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# RESPONSES AND STRUCTURAL RECOVERY OF PERIPHYTIC DIATOM COMMUNITIES AFTER SHORT-TERM ENVIRONMENTAL DISTURBANCE IN SOME RIVERS (HANOI, VIETNAM)

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## 1. Introduction

Diatoms have been widely used as organisms for monitoring and assessing water quality owing to their wide distribution and well-studied ecology (Potapova and Charles 2005). Diatoms are considered to be excellent biological indicators for many types of pollution in aquatic systems such as organic pollution (Descy and Coste, 1991), acidification, metallic pollution (Cattaneo et al., 2004) and eutrophication (Dixit et al., 1992). Diatom indices based on selected sensitivities or tolerance of diatom species to organic pollution or acidity are being routinely and widely used in many European and Asian countries (Whitton and Rott, 1996; Watanabe et al., 1986) for rivers quality assessment. Communities in streams and rivers are subjected too to natural stress such as floods besides their exposition to multiple anthropogenic inputs as well.

It has been well documented that multi-sources of pollution greatly affect structure and function of periphytic algae communities. These effects include reduced photosynthetic ability (Barranguet et al., 2000), reduced growth rate (Genter, 1996), interruption of cell division and deformation of diatom frustules (Gold et al., 2003a, Cattaneo et al., 2004). At a cellular level, algae may tolerate pollutant stress by showing a decreased number of binding sites at the cell surface, physiological development of exclusion mechanisms, genetic adaptation, morphological changes, and internal detoxifying mechanisms (Genter, 1996). At a community level, periphytic algae increase their tolerance by shifting its composition to more tolerant species when they are exposed to pollution (Kasai, 1999), which can result in structural changes such as decreased species diversity and richness (Genter, 1996). Many manipulative stream experiments have been extensively performed in both natural and laboratory conditions to assess the impacts of short and long term environmental changes by using periphytic algae communities. Barranguet et al (2000) used indoor experiment to assess the short term metal effect on communities' tolerance in photosynthesis process. Pan et al (2000) used mesocosms to study structural and functional changes of epiphytic algal assemblages due to increased P loading. Up to now, a few transfer experiments have been performed to assess alters of periphytic diatom assemblages caused by changes in water quality. Earlier studies have suggested that translocation of periphytic diatom communities on artificial substrates between different pollution sites was a suitable method to assess *in situ* effects of metallic pollution. (Ivorra et al., 1999). Differences or changes in the biomass, productivity, and structure of periphyton communities can provide a sensitive measure of the trophic and wholeness status of streams and rivers.

In the present study, through transfer outdoor experiments, we aimed to test the appropriativity of periphytic diatom communities as a reliable and efficient tool for a fast diagnosis of biological

conditions in streams and rivers, first by assessing the response of benthic diatom communities to environmental stress via transfers of early stages of benthic diatom communities developed on artificial substrates from reference site to heavily polluted site and moderately polluted site and reversely; then by estimating the time needed for diatom communities to integrate a change of environmental conditions in their structures; and finally by studying how benthic diatom communities recover their structure from pollution stress.

## **2. Materials and methods**

### ***Study area and design***

Three study sites, presenting different water pollution levels, were selected and subjected to transfer experiment during dry season: (i) the comparatively unpolluted reference site (Red) situated at Sontay (Sontay district, Hatay province) in the Red River, about 8 km upstream from the Nhue River and Hanoi city, shows low nutrients and metal concentrations (Trinh, 2003); (ii) the heavily polluted site (TL) located upper the Thanhliet dam (*TL dam*) and downstream Tolich River, is characterized by low dissolved oxygen, high nutrients and metal concentrations with black, foul-smelling waters; (iii) the moderately polluted site (NT<sub>2</sub>) is positioned downstream Nhue River about 7 km below its confluence with the Tolich River.

