APPLICATION OF CANONICAL CORRELATION ANALYSIS IN SOLAR SALTWORKS ENZYMATIC ACTIVITIES EVOLUTION: PROTEOLYTIC ACTIVITY CASE

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EXTENDED ABSTRACT

The physics and the geochemistry of formation of common salt deposits, hálita, produced by evaporation of seawater, is very established. Field and laboratory studies have demonstrated that the quantity and quality of sea salt produced in solar saltworks are modulated by the participation of bacterial, cyanobacterial and crustacean. And that the clarification of the brines is fundamentally consequence of the organic matter degradation for bacterial activity. In this degradation process of the components of organic matter is necessary that the different microorganisms that habit in water column and biofilms in the bottom of the ponds, synthesize and excrete enzymes with capacity to carry out the hydrolysis of proteins, lipids, chitin and cellulose.

Since the existent conditions in each one of the ponds can fluctuate responding to changes in the environment or for effect to transfer the content of one pond to other, is expected that the biosynthesis and activity of these enzymes and in consequence the speed of degradation of the organic matter presents fluctuations.

The mean values of proteolytic activity in subsurface samples, spacely fluctuated in the range of 3.63±4.57 to 0.77±0.61 (µg of Tyrosine/ml), corresponding the biggest value to the pond “Vaso 6” and smaller to “Zapote”. Temporarily this interval was from 4.69±5.26 to 0.88±0.39, corresponding the biggest value to September of 2001 and the minor to February of 2001. For the bottom samples, spacely the range was from 3.31±4.6 to 0.99±0.5, corresponding the biggest value to the pond “Chocolatera” and the minor to “Nohoch”; temporarily this interval was from 3.97±5.42 to 0.70±0.62, corresponding the biggest value to September of 2001 and the minor to February of 2001.

KEYWORDS: proteolytic activity, solar saltworks

INTRODUCTION

In general the solar saltworks are located in arid areas of tropical and subtropical regions, and they are characterized to have low leves of water and a high evaporation rate.

Production of the sea salt in evaporation solar ponds is more than a simple process of solar evaporation; because in a simultaneous form a chemical processes are developed, as the precipitation of mineral salts different from sodium chloride in its saturation point; physical, as the increment of temperature in the system that falls when salinity increases ; and biological, developed by microbial consortia that are present the water column and microbial mats that besides sealing the bottom of the evaporation ponds, they favor the retention of the solar energy that increases the temperature of the brine, besides playing an important paper in the iron mobilization (Bremen, J., 1983: López-Cortés, A.R., 1990).

The systems of solar saltworks ponds for the sea salt production are constituted for sequential interconnected evaporation ponds that are fed with seawater, which evaporates taking advantage of the solar energy. In these ponds, they inhabit bacterial consortia adapted to the environmental conditions (temperature, salinity, pH, etc.), that they contribute from an important way to the degradation of the organic matter present in the sea water, this process is very important to the obtain sea salt of better quality. This degradation process, is consequence of the activity of extracellular enzymes produced by microorganisms presents in the water column and mats in the bottom of ponds. Proteases, cellulases, chitinases and lipases contribute to the elimination of the organic matter organic present in the feeding water.
The aim of this work was to evaluate the possibility to use the canonical correlation analysis to establish the existent relationship between the environmental conditions and the evolution of the enzymatic activities in a system of solar saltworks, using as model of study the proteolytic activity evolution in the system of solar saltworks of the company, Industria Salinera de Yucatán, S.A., located in "Las Coloradas" municipality of "Rio Lagartos", Yucatán, México.

AREA STUDIED

"Las Coloradas" is a community 100% focused to sea salt production, dedicated to the production of salt in the same place in which the Mayan civilization harvested this mineral at least 2100 years ago (COSYSA, 08/07/2008).
The activities for sea salt production are developed in an ecosystem that together with "El Cuyo" form the internal basins of the complex coastal lagunar system of "Rio Lagartos", located in the coast of the state of Yucatan, their geographical situation is 21º 34´ and 21º 36´ North latitude, and 87º 51´ and 88º 13´ West longitude (Castañeda, L.O., 1994). The scarce exchange of water between these internal basins and that of "Rio Lagartos", generate favorable conditions that increase the salinity of the internal parts, being formed this way, an hypersaline environment with a content of salt that fluctuates between the 40 and 180 g/liter), suitable condition for the production of salt by evaporation process (Herrera-Silveira, G.A., 1995).

