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Use of diatoms (Bacillariophyceae) for water quality assessment in two tropical streams in Costa Rica

Astrid Michels
Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), Universidad de Costa Rica, 2060 San José, Costa Rica. Fax: (506) 253-5661. E-mail: Astrid.Michels@t-online.de
Present address: TU-Munich, Limnologicallnstitute Iffeldorf, Hofmark 3, 82 393 Iffeldorf, Germany.

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Abstract: Effects of pig farm sewage water in two tropical streams were studied for a five month period considering physical-chemical and biological parameters. The method of Lange-Bertalot and the method of Steinberg & Schiefele, both based on benthic diatoms, were applied for the biological water assessment. Results were compared to indices calculated from the physical-chemical variables (oxygen, nitrate, nitrite, ammonia and phosphate). According to both methods the percentages of the diatom species groups reflected the increase and subsequent decrease of pollution. However, in both methods, species groups did often not fit the criteria of the classification schemes for calculation of water quality. The sources of these problems were discussed in this paper. More information on the ecological requirements of diatoms in tropical areas is needed to design procedures for water quality assessment for tropical streams.

Key words: Diatoms, bioindicator, organic pollution, water quality assessment, tropical stream.

Biological indicators are particularly important for monitoring water quality because they show the cumulative effects of present and past conditions, whereas chemical and physical measures apply only to the moment of sampling. Therefore, biological indicators are extremely useful in evaluating impacts of anthropogenic stress on aquatic environments and assessments should include these biological aspects.

Diatoms (Bacillariophyceae) are particularly interesting as potential indicators of water quality. They occur in all aquatic environments and are found at almost all levels of pollution (Hürlimann 1993, Hofmann 1994). The following characteristics qualify diatoms as suitable bio-indicators: they have worldwide distribution, high reproduction rate and individual species high sensitivity towards different levels of organic polluted waters (Hürlimann 1993, Hofmann 1994, Reid *et al.* 1995). Thus, they are used as indicators for saprobic conditions (Lange-Bertalot 1978, 1979a), salinity (Ziemann 1971), acidification (Arzet 1987) and eutrophication in lakes and rivers (Steinberg & Schiefele 1988, Hofmann 1994). Besides easy sampling, diatoms have the great advantage that their siliceous walls provide the detail from which identification is made, and every sample ends up permanently mounted on a microscope slide (Reid *et al.* 1995). Thus, records can be re-checked, species compared with other collections, and samples compared over periods of time. Because of their great importance in aquatic systems, considerable research effort has been carried out in temperate regions.

Several indices using diatoms have been developed: the saprobic system and its derivatives (Kolkwitz & Marsson 1909, Liebmann