### ***Field sampling***

The experiment was performed during the dry season from 9<sup>th</sup> January to 20<sup>th</sup> February, 2005. At both sites, Red and TL, one set of two plastic baskets equipped with floaters were immersed in water column parallel to the current at a depth of 15-20 cm below the surface and tied to the bank with a rope. In each plastic basket, 18 glass slides were placed separately and vertically to be used as artificial substrates for algae attachment. One set was left at Red and one at TL sites, at the beginning of the experiment, for a period of two weeks to allow the development of biofilms on glass slides. After two weeks of colonization at Red site, one of the two plastic baskets containing glass slides was transferred to heavy polluted site (TL); the other basket was moved to the moderate site (NT<sub>2</sub>). Same procedure was followed at TL site, with one plastic basket containing glass slides transferred to reference site (Red) and one basket to moderate site (NT<sub>2</sub>). During transportation, glass slides were kept immersed in their initial river water within a container box (1-2 h travel time). After transfer, glass slides were left in their new locations for an addition period of four weeks. Periphytic samples developed on glass slides were sampled before transfer at week 2 (W<sub>2</sub>), and after transfer at week 4 (W<sub>4</sub>) and week 6 (W<sub>6</sub>). On the day of sampling, at each site, three glass slides, considered as independent samples, were randomly removed from each plastic basket. Biofilms were collected from the glass slides by using a nylon brush, and then washed and diluted in a known volume (100 or 200 mL) of distilled water depending on the biofilms thickness. Biofilm samples were preserved in labelled glass bottles with 5% formalin solution for delayed identification of diatoms composition.

### ***Laboratory analyses***

#### ***Diatom preparation***

After homogenization, 2 ml aliquot of each diatom sample from each site were digested with concentrated hydrogen peroxide (30%) and hydrochloric acid (35%) to remove organic matter and dissolved calcium carbonates, then rinsed several times and diluted with deionized water. The cleaned diatom frustules were then mounted on a microscope glass slide using Naphrax, high resolution mounting medium. Diatoms were identified with a Leitz DMRD light microscope at 1000x

magnification. Approximately 400 valves were identified to species level from each slide of the three replicates. The Süßwasserflora nomenclatures (Krammer and Lange-Bertalot, 1986-1991) were used as references for diatom taxonomy. Relative abundance of diatom species (in percentage) was estimated. Species richness (S) was calculated and biological diversity was estimated through Shannon-Weaver diversity index ( $H'$ ). Diatom indices (IPS and DAIPo index) were applied in order to classify water quality in each sampling site. A Nageotte counting chamber (Marienfeld, Germany) was used to estimate diatom density in each sample by counting the total number of diatoms in 30 fields (1.25  $\mu\text{l}$  each, 0.5 mm depth) using a light microscope (Olympus BX x 50) at 200x magnification. Data were expressed in cells per unit area of glass slide (number diatom cells/  $\text{cm}^2$ ).

