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Humic Substances from sediments of Lobos Pond (Argentina). Isolation, Characterization and Limnological Implications.

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Abstract.

Humic substances (HS) were isolated from the sediments of Lobos Pond (Argentina) using mild conditions to preserve their native structure. The HS (humic and fulvic acids) were characterized by means of elemental analysis and FTIR spectroscopy. Also a by-product obtained during fulvic acids (FA) fractionation (an amorphous white solid residue) was analyzed. Results revealed possible interactions between FA and inorganic-organic substances that may have implications referring to bioavailability. Other limnological implications, such as autochtonous origin of HS linked with the hydrology, and change of pH during stormy weather that affects HS interactions, are discussed.

Keywords: humic substances, isolation, sediment, pampasic ponds, Argentina.

Introduction

Lobos Pond (35º 17´ S, 59º 07´ W) is a pampasic pond located in the Salado River drainage basin, Buenos Aires Province (Fig. 1 a, b, c), which is placed in the geomorphologic unit called “Pampa deprimida”. This pond is a permanent shallow water body (mean depth: 1.07 m, surface area: 7.51 Km^2) with a main tributary, Las Garzas Stream, and an outlet, Saladillo Stream, which is regulated by a dam, that drains to the wetland Cañada del Toro (Fig. 1 d). Factors involved in the water quality of the pond are the stable population of Loguercio Village

