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CHOICE OF MACROPHYTE SUBSTRATE IN THE USE OF DIATOMS AS INDICATORS OF POND WATER QUALITY ASSESSMENT: PRELIMINARY DATA ON THE CASE OF ALALAY POND (COCHABAMBA, BOLIVIA)

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Abstract
Alalay Pond is a 230-hectare, shallow aquatic ecosystem within Cochabamba, the third largest city in Bolivia. With the aim to determine the suitability of epiphytic diatoms for water quality assessment in the pond and to choose a substrate that would hold a representative bioindicator epiphytic community, the macrophytes *Schoenoplectus californicus* subsp. *tatora* (Kunth) T. Koyama, *Typha dominguensis* Pers., *Myriophyllum verticillatum* L. and *Azolla filiculoides* Lam. were selected and differences in diatom community composition and structure were tested. Diatoms were collected during four sampling campaigns in the March-September, 2011 period, from three stations contiguous to the pelagic zone and prepared for analysis using standard, internationally used protocols. In all, 27 samples were collected from which 28 taxa characteristic of eutrophic environments were identified and 17 others could not be assigned names from the literature. Although many of the species are shared among sampling sites, the epiphytic communities developing at each station were different in structure and composition. Although, there are marked temporal variations in community features on each of the macrophytes, Shannon-Wiener and Pielou indexes, as well as canonical correspondence analysis, showed no marked differences within a single campaign and station among the 4 macrophytes. Shifts in structure and composition are denoted less commonly by species replacement and more often by changes in percent relative abundance of dominant and rare species. Very few species are restricted to a station or seem to show strong preference for a particular substrate. Taking into account growth, structural and ecological characteristics, as well as some phenological features of the macrophytes, *S. californicus* subsp. *tatora* and *T. dominguensis* are the most suitable substrates for water quality assessments in the pond. As demonstrated by multivariate analyses, among-site and time-scale differences in community composition and structure

were attributable to alkalinity, chemical oxygen demand (COD), ammonium-nitrogen, conductivity and dissolved oxygen (DO). More frequent sampling, diversification of substrates and extending the study for a longer period are recommended to establish a monitoring program during any future restoration of the pond.

Keywords: Diatoms, Bacillariophyta, epiphytes, substrate, water quality, Alalay Pond, Bolivia

1 INTRODUCTION

The world´s water resources are being degraded at accelerated rates mainly due to human population growth and anthropogenic activities such as industry, urbanization and agricultural land use. As a byproduct of these activities, organic and inorganic waste is constantly deposited directly or indirectly into aquatic systems altering their structure and function (Moreno & colab., 2010; Wetzel, 2001). Thus, anthropogenic activities are a major threat to aquatic ecosystem stability and the magnitude of this threat is related to the volume of discharged effluents and the volume and flux of the water held in the receiving aquatic system (Gómez & Ramírez, 2004; Salomoni & Torgan, 2008). Besides restricting the availability of drinking water, river and lake contamination also reduces the quantity and quality of ecosystem services offered by them (e.g., as habitats, climate regulators, sites for recreation, fishing, etc.) (Jill & colab., 2003). In urban scenarios, discharge of residual waters without previous treatment and rich in nitrogen and phosphorus compounds leads to eutrophication with the implied overgrowth of algae and macrophytes, which later decomposition depletes oxygen in the water column killing aerobic organisms (Brahi, 2004; Camargo & Alonso, 2006; López Cortez & colab., 2003; Moss, 1998). Also, untreated industrial discharges may cause contamination by heavy metals and organic compounds that are persistent in the ecosystem causing distress in food webs and ecosystem functioning.

As a response to water quality degradation, there is an increased interest in the protection, restoration and management of aquatic systems. With the aim to estimate the impact of human activities on water quality, physical, chemical and biological criteria have been used either combined or in isolation. Within the biological criteria, biomonitoring is considered as an effective tool because: a) individuals, populations and communities show integrated responses to their surroundings, thus reflecting continuous fluctuations in water quality; b) biological responses to environmental change are a direct measure of the ecological integrity of an ecosystem; and

c) because the maintenance of biological diversity and integrity is usually sought during environmental management activities, the monitoring of these characteristics (rather than physical and chemical aspects) is desired (Blanco & colab., 2004; Lobo & colab., 2004).

In order to better visualize change in an aquatic system and to summarize great amounts of information into single numbers that are easier to understand, a plethora of indexes of biological integrity have been proposed. These indexes make use of autecological characteristics of individual species or sinecological traits of communities or higher taxonomic groups (Stoermer & Smol, 2001). Index calculation is usually based on taxa relative abundance and optima and tolerances to water chemistry and other habitat conditions (Bere & Tundisi, 2010; de la Rey & colab., 2004; Patrick, 1986; Potapova & colab., 2004). Diatoms – unicellular, microscopic, photosynthetic eukaryotes in the Kingdom Chromalveolata– are one of the groups whose optima and tolerances to environmental factors are relatively easy to determine, thus being excellent indicators of ecosystem health (Bere & Tundisi, 2010; Pan & colab., 1996; Rimet & colab., 2005;). Dozens of studies show the suitability of diatoms in the calculation of indexes (Blanco & colab., 2004; CEMAGREF, 1982; Dell'Uomo, 2004; Dell'Uomo & colab., 1999; Descy, 1979; Gómez & Licursi, 2001; Kelly & Witthon, 1995; Prygiel & Coste, 1993; Sládecek, 1973, among many others). In the case of lentic water bodies, the following indexes have been used: Trophic Index (Hofmann, 1994), Trophic Diatom Index for Lakes (Stenger-Kovács & colab., 2007), the S Index (Sgro & colab., 2007), and the Sodic Conductivity Index for Lakes (Ács & colab., 2005). Schaumburg & colab., (2004) and Kelly & colab., (2008) pondered the use of epiphytic diatoms (those growing on plant submersed substrates) as effective bioindicators. Among the characteristics of this community favorable to the calculation of indexes are that a) they are usually diverse and well structured; b) they reach ecological climax in short periods of time; c) may be in contact with the water column, rather than the sediments; and d) their collection is rather easy.

Because there are usually several macrophytes growing in an aquatic system and because there are differences in their structure (e.g., leaf and stem surface area and relief), choice of macrophyte as a substrate becomes a critical issue in the selection of the most suitable epiphytic community for bioindication purposes. The present work deals with the selection of such substrate among four macrophytes growing in Alalay Pond, a eutrophic aquatic system located within the urban limits of the city of Cochabamba, Bolivia. The selection of the best substrate is based on epiphytic community compositional and structural characteristics, as well as structural and biological features of the selected macrophytes.

2 MATERIALS AND METHODS

2.1 Study area

Alalay Pond is located in the city of Cochabamba, Bolivia (latitude: 17°23’43” S, longitude: 66°09’35’’ W) (Figure 1). Situated at an altitude of 2560 m a.s.l., it has a surface of 230 ha, a mean depth of 0.5 to 3 m and it is the largest shallow pond in the South American Interandean Dry Valleys. The pond is an artificial system dug during the 1930s in order to serve as a relief from the frequent flooding produced by the Rocha River, the man lotic system crossing the city (Maldonado & colab., 1998). In order to augment the level of water to be used for recreational, fishing and agricultural purposes, a second inlet was built based on waters from La Angostura, a large reservoir located south of the pond (Maldonado & colab., 1998). The pond has a unique outlet, which conducts excess water back to the Rocha River. The uncontrolled urban growth that has characterized Cochabamba in the last fifty years has seen a dramatic shift in the land use around the pond. Nowadays, the pond is completely surrounded by urban developments with a very thin belt of surrounding vegetation, which in some parts of the pond´s perimeter is completely absent.