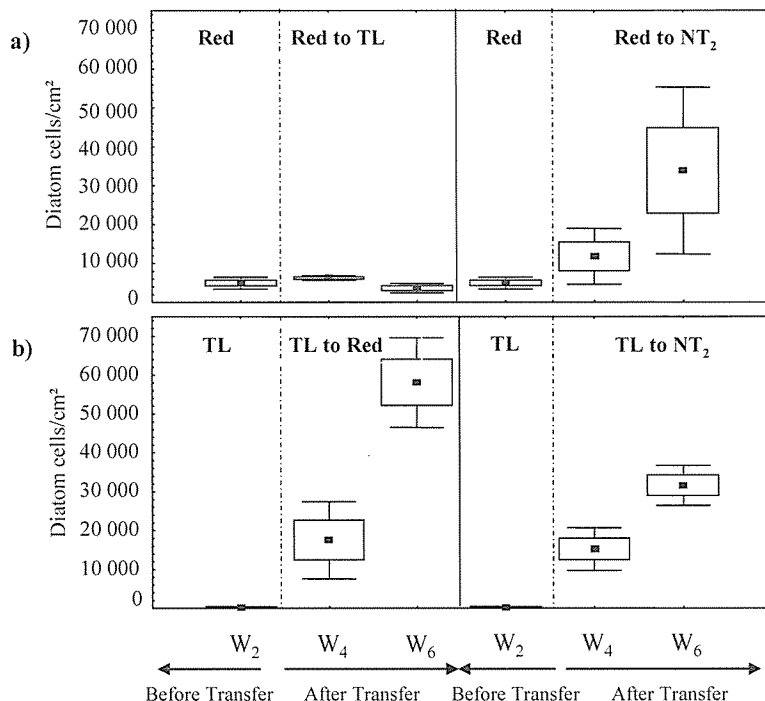
### ***Data treatment***

Diatom indices IPS (Index of Polluosensitivity Specific) (Cemagref, 1982) and DAIPo (Diatom Assemblage Index to organic Pollution) (Watanabe et al., 1986) were calculated using the OMNIDIA software (Lecointe et al., 1993). These diatom indices were transformed to range from 1 to 20 to be comparable. In order to study significant differences in diatom density, structure of diatom communities (species richness, diversity index) and diatom indices between the local and transferred diatom communities, we first performed ANOVA method (one way or two way) using STATISTICA software (StatSoft, 2004) after checking assumptions (normality and homoscedasticity of the error term). If the null hypothesis was rejected, *post-hoc* tests (Least Significant Difference test (LSD)) were applied in order to find significant differences between groups. For all statistical results, a probability of  $p < 0.05$  was considered significant. Taxonomic differences between the sites were displayed using Principal Component Analysis (PCA) with PC-ORD Software (McCune and Mefford 1999). PCA was performed on relative abundances of only 53 species out of a total of 277 identified diatom species, which had the highest cumulative relative abundances within local and transferred diatom communities collected throughout the experiment.

## **3. Results**

### ***Diatom density***

Quantitative characterization of diatom communities assemblages are reflected by numeration of diatom cells. Diatom densities developed on glass substrates before ( $W_2$ ) and after transfer ( $W_4$  and  $W_6$ ) are illustrated in figure 1 for the three sites. Figure 1a presents diatom density coming from biofilms previously colonized in Red site during two weeks, then transferred to either TL site or NT<sub>2</sub> site during four extra weeks. Figure 1b shows same evolution, but for biofilms previously developed at TL site then transferred to Red or NT<sub>2</sub> site. Total diatom density at Red site shows a higher number of cells during the two weeks initial period of colonisation than those at TL site ( $4,928 \pm 446 \text{ cells.cm}^{-2}$ ;  $240 \pm 19 \text{ cells.cm}^{-2}$ , respectively). A yellow-brown colour layer including detritus, algae, bacteria, and suspended particular matters covered glass substrates at Red site after two weeks, whereas a thin black coloured layer was visible on its substrates at TL site. After transfer of the glass substrates from Red site to NT<sub>2</sub> and TL sites, development of diatoms at these two sites are different (figure 1a). Densities at TL site range from  $3,595 \pm 351 \text{ cells.cm}^{-2}$  to  $6,248 \pm 182 \text{ cells.cm}^{-2}$ , showing low development of diatom communities. Meanwhile at NT<sub>2</sub> site, diatom densities developments after transfer from Red site increase significantly ( $p < 0.05$ ) till the end of the experiment. For glass substrates transferred from TL site to Red and NT<sub>2</sub> sites, densities of diatoms on substrates grow a lot at their new location and reach maximum values during the last week with  $31,539 \pm 1511 \text{ cells.cm}^{-2}$  at NT<sub>2</sub> site and  $58,034 \pm 3401 \text{ cells.cm}^{-2}$  at Red site.



**Figure 1a and b.** Diatom densities on artificial substrates (cells.cm<sup>-2</sup>) collected from the three stations Red, NT<sub>2</sub> and TL at the week 2 (W<sub>2</sub>) (during initial colonization on site before transfer), at week 4 (W<sub>4</sub>) and week 6 (W<sub>6</sub>) (after transfer at their new locations) of the experiment. Boxplot: mean value (n = 3) with its confidence interval at 95 % denoted by the two horizontal lines outside the box.

Despite the difference in diatom density from the initial period of colonization in Red or TL site, diatom growth at NT<sub>2</sub> site after both translocations shows similar trend of evolution, ranging from 11,786±2,110 cells.cm<sup>-2</sup> at W<sub>4</sub> to 33,810±6,313 cells.cm<sup>-2</sup> at the W<sub>6</sub> for transferred diatom communities from Red site to NT<sub>2</sub> site, and from 15,176±1,621 cells.cm<sup>-2</sup> at W<sub>4</sub> and 31,539±1,511 cells.cm<sup>-2</sup> at the W<sub>6</sub> for transferred diatom communities from TL site to NT<sub>2</sub> site. Qualitative observations of biofilms at NT<sub>2</sub>, after 4 weeks of transfer, indicated the development of a thick, yellow-brown and mucilaginous coat on their outside, black and colored aspect on their inside. According to two-way ANOVA results, significant differences in diatom density between sites and colonization durations are observed ( $p < 0.05$ ).