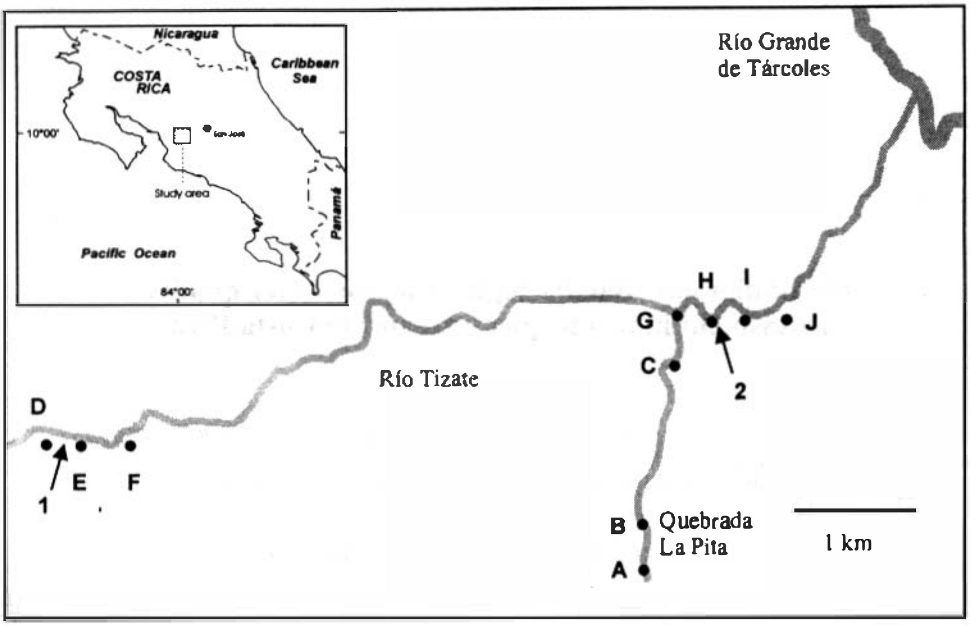

Fig. 1. Location of sampling sites along Quebrada La Pita (A-C) and Río Tizate (D-J). Arrow mark sewage inflows from pig farm. Inset: Map showing the study area in Costa Rica.

1962, Sládecek 1973), indices based on diversity changes in diatom communities (Patrick 1964), classifications according to sensitivity to pollution (Coste 1974, Descy 1979, Lange-Bertalot 1979a, Steinberg & Schiefele 1988, Kelly *et al.* 1995). One of the most universal methods is that of Lange-Bertalot (1979a), which uses groups of species that differentiate towards different degrees of pollution by substances from domestic as well as industrial sewage (Sullivan 1984). Comparable studies from the tropics are few, and diatoms have received little attention on the overall context of water quality monitoring.

In this study the epilithic diatom flora of two streams in Costa Rica has been investigated over a five-month period. Changes in diatoms species composition was monitored at ten sites, which differed in their degree of anthropogenic influence. A detailed description of the physical-chemical properties of the streams and the diatom species composition is given by Michels (1998).

The major objective of this study was to test whether methods for water quality assessment using diatoms developed in temperate zones can be applied to tropical environments, and to discuss the problems involved.

MATERIALS AND METHODS

Site description: The two streams, Quebrada La Pita and Río Tizate, were located in the Central Valley, Costa Rica, 15 km SW from Alajuela (9°50' N, 84°20' W) (Fig. 1) near the larger town of Turrucares at an altitude of 450-650 m a.s.l. Along the two streams ten sites were selected and sampled in a three-week interval from February to June 1995. Along the undisturbed Quebrada La Pita (A-C), three sampling sites were chosen (Fig. 1). Along Río Tizate, which was affected by sewage water of two pig farms, seven sites (D-I) were selected, with sites E, F, I, and J affected by direct sewage water inflows (Fig. 1).

Sampling methods for physical and chemical analyses, diatom slide preparation, identification and counting are described in Michels (1998).

Diatom assessment: The biological water quality assessment was performed following two methods, based on categorizing diatoms into three groups according to their sensitivity or resistance towards pollution: 1) pollution-classification for estimation of limnosaprobity according to Lange-Bertalot (1979a) and 2) pollution/trophy classification according to Steinberg & Schiefele (1988) for estimation of trophy.

1. Diatom analyses according to Lange-Bertalot (1979a): This method is based on the tolerance of diatoms to different stages of organic pollution. "By grouping diatom species with similar tolerances in three categories, their percentages within the communities can be used as an indicator for the ecological conditions of different habitats." (Lange-Bertalot & Lorbach 1979). This method distinguishes three species groups:

a) species sensitive to pollution: occurring only in oligotrophic and katharobic situations;

b) species tolerant to pollution: occurring in lightly polluted waters (b- to α-mesosaprobic conditions; II-III, III);

c) species most tolerant to pollution; occurring in very heavily polluted waters (α-mesosaprobic to polysaprobic; III-IV, IV).

The relative proportions of the three species groups are used to determine water quality (Table 1).

2. Diatom analyses according to Steinberg & Schiefele (1988): Steinberg & Schiefele expanded the method of Lange-Bertalot (1979a) by introducing an extra group, the eutraphent species, which indicate nutrient-rich conditions. From the percentages of each group the classification into four trophy (T=loading with nutrients) and three pollution (P=pollution) classes results, ranging from unpolluted, nutrient poor but oxygen rich waters to heavily industrialized and organic oxygen consuming-waters (Table 2).