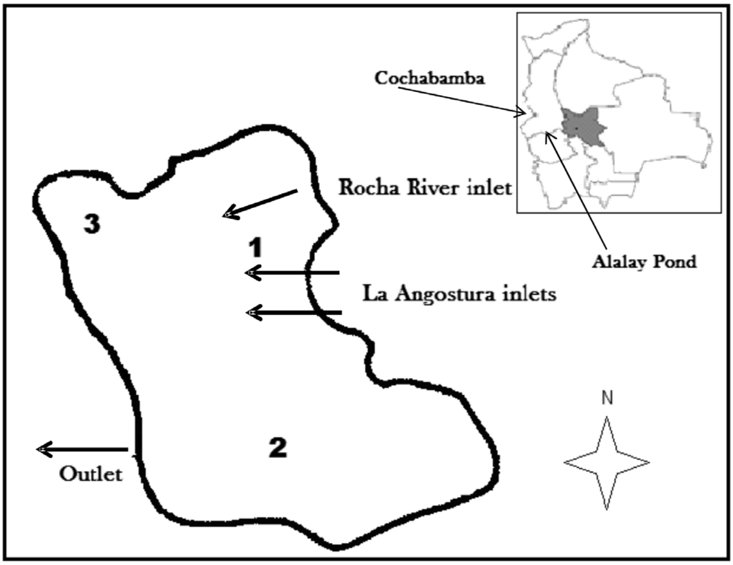

Figure 1. Location of Alalay Pond in the city of Cochabamba, capital of the Department of the same name. Numbers in pond outline depict sampling stations.

The pond itself contains profuse growths of emergent macrophytes (*Schoenoplectus californicus* subsp. *tatora* (Kunth) T. Koyama and *Typha dominguensis* Pers.), submersed macrophytes (e.g., *Myriophyllum verticillatum* L.), and floating macrophytes (*Azolla filiculoides* Lam. and *Pistia stratiotes* L.) (Arrazola, 2000; Ayala & colab., 2006; Bayro, 2000; HAMC, 2003; HAMC, 2006). These plants favor the development of a diverse community of birds, insects, and some amphibians and reptiles, thus highly contributing to the maintenance of the biodiversity found in the pond. Additionally, it is possible, although yet undemonstrated, that *Schoenoplectus californicus* subsp. *tatora* and *Typha dominguensis* act as barriers to contaminants entering from the watershed.

The pond is currently highly eutrophic and contaminated due to human-related effects such as sewage discharge, surface runoff carrying, household garbage pollutants (all affecting sampling stations 1-3, see Figure 1), and inputs from clothing and plastic factories (affecting mainly station 2). The Rocha River waters also hold large amounts of nutrients and organic matter, mainly affecting station 1. Despite several attempts to recover and restore the pond, its eutrophic and contaminated condition has been continuous for at least the last four decades. Recent studies show that water discharges into the pond contain traces of heavy metals (mainly zinc, led, copper, chromium and mercury), although their effects on the biota and ecosystem integrity have yet to be studied (Pérez, 2005).

The first monitoring studies in Alalay Pond commenced in 1979 and since then, several other studies have been performed on diverse aspects of the biology and limnology of the system, but none of them have derived in a continuous monitoring or an effective recuperation program (Ayala & colab., 2007; Balderrama & colab., 1998; Barra & colab., 1983, 1993; Cadima, 1998; Castellón, 2001; Maldonado & Goitia, 1993; Maldonado & colab., 1998; Meneses, 1996, 1998; Morales, 1993; Morales & Trainor, 1996; Arias, 1998; Romero & colab., 1998; UMSS, 1990; Van Damme & colab., 1997).

2.2 Sample collection, preparation and analysis

Three sampling stations were set for the collection of biological samples and physico-chemistry based on the distribution patterns of the macrophyte vegetation around or within the open waters of the pond, since the main interest was the determination of open water quality, rather than the conditions of the water among the aquatic vegetation belt along the coast of the pond. Four macrophytes were selected for the study, two emergent plants (*S. californicus* subsp. *tatora* and *T. dominguensis*), one submersed

(*M. verticillatum*) and the floating macrophyte *A. filiculoides*. Sample collection generally followed Blanco & Bécares (2006) and was done four times in the March-September, 2011 period at intervals of 60 days among samplings. Two of the sampling campaigns fell within the dry period and the other two during the wet period of the year; being close to the Equator, the climate in Bolivia is typically tropical and the four seasons of higher latitudes are practically replaced by a warm rainy season (October to April) and a dry cold season (May to September). At each site 20 cm stem cuttings of each macrophyte were extracted from 20 cm below the water surface, stored separately in plastic bags and transported to the laboratory where epiphytes were removed with a brush and oxidized with hot nitric acid. In the case of *A. filiculoides*, a handful of plants (about 50 individuals on average) were collected and treated in the same manner as the other plants. After repeated rinsing and decantation with distilled water, air-dried aliquots were mounted on permanent glass slides using Naphrax®. At least 600 valves were identified and counted under the light microscope at a magnification of 1250X using a Zeiss Universal microscope equipped with Nomarski interference contrast and a Plan 100X, 1.25 NA, immersion objective. Identification was done based on South American floras such as Metzeltin & Lange-Bertalot (1998), Metzeltin & colab. (2005), Metzeltin & Lange-Bertalot (2007), Rumrich & colab. (2000). Taxonomic articles by Frenguelli (1939), Manguin (1964), Morales & Vis (2007), Morales & colab. (2007), Servant-Vildary (1986), and the annotated checklist by Hohn (1966), were also utilized. In limited cases, European references were also used especially for the identification of cosmopolitan taxa or species characteristic of eutrophic habitats (Krammer 1997a, 1997b, 2000; Krammer & Lange-Bertalot 1986, 1988, 1991a, 1991b; Lange-Bertalot 1993, 2001; Lange-Bertalot & Moser 1994).

The structure of the epiphytic diatom communities was determined by calculation of the Shannon-Wiener diversity index and the Pielou uniformity index. Statistical analysis and graphics were produced using CANOCO v. 4.5 and EXCEL 2010. The plate with digitally handled micrographs of representative specimens was elaborated using Photoshop CS3.

3 RESULTS AND DISCUSSION

3.1 Growth characteristics and other aspects of the four macrophytes

Azolla filiculoides provides a great surface area susceptible to colonization by diatoms and other algae. The undersides of leaves and the profuse root system, both with a rugged relief, are always in contact with the water, thus favoring nutrient exchange with the medium. *Azolla* is also known to be in close association with cyanobacteria (Armstrong, 1979, 1985), thus being able to trap nitrogen, which further favors its colonization by other nitrophilous groups such as diatoms. The floating growths of this macrophyte never settle in just one place. Strong N-S winds present in the pond catchment area constantly push these floating mats towards the eastern or southern portions of the pond. Proof of this is the complete absence of *Azolla* from the third station (to the northwest) during the whole period of study. Additionally, this macrophyte is not perennial and it is present only during a portion of the year, mainly during the wet season, dying off as the wintery dry season sets on (as also reported by Ferentinos & colab., 2002). Although present in the first sampling station during the period of study, *Azolla* was only present in station 2 during the third sampling and it was completely absent from the third station. This macrophyte is adapted to live in a range of trophic conditions, but seems to be more frequent in eutrophic habitats (Serag & colab., 2000). Although it might be thought that profuse growths of *Azolla* could reduce algal development by shading (Ferentinos & colab., 2002), the epiphytic communities found on this macrophyte in Alalay Pond were always well developed and 600 valve counts were always reached from this substratum.