### *Species richness, diversity of diatom communities and diatom indices*

Evolution of species richness (S), diversity index (H') and diatom indices occurring along the experiment are shown in table 1. A significant difference is noted between communities previously developed at Red and TL site during the first two weeks with 22% more species initially developed at Red site than at TL site. On the other hand, no significant difference is detected in S between local diatom communities (Red, TL) at W<sub>2</sub> and transferred diatom communities during the four last weeks of experiment, although S seems to decrease for the condition Red to NT<sub>2</sub>, and to increase for both transfers from TL when compared to week 2 values. Concerning Shannon diversity index (H'), only transferred diatom communities from Red to NT<sub>2</sub> shows a significant difference during the course of the experiment, when compared to local diatom communities according to one-way ANOVA result ( $p < 0.05$ ).

Stations	Weeks	S	H'	IPS	DAIPo
Red to NT <sub>2</sub>	W <sub>2</sub>	80 (1)	4.9 (0.04)	11.1 (0.3)	11.3 (0.1)
	W <sub>4</sub>	73 (6)	4.3 (0.1)	6.4 (0.5)	8.6 (0.2)
	W <sub>6</sub>	70 (2)	4.2 (0.03)	4.5 (0.2)	7.3 (0.2)
Red to TL	W <sub>2</sub>	80 (1)	4.9 (0.04)	11.1 (0.3)	11.3 (0.1)
	W <sub>4</sub>	81 (3)	5.1 (0.2)	8.3 (0.4)	9.1 (0.3)
	W <sub>6</sub>	83 (2)	5.1 (0.1)	7.9 (0.4)	8.6 (0.4)
TL to NT <sub>2</sub>	W <sub>2</sub>	52 (7)	4.2 (0.23)	4.6 (0.6)	6.3 (1)
	W <sub>4</sub>	67 (2)	4 (0.1)	7.0 (0.5)	9.3 (0.3)
	W <sub>6</sub>	62 (2)	3.9 (0.1)	4.2 (0.5)	10 (0.1)
TL to Red	W <sub>2</sub>	52 (7)	4.2 (0.23)	4.6 (0.6)	6.3 (1)
	W <sub>4</sub>	69 (1)	4.2 (0.03)	8.1 (0.4)	7.9 (0.9)
	W <sub>6</sub>	64 (3)	3.8 (0.1)	8.6 (0.2)	7.1 (0.5)

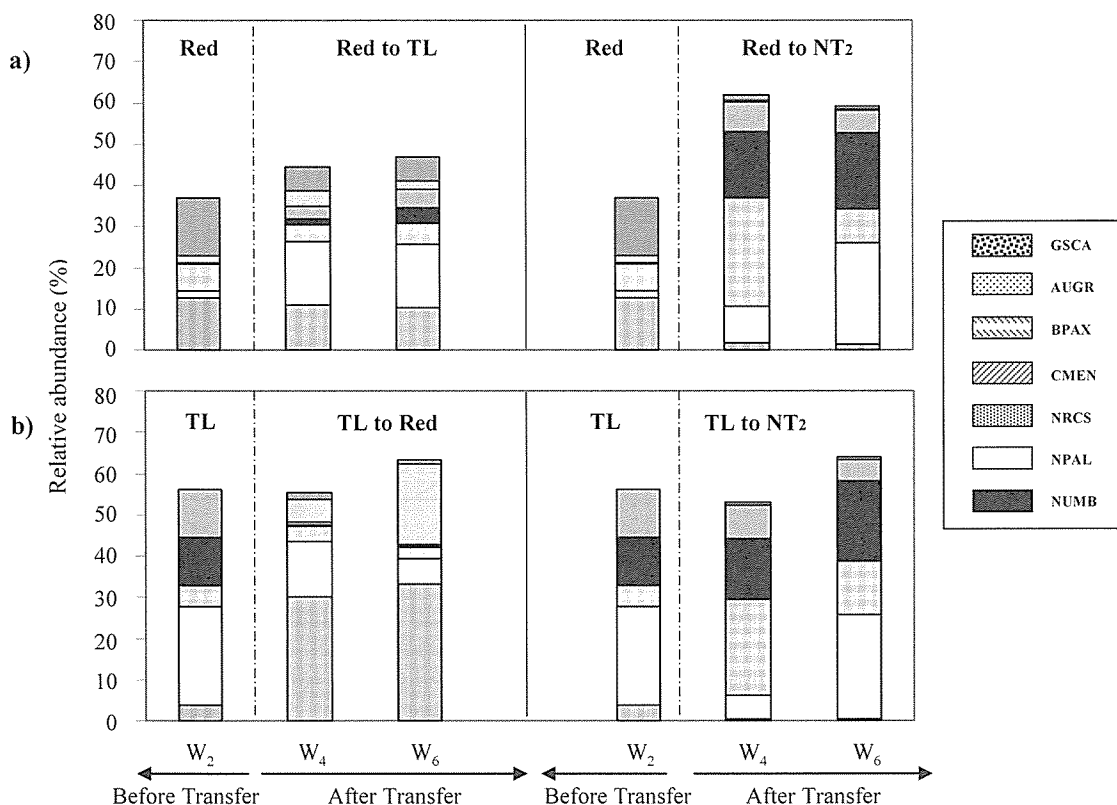
**Table 1.** Values of Species richness (S), Diversity index (H') of diatom communities and values for the two diatom indices IPS and DAIPo collected from the three stations Red, NT<sub>2</sub> and TL at the week 2 (W<sub>2</sub>) (during initial colonization on site before transfer), at week 4 (W<sub>4</sub>) and week 6 (W<sub>6</sub>) (after transfer at their new locations) of the experiment (mean value and standard error, n = 3)