Classification of species into the ecologically different species groups followed (Lange-Bertalot 1978, 1979a, 1979b, Lange-Bertalot & Lorbach 1979, Krammer & Lange-Bertalot 1986-1991, Steinberg & Schiefele 1988, van Dam *et al.* 1994).

Water quality was calculated according to both classification methods. Problems occurred, as in some cases the three species groups were represented in equal portions, and thus did not fit any of the cases specified in Table 1 and Table 2. In this case water quality was classified with respect to the sensitive species: according to Lange-Bertalot (1979a), the sensitive species do not occur in water conditions worse than the critical pollution level and their presence or absence can serve

TABLE 1

Classification scheme for assessing limnosaprobity according to Lange-Bertalot (1979a)

	Description		Proportions
II	b-mesosaprob	Moderably polluted	s > 50%
II-III	b-a-mesosaprob	Critically polluted	10% < s < 50%
III	a-mesosaprob	Heavily polluted	s < 10% and t > 50%
III-IV	a-meso-polysaprob	Very heavily polluted	s + t > 10%
IV	polysaprob	Excessively polluted	s + t < 10% and mt > 90%

s: sensitive species; t: tolerant species; mt: most tolerant species

TABLE 2

Classification scheme for assessing trophy/pollution according to Steinberg & Schiefele (1988)

Pollution Class	Proportions		
T1	o ≥ 50%, hs ≥ 10%	eu < 10%	t + ht + mt + s < 10%
T2	o ≥ 50%, hs ≥ 10%	eu < 50%	t + ht + mt + s < 10%
T3	o < 50%, hs ≥ 10%	eu ≥ 50%	t + ht + mt + s < 10%
T4	o < 50%, hs < 10%	eu ≥ 50%	
P1	o + hs < 10%	eu < 50%	t + ht + mt + s ≥ 10%
P2	o + hs < 10%	eu < 50%	t + ht + mt + s ≥ 50%
P3	o + hs < 10%	eu < 10%	t + ht + mt + s ≥ 50%

o: oligotraphent; eu: eutraphent; hs: highly sensitive; s: sensitive; t: tolerant; ht: highly tolerant; mt: most tolerant

TABLE 3

Classification scheme for assessing water quality according to physico-chemical parameters (Barndt & Bohn 1992)

Water Quality class	Oxygen %	BOD_5 (mg/l)	NH_4-N mg/l	NO_2-N mg/l	NO_3-N mg/l	PO_4-P mg/l
I, I-II	100 – 85	1 - 2	< 0,1	< 0,1	< 1,0	< 0,015
II, II-III	50 – 85	2 - 10	< 0,3		1 – 5	0,015 – 0,15
III, III-IV	10 – 50	10 – 20	< 1,0	0,4 – 6,0	> 5,0	0,15 – 1,5
IV	< 10	> 15	> 1,0	> 6,0		> 1,5

to distinguish between water qualities. The pollution tolerant species, however, can also occur in unpolluted water and therefore their occurrence alone do not serve to indicate a distinct level of water quality (Lange-Bertalot 1979b).

Chemical assessment: The chemical water quality assessment is based on the classification of commonly accepted concentration ranges (Barndt & Bohn 1992) of the most important aquatic parameters: Nitrate, nitrite, soluble reactive phosphorus, ammonia, oxygen and biochemical oxygen demand in five days (Table 3). While phosphorus and nitrogen are the relevant parameters for the assessment of nutrient loading, ammonia and oxygen saturation are the pertinent criteria with respect to saprobity levels.

RESULTS

A total of 127 species of diatoms were recorded in this study. According to the method of Lange-Bertalot, 46 species could be assigned to any of the three species groups: 17 species could be attributed to the sensitive group, 16 to the tolerant group and 13 to the most tolerant group. According to the modified method of Schiefele & Steinberg, 38 species could be classified: eight were regarded as sensitive, 20 as tolerant, and ten as eutraphent. On average 75 % of total species abundance could be considered for water quality classification. Species used for the water quality assessment can be seen in Fig 2.

In Fig. 3 the fluctuations of the ecologically different diatom species groups according to both methods were presented.