MATERIALS AND METHODS

Sampling stations

This study was carried out in the system of solar salt ponds of the company, Industria Salinera de Yucatán S. A. (ISYSA), figure 1. Which is located in "Las Coloradas",
municipality of "Rio Lagartos", in the state of Yucatan, México. The system has 31 evaporation ponds, of which 26 are only active, and in 13 of these last ponds, the sampling stations settled down. Taking as selection approaches the fact that the Dr. Joseph S. Davis of the Department of Botany of the University of Florida has carried out in them studies related with the structure and function of the biological communities and that in these ponds areas are located areas of low, medium and high salinity.
The sampling stations were: "San Fernando", "Chocolatera", "Vaso 6", "Via 3", "Chel", "Polkos", "Chojón", "Anteojo Morano", "Poceb Xac", "Soledad", "Nohoch", "Chichan" and "Zapote".
"San Fernando" is the feeding pond in consequence of low salinity, the last pond, "Zapote", corresponds to the pond of high salinity.

Sampling System

The period of sampling was of nine months for the 2001 and three for the 2002. The samplings were monthly, excepting in the month of April of the 2001 that includes two dates. Two samples for date, one in the subsurface and the other in the bottom of the ponds. Temperature was determined in situ, the salinity was determinated in the laboratory of ISYSA, pH and the proteolytic activities were determined in the laboratory of Instituto Tecnológico de Mérida.

Proteolytic Activity
For proteolytic activity determination, 10 ml of sample of water was centrifuged at 15 000 rpm and 4°C for 15 minutes for obtain the cell free supernatat. Which was used for proteolytic activity determination according to the method of Kunitz, (1947) using as substrate casein, liberated tyrosine was quantifies following the method of Lowry, (1951)

Canonical analysis of Correspondences (ACC).
To determine the relationships between the proteolytic activities and environmental conditions an Analysis of Canonical Correspondence (ACC) was applied (Ter Braak, F.C. 1995), by means of the package CANOCO (Ter Braak, F.C.1991).

RESULTS
Environmental Setting
This study was carried out in 13 evaporation ponds; of variable surface, depth and volume, figure 2. “Chichan” was the pond of smaller surface (293 000 m2) and “Chel” was the bigger (2 292 350 m2). The smallest depth corresponded to the pond “San Fernando” (0.34 m) and the bigger to “Vaso 6” (0.77 m). The pond of more volume (1 260 793 m3) was “Chel” and the smaller (124 479 m3) was “Anteojo Morano”.

The spatial variation of the mean values of temperature, showed a slow increment of the first pond “San Fernando”, with 27.12 ºC until the last “Zapote”, with 31.23 ºC; reaching values bigger than 30 ºC from the sixth pond “Polkos.” The temporal variation of this parameter, presented the lowest mean values of temperature for the months of February and March of 2001 (22.85 and 26.35 ºC respectively) and the highest mean values corresponded to October of 2001 (33.09 ºC) and July of 2002 (34.32 ºC); the temperature in other ponds varied from 28.11 to 31.27 ºC.

The spatial variation of salinity, presented an increment in its mean values from 9.22 to 26.16 ºBe, of the first pond “San Fernando” until the last one “Zapote” and it showed a weak temporary variation; the biggest salinities (of 18.03 to 18.89 ºB) were observed in April and November of 2001 and May of the 2002, and the minor (of 15.27 ºB) in May of 2001; in the other months salinity varied from 16.93 to 17,94 ºB

The spatial mean values variation of pH, showed in the first five ponds lightly alkaline values, of 7.52 at 8.05, and descend in the following five ponds, of 7.48 at 7.42, in the last three ponds these values descended to 7.33. The Temporal variation of pH mean values were bigger in the samples corresponding to the months of principle (of 7.69 at 7.77) and end (of 7.61 at 7.84) of year, in comparison with the other months (of 7.36 at 7.57).

Proteolytic Activity
The maximum levels of proteolytic activity in the surface, figure 3A, were in the ponds “Vaso 6” with 29.26 µg tyrosine / ml and “Polkos” with 22.19 µg tyirosine/ml in the month of September of 2001 Other considerably high values were found in the ponds “Vaso 6” with 10.77 µg tyrosine / ml in March of 2001, “Poceb Xac” with 8.56 µg tyrosine/ml in April of 2001 and “San Fernando” with 7.53 tyrosine µg / ml in March of 2002. The other activities were not bigger than 3.92 µtyrosine / ml g.
The maximum value of proteolytic activity in the bottom, figure 3B, was found in the pond “Chocolatera” with 30.81 µg tyrosine / ml in April of 2001. Other medium values were measured in the ponds “Via 3” with 15.78 µg tyrosine / ml in March of 2001 and “Chel” with 6.34 µg tyrosine / ml in April of 2001. The other activities were not bigger than 4.39 µg tyrosine / ml .