Both water quality diatom indices, IPS and DAIPo, reveal fairly similar profiles (table 1). After transfer from Red site, IPS and DAIPo indices gradually and significantly ( $p < 0.05$ ) decrease with exposition duration to reach a minimum value at W<sub>6</sub> (4,5 and 7,3 at NT<sub>2</sub> site and 7,9 and 8,6 at TL site, respectively). Table 5.2 results also show a considerable increase of the indices (mainly IPS) after transfer from TL site to Red site, although they do not reach the high values obtained at the local site (Red) during the first period of colonization. After transfer from TL to NT<sub>2</sub>, diatom indices increase too at W<sub>4</sub> but show different patterns after, with a decrease during the last week of experiment for IPS and a stabilization for DAIPo.

### Diatom composition

Composition of diatom communities of local and transferred diatom assemblages which colonized the substrates through out the experiment is presented through relative abundances of 7 diatom species (mean relative abundances  $\geq 8\%$ ) in figure 2 a and b. Diatoms community of Red site at W<sub>2</sub> is dominated by *Gyrosigma scalproides* (GSCA) and *Navicula recens* (NRCS) in the range from 14.1% to 12.5% respectively (figure 2a). After transfer, the composition of transferred diatom communities is modified. When periphytic diatom communities from Red site are transferred to NT<sub>2</sub> site, high proportion of *Gyrosigma scalproides* (GSCA) and *Navicula recens* (NRCS) are rapidly replaced by polysaprobic taxa like *Nitzschia palea* (NPAL) (W<sub>4</sub>: 8.9 %; W<sub>6</sub>: 24.6%) *Nitzschia umbonata* (NUMB) (W<sub>4</sub>: 16.7%; W<sub>6</sub>: 18.3%), the planktonic taxa *Cyclotella meneghiniana* (CMEN) (W<sub>4</sub>: 7%; W<sub>6</sub>: 5.4%) and *Aulacoseira granulata* (AUGR) (W<sub>4</sub>: 26.2%; W<sub>6</sub>: 8.4%). In contrast, initial diatom communities of Red site transferred to TL site are still present till the experiment ends; pollution tolerant taxa *Nitzschia palea* (NPAL) and *Nitzschia umbonata* (NUMB) being clearly settled with mean relative abundances around 15.6% and 2% respectively. Besides, these two species *Nitzschia umbonata* (NUMB) and