Results after the method of Lange-Bertalot (Fig. 3a-3c):

a) The species classified as "sensitive to pollution" were fairly abundant at all sites (> 20 %).

b) Species assigned as "tolerant to pollution" were abundant at all sampling sites (10 – 20 %).

c) The species regarded as "most tolerant to pollution" increased at sites E, F, downstream pig farm 1, decreased subsequently at sites G, H and increased again downstream pig farm 2 at sites I, J.

Results after the method of Steinberg & Schiefele (Fig. 3d-3f):

d) The species group categorized as "sensitive to pollution" was abundant (> 10 %) at non-polluted sites (A-C) and disappeared at polluted ones (E, F, I, J).

e) The species classified as "eutraphent" were present at all sites, except the spring-site, reaching abundance of more than 30 %.

f) The species belonging to the "most tolerant, highly tolerant, and tolerant to pollution" - group were highly abundant (> 30 %) at sites E, F downstream pig farm 1, decreased subsequently at sites G, H and increased again downstream pig farm 2 at sites I, J.

Comparison of diatom assessment with chemical assessment:

Based on the percentages of the species groups water quality was calculated for both diatom based methods according to Table 1 and Table 2, and then compared to the classification based on the physical and chemical data (Table 3). Results were presented in Fig. 4.

According to nitrate/nitrite concentration water quality was classified as nutrient poor (water class I, I-II) at all sites.

Phosphate levels indicated nutrient rich conditions (water class III-IV, IV) at all sites.