Myriophyllum verticillatum is more common in Alalay Pond in areas where the two emergent macrophytes were not present (see below). Profuse growths of this macrophyte were more evident toward the second sampling campaign. During the third and fourth campaigns, navigation was impossible due to overgrowth of *M. verticillatum* beds and sampling stations had to be reached by accessing the pond directly from adjacent coasts. This macrophyte lives in nutrient enriched habitats (Klinkenberg, 2012), it grows submersed (except for the inflorescences) and its thallus structure (erect round stems and highly dissected leaves) offers an extensive surface area to be colonized by algae. The diatom communities growing on this substrate were mostly well developed, although site 2 and 3 yielded counts lower than 600 valves during the first sampling. This was probably due to the presence of contaminants preventing epiphytic growth, as suggested by COD values (170 and 259 mg O_2/L, respectively). Although present during the 4 sampling campaigns at stations 1 and 2, *Myriophyllum* appeared only during the first and fourth samplings at station 3. This is probably explained by a contamination-related loss of the submersed vegetation cover during the second and third samplings and a re-colonization of this area by population dispersal and receding of the contamination state towards the fourth campaign. Although, *M. verticillatum* is a perennial plant (Margalef, 1981;

Sánchez, 2011), its populations might be drastically reduced in Alalay Pond mainly due to competition by *P. stratiotes*, which flourishes during the earlier part of the year.

Schoenoplectus californicus subsp. *tatora* is probably the macrophyte with the highest biomass in Alalay Pond; it presents extensive continuous growths around the coast of the pond, except in the northern and northwestern shores. Especially in the eastern and southeastern parts of the pond, the growths have gained terrain in the last few years reducing considerably the pelagic zone. As reported for other parts of Bolivia, this emergent macrophyte grows in eutrophic conditions (Fontúrbel, 2005; Fontúrbel & colab., 2006). Being perennial, *S. californicus* subsp. *tatora* provides substrate for epiphytes all year round. Even with water level fluctuations, a considerable portion of the stems of this macrophyte remains under water, thus assuring a constant presence of epiphytes. There is a certain ruggedness to the surface of the stems of this macrophyte, which might favor the establishment of complex epiphytic communities. It is unclear why during sampling campaigns 1, 2 and 4 valve counts were lower than 600 valves, but it might be due to a higher predation rate on this substrate that usually hosts a higher diversity of benthic feeders (insect larvae and other invertebrates, as well as ciliates) than the other three substrates, although this is only a pure observational argument that merits further research.

Typha dominguensis is restricted to the northern and northwestern parts of the pond, where growths are not extensive, but sufficient to provide nesting sites for birds. *T. dominguensis* is well adapted to live in nutrient enriched situations and forms dense growths in eutrophic conditions (Macek & colab., 2010; Marotta & colab., 2009). Since the habit of this macrophyte is perennial, a substrate for epiphytic growth is assured during the whole year, water level change being of little concern also in the case of this macrophyte. Although somewhat smooth to touch, microscopically (as evidenced by a dissecting microscope), the stems of this macrophyte also present a certain ruggedness, thus favoring the attachment of algae, bacteria and other microorganisms. The diatom communities growing on this macrophyte were profuse during the period of study, and counts of 600 valves were reached with most samples, except that collected from the third sampling station during the first campaign. This lack of diatoms appeared to be correlated to the hypoxic, strongly contaminated conditions of this station as denoted by DO (1.83 mg O_2/L) and COD (259 mg O_2/L) values.

3.2 Taxonomic composition and structure of epiphytic diatom communities

A total of 20 diatom genera containing 45 species were recorded from the three sampling stations during the four campaigns (a complete list of taxa will be published later by the authors). This richness is low and comparable to reports of epiphytic diatoms from other parts of the world (e.g., Himalayas: Simkhada (2006), Patagonian Andes: Díaz Villanueva (2006), Carpathians: Marosi & colab. (2006), Carolina Bays, North America: Gaiser & Johansen (2000). From the 45 species, 39 appeared in counts and the remaining were detected during qualitative analyses performed prior to each count. Twenty eight species were assigned names from the literature, while 17 corresponded to unknown taxa, which after future detailed analysis under light and electron microscopy could yield species new to science. Nine of the most abundant species are represented in Figure 2.

The best represented genera were *Nitzschia* Hassall with 19 species and *Gomphonema* Ehrenberg with 5 taxa. The rest of the genera were represented by only 1 or 2 species. *Nitzschia* and *Gomphonema* have been reported by other authors as among the most specious taxa in Bolivian Andean ecosystems (Frenguelli, 1939; Morales & colab, 2010; Servant-Vildary, 1978). *Nitzschia* (10 morphotypes) and *Gomphonema* (4 morphotypes) are also the two genera with the maximum amount of undetermined species, which agrees with previous records in the literature for Bolivia (Álvarez-Blanco & colab. 2011; Morales & colab., 2010). This also suggests that despite the importance of these two genera for the diatom flora of Bolivia, they are among the less studied diatoms, with obvious negative repercussions on water quality studies.

Regarding dominance and abundance, 7 taxa had relative abundances higher than 5% (*Epithemia adnata* var. *proboscidea* (Kützing) Hendey, *Amphora paraveneta* Lange-Bertalot et al., *Gomphonema* sp. 1 ALALAY *Gomphonema* sp. 2 ALALAY, *Synedra tabulata* var. *gracillima* Tempere & Peragallo, *Nitzschia* sp. 1 ROCHA, *Lemnicola hungarica* (Grunow) Round & Basson) (Figure 2), 2 had relative abundances between 2 and 5% (*Epithemia goeppertiana* Hilse and *Nitzschia amphibia* Grunow) (Figure 2), and the remaining 36 had relative abundances less that 1%.

During the study, the Shannon-Wiener diversity index fluctuated between 0.03 and 3.03, while the Pielou index oscillated between 0.03 and 0.96, showing that diatom epiphytic diversity in the pond is low (Table 1). There is no consistent pattern showing a higher diversity of epiphytes on either macrophyte throughout the period of study or at any given collecting site (Figure 3). What is clear is the temporal variation in the structure of the epiphytic communities within each site (temporal) and among sites (spatial) as the collecting campaigns progressed (Figures 3 and 4; see discussion in the next subsection below).