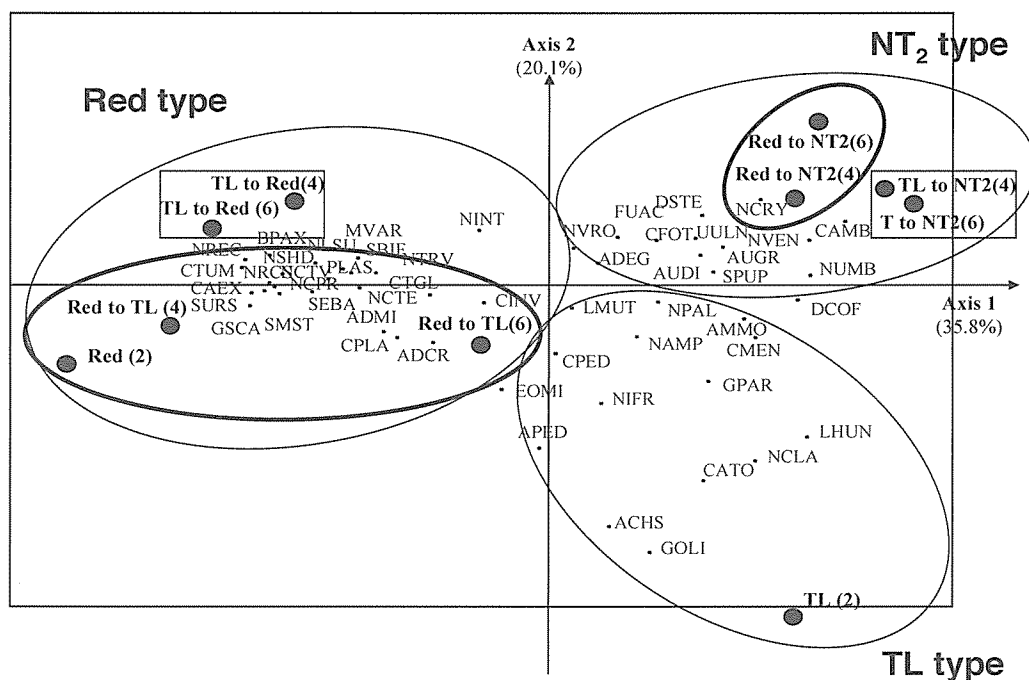
*Cyclotella meneghiniana* (CMEN) appear as predominant species at TL site on the week 2 (before transfer), with mean relative abundance  $\geq 10\%$  (figure 2b).



**Figure 2a and b.** Relative abundances of diatom species (mean value,  $n = 3$ ) with mean relative abundances  $\geq 8\%$  at week 2 (W<sub>2</sub>) (before transfer), at week 4 (W<sub>4</sub>) and 6 week (W<sub>6</sub>) (after transfer) of the experiment for glass substrates collected at Red, NT<sub>2</sub> and TL stations. (NUMB: *Nitzschia umbonata*, NPAL: *Nitzschia palea*, AUGR: *Aulacoseira granulata*, GSCA: *Gyrosigma scalproides*, NRCS: *Navicula recens*, CMEN: *Cyclotella meneghiniana*, BPAX: *Bacillaria paxillifera*)

After transfer from TL to Red and from TL to NT<sub>2</sub>, diatom composition of the communities is manifestly modified. For the diatom assemblages transferred to Red site, the initial dominant taxa *Nitzschia palea* (NPAL) is still persistent on substrates, but with lower relative abundances (ranged from 13.5% to 6.2%), until the end of the experiment, whereas *Nitzschia umbonata* (NUMB) and *Cyclotella meneghiniana* (CMEN) are replaced by the dominance of *Navicula recens* (NRCS) (30% at W<sub>4</sub> and 33 % at W<sub>6</sub>) and *Bacillaria paxillifera* (BPAX) (5.4% at W<sub>4</sub> and 19.5 % W<sub>6</sub>). The assemblages of the biofilms transferred from TL site to NT<sub>2</sub> are modified too and proportions of the 4 main diatom species such as *Nitzschia palea* (NPAL), *Nitzschia umbonata* (NUMB), *Cyclotella meneghiniana* (CMEN) and *Aulacoseira granulata* (AUGR) differ from initial colonization period.

Through Principal Component Analysis (PCA) of 53 diatom species upon a total of 277, modifications in the composition of transferred diatom communities in comparison with local diatom communities are figured in terms of duration time and sampling site (figure 3). The graph shows a projection of plan 1/2 explaining 53.9% of total variability, and includes all species data and transfers conditions. Communities from Red site are more rapidly discriminated when transferred to NT<sub>2</sub> site than to TL site



**Figure 3.** Principal Component Analysis (PCA) based on relative abundances of 53 diatom species (>1% and 3 replicates per site) at week 2 (W<sub>2</sub>) (before transfer) and week 4 (W<sub>4</sub>), week 6 (W<sub>6</sub>) (after transfer) of the experiment on glass substrates collected at Red, NT<sub>2</sub> and TL stations. (week is indicated in bracket)