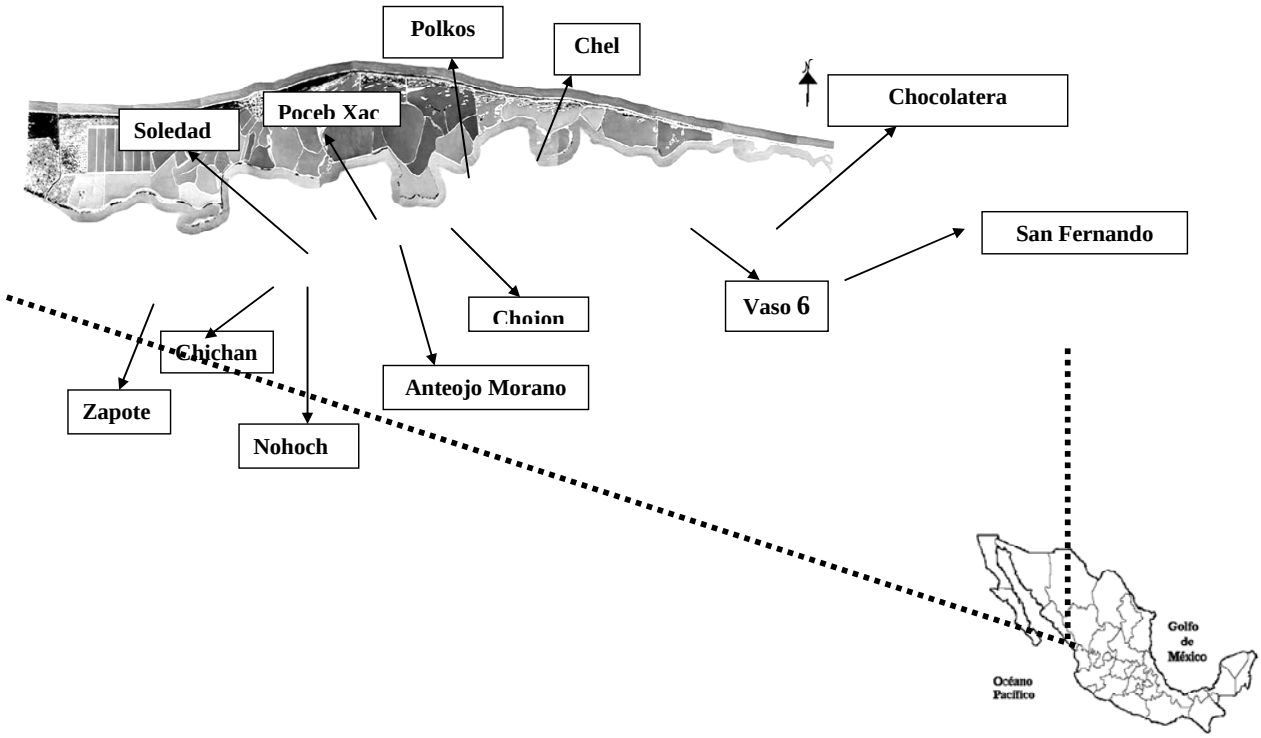

Figure 1. Solar saltworks system of the company Intustria Salinera de Yacatan, S.A.

Analysis of Canonical Correspondence (ACC).

The proteolytic activities of surface and bottom samples showed a tendency to be stabilized in their lower level of the pond 7 (Chojon) from now on, this effect may be was ly owed by the strong environmental stress caused by the increment of the salinity (of 9.22 to 14.82 º B) and changes of the temperature (of 27.12 to 28.97 ºC); as well as for the descent of the level of the pH (of 7.85 at 7.52). The high proteolytic activities in the first ponds, denote the absence of this environmental stress and the presence of microbial consortia which capacity to produce this enzymes.

To determine the tendency of the proteolytic activity with regarding the environmental parameters, an was applied (ACC). This technique provides a general structure for the estimate and statistical confirmation of the effects of the environmental variables; allowing to settle down that the proteolytics activities synthesized simultaneously, are affected by the changes of environmental conditions. When relating the component “environmental variables” with the component “proteolytic activities” the analysis show an explained variance of 71.1 and 60.7 for subsuperficial and bottom samples respectively, that which indicated a very high relationship between both components, Figure 4.