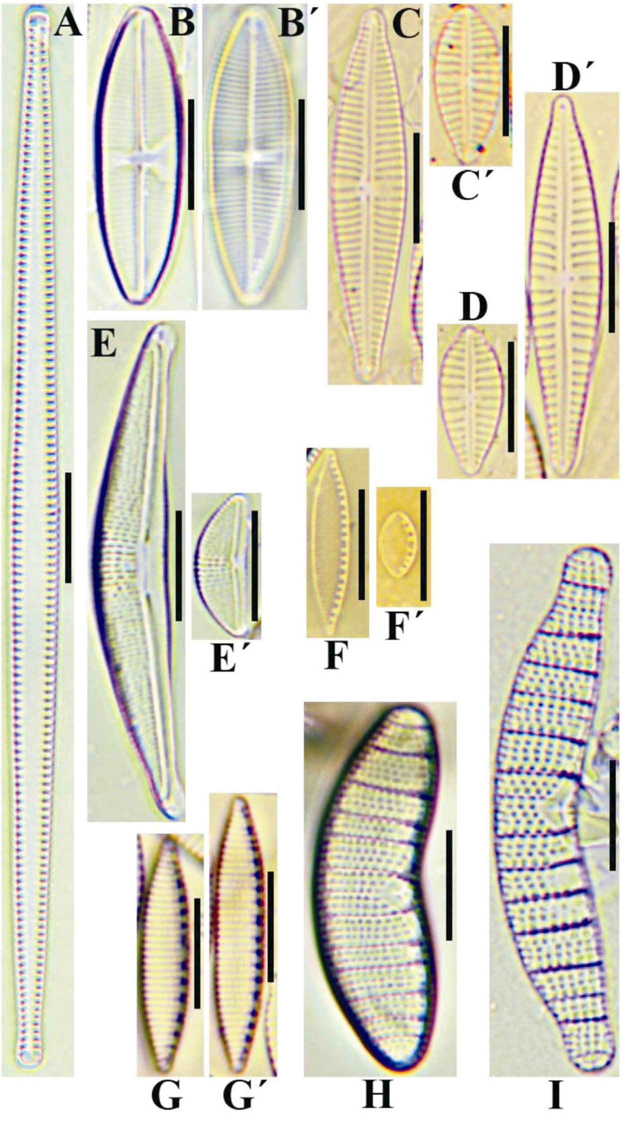

Figure 2. LM micrographs of nine of the most common epiphytes found during the study. A. *Synedra tabulata* var. *gracillima*. B-B´. *Lemnicola hungarica*, rapho and araphovalves, respectively. C-C´. *Gomphonema* sp. 1 ALALAY. D. *Gomphonema* sp. 2 ALALAY. E-E´. *Amphora paraveneta*. F-F´. *Nitzschia* sp. 1 ALALAY. G-G´. *Nitzschia amphibia*. H. *Epithemia goeppertiana*. I. *Epithemia adnata* var. *proboscidea*. Scale bars=10 µm.

Table 1. Shannon-Wiener (H´) and Pielou (J) indexes for each of the 4 macrophytes growing at the 3 sampling stations. Each column shows the change of an index over time as sampling campaigns proceed. Highest and lowest index values for each macrophyte are highlighted in darker gray. NP=Not present.

		SCHOcalif		MYvertic		AZfilic		TYdoming	
		H´	J	H´	J	H´	J	H´	J
Station 1	**SC1**	0.72	0.28	0.94	0.28	1.32	0.42	NP	NP
	SC2	2.48	0.61	1.48	0.47	2.71	0.73	NP	NP
	SC3	3.03	0.76	2.84	0.73	2.66	0.72	NP	NP
	SC4	2.64	0.65	2.56	0.71	2.34	0.66	NP	NP
Station 2	**SC1**	1.52	0.96	0.99	0.32	NP	NP	NP	NP
	SC2	0.88	0.27	1.3	0.36	NP	NP	NP	NP
	SC3	0.99	0.33	0.66	0.29	1.56	0.77	NP	NP
	SC4	2.06	0.88	0.91	0.3	NP	NP	NP	NP
Station 3	**SC1**	NP	NP	0.76	0.28	NP	NP	1.44	0.62
	SC2	NP	NP	NP	NP	NP	NP	0.03	0.03
	SC3	NP	NP	NP	NP	NP	NP	1.07	0.67
	SC4	NP	NP	0.23	0.27	NP	NP	1.67	0.83

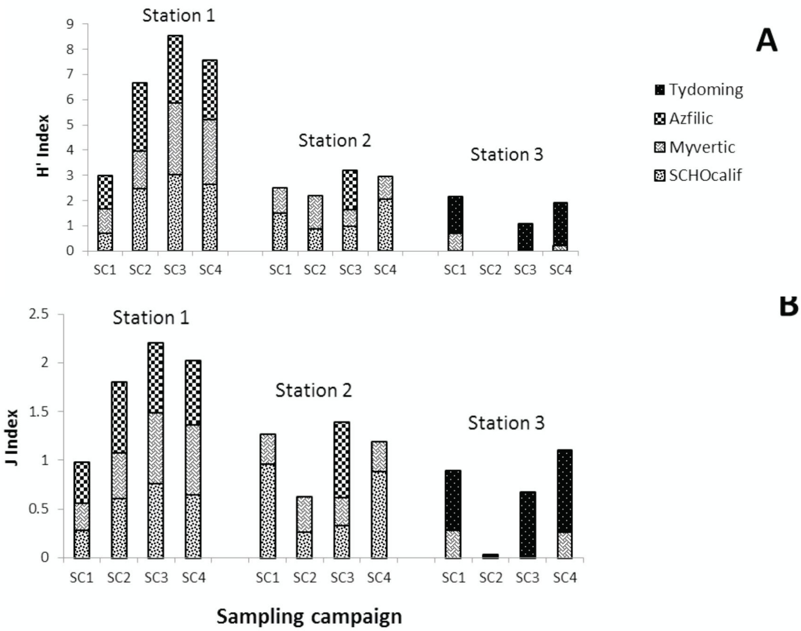

Figure 3. Temporal and spatial variations in the values of the Shannon-Wiener (A) and Pielou (B) indexes during the period of study. Each pattern represents a macrophyte

Of the 28 known taxa, 19 are reported as eutraphentic to hypereutraphentic by Van Dam & colab. (1994), while the 9 remaining taxa were not characterized in this reference. From the unknown morphotypes, *Gomphonema* sp. 2 ALALAY (15.5%), *Nitzschia* sp. 1 ROCHA (7.7%) and *Gomphonema* sp. 1 ALALAY (5.2%) presented abundances higher that 5%, which might be an indication of at least a tendency of these entities to occur in eutrophic habitats.

3.3 Spatial and temporal variation of epiphytic diatom communities

The composition of epiphytic communities developing on the 4 macrophytes of any given site did not vary significantly. This pattern holds true for all sampling dates, thus suggesting that at least the 7 taxa having abundances higher than 5% do not seem to strongly prefer a particular substratum. This is corroborated by the ANOVA analyses (Table 2), as well as the diversity and uniformity indexes as discussed above (Table 1).

Table 2. EXCEL results of F test comparing epiphytic community variances on the macrophytes present at each station during each of the sampling campaigns. If values in the F column are lower than those in Fc one, then the compared communities are not different from each other. NC=Not calculated because only 1 macrophyte was present at site.