In all the other cases, discrimination from initial colonization site occurs as soon as week 4 (after 2 weeks of transfer), indicating a clear shift of the communities to adopt same type of assemblages than those of their transfer site. Diatom communities transferred to NT<sub>2</sub> (from Red and TL) are characterized by species such as *Nitzschia umbonata* (NUMB), *Navicula veneta* (NVEN), *Sellaphora pupula* (SPUP), *Navicula cryptocephala* (NCRY), *Aulacoseira granulata* (AUGR), *Ulnaria ulna* (UULN). Group of diatom assemblages positioned on the left half plane are representative of Red site, communities transferred from TL to Red and from Red to TL such as *Gyrosigma scalpoides* (GSCA), *Navicula recens* (NRCS), *Seminavis strigosa* (SMST), *Cymbella excisa* (CAEX), *Achnanthyidium minutissimum* (ADMI), *Sellaphora bacillum* (SEBA), *Bacillaria paxillifera* (BPAX). Meanwhile, planktonic and polysabrobic taxa e.g. *Cyclotella atomus* (CATO), *Cyclotella meneghiniana* (CMEN), *Lemnicola hungarica* (LHUN), *Nitzschia palea* (NPAL), *Gomphonema parvulum* (GPAR) and *Eolimna minima* (EOMI) of the TL site (W<sub>2</sub>) mainly separate communities from Red and NT sites.

#### 4. Discussion

The structural adaptability of periphytic diatom communities to environmental disturbances was observed after transfer of early stages of periphytic diatom communities developed on artificial substrates from comparatively unpolluted site (Red) or heavily polluted site (TL); to moderate polluted site (NT<sub>2</sub>); and transfer between Red and TL site conversely. In our study, multiple sources of pollution in the rivers and directions of transfer determine responses of structural periphytic diatom communities are clearly illustrated by densities of diatom. Significant increase of diatom densities after transfer are already observed when periphytic diatom communities are moved from TL site to Red site and to NT<sub>2</sub>



site and from Red site to NT<sub>2</sub> site. Some communities are differently altered and increase their biomass; others show reduction in cells density after short time period of disturbance (figure 1a and b). Equally, an active immigration and growth rate of cells could also play an important role and contribute to increase the number of diatoms cells on substrates, in both early and late stage of colonization in our study (Stevenson and Peterson, 1991; Stevenson et al., 1991). Conversely, high concentrations of nutrients and other contaminants accompanied by low dissolved oxygen showed a marked influence on the accumulation of diatom on substrates in TL site where a slow development was reported in diatom density communities transferred to TL, though they were higher than those at local site before transfer. Same observations were made in previous experiment when dynamic of diatom colonization was followed in similar conditions (Duong et al., 2006b). In Red site before transfer (W<sub>2</sub>) a complex layer containing algae, bacteria, detritus and polysaccharide exudates which constitutes the biofilm matrix covering densely the substrates and permitted the development of higher number of diatoms cells in comparison with local site (TL).

Diversity and species richness are classical indicators of changes in communities structure to disturbances (Jüttner et al., 1996; Sabater, 2000). Sabater (2000) showed a marked decrease in diversity between references sites in comparison with affected sites of the Guadiamar River, S-W Spain. Stress effects of from mine drainage on diversity of primary producers in mountain stream were observed by Niyogi et al (2002), who suggested that physical stress did not strongly affect diversity of primary producers in streams like chemical stress. In our study, we found that response of structural communities (diversity index and species richness) to water quality changes was not clear (table 1). No significant difference in species richness and diversity index was found between local and transferred diatom communities in all cases except transferred communities from Red to NT<sub>2</sub>. This result is not surprising as diatom communities are deeply transferred after a disturbance. The assemblages are rearranged by changing their composition from sensitive species to more tolerant species to cope environmental altered condition. In this case, numerous replacements occur as long as polluted condition stays bearable for newly assemblages to settle without conducting to a notable and significant decrease of diversity. This adaptation can reversely happen with the replacement of resistant taxa by more sensitive taxa in aquatic stress (Kassai, 1999) and in return communities facing stress may modify their structure and function on their own (Niederlehner and Cairns, 1992). Thus, diatom communities transferred from Red to NT<sub>2</sub>, TL to Red and TL to NT<sub>2</sub> changed quickly their composition to adapt themselves to new conditions (figure 2a and b). Communities characterized by rich nutrients, high conductivity and low dissolved oxygen with dominant taxa such as *Nitzschia umbonata* (NUMB), *Cyclotella meneghiniana* (CMEN), *Nitzschia palea* (NPAL) are replaced by less tolerant taxa to pollution such as *Navicula recens* (NRCS), *Bacillaria paxillifera* (BPAX) and sensitive taxa *Achnanthes minutissimum* (ADMI) when diatom assemblages are moved from TL to Red site. The increase of diatom density and taxa less tolerant to pollution in communities transferred to Red site reflect considerable improvement of water quality. On the other hand, complete replacement of pollution-tolerant taxa in transferred communities to NT<sub>2</sub> site (Red to NT<sub>2</sub> and TL to NT<sub>2</sub>) showed capacity rapid recovery of periphytic diatoms which succeed to a structural stability after environmental changes. Our results are in agreement with those of Gold et al (2002), who observed structural community adaptation to new environmental conditions within two weeks after exposure to metal pollution. However, after four weeks of transfer, diatom communities did modify their structure but not completely for assemblages transferred from Red site to TL site (figure 3). At Red site before transfer, biofilm matrix densely covering substrates seems to limit the settlement of indigenous diatom species of polluted site (TL) onto to substrates. This results in the retention of initial species of Red site, *Navicula recens* (NRCS), *Bacillaria paxillifera* (BPAX), *Gyrosigma scalproides* (GSCA) still represented in a minor proportion of transferred communities at polluted site although some native diatom species of the TL site were already settled and gradually increased at W<sub>6</sub> (after 4 weeks