Oxygen, biochemical oxygen demand, and ammonia reflected a decrease of water

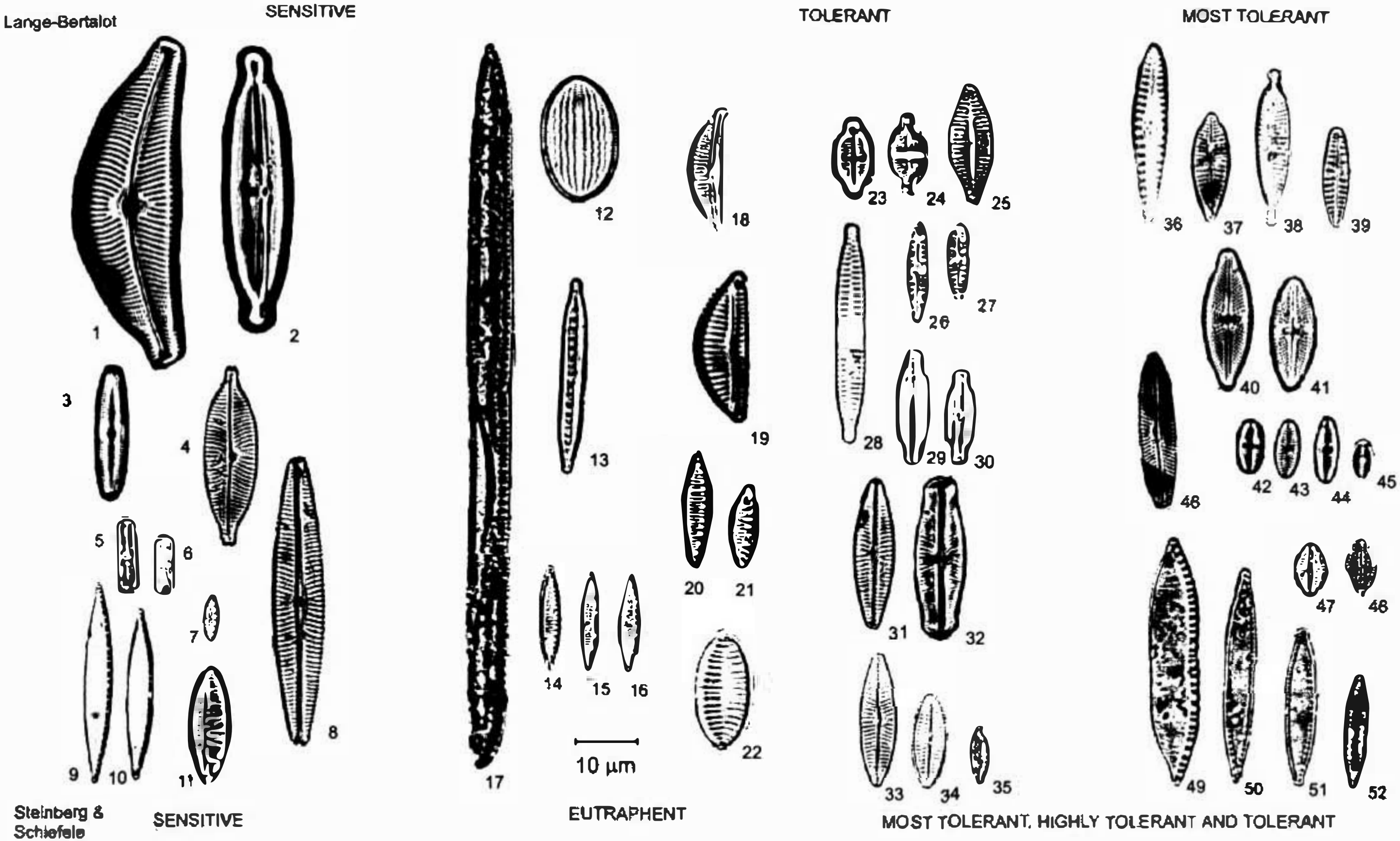

Fig. 2. Light micrographs of representatives of the ecologically different groups (800 X). 1. *Cymbella tumida* Brébisson; 2. *Frustulia rhomboides* Ehrenberg; 3. *Caloneis bacillum* Grunow; 4. *Navicula viridula var. rostellata* Cleve; Fig. 5-6. *Navicula contenta* Grunow; 7. *Nitzschia inconspicua* Grunow; 8. *Navicula radiosa* Kützing; Fig. 9-10. *Nitzschia fonticola* Grunow; 11. *Nitzschia sinuata* Grunow; 12. *Cocconeis placentula* Ehrenberg; 13. *Nitzschia dissipata* Grunow; 14-16. *Nitzschia frustulum* Grunow; 17. *Nitzschia linearis* Smith; 18. *Amphora acutiuscula* Kützing; 19. *Cymbella silesiaca* Bleisch; 20-21. *Nitzschia amphibia* Grunow; 22. *Nitzschia levidensis* Grunow; 23-24. *Achnanthes exigua* Grunow; 25. *Achnanthes lanceolata* ssp. *frequentissima* Lange-Bertalot; 26-27. *Gomphonema pumilum var. rigidum* Reichardt; 28. *Fragilaria capucina var. vaucheriae* (Kützing) Lange-Bertalot; 29-30. *Navicula arvensis var. maior* Lange-Bertalot; 31. *Navicula cryptocephala* Kützing; 32. *Navicula pupula* Kützing; 33-34. *Navicula erifuga* Lange-Bertalot; 35. *Nitzschia microcephala* Grunow; 36-37. *Gomphonema parvulum* Kützing; 38-39. *Gomphonema parvulum var. lagenula* Kützing; 40-41. *Navicula c.f. mutica var. intermedia* Kützing; 42-45. *Navicula seminulum* Grunow; 46. *Navicula schroeterii* Meister; 47-48. *Navicula subminuscula* Manguin; 49. *Nitzschia umbonata* Lange-Bertalot; 50-52. *Nitzschia palea* Smith.