	F	p	Fc
SC1-Station 1	3.55 E-15	0.99	3.29
SC1- Station 2	0	0.99	7.71
SC1- Station 3	2.18 E-15	0.99	4.75
SC2- Station 1	0	0.99	3.15
SC2- Station 2	0.68	0.42	4.2
SC2- Station 3	NC	NC	NC
SC3- Station 1	0	0.99	3.16
SC3- Station 2	1.3 E-15	0.99	3.47
SC3- Station 3	NC	NC	NC
SC4- Station 1	0.01	0.99	3.17
SC4- Station 2	1.66 E-15	0.99	4.6
SC4- Station 3	0	1	4.97

From the taxa having abundances higher than 5%, *Amphora paraveneta* was the only taxon that occurred in all substrata at the 3 stations during the four campaigns, *Lemnicola hungarica* was restricted to the first station, but it occurred on the 3 macrophytes present there from the second to the fourth campaigns (Figure 4). From the species reaching abundances lower than 5%, *Amphora coffeaeformis* (Agardh) Kützing and *Cymbella excisa* Kützing grew only on *S. californicus* subsp. *tatora* and were registered only during the second sampling campaign. Also restricted to this same macrophyte were *Cyclotella meneghiniana* Kützing, *Nitzschia angusteforaminata* Lange-Bertalot and *Nitzschia communis* Rabenhorst, but they were found during all sampling campaigns at the 3 sampling stations. There are no clear patterns of occurrence for the remaining identified taxa.

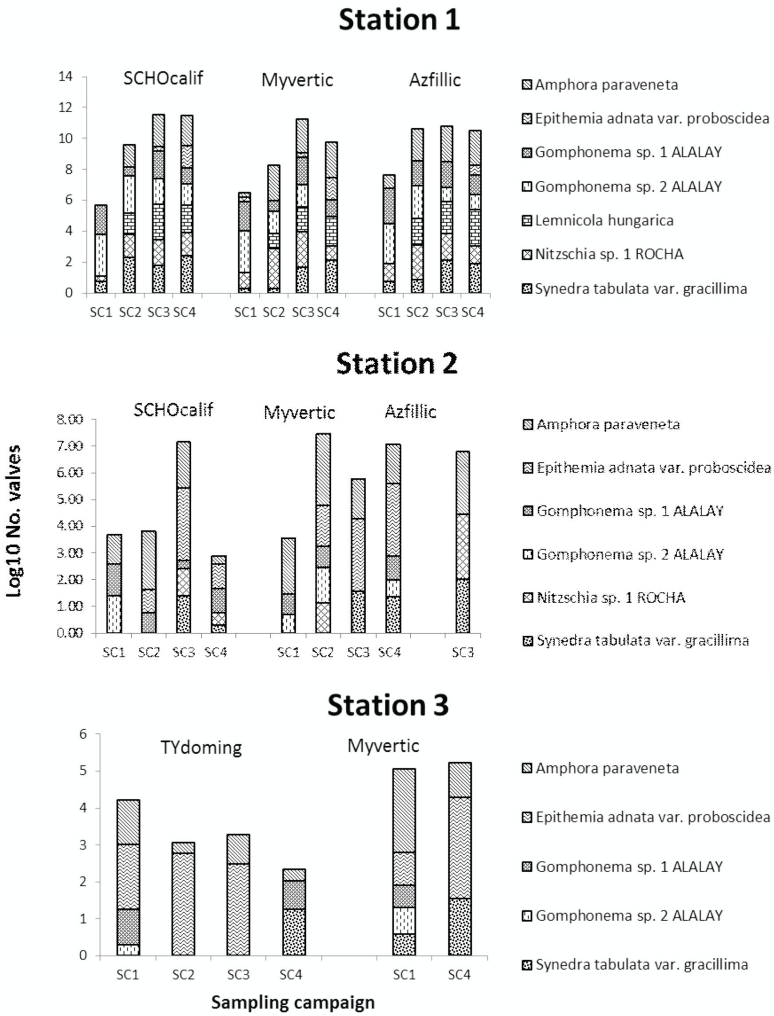

Figure 4. Spatial and temporal variation of the 7 taxa with abundances higher than 5%

Comparisons among sites reveal that there are differences in the structure of the communities developed and that these differences are attributable to water chemistry variations over time (Figure 5). In Figure 5, different substrata appear plotted together by station and by date of collection (a further demonstration that there is not a typical community associated to any of the substrata) and that they are arranged following values in measured environmental variables, being COD, alkalinity and specific conductance the main drivers of the observed pattern. It is not surprising to see shorter vectors for N, P, and S in Figure 5 since eutrophic conditions must be supplying excess amounts of these nutrients, under which conditions other than water chemistry variables become responsible for observed community responses (Wetzel, 2001).

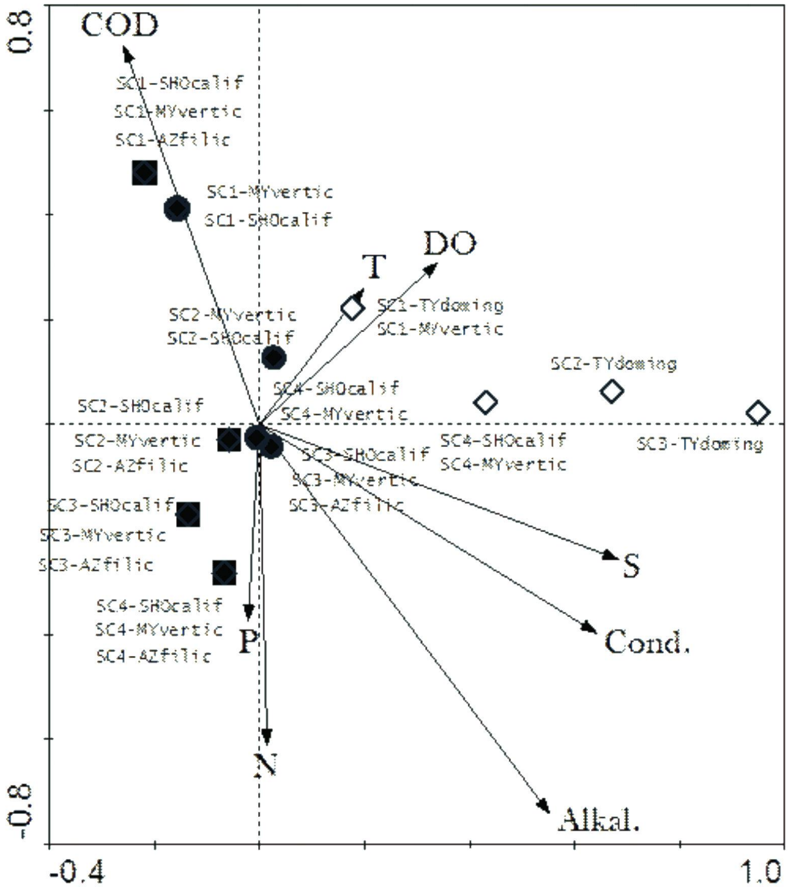

Figure 5. CCA analysis showing the relationship of the communities developing at each stations, on each macrophyte and in each of the sampling campaigns as a function of measured water physic-chemistry.

At any given site, changes can also be seen in community structure over time (Figures 3 and 4). First, there is a replacement of taxa by newcomers as in the case of Figure 4B on *Schoenoplectus californicus* subsp. *tatora*, where *Gomphonema* sp. 1 ALALAY is replaced by *Epithemia adnata* var. *proboscidea* from the first to the second campaigns and by the third and fourth samplings, *Nitzschia* sp. 1 ROCHA and *Synedra tabulata* var. *gracillima* had been added to the set of most abundant species. Second, there are shifts in dominance among the most common species, as can be observed for all macrophytes at all stations in Figure 4. Replacements and shifts also occurred among less common species, causing part of the community structure variation.

