1.4 ± 0.00	1.18± 0.14
SUVA	2.19	3.41	6.97	5.09	5.46	2.14	10.7	10.2	12.9
A 254/436	26.0	24.5	12.0	15.0	11.2	8.50	31.5	15.1	14.6
A 254/204	0.117	0.119	0.116	0.170	0.251	0.111	0.034	0.228	0.128
A 250/365	4.19	5.23	4.67	3.93	4.33	3.47	5.27	5.12	5.18

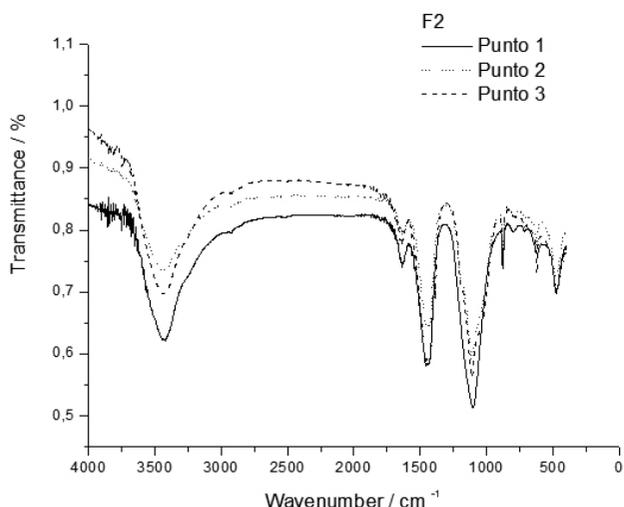


Figure 6: FT-IR spectra of DOM (F2) fraction lower than 1 kDa.

From the fractionation of the MOD by polarity two fractions were recognized: a hydrophilic fraction composed of simple organic acids, polyhydroxylated phenols, carbohydrates, amino acids and amino sugars, and a hydrophobic fraction that may contain phenols, hydrocarbons, fats, nucleic acids, and quinones.

The organic carbon content in the extracts fractionated by polarity (Figure 7) showed that the predominant fraction is hydrophobic, corresponding specifically to the hydrophobic base (F3), with a proportion of organic carbon between 55 and 84% with respect to the sum of the organic carbon of the three fractions, for each sampling point. The highest organic carbon content was that

of F3 of the fraction smaller than 1 kDa from point 1, with a mainly natural origin, followed by F5 and finally F4.

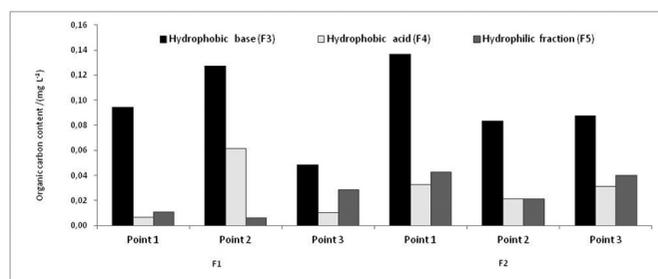


Figure 7: Organic carbon content of F1 and F2 fractionated by polarity.

When the organic carbon results of the different polarity fractions and the FT-IR spectra of F1 and F2 are compared, it is seen that for F1 from point 2 the signals show a DOM with a highly hydrophobic character, because of the signal corresponding to hydrocarbons at 2930-2860 and 1455  $cm^{-1}$ , in agreement with the higher organic carbon content present in F3.<sup>24</sup>

The SUVA parameter (Table 2), which allows describing the hydrophobic and hydrophilic character of the DOM obtained from natural water, was determined from the results of UV-Vis spectroscopy and the organic carbon content of each of the fractions. The results obtained for the DOM fraction were 2.19, 3.41 and 6.97 for points 1, 2 and 3, respectively, where a SUVA >4 indicates mainly hydrophobic, high molecular weight organic material and especially aromatic matter, while a SUVA <3 refers to mainly organic compounds which are hydrophilic, of low molecular weight and have low charge density.<sup>34, 35, 36</sup>

The results of this study indicate a higher anthropic influence from the point 1 to 3 implying an increase in hydrophobicity; however, the increase of Suva also been associated with an increase in concentrations of Fe or nitrate.<sup>37</sup>

Though, the effect of nitrate has been significantly associated with groundwater and nitrate treated samples,<sup>38</sup> while the effect of Fe would be marginal in water with concentrations between 0-0.5 mg / L of this element, as in this study; Therefore, it is more likely that the increase in the value of Suva corresponds to changes in the pattern of organic compounds that are being incorporated into the waters of the river by human activity on its way to the sea.