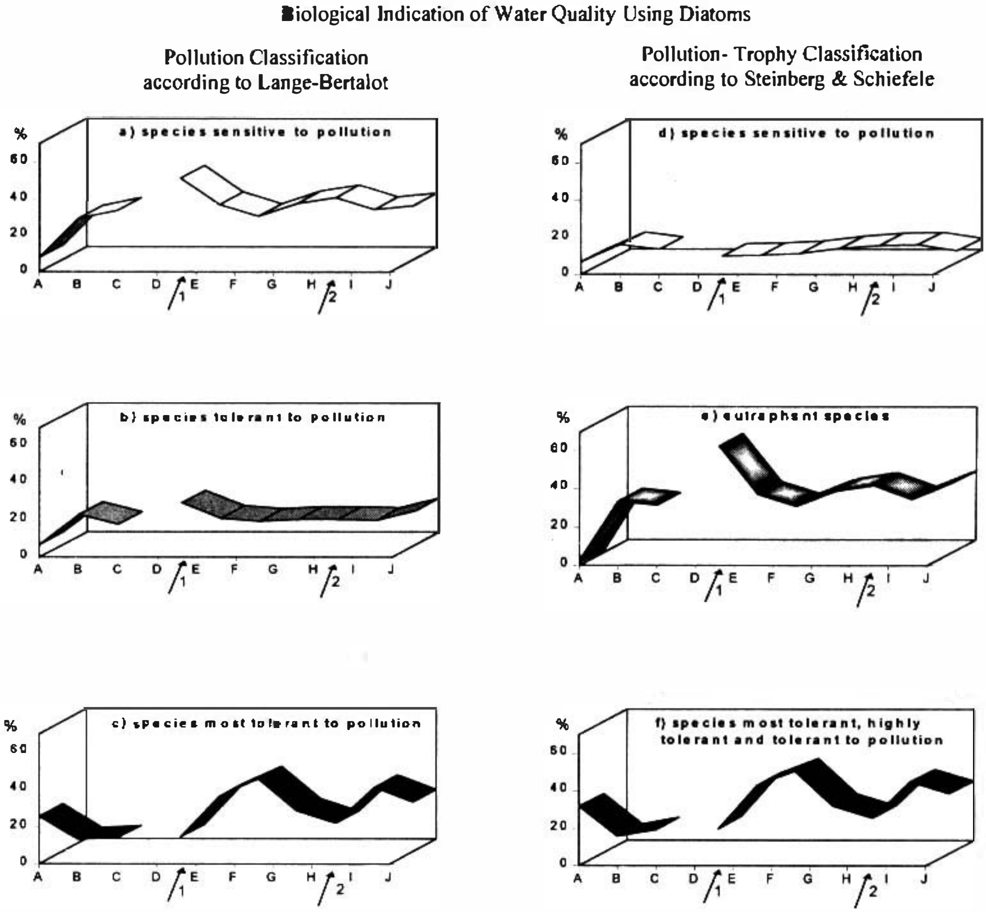

Fig. 3 a-f. Percentages of the ecologically different diatom species groups (average values of the period February to June 1995, n=7) classified according to Lange-Bertalot (a-c) and to Steinberg & Schiefelc (d-f) at the sampling sites along Quebrada la Pita (A-C) and Río Tizate (D-J) The percentages represent the specific proportion in the total diatom community. Arrow marks sewage inflows from two pig-farms.

quality to heavily polluted (water class III, III-IV) downstream pig farm 1 (site E, F) and pig farm 2 (sites I, J).

The diatom assessment according to Lange-Bertalot suggested critical pollution (water class II-III) for all sites. Site A could not be assigned to any water quality, as less than 50 % of the diatom species could be classified.

The method of Schiefele & Steinberg distinguished between low trophic levels (T2) at the unpolluted sites B, C, nutrient rich sections (T4/P1) at site D and polluted conditions (P1, P2) at sewage affected sites E, F, G, H, I and J. Site A could not be assigned to any water quality, because less than 50 % of the diatom species could be classified.

DISCUSSION

In several studies diatoms were used for estimation of saprobity and they were regarded as good bioindicators for water quality (Lange-Bertalot 1978, Lange-Bertalot 1979b, Lange-Bertalot 1979a, Fabri & Leclercq 1984).

The empirical method of Lange-Bertalot was established from studies on the Rivers Main and Rhine in Europe, and it was also valid for several other comparable European rivers (Lange-Bertalot 1979a). Even for rivers