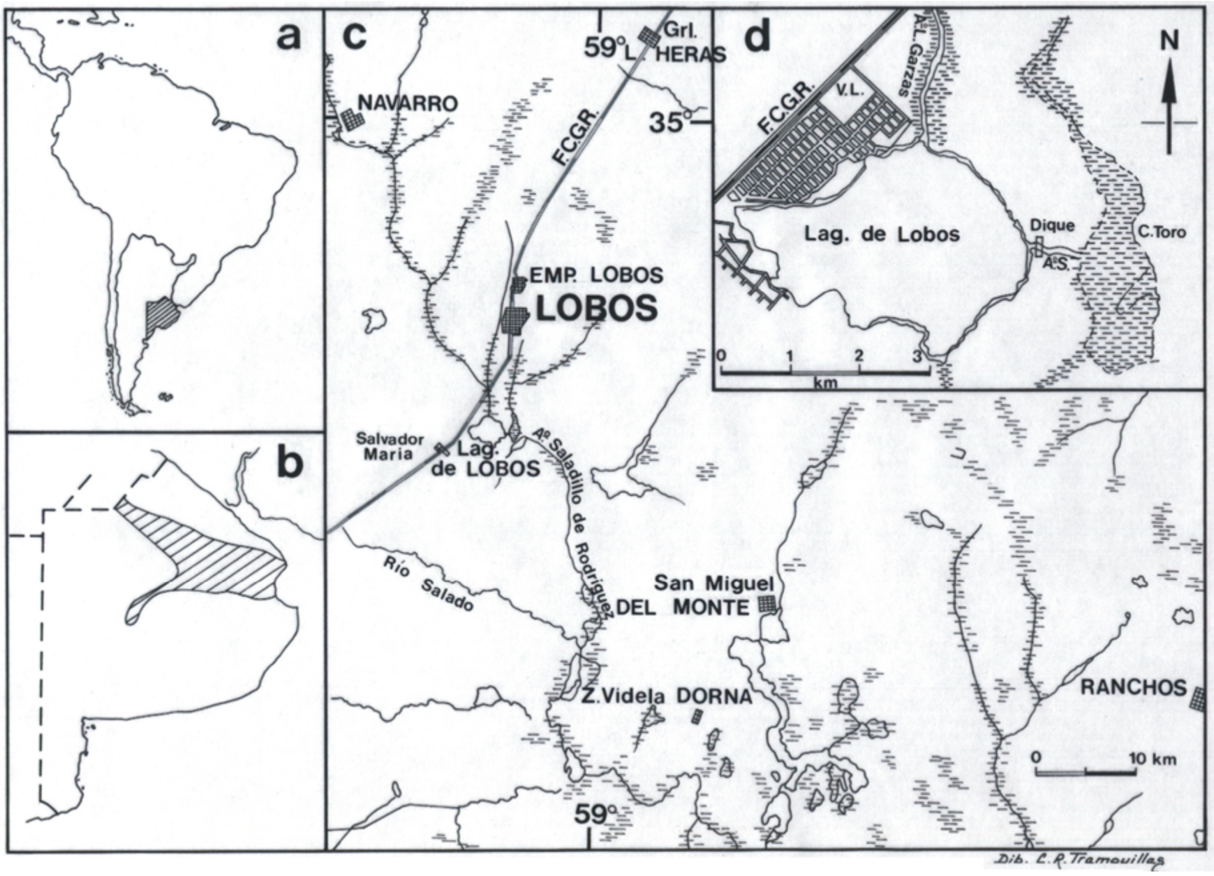

Fig. 1: (a) South America, Argentina and Buenos Aires Province. (b) Buenos Aires Province and the Salado River drainage basin. (c) Location of Lobos Pond (Lag. de Lobos) in the Salado River drainage basin. (d) Lobos Pond (Lag. de Lobos), Las Garzas Stream (A° L. Garzas), Saladillo Stream (A°. S.), dam (Dique), Cañada del Toro (C. Toro), Loguercio Village (V.L.) and Railway General Roca (F.C.G.R.).

(about 500 inhabitants), placed in the shoreline, tourism and recreational activities (fishing, boating and sailing) and Las Garzas Stream that collects upstream the raw sewage of Navarro City (about 10000 inhabitants) and during the eighty decade also the effluent of the wastewater secondary treatment plant of Lobos City (about 30000 inhabitants) (Fig. 1 c, d). All through the eighty decade problems associated to eutrophication, like algal blooms, moved the government to put in practice a limnological project. Main results that emerged from it[1] are indicated in Table 1. High pH and chlorophyll *a* concentration observed in the pond as a product of autotrophic activity, put on evidence the impact of the high concentrations of nutrients coming from Las Garzas Stream (Table 1).

	Lobos Pond	Las Garzas Stream
pH	8.40	8.61
TDS (g/l)	1.4	3.2
T N (mg N/l)	2.0	1.3
T P (mg P/l)	0.36	0.46
T N/T P atomic ratio	12.3	6.3
Secchi disk depth (m)	0.18	0.24
Seston (mg/l)	90.5	49.4
Chlorophyll *a* (µg/l)	115.8	-

Table 1: Average values obtained monthly from March 1986 to April 1987 according to Mariñelarena and Conzonno (1997).

On the other hand, in accordance with the nitrogen limitation in relation to phosphorus as revealed by N/P relationships,[2] phytoplankton studies[3] showed that blue-green algae are the dominant species, which was other indicator of the eutrophication process, followed in abundance by the group of diatoms. Conclusions of those studies made the authorities to decide the diversion of the effluent of the wastewater secondary treatment plant of Lobos City, which was considered the main factor of eutrophication, to the wetland Cañada del Toro (Fig. 1 d). Although there were no other studies, restoration process in Lobos Pond, if really occurred because other sources still remain, was probably long because of the low renewal of water promoted by the dam.

Lake sediments contributes to the knowledge of all that occurred in the drainage basin, airsheds and waters. They contain many kinds of organic compounds, being humic substances (HS) always present. Therefore studies on HS from Lobos Pond were begun, taking into account that this pond had the impact of cultural eutrophication nearly twenty years ago as above mentioned.

These substances are formed during the decomposition of biogenic materials and are biochemically resistant to further decomposition. The classification of HS in humic acids (HA) and fulvic acids (FA) is generally based on their behavior in alkaline solution. Previous studies in our laboratory dealed with aquatic HS in ponds of the same drainage basin [4-6].

In the present study a method that allows the isolation of HS in sediment in mild conditions is described, chemical characterization of the HS was performed on the basis of FTIR spectroscopy and elemental composition. Limnological implications are also discussed.