Some authors have attempted to relate the value of Suva with the reactivity of the DOM.<sup>39</sup> However, in experiments that have proven reactivity with chlorine and tetramethylammonium hydroxide, it has been observed a wide range of responses for samples with similar Suva; in view of this the authors point to the measure of Suva as a good predictor of the general chemical characteristics of the DOM, but does not provide information about the reactivity of the DOM from different sources.

The results obtained for the extract fractionated by molecular weight showed that F2 has a strongly hydrophobic character for the three sampling points, but not so F1, where a hydrophobic character is found only for sampling points 1 and 2 (Table 2).

The 254/436 absorbance ratios (Table 2) have been described in the range of 4.37 to 11.34 in the river water, associated with a terrestrial/ allocthonous DOM that has a higher aromatic carbon content, associated with the presence of substances such as tannins and/or humic compounds derived from higher plants and organic matter from the soil.<sup>40</sup> The result obtained for the DOM from point 3 is within this range, but not so for points 1 and 2, whose average value was 25, indicating that the DOM is derived from a microbial source.<sup>41</sup> In relation to the extracts fractionated by molecular weight, F2 had higher A254/A436 ratios than F1 for the three sampling points.

The 254/204 and 250/365 absorbance ratios have been reported to be useful for the characterization of the DOM, whose A254/A204 ratio allows the correlation with the amount of aromatic carbon present in the DOM,<sup>42</sup> and the A250/A365 ratio is used as an indicator of molecular size. The results show that the A254/A204 values (Table 2) were similar for the DOM from the three sampling points, but not so for the different molecular weight fractions, where the ratio is greater in F1 and F2 from sampling point 2.

As to the A250/A365 ratio (Table 2), the values obtained for the different molecular weight fractions coincide, pointing to the efficiency of the fractionating procedure with the ultrafiltration cell. The A250/A365 results of the DOM indicate that the one from sampling point 1 has a lower molecular weight, followed by the DOM from sampling points 3 and 2.

## CONCLUSIONS

From the results it can be concluded that the DOM present in natural water of the Bio-Bío river, VIII Region of Chile, of the Rucalhue sector, Laja and Concepción, is composed of macromolecules with a molecular weight range, chemical composition, and a specified spectroscopic profile that are similar for the three sampling points, as well as between the fractions which have the same molecular weight.

From the fractionation by molecular weight it was possible to determine for the three sampling points that the DOM has mainly a molecular weight greater than 1 kDa, with a hydrophobic character, where the molecular size is greater for point 2, a sector with a high anthropic impact, followed by point 3, and finally point 1.

The effect of human activity is reflected in increased molecular size of the DOM, as well as increased organic carbon content and conductivity.

From the spectroscopic results it can be concluded that the DOM of the Rucalhue sector (point 1) has a terrestrial origin, with a high aromatic carbon content, distinguishing it from the DOM of the Laja and Concepción sectors (points 2 and 3), which have a greater anthropic impact.

## ACKNOWLEDGEMENTS

This study was supported by National Fund for Scientific and Technological Development (FONDECYT), project N° 11110223.

## REFERENCES

- 1.- J. Leenheer, J. Croué, *Environ. Sci. Technol.* 1, 19, (2003)
- 2.- A. Nebbioso, A. Piccolo, *Anal. Bioanal. Chem.* 405, 109, (2013)
- 3.- EPA; Agencia de Protección Ambiental de Estados Unidos. Folleto informativo de tecnología de biosólidos: Aplicación de biosólidos al terreno, EPA 832-F-00-064, 2000.
- 4.- S. Hernandez-Ruiz, L. Abrell, S. Wickramasekara, B. Chefetz, J. Chorover, *Water Res.* 46, 943, (2012)