transfer) such as *Nitzschia palea* (NPAL) and *Cyclotella meneghiniana* (CMEN). In this case, four weeks prove to be necessary for transferred diatom communities to TL site to acclimate to TL water quality conditions. Nutrient enrichments are not only parameters affecting diatom assemblages communities, increased metals concentration and other contaminants present in TL site play a possible and important role in the responses of communities structure (Soldo and Behra, 2000), nature and source of wastewater effluents being diversified but with no precise data.

Diatom indices (IPS and DAIPo) have already been applied successfully to assess water quality rivers of Vietnam (Duong et al., 2006a and b). In this study, both of them give a similar trend of improvement in water quality when diatom communities were transferred from polluted site to unpolluted and to moderate sites; and worsening of water quality when communities were transferred from unpolluted site to heavily polluted and moderate polluted sites (table 1). Recovery of water quality was clearly observed very soon only after 2 weeks transfer. A slow decrease of diatom indices occurred when diatom communities were moved from Red to TL even if a proportion of diatoms taxa characterized of Red site still remained in its biofilm. Changes of diatom indices according to assemblages transfer direction showed a sensitivity of indices to notice alteration of water quality. This result is in agreement with observation of Rimet et al (2005) who reported diatom indices sensitivity to water quality changes by using transferred biofilms from several polluted rivers to an unpolluted stream. According to them, several indices such as IPS (Index of pollution sensitivity), ROT (Saprobic index of Rott), SHE (Schiefele and Schreiner index), EPI (Eutrophication Pollution Index) and CEE (European index) are well reflecting the improvement in water quality. Thus, diatom indices appear as relevant global criteria to assess water quality in rivers.

## 5. Conclusion

1. Responses of periphytic diatom communities to environmental changes varied and greatly depended on specific environment of each site.
2. Species richness and diversity index did not clearly reflected responses of periphytic diatom to disturbance. Shifts in values of IPS and DAIPo indices throughout the experiment indicate sensitivity of these indices to water quality changes.
3. Recovery of periphytic diatoms to new conditions appeared from two weeks in unpolluted site and moderate polluted sites. During this two week periods necessary for the shift in communities, diatom assemblages transferred from TL still keep initial characteristics of tolerant species; they are accompanied in their new location by new colonization of more sensitive species which can incorporate contaminant adsorbed within biofilm. This possible availability of contaminant could lead to a favored incorporation of contaminants in sensitive taxa newly settled which do not have the same defense or tolerance mechanisms against pollutants than tolerant taxa. Trophic transfer could take in charge contamination and transfer it to higher trophic levels for contaminants like metals. Set up of new transfer experiment including contaminant quantification at the different steps of the process that is: in polluted site before transfer and after transfer to non polluted site and reversely could be great interest to evaluate the importance of the potential of trophic transfers during the period of time necessary to the assemblages to adapt to new conditions. *In situ* experiment with declared metal pollution and indoor laboratory experiment are question to study thoroughly.

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