For the subsuperficial samples it was observed a clear relationship of the temperature and salinity with the proteolytic activities in the ponds “Zapote”, “Nohoch”, “Chojon”, “Chichan” and “Soledad”, as long as the volume, area and depth of the bodies of water was related in “Via 3”, “Chel” and “Poceb Chac”, and the pH in “Polkos”, “Anteojo Morano”, “San Fernando” and “Vaso 6”; and for the bottom samples, the biggest volumes in the ponds were related with the proteolytic activities in “Chel” and “Chocolatera”, as long as smaller volume in “Chojon” and “Polkos”, the depth in “Via 3”, the area and pH in “Vaso 6” and finally the temperature and salinity in “Anteojo Morano”, “Nohoch”, “San Fernado”, “Poceb Chac” and “Chichan”.

In both, the subsurfaces and bottom samples, the low levels of temperature and salinity, and high values of pH, were related with high proteolytic activities.

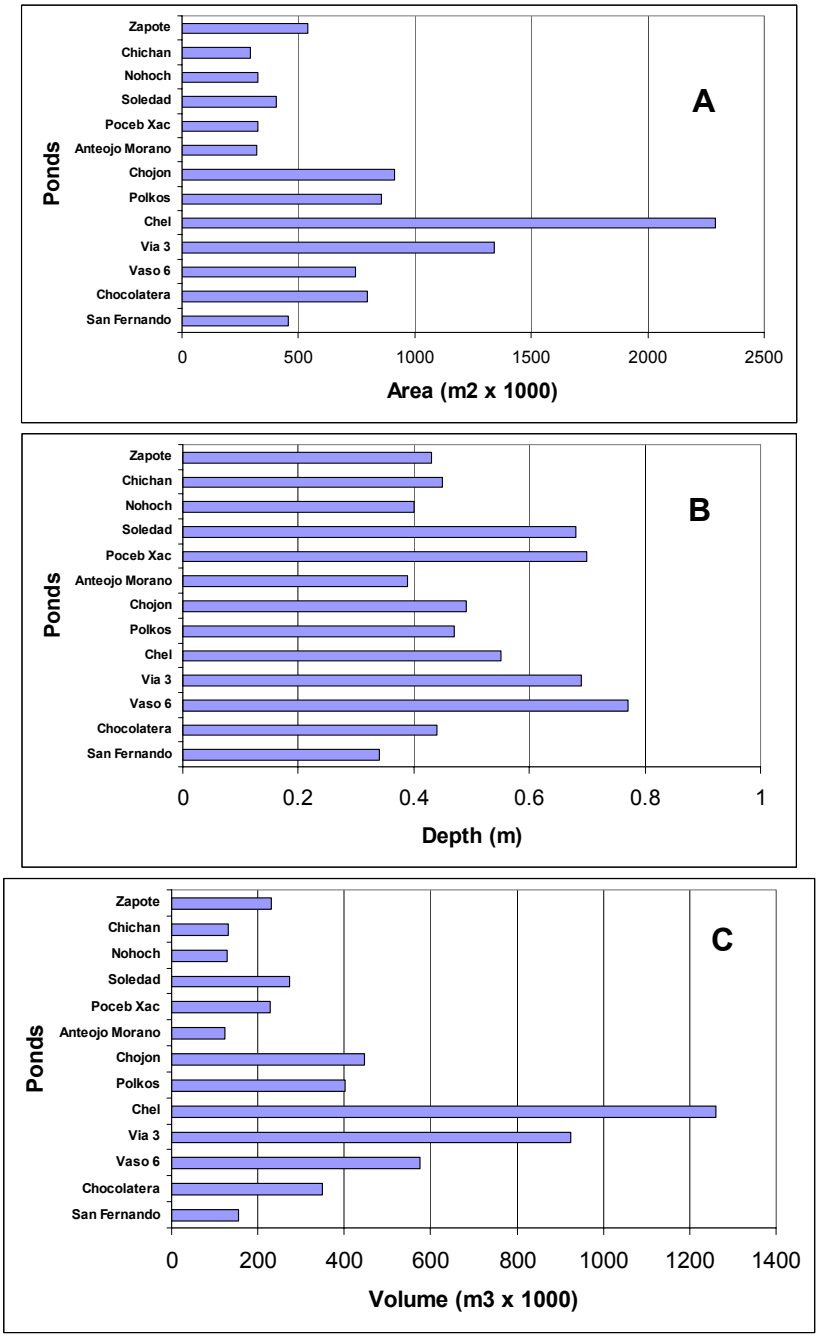

Figure 2. Characteristics of the solar saltworks of the company Industria Salinera de Yucatán, S.A., area (A); depth (B) and volume (C)

While high temperatures and salinities, and low pH values were related with low proteolytic activities.