Materials and methods

Superficial sediment samples (5-6 cm of the upper layer) were collected from the centre of Lobos Pond (three sampling points) with a sediment grab in March, 2003. After being air dried at room temperature (20º-25º C) for 7 days, the sediments samples were ground and sieved through 1mm sieve. Moisture, ash and total organic matter (TOM) of the sediment were determinated following Belzile et al.[7]

Sequential extraction of organic matter:

The sediments obtained from the three points of the centre of the pond (Fig. 1 d) were mixed. A sample (10g) was extracted in a Soxhlet apparatus for 16 h with 200 ml of spectroscopic grade n-hexane, n-hexane–acetone (4:1), acetone, acetone-methanol (4:1). The solvent was evaporated at room temperature under N_2 and transferred to a 500 ml erlenmeyer containing 200 ml of a 0.5 M NaOH solution and shaken under N_2 for 24 h. [7] The supernatant was separated from the pellet by centrifugation at 8000 rpm followed by vacuum filtration using a Millipore filter (0.45 □m).

Isolation of Humic Substances:

The NaOH solution was transferred to a separator funnel and pH was adjusted to 1 with 6M HCl, after 1h at room temperature Methyl isobutyl ketone (MIBK; 150 ml) was added. The mixture was shaken vigorously to allow the precipitation of HA in the interphase.[8] The aqueous phase (containing most of FA) was separated and worked up following procedure A.

Dissolution of HA to separate it from the organic phase, was performed with 0.5 M NaOH solution (100 ml). The organic phase (MIBK extract) was separated and evaporated to dryness under vacuum.

The NaOH solution was acidified (pH 1) to allow the precipitation of HA. After 1h, it was centrifuged at 8000 rpm. The pellet (HA) was treated following procedure B.

Procedure A:

The FA fraction was filtered (Millipore 0.45) and was adjusted to pH 8 with a 1M solution of NaOH. After 4h, it was centrifuged at 8000 rpm. An amorphous white solid residue was obtained, and analysed by mass spectrometry and FTIR. The solution was evaporated to 50 ml under vacuum and filtered. After dialysis (molecular weight cutoff point of 3500 Da) during 24h it was lyophilized. [6]

Procedure B:

The HA pellet was dissolved in 20 ml of a 0.1 M KOH solution under N_2 and KCl (0.3g) was added to make the system 0.3 M in K.[7] After 12 h (N_2 atmosphere) the solution was centrifuged at 12000 rpm for 20 min. The clear HA supernatant was acidified with 6 M HCl to pH 1 and the HA allowed to precipitate. The solid was separated from the liquid phase by centrifugation at 12000 rpm for 20 min. The pellet was dried by lyophilization.

FTIR spectra of the material extracted with solvents were recorded in the form of a film, and HA and FA were recorded in the form of KBr pellets; all of them in a FT-IR NICOLET 510 equipment. The electron impact mass spectra (EIMS) were measured on a Shimadzu QP-5000 mass spectrometer at 70 eV by direct inlet. Elemental analyses were performed with Carlo Erba EA 1108 equipment.

Results and Discussion

Isolation and Purification

Air dried sediment samples showed moisture: 5.3%, ash: 81.83% and Total Organic Matter (TOM): 12.87% (average data); in accordance with previous reports.[9] High amounts of organic matter in the sediments are probably the result of the contribution of autochtonous source as well as the supply of organic matter of external raw sewages.

Sequential extraction with organic solvents rendered: Hexane extract (14 mg), acetone extract (19 mg), hexane – acetone extract (30 mg), and acetone – methanol extract (20.5 mg). Fractions were analyzed by IR spectroscopy. As expected, paraffins were more abundant in the hexane extract, while esters of fatty acids; long chain alcohols and wax esters were predominant when more polar solvents were used. These compounds were also present in the MIBK extract (13 mg). Humic substances extracted from the sample after extraction with organic solvents, rendered: HA: 64 mg and FA: 89 mg; representing more than 10% of sediments' TOM.

FTIR spectra of both fractions (Figs. 2.a,b) showed typical bands (HA: 3400 cm-1 – 2900 cm-1 – 1670 cm-1 – 1540 cm-1 ; FA: 3408,3 cm-1 - 2930.7 cm-1 - 1642.6 cm-1 - 1402.6 cm-1 - 1138.5 cm-1) and are comparable to previous reports in the literature. [7] No signals attributable to artifacts were detected; suggesting that HA and FA are free from organic impurities.