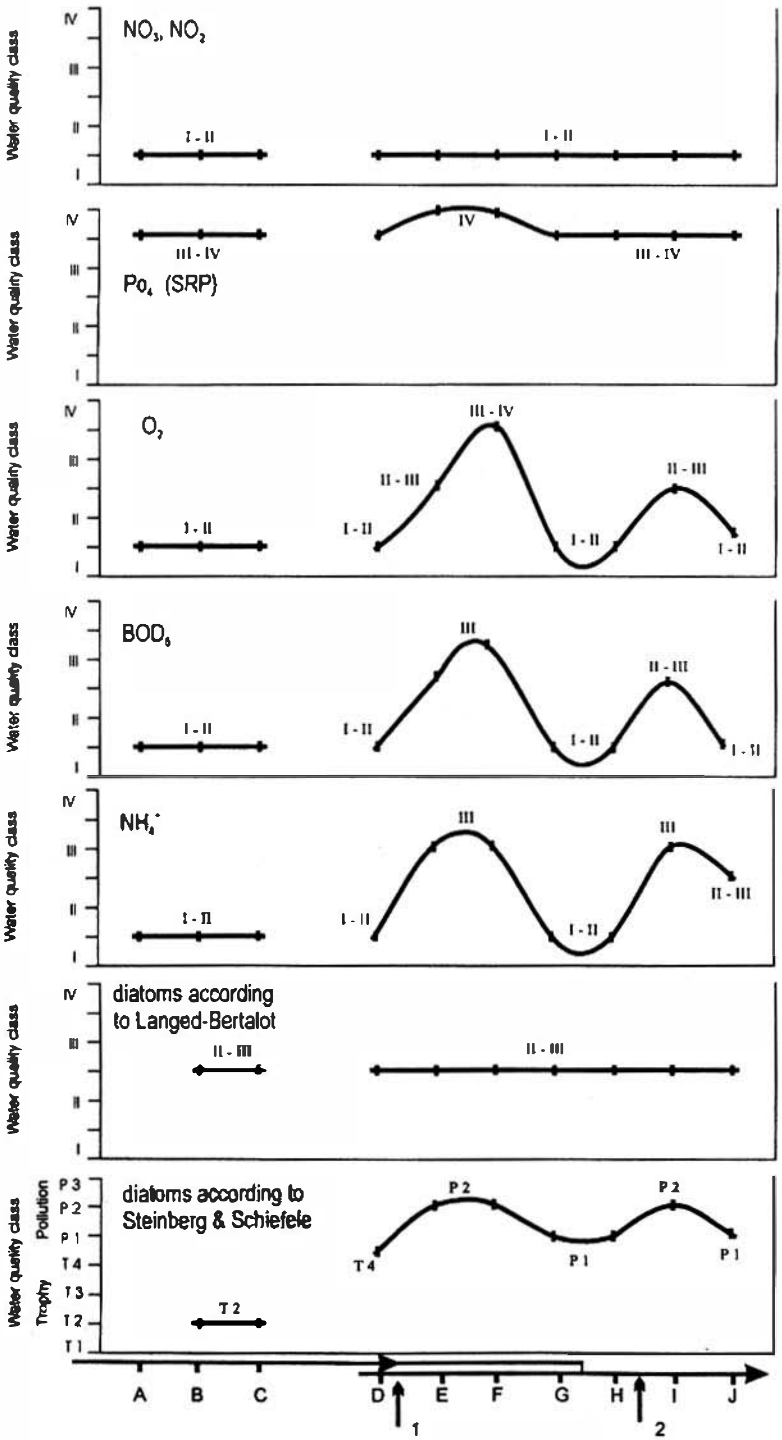

Fig. 4. Comparison of chemical water quality estimation with diatom assessments for the sampling sites on Quebrada La Pita (A-C) and Rio Tizate (D-J). Arrow mark sewage inflows from two pig-farms.

in other parts of the world, reliable results for water quality determination were achieved. Schoeman (1984) for instance, applied Lange-Bertalots' method successfully to the highly polluted Hennops River in South Africa. Similar results were reported from Japan, where this method was used for assessing water quality of some heavily polluted rivers close to Tokyo (Kobayasi & Mayami 1982, Sullivan 1984). Silva-Benavides (1996) attained good results with this method to estimate water quality of the heavily polluted Río Grande de Tárcoles in Costa Rica. However there are also some examples in which the Lange-Bertalot method did not provide reliable results. In the present study, as well as in the works of Engelberg (1987), Schiefele (1987), Reichardt (1991) in Europe, diatom analyses indicated for almost all stream sections "critically polluted" (water class II-III), although the real conditions were much more varied. Even in a more recent investigation on the river Main, the method did not fit anymore the conditions (Schmidt 1991). This is probably, because, when the method was developed in the 1970's, water contamination was mostly due to organic, oxygen-consuming substances, whereas nowadays pollution consists mainly of inorganic nutrients from diverged purified sewage waters (Schmidt 1991).