- 5.- B. Pan, B. Xing, W. Liu, G. Xing, S. Tao, *Chemosphere*, 69, 1555, (2007)
- 6.- L. Settimo, M. McLaughlin, J. Kirby, K. Langdon, L. Janik, S. Smith, *Environ. Pollution* 199, 174, (2015)
- 7.- A. Matilainen, E. Gjessing, T. Lahtinen, L. Hed, A. Bhatnagar, M. Sillanpää, *Chemosphere*, 83, 1431, (2011)
- 8.- G. Zheng, W. Price, *Environ. Sci. & Technol.* 46, 1675, (2012)
- 9.- Y. Chen, G. Yang, G. Wu, X. Gao, Q. Xia, *Cont. Shelf Res.* 52, 97, (2013)
- 10.- N. Her, G. Amy, D. Foss, J. Cho, *Environ. Sci. & Technol.* 36, 3393, (2002)
- 11.- E. Ged, T. Boyer, *Chemosphere*, 91, 921, (2013)
- 12.- H. Kim, M. Yu, I. Han, *Appl. Geochem.* 21, 1226, (2006)
- 13.- A. Costa, E. Passos, C. Garcia, J. Alves, *J. Braz. Chem. Soc.* 22, 11, 2139, (2011)
- 14.- H. Schulten, *Environ. Toxicol. Chem.* 18, 8, 1643, (1999)
- 15.- S. Hernandez, S. Wickramasekara, L. Abrell, X. Gao, B. Chefetz, J. Chorover, *Chemosphere* 91, 344, (2013)
- 16.- M. Wei-Haas, K. Hageman, Y. Chin, *Environ. Sci. Technol.* 48, 4852, (2014)
- 17.- G. Palma, A. Sánchez, Y. Olave, F. Encina, R. Palma, R. Barra, *Chemosphere* 57, 763, (2004)
- 18.- J. Narváez, P. Richter, I. Toral, *J. Chil. Chem. Soc.* 52, 3, 1261, (2007)
- 19.- R. Barra, P. Popp, R. Quiroz, C. Bauer, H. Cid, W. Tumpling, *Chemosphere* 58, 905, (2005)
- 20.- R. Orrego, B. Jimenez, L. Bordajandi, J. Gavilan, B. Inzunza, E. Abad, M. Gonzalez, J. Rivera, R. Barra, *Chemosphere* 60, 829, (2005)
- 21.- A. Tadini, M. Campanha, A. Moreira, M. Bisinoti, *J. Braz. Chem. Soc.*, 24, 1789, (2013)
- 22.- E. Fagnani, J. Guimaraes, P. Fadini, *Aquat. Geochem.* 18, 445, (2012)
- 23.- A. Costa, E. Passos, C. Garcia, J. Alves, *J. Braz. Chem. Soc.* 22, 2139, (2011) [www.astm.org/Standards/D5907.htm](http://www.astm.org/Standards/D5907.htm)<sup>9</sup>
- 24.- J. Leenheer, *Environ. Sci. Technol.* 15, 578, (1981)
- 25.- [www.astm.org/Standards/D5907.htm](http://www.astm.org/Standards/D5907.htm), accessed in June 2015.
- 26.- DGA, 2004, Dirección general de aguas. Diagnóstico y clasificación de los cursos y cuerpos de agua. <http://documentos.dga.cl/CQA4432v1.pdf>
- 27.- R. Quails, B. Raines, *Soil Sci. Soc. Am. J.* 56, 578, (1992)
- 28.- A. Charriau, L. Lesven, Y. Gao, M. Leermakers, W. Baeyens, B. Ouddane, G. Billon, *Appl. Geochem.* 26, 80, (2011)
- 29.- A. Kaschl, V. Romhelda, Y. Chen, *Sci. Total Environ.* 291, 45, (2002)
- 30.- H. Ma, H. Allen, Y. Yin, *Wat. Res.* 35, 4, 985, (2001)
- 31.- B. Lam, A. Simpson, *Analyst.* 133, 263, (2008)
- 32.- M. Dignac, P. Ginestet, A. Bruchet, J. Audic, S. Derenne, C. Largeau, *Water Sci. Technol.* 43, 2, 51, (2001)
- 33.- H. Shin, J. Monsallier, G. Choppin, *Talanta* 50, 641, (1999)
- 34.- J. Edzwald, J. Tobiasson, *Water Sci. Technol.* 40, 9, 63, (1999)
- 35.- E. Sharp, S. Parsons, B. Jefferson, *Sci. Total Environ.* 363, 183, (2006)
- 36.- E. Sharp, P. Jarvis, S. Parsons, B. Jefferson, *Colloids and Surfaces A: Physicochem. Eng. Aspects* 286, 104, (2006)
- 37.- F. Van der Leeden, F. Troise, D. Todd, *The Water Encyclopedia*, 2nd ed.; Lewis Publishers, Inc.: Chelsea, MI, 1990.
- 38.- D. Goolsby, W. Battaglin, *Nitrogen in the Mississippi Basin - Estimating Sources and Predicting Flux to the Gulf of Mexico, 135-00*, United States Geological Survey (2000).
- 39.- A. Weishaar, G. Aiken, B. Bergamaschi, M. Fram, R. Fujii, K. Mopper, *Environ. Sci. Technol.* 37, 4702, (2003)
- 40.- T. Battin, *Org. Geochem.* 28, 561, (1998)
- 41.- R. Jaffe', J. Boyera, X. Lua, N. Maiea, C. Yang, N. Scullya, S. Mock, *Mar. Chem.* 84, 195, (2004)
- 42.- J. Hur, M. Williams, M. Schlautman, *Chemosphere* 63, 387, (2006)