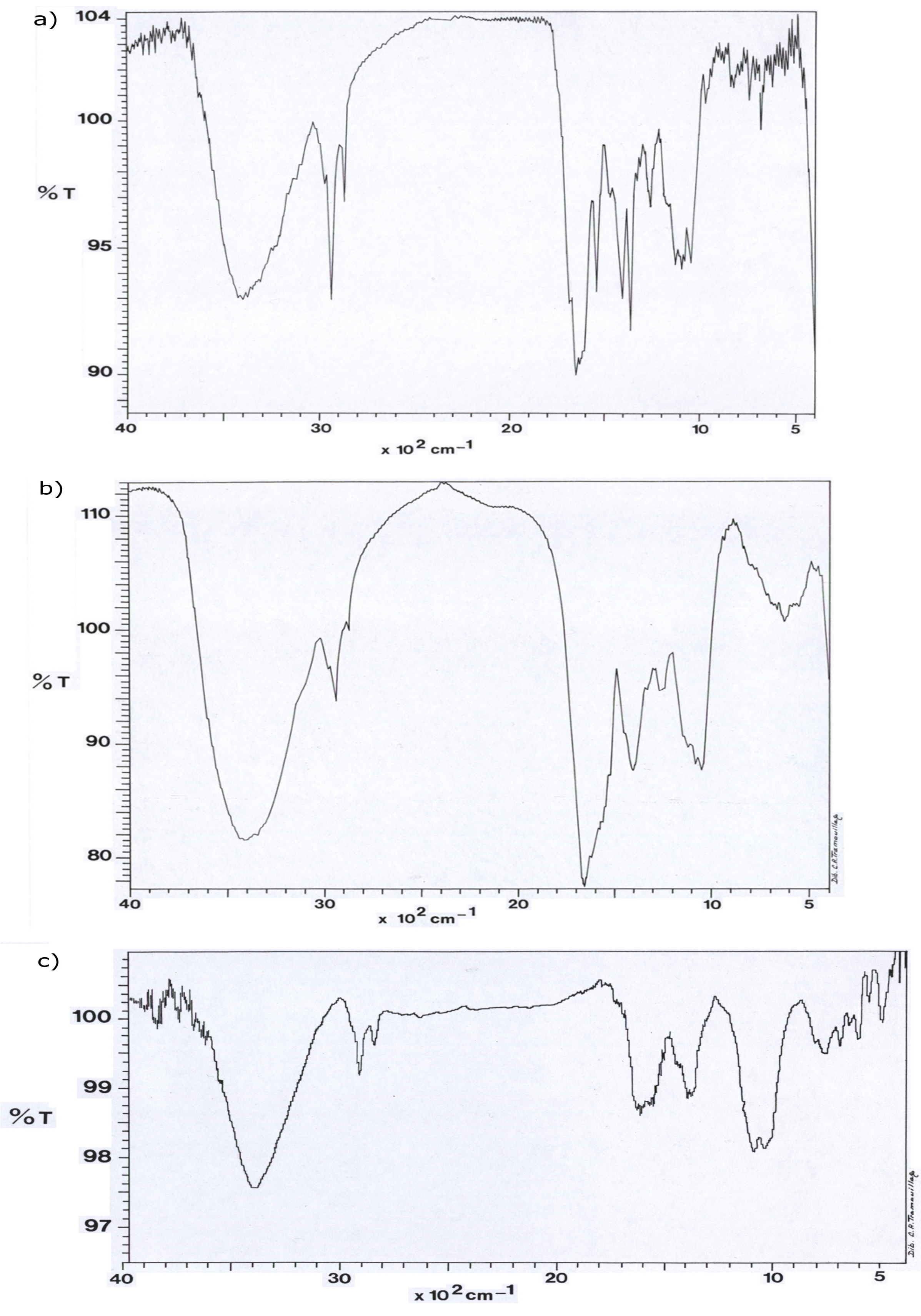
a)
104
100
%T
95
90
40
30
20
10
5
x 10² cm⁻¹
b)
110
100
%T
90
80
40
30
20
10
5
x 10² cm⁻¹
c)
100
99
%T
98
97
40
30
20
10
5
x 10² cm⁻¹

Fig. 2: FTIR spectrum of fractions isolated from Lobos Pond sediments.

a. HA; **b.** FA; **c.** amorphous white solid residue (WSR) isolated from FA fractionation

Elemental analyses of HA and FA isolated from Lobos Pond sediment are shown in Table 2. The results obtained were compared with literature data[7,10] since this is the first study of humic substances from sediments of pampasic ponds in Argentina. It can be observed that %C and %H for HA and FA from Lobos Pond are similar to those reported for sediments from other sources. The comparison between HA and FA in this pond indicated that higher H/C and O/C found for FA, suggests higher content of methylene, methyl groups and hydroxyl, carboxyl groups respectively. The N/C ratios are similar for both HA and FA.