4 CONCLUSIONS

Epiphytic diatom community structure and composition is similar on the 4 selected macrophytes for each sampling date and station. There are marked differences in the structure of the epiphytes among stations due to different stressors affecting each sampling station. At any given station, there are temporal changes in community structure and composition related to shifts in environmental variables and these changes are similar on the four selected macrophytes.

Azolla filiculoides is restricted to only two of the sampling stations, distribution that is affected by the wind pattern in the pond watershed. The colonization of station 3 by *M. verticillatum* seems to be intermittent and it is probably related to shifts in water quality. Therefore, these two macrophytes can not be reliably used as substrates of epiphytic diatoms.

The macrophytes *S. californicus* subsp. *tatora* and *T. dominguensis*, on the other hand, expose permanent suitable stands for epiphytic diatom development, are well adapted to withstand the eutrophic conditions of the pond, and their growths are easy to access and sample. Epiphytic diatoms are taxonomically and structurally well represented on these two macrophytes, thus becoming the preferred substrata to be used in water quality assessments in Alalay Pond. Both macrophytes should be used in combination since neither occurs in the 3 sampling sites at once.

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6 REFERENCES

Ács, É., Reskónó, NM., Szabó, Taba & Kiss, KT. (2005), Application of benthic diatoms in water quality monitoring of lake Velence. Recommendations and assignments. Acta Botanica Hungarica **47**:211-223.

Álvarez-Blanco, I., Cejudo-Figueiras, C., de Godos, Muñoz & Blanco, S. (2011), *Las diatomeas de los salares del Altiplano boliviano: singularidades florísticas*. Boletín de la Real Sociedad Española de Historia Natural, Sección Biología **105**(1-4): 67-82.

Arias, S. (1998), Proyecto: *Conservación de la Ornitofauna de la Laguna Alalay*. CUEMAD - UMSS: 57 p.

Armstrong, WP. (1979), *A marriage between a fern and an alga*. Environment Southwest **500**: 20-24.

Armstrong, WP. (1985), *A fern-alga symbiosis*. Carolina Tips **48**: 9-11.

Arrazola, V. (2000), *Estudio de comunidades vegetales acuáticas de seis lagunas de los pisos mesotropical, supratropical y orotropical de Cochabamba*. Tesis de Licenciatura en Biología. Universidad Mayor de San Simón, Cochabamba: 106p.

Ayala, R., Castro, M., Bayro, Acosta & Rejas, D. (2006), *Interacciones fitoplancton-zooplancton en una laguna eutrofizada del valle del Cochabamba (Bolivia)*. Ciencias y Tecnología. Facultad de Ciencias y Tecnología. Revista Facultativa **5**: 43-50.

Ayala, R., Acosta & Rejas Danny (2007), *Management of Laguna Alalay: a case study of lake restoration in Andean valleys in Bolivia.* Aquatic Ecology **41**: 621-630.

Balderrama, JA., Sahonero & Arias Saúl (1998), Charadriiformes de la laguna Alalay (Cochabamba, Bolivia). Revista Boliviana de Ecología y Conservación Ambiental **3**:73-78.

Barra, C., del Castillo, M., Maldonado & Sahonero, R. (1983), Sinopsis limnólogica de la laguna Alalay (Cochabamba-Bolivia). Informe Técnico. Departamento de Biología, Universidad Mayor de San Simón: 41p.

Barra, C., Mirtha C., Maldonado, Goitia & Acosta., F. (1993), Diagnóstico limnológico de la laguna Alalay (Cochamba-Bolivia). Publicaciones Facultad de Ciencias y Tecnología. Universidad Mayor de San Simón. Serie Científica **3**: 73-78.

Bayro, V. (2000), *Interacción de la macrofitia y el fitoplancton en la laguna Alalay (Cochabamba-Bolivia)*. Tesis de Licenciatura en Biología. Universidad Mayor de San Simón, Cochabamba: 102p.

Bere, J. & Tundisi, JG. (2010), *Biological monitoring of lotic ecosystems: the role of diatoms*. Brazilian Journal of Biology **70**(3): 493-502.

Blanco, S. & Bécares, E. (2006), *Método de muestreo de diatomeas epífitas en lagunas para la aplicación de la Directiva Marco del Agua*. Tecnología del Agua **277**: 42-47.

Blanco, S., Ector & Bécares Eloy (2004), *Epiphytic diatoms as water quality indicators in Spanish shallow lakes*. Vie Milieu **54**(2-3): 71-79.

Brahi, V. (2004*), L'eau et l'environnement aquatique: l'eutrophisation et l'enrichissement en nutriments*. Chapter 10. Rapport Analytique 2006-2007: 378-395.

Cadima, M. (1998), *Algas y macrófitas de la laguna Alalay (Cochabamba-Bolivia)*. Revista Boliviana de Ecología y Conservación Ambiental **3**: 35-46.

Camargo, J. & Alonso, A. (2006), *Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment*. Environment International **32**: 831-849.

Castellón, D. (2001). *Estrategias de manipulación de la laguna Alalay (Cochabamba)*. Tesis de Maestría en Ciencias Ambientales. Universidad Mayor de San Simón, Cochabamba: 90p.

CEMAGREF. (1982), *Etude des méthodes biologiques d'appréciation quantitative de la qualité des eaux. Rapport Q.E. Lyon*. Agence de l'Eau Rhône-Méditerranée-Corse-Cemagref, Lyon. 218p.

de la Rey, PA., Taylor, JC., Laas, Rensburg & Vosloo A. (2004), *Determining the possible application value of diatoms as indicators of general water quality: A comparison with SASS 5*.Water SA **30**(3): 325-332.

Dell'Uomo, A. Pensiere & Corradetti Daniela. (1999), *Diatomées épilithiques du fleuve Esino (Italie centrale) et leur utilisation pour l'evaluation de la qualité biologique de l'eau*. Criptogamie Algologie **20**(3): 253-269.

Dell'Uomo, A. (2004), *L'indice diatomico di eutrofizzazione/polluzione (EPI-D) nel monitoraggio delle acque correnti.* Linee guida. A.P.A.T.,A.R.P.A.T. 101p.

Descy, JP. (1979), *A new approach to water quality estimation using diatoms*. Nova Hedwigia Beiheft **64**: 305-323.

Díaz Villanueva, V. (2006), *Diatom distribution in a temporary pond from the Patagonian Andes (Fantasma Pond) during drought*. Nova Hedwigia **83**: 459-472.

Ferentinos, L., Smith & Valenzuela Hector (2002), Azolla*. Sustainable Agriculture/Green Manure Crops.* SA-GM-2: 1-3.

Fontúrbel, F. (2005), *Indicadores fisicoquímicos y biológicos del proceso de eutrofización del Lago Titikaka (Bolivia).* Ecología Aplicada **4**(1, 2): 135-141.

Fontúrbel, F., Richard & Crispieri Gónzalo (2006), *Diversidad de flora fanerógama y aspectos ecobiológicos de la unidad vegetal de Aygachi-Cumana-Bahía Cohana, Lago Titikaka (La Paz, Bolivia).* Ciencia Abierta Internacional **30**: 1-19.