In this study, the method of Lange-Bertalot did not show a clear differentiation of the monitored sites, although this did occur with the water quality assessment based on physical-chemical variables. This is partly due to the lack of information on the autecological requirements of some species. On average only 75% of all species abundance could be considered. For example, *Achnanthes praecipua*, one of the dominant species, could not be classified in any of the differentiating species groups and therefore in some samples, like the spring-site, less than 50% of the whole diatom community could be categorized; thus not allowing any assessment of water quality.

The classification into differential species groups differs from the classical studies of Lange-Bertalot for some species. For example, *Cocconeis placentula*, a species regarded as sensitive by Lange-Bertalot, was the most abundant species in this study, even at highly polluted sites (Michels 1998). In several studies this species seems to be common at nutrient-rich, well oxygenated sites (Steinberg & Schiefele 1988, Hofmann 1994, Silva-Benavides 1994). Fabri *et al.* (1984) found that *Cocconeis placentula* was able to tolerate moderately polluted conditions if oxygen saturation levels were high. According to Reichardt (1991), an essential reason for the failure of the method of Lange-Bertalot is, that there are a number of species like *Cocconeis placentula* which are classified as sensitive, but are able to live under much worse conditions at high oxygen supply. Costa Rican streams are generally characterized by high gradients and high flows, which cause high oxygen saturation levels.

In general terms the method of Steinberg & Schiefele seemed to be in fairly good concordance with the physical-chemical parameters. Remarkably the difference was mainly due to the classification of a single species, the most abundant in this study, *Cocconeis placentula*, which is classified as sensitive by Lange-Bertalot and as eutraphent by Steinberg & Schiefele.

According to Steinberg & Schiefele, the sensitive group was mainly made up by *Nitzschia fonticola,* which hitherto has commonly been regarded as eutraphent (Hofmann 1994). If this species has to be assigned to the eutraphent group, then the sampling sites classified as low levels of trophy (T4), would be classified as eutrophic (T4). However, the eutrophic state can go along with different degrees of saprobity. Hence, Steinberg & Schiefele's method also does not fit the conditions of the tropical rivers. Therefore diatoms have to be assigned to both trophic and saprobic requirements, and hence methods for water quality assessments should be based on the combined application of a saprobity indication system with a trophy indication system (Hofmann 1994).

Nevertheless this study confirms that diatoms are useful indicators of pollution and trophy: the changes in the percentages of the ecologically different species groups reflect the increase and subsequent decrease of pollution and the eutraphent species reflecting the phosphate-rich conditions of the streams, which were possibly due to the weathering of young volcanic rocks (Talling & Talling 1965, Pringle *et al.* 1990). But so far, indication of water quality is insufficiently, and according to the data the above-presented methods cannot be applied to tropical streams.

More ecological studies of tropical diatom communities based on long-term data are necessary in order to get information about their specific ecological requirements, and to improve methods for water quality assessments. Future studies should focus particularly on sites in undisturbed, pristine tropical environments, where sensitive species occur, to be able to differentiate between unpolluted and polluted sites.

RESUMEN

Se estudió el efecto de aguas negras de porquerizas en dos ríos tropicales considerando parámetros físico-químicos y biológicos. Se aplicaron los métodos de Lange-Bertalot y Steinberg & Schiefele, utilizando las diatomeas epilíticas, para el monitoreo biológico. Los resultados se compararon con índices calculados de los parámetros físico-químicos (oxígeno, nitato, nitrito, amonio, fosfato). Grupos de especies con la misma tolerancia a la contaminación reflejaron el aumento y la siguente disminución de contaminación y por lo tanto proveen información útil con respecto a la calidad del agua. Sin embargo, las diatomeas no correspondían a los criterios de clasificación y por lo tanto la indicación de la calidad del agua fue insuficiente. Se necesitarán más estudios sobre la autecología de las diatomeas en regiones tropicales para hacer los metodos de monitoreo de calidad del agua más apropiados para ríos de esta zona.

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