	%C	%H	%O	%N	H/C	O/C	N/C
HA[A]	40.8	5.5	39.2	6.5	1.6	0.72	0.14
FA[A]	34.4	6.2	40.6	6.0	2.2	0.88	0.15
HA[B]	57.06	3.94	35.08	3.37	0.83	0.46	0.05
FA[B]	38.19	3.48	55.98	1.77	1.09	1.10	0.04
HA[C]*	---	---	---	---	1.17- 1.43	0.07 - 0.11	0.44 - 0.63
FA[C]*	---	---	---	---	1.10 - 1.58	0.13 - 0.16	0.57 - 0.86
HA[D]*	52.7- 54.6	5.83 - 6.27	34.2 - 36.3	4.85 - 5.23	1.05 - 1.43	0.07 - 0.08	0.47 - 0.64
FA[D]*	45.1– 48.0	6.59 - 6.94	40.4 - 42.7	4.94– 5.76	1.66 - 2.06	0.06 - 0.11	0.63 - 1.07

[A] This work; [B] Stream sediment of South Clear Creek, USA (reference 8); [C,D] Sediment from lake (reference 7); *data range.

Table 2: Elemental analyses of humic substances from different sediment sources.

Fulvic Acids interactions

In previous reports [11] a by-product of FA fractionation has been mentioned, but it has not been analyzed. Therefore, attention was drawn on the amorphous white solid residue (WSR) (308 mg) obtained from FA fraction following procedure A. Its low organic matter content (TOM: 26.3%), and high ash content (57.5%) suggested the presence of mineral compounds as major components. The lack of solubility of this material, both in polar and non-polar solvents, was in accordance with an organic-mineral mixed composition. After calcination (muffle furnace at 750ºC, 4h) the residue obtained was insoluble in mineral acids, suggesting the presence of silicates. The fact that the treatment of this residue with fluorhydric acid produced gaseous SiF_4 confirms that hypothesis.

On the other hand WSR was analyzed by mass spectrometry and FTIR. Although it is not a homogeneous product, the mass spectrum showed molecular ions (ranging from 200 to 300 Da) and fragmentation pattern (base peak = 43) characteristic of organic compounds related with fatty acids and its derivatives (i.e. palmitaldehyde) or long chain alcohols derived from octadecatrienoic acid. In accordance, the IR spectrum showed strong absorption bands at 3400 cm^{-1} (broad band), and 1630 cm^{-1} corresponding respectively to hydroxyl and carbonyl groups present in these compounds (Fig. 2.c). It is noteworthy that this white material may be liberated from FA even after a second repetition of the process (pH shift in procedure A). In our case we repeated the operation 4 times until this white residue was no longer observed. In this sense FA might behave in the sediments like hydrophobic microenvironments, in which this white material could grow or accumulate. The dynamics of HS aggregation / disaggregation is likely an important process controlling the transport and fate of pollutants, because aggregating molecules could trap inorganic or organic contaminants. [12]

Figura 2.c (FTIR spectrum of the WSR)

Interaction of FA with silica is apparently similar to the observed between FA and calcium carbonate, where as a result of such interaction inhibition of precipitation of the latter takes place. [5, 13] Also the presence of fatty acids contributes to the formation of a coating that prevents the precipitation of calcite. [14] On the basis of these facts, it may be suggested that those fatty acids may influence silica to keep in some kind of state like dissolved amorphous silica.