Frenguelli, J. (1939), *Diatomeas del Lago Titicaca*. Notas del Museo de La Plata, Botánica **4**: 175-196.

Gaiser, EE. & Johansen, J. (2000), *Freshwater diatoms from Carolina Bays and other isolated wetlands on the Atlantic Coastal Plane of South Carolina, USA., with description of seven taxa new to science.* Diatom Research **15**: 75-130.

Gómez, LM. & Ramíres, Z. (2004), *Microalgas como biomonitores de contaminación*. Revista Cubana de Química **16**(2): 34-48.

Gómez, N. & Licursi, M. (2001), *The Pampean Diatom Index (IDP) for assessment of rivers and streams in Argentina*. Aquatic Ecology **35**: 173-181.

(HAMC) Honorable Alcaldía Municipal de Cochabamba (2003), *Proyecto MANLAKE. Manejo de lagunas urbanas en el Valle de Cochabamba*: 10p.

(HAMC) Honorable Alcaldía Municipal de Cochabamba (2006), *Plan de manejo integral para la conservación y mejoramiento de la laguna Alalay*: 13p.

Hofmann, G. (1994), *Aufwuchs-Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie*. Bibliotheca Diatomologica **30:** 1-241.

Hohn, MH. (1996), *Bacillariphyta*. In: Patrick, R., Aldrich, FA., Cairns, JJr., Druet, F., Hohn, MH., Roback, SS., Skuja, H., Spanger, PJ., Swabey, YH & Whitford, LA. (eds): The Cartberwood Foundation Peruvian-Amazon expedition: limnological and systematic studies. Monographs of the Academy of Natural Sciences of Philadelphia **14**: 459-495.

Jill, S., LeRoy, N., Angermeier, Dahm, Gleick, Hairston, Jackson, Johnston, Richter & Steinman, A. (2003), *Ecosistemas de Agua Dulce Sustentables*. Tópicos en Ecología **10**: 1-15.

Kelly, MG. & Whitton, BA. (1995), *The Trophic Diatom Index; a new index for monitoring eutrophication in rivers*. Journal of Applied Phycology **7**: 433-444.

Kelly, MG., King, L., Jones, Barker & Jamieson, BJ. (2008), *Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes*. Hydrobiologia **610**(1): 125-129.

Klinkenberg, B. (ed). (2012), *E-Flora BC:* Electronic Atlas of the Plants of British Columbia [eflora.bc.ca]. Lab for Advanced Spatial Analysis, Department of Geography, University of British Columbia, Vancouver. [Accessed: 6/7/2012 7:49:28 PM]

Krammer, K. (1997a), *Die cymbelloinden Diatomeen.* Eine Monographie der weltweit bekannten Taxa. Teil 1. Allgemeines und Encyonema Part. Bibliotheca Diatomologica **36**: 1-382.

Krammer, K. (1997b), *Die cymbelloinden Diatomeen.* Eine Monographie der weltweit bekannten Taxa. Teil 2. Encyonema Part., Encyonopsis *und* Cymbellopsis. Bibliotheca Diatomologica **37**: 1-469.

Krammer, K. (2000), *The genus* Pinnularia. In Lange-Bertalot, H. (ed.): Diatoms of Europe. Diatoms of the European inland waters and comparable habitats: 703p.

Krammer, K. & Lange-Bertalot, H. (1986), *Bacillariophyceae 1: Naviculae.* In: Ettl, H.; Gerloff, J.; Heynig, H. & Mollenhauer, d. (eds): Süsswasserflora von Mitteleuropa **2/1**: 876p.

Krammer, K. & Lange-Bertalot, H. (1988), *Bacillariophyceae 2: Bacillariaceae, Epithemiaceae, Surirellaceae*. In: Ettl, H.; Gerloff, J.; Heying, H. & Mollenhauer, D. (eds): Süsswasserflora von Mitteleuropa **2/2**: 596p.

Krammer, K. & Lange-Bertalot, H. (1991a), *Bacillariophyceae 3:* Centrales, Fragilariaceae, Eunotiaceae. In: Ett, H.; Gerloff, J.; Heynig, H. & Mollenhauer, D. (eds): Süsswasserflora von Mitteleuropa **2/3**: 576p.

Krammer, K. & Lange-Bertalot, H. (1991b), *Bacillariophyceae 4:* Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. In: Ettl, H.; Gärtner, G.; Gerloff, J.; Heynig, H. & Mollenhauer, D. (eds): Süsswasserflora von Mitteleuropa **2/4**: 437p.

Lange-Bertalot, H. (1993), *85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süsswasserflora von Mitteleuropa 2/1*. Bibliotheca Diatomologica **27**:1-454.

Lange-Bertalot, H. (2001), Navicula *sensu stricto, 10 genera separated from* Navicula *sensu lato,* Frustulia. In: Lange-Bertalot, H. (ed.): Diatoms of Europe. Diatoms of the European inland waters and comparable habitats 2: 256p.

Lange-Bertalot, H. & Moser, G. (1994), Brachysira. *Monographie der Gattung*. Bibliotheca Diatomologica **29**, 1-212.

Lobo, E., Callegaro, V., Wetzel, Hermany & Bes, D. (2004), *Water quality study of Condor and Capivara streams, Porto Alegre municipal district, RS, Brazil, using epilithic diatoms biocenoses as bioindicators*. Oceanological and Hydrobiological Studies **33**(2): 77-93. (2) usually omitted

López Cortés, D., Lizárraga I., Bustillos, Rodríguez & Murillo, I. (2003), *Variabilidad del estado trófico y la biomasa del fitoplacton de Bahía Concepción, Golfo de California*. Hidrobiológia **13**(3): 195-206.

Macek, P., Rejmánková & Lepš Jan (2010), *Dynamics of* Typha dominguensis *spread in* Eleocharis *dominated oligotrophic tropical wetlands following nutrient enrichment*. Evolutionary Ecology, **24**(6):1505-1519.

Maldonado, M. & Goitia, E. (1993), *Bases limnológicas para la recuperación de la laguna Alalay (Cochabamba)*. Publicaciones Facultad de Ciencias y Tecnología. Universidad Mayor de San Simón. Serie Científica **3**: 1-35.

Maldonado, M., Van Damme & Rojas Jenny (1998), *Estrategias para la recuperación y la conservación de la laguna Alalay (Cochabamba, Bolivia).* Revista Boliviana de Ecología y Conservación Ambiental **3**: 3-9.

Manguin, E. (1964), *Contribution á la connaissance des diatomées des Andes du Pérou*. Mémoires du Muséum d'Histoire Naturelle. Série B, Botanique **12**: 41-48.

Margalef, R. (1981), *Limnología*. Ed. Omega. 1010p.

Marosi, BA., Kiss & Péterfi Leontin (2006), *Preliminary studies on the structure of diatom communities in the ponds of the nature reserve Fânațele Clujului (Transylvania, Romania)*. Contribuții Bonatice **41**: 55-61.

Marotta, H., Bento, L., Esteves & Enrich-Prast Alex (2009), *Whole Ecosystem Evidence of Eutrophication Enhancement by Wetland Dredging in a Shallow Tropical Lake*. Estuaries and Coasts **32**(4): 654-660.