The drops concentrations of proteolytic activity proteolítica in March, could be due to the low concentration of protein in organic matter; and the high values of this activity in September could be due to high levels of organic matter with protein. In general, the reading of the graphs suggests that pH is the most important factor that affects the proteolytic activity. With regard to higher proteolytic activity in bottom that those of the surface, could be related with the factor temperature; that usually stratifies in the colum of water; this way, in the months of March the enviroment in the bottom of ponds could have been warmer than in the surface; while this you could have been invested in September, where higger proteolytic activity was observed in the surface.

CONCLUSIONS

When proteolytic activity was determined it was found that in the ponds with more environmental stress were presented low activity. This suggests that besides the concentration of organic matter with high concentration of protein, the environmental stress affects from a negative way the proteolytic activity.

The Analysis Canonical of Correspondence shows that relation between the component "variable environmental" with the component "proteolytic activities" have to very high relationship between both components in subsurfaces and bottom samples.

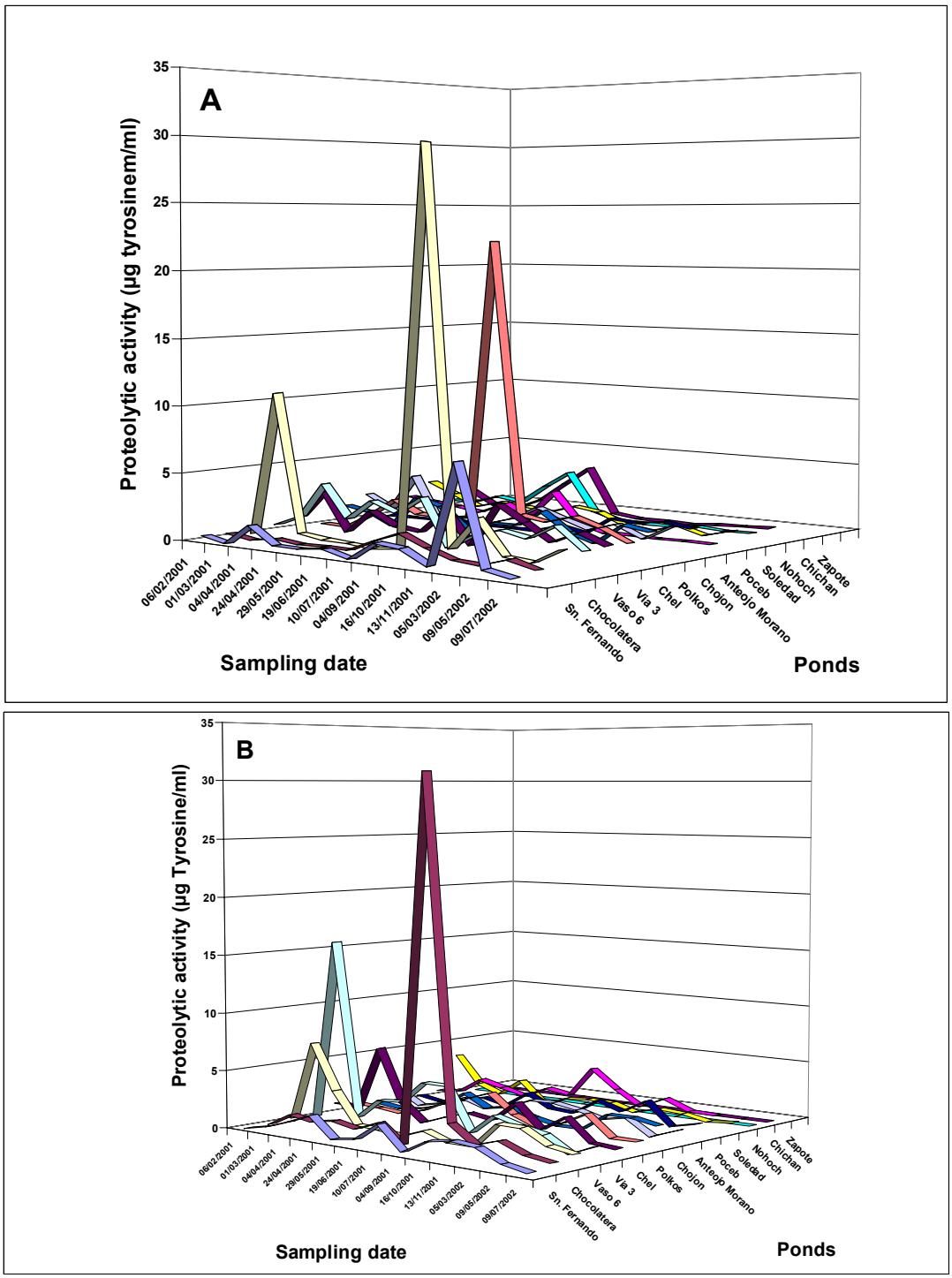

Figure 3. Temporal and spatial variation of proteolytic activity on the surface (A) and the bottom (B) of the solar saltworks of the company Industria Salinera de Yucatán, S.A.

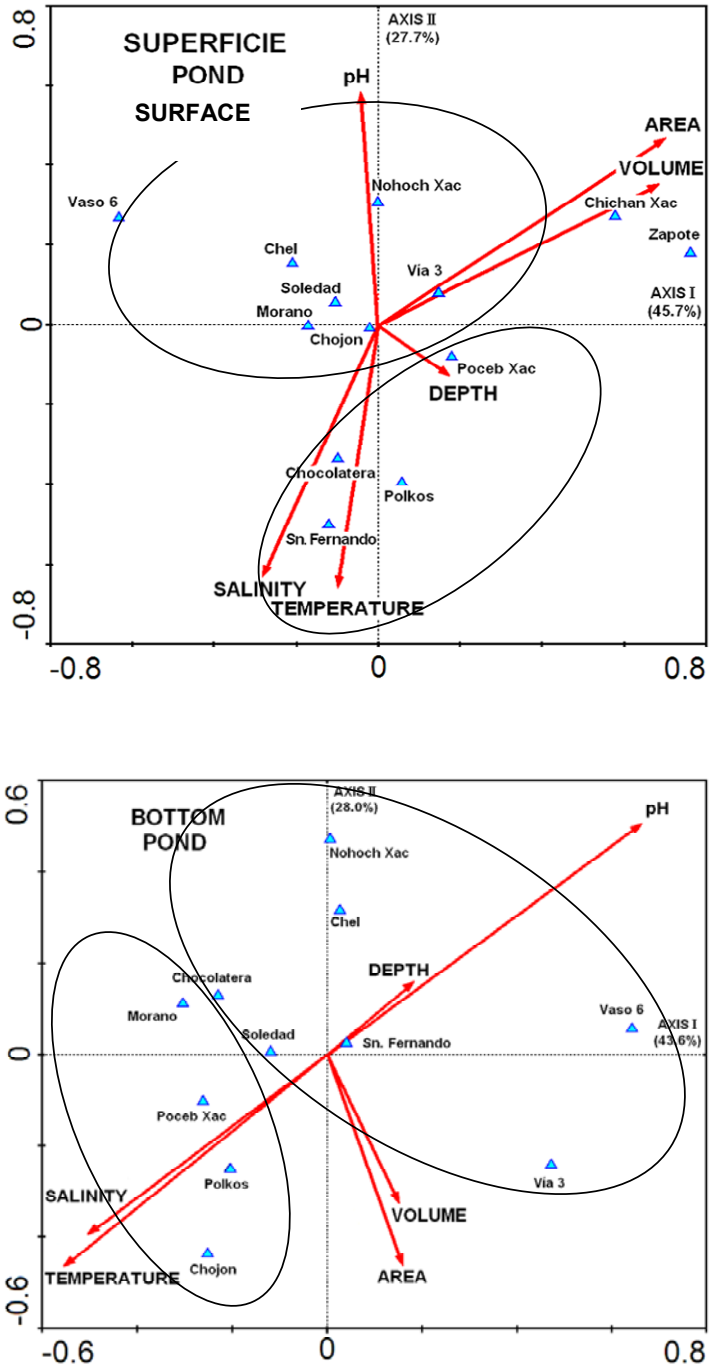

Figure 4. Diagram ACC for proteolytic activity and environmental conditions on the surface (A) and the bottom (B) of the solar slatworks of the company Industria Salinera de Yucatán, S.A

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