Furthermore the entrapment capability showed by FA to this WSR (a silicates- fatty acids core), could be related with two models. One of them, the model suggested by Piccolo, [15] is based on the reversible self-association of small molecules, in which interior hydrophobic substances could be trapped. The stabilization of such conformation should be attributed to the entropy-driven tendency to exclude water molecules from humic association decreasing the total molecular energy. On the other hand, Jansen [16] based in molecular modeling, describe the HS like a helical structure with a semi-hydrophilic surface and hydrophobic interior. Besides, this entrapment capability show a correlation with our previous reports about the displacement experiments carried out by the addition of label and unlabeled carbohydrates to evaluate the interaction between HS and carbohydrates. [6] We observed that, upon lowering the pH, the interaction decreases in relation to the steric arrangement that causes the diminution of the accessibility of the available sites in the HA molecules. This molecular behavior of the FA may be related with the model proposed by Jansen.[16]

Limnological implications

Hydrology of pampasic ponds depends mostly in the supply of groundwater drainage. In Lobos Pond this fact became evident taking into account the salinity of Las Garzas Stream, which was more than two times higher than that of the pond (Table 1).Therefore it may be expected that the input of allochthonous HS to the sediment might not be so important in relation to the HS derived from the biota of the pond. Elemental composition in particular the H/C relation (Table

2), that implies high aliphatic respect to aromatic (normally derived from lignin) structures may reinforce the autochtonous character of HS from Lobos Pond.

Another factor to be taken into account is the interaction of fatty acids with silica favouring its bioavailability for diatoms, which is the second group of dominant algae as previously mentioned. The availability is further enhanced by the nearly permanent vertical mixing in the pond as evidenced by the low Secchi disk depth and the high values of seston (Table 1), which permits bottom sediments to reach the euphotic layer.

In relation to interactions of HS with organic compounds, which in this case seem to be with fatty acids, previous studies in Chascomús Pond with aquatic HS and carbohydrates, [6] put on evidence the important role of these interactions, since that research indicated that they may influence the distribution and transport in the aquatic ecosystem and at the same time is weak enough to let them be available to the organisms involved in heterothrophic activity.

Under normal conditions, vertical mixing, which is common in pampasic ponds, has the effect of preventing stable stratification, as well as in maintaining high turbidity causing the limitation of light and consequently the limitation of primary production. Nevertheless, climatic events, such as rainstorms or flooded periods, drastically change physical and chemical conditions. [4,5] This happened during the sampling period referred in Lobos Pond,[1] when a heavy rainstorm (100 mm in two days) occurred at the end of September which caused pH to reach a value of 7.78, Secchi disc reading 0.30 m, seston 16.5 mg/l and chlorophyll *a* 12.0 µg/l, that is to say very apart from the conditions indicated for average values in Table 1. Although arriving to normal conditions could take only few days, these kinds of events produce drastic changes in the physico-chemical characteristic of the pond. This fact has strong influence in HS, especially in what is referred to pH as already described in FA-interactions related with bioavailability of inorganic-organic substances.

Conclusions

Humic Substances were isolated from sediments of Lobos Pond with a mild technique avoiding drastic chemical treatments that could alter their native structure. FTIR spectra showing the lack of contaminant material indicates that HA and FA obtained were efficiently isolated from the organic matter matrix present in the sediments.

Due to vertical mixing, sediments participate in the overall functioning of the system (to begin with light limitation in the water column) and in consequence the HS associated with. Important results that arise from this work are the interactions of HS with silica and other organic compounds that may have to do in both autothrophic and heterothrophic activities. These phenomena undoubtedly must be a matter of other studies in this pond.

Acknowledgements

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Substâncias Húmicas de sedimentos da Lagoa de Lobos (Argentina). Isolamento, Caracterização, e as implicações limnológicas.

Resumo. Substâncias Húmicas (HS) foram isoladas a partir de amostras de sedimentos da Lagoa de Lobos (Argentina). Para preservar a estrutura nativa, foram utilizadas condições brandas. As HS (ácidos húmicos e fúlvicos) foram caracterizadas por meio de análise elementar e espectroscopia de infravermelho. Foi analisado um subproduto obtido do fracionamento dos ácidos fúlvicos (FA) (resíduo sólido, branco e amorfo). Os resultados revelaram as possíveis interações entre a FA e substâncias inorgânicas e orgânicas, que podem ter implicações referentes à biodisponibilidade. São discutidas ainda outras implicações limnológicas que podem afetar as interações das mesmas, tais como a origem nativa das HS relacionadas com a hidrologia e a mudança do pH durante as tempestades.

Palavras-chave: substâncias húmicas, isolamento, sedimentos, lagoas pampásicas, Argentina.