Meneses, L. (1996), *Descripción taxonómica y composición de la comunidad de Cladóceros (*Crustacea*) de la laguna Alalay*. Tesis de Licenciatura en Biología. Universidad Mayor de San Simón.

Meneses, L. (1998), *Estructura de la comunidad de Cladóceros en la laguna Alalay (Cochabamba, Bolivia)*. Revista Boliviana de Ecología y Conservación Ambiental **3**: 47-58.

Metzeltin, D. & Lange-Bertalot, H. (1998), *Tropical Diatoms of South America: About 700 predominantly rarely know or new taxa representative of the Neotropical flora*. Iconographia Diatomologica **5**: 1-695.

Metzeltin, D. & Lange-Bertalot, H. (2007), *Tropical Diatoms of South America II. Special remarks on biogeographic disjunction*. Iconographia Diatomologica **18**:1-877.

Metzeltin D., Lange-Bertalot & Gracía-Rodríguez Felipe (2005), *Diatoms of Uruguay compared with other taxa form South America and elsewhere*. Iconographia Diatomologica **15**:1-736.

Morales, A. (1993), *La comunidad de fitoplancton en la laguna Alalay (Cochabamba, Bolivia)*. Tesis de Licenciatura en Biología. Universidad Mayor de San Simón: 124p.

Morales, A., & Trainor, F. (1996), *Phytoplankton community of Alalay Pond, Cochabamba, Bolivia*. Algae (The Korean Journal of Phycology) **11**(2):207-215.

Morales, EA. & Vis, ML. (2007), *Epilithic diatoms (Bacillariophyceae) from cloud forest and alpine streams in Bolivia, South America.* Proceedings of the Academy of Natural Sciences of Philadelphia **156**: 123-155.

Morales, EA., Vis, ML., Férnandez & Kociolek, JP. (2007), *Epilithic diatoms (Bacillariophyta) from cloud forest and alpine streams in Bolivia, South America 2: A preliminary report on the diatoms from Sorata, Department of La Paz*. Acta Nova **3**: 680-696.

Morales, E.A., Fernández & Chávez, Vania (2010), *Diatomeas (Bacillariophyta): ¿Por qué debemos incorporarlas en estudios de la biodiversidad boliviana?*. In: Beck, S. G. et al. (Eds.). Biodiversidad y Ecología en Bolivia. Simposio XXX Aniversario Instituto de Ecología Universidad Mayor de San Andrés, La Paz. Bolivia: 31-54.

Moreno, P., Quintero & López Armando (2010), *Métodos para identificar, diagnosticar y evaluar el grado de eutrofía*. ContactoS **78:** 25-23.

Moss, B. (1998), *Shallow lakes: biomanipulation and eutrophication*. SCOPE Newsletter **29**: 45 p.

Pan, Y., Steevenson, B., Hill, Herlihy & Collins, GB. (1996), *Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment*. Journal of the North American Benthological Society 15: 481-495.

Patrick, R. (1986), *Diatoms as Indicators of Changes in Water Quality*. Proceedings of Eighth International Diatom Symposium. Koeltz Scientific Books. Koenigstein, Germany: 759-766.

Pérez, M. (2005), *Especiación de metales pesados en sedimentos de la laguna Alalay*. Tesis de Maestría en Ingeniería Ambiental. Universidad Mayor de San Simón, Cochabamba: 90p.

Potapova, M., Charles, D., Karin, Ponader & Winter, D. (2004), *Quantifying species indicator values for trophic diatom indices: a comparison of approaches*. Hydrobiologia **517**: 25-41.

Prygiel, J. & Coste, M. (1993), *The assessment of water quality in the Artois-Picardie water basin (France) by the use of diatom índices*. Hydrobiologia **269/279**(1): 343-349.

Rimet, F., Cauchie, HM., Hoffmann & Ector, L. (2005), *Response of diatoms indices to simulates water quality improvements in a river*. Journal of Applied Phycology **17**:119-128.

Romero, AM., Van Damme & Goitia Edgar (1998), *Contaminación orgánica en el río Rocha (Cochabamba, Bolivia)*. Revista Boliviana de Ecología y Conservación Ambiental **3**: 11-23.

Rumrich, U., Lange-Bertalot & Rumrich Manfred (2000), *Diatoms of the Andes (from Venezuela to Patagonia/ Tierra del Fuego)*. Iconographia Diatomologica **9**: 1-673.

Salomoni, SE. & Torgan, LC. (2008), *Diatomáceas epilíticas indicadoras de graus de contaminação no Lago Guaíba do extremo sul do Brasil*. Acta Limnológica Brasiliensa **20**(4): 313-324.

Sánchez, JJ. (2001), Estudio de la implantación de piscinas ecológicas en las piscinas municipales de Cataluña. Departament de Projectes d'Enginyeria. Universitat Politècnica de Catalunya: 34p.

Schaumburg, J., Schranz, CH., Hofmann & Stelzer, D. (2004), *Macrophytes and phytobenthos as indicators of ecological status in German lakes- a contribution to the implementation of the Water Framework Directive*. Limnologica **34**: 302-314.

Servant-Vildary, S. (1978), *Les diatomées des sédiments superficiels d'un lac salé, chloruré, sulfaté sodique de l'altiplano bolivien, le lac Poopó*. Cahiers ORSTOM, Série Géologique 10: 79-90.

Servant-Vildary, S. (1986), *Les diatomées actuelles des Andes de Bolivie (Taxonomie, écologie*). Cahiers de
Micropaléontologie, CNRS 1: 99-124

Serag, MS., El-Hakeem, A., Badway & Mousa MA. (2000), *On the ecology of* Azolla filiculoides *Lam. In Damietta disctrict*, Egypt. Limnologica **30**: 73-81.

Sgro, GV., Reavie, ED., Kingston & Kireta, A. (2007), *A diatom quality index from a diatom-based total phosphorus inference model*. Environmental Bioindicators **2**:15-34.

Simkhada, B. (2006), *Diatoms as indicators of environmental changes in lakes and ponds of the lowlands, middle hills and high Himalaya of Nepal*. Ph.D. Thesis, University of Bielfeld: 247 pp.

Sládecek, V. (1973), *System of water quality from the biological point of view*. Archiv für Hydrobiologie Beiheft Ergegnisse der Limnologie **7**:1-218.

Stenger-Kovács, C., Padisák, J., Hajnal & Buczkó, K. (2007), *Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic Diatom Index for Lakes (TDIL= developed in Hungary)*. Hidrobiologia **589:** 141-154.

Stoermer, E. & Smol, J. (2001), *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press: 469 p.

UMSS. (1990), *Informe final técnico de la laguna Alalay*. Cochabamba, Bolivia: 120p.

Van Damme, PA., Romero, AM., Goitia, Rojas, Cadima, Arce & Romero M. (1997), *Evaluación de estrategias actuales y alternativas para la recuperación de la laguna Alalay (Cochabamba)*. Centro de Medio Ambiente y Recursos Renovables CEMAR. Universidad Mayor de San Simón. Cochabamba: 91p.

Van Dam, H., Mertens & Sinkeldam Jos (1994), *A coded checklist and ecological indicator values of freshwater diatoms from The Netherlands*. Journal of Aquatic Ecology **28**(1): 117-133.

Wetzel, RG. (2001), *Limnology: Lake and River Ecosystems*. Third Edition. AcademicPress: 1006p.