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Using Benthic Diatoms as a Bioindicator to Assess Rural-urban River Conditions in Tropical Area: A Case Study in the Sai Gon River, Vietnam

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ABSTRACT: The changes in diatom assemblages along an urban-to-rural gradient were characterized to assess the ecological status of the Sai Gon River, Vietnam. Diatoms and physico-chemical variables were measured at 10 stations during dry and rainy season. One-way ANOVA showed that diatom metrics and physicochemical variables were significantly different ($p < 0.05$) between the upper course sites and both the middle- and the lower sites. However, no significant differences were observed between the middle course sites and the lower course sites. *Achnanthidium minutissimum* and *A. exigua* were potential indicators of low nutrient in the upper course sites; *Melosira granulata* and *Navicula viridula* were preferred moderately eutrophic water in the middle course sites; while *Navicula cryptocephala* and *Nitzschia palea* were tolerant to very heavy pollution and dominant in the lower course sites. Canonical correlation analysis (CCA) results showed that concentration of TSS, TN, TP, BOD_5 and COD were the most important factors in structuring benthic diatom communities in the Sai Gon River. The results of this study indicated that diatom community was sensitive to changes in urban condition and could be used as an indicator of urbanization.

Keywords: phytoplankton, biological monitoring, water quality, urbanization.

INTRODUCTION

Ho Chi Minh City (HCMC) is the biggest city in Vietnam that will become a mega-city in the near future (Le et al., 2015). It began a period of rapid urbanization with the reform opening in the 1990s (Kontgis et al., 2014). The population has been continually increasing, as for population of 4.6 million in 1995 rose to 8.5 million in 2012, with the annual growth rate 3.5% per year, of which migrants make up about a third (Huynh, 2015). Many industrial and export-processing zones, as well as new residential areas, have emerged in recent years accelerating urbanization and development of HCMC. The rapid urbanization has increased impervious surface cover results in the increase of storm-water runoff and high temperature (Miller et al., 2014). Rivers and creeks in urban areas have been used for the disposals of both solid wastes and wastewaters, usually untreated, and are consequently severely polluted (Huy et al., 2003; Hien et al., 2007; Nguyen et al., 2011). This high pollution status has already damaged the ecological balance of the rivers and caused a series of environmental concerns, for example, alteration of river morphology, decline of biodiversity,

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increasing eutrophication and deterioration of surface water quality (Khai & Yabe, 2015; Le et al., 2015; Vo et al., 2015). Therefore, it is necessary to measure the human impacts on aquatic community structure.

To assess of urbanization impacts on aquatic biota, the microbial, macro-invertebrate, and fish assemblages have typically used (Shehane et al., 2005; Mangadze et al., 2016). Epilithic diatoms are usually the most prevalent and diverse algal group in running waters (Mangadze et al., 2016). They have a short reproductive cycle, allowing species assemblage compositions to quickly reflect changes in environmental condition. They could be used as indicators for heavily polluted sites where fish and macro-invertebrates are entirely absent or less diverse (Mangadze et al., 2016). Therefore, epilithic diatoms are the most potent bio-indicators for assessment of human impact and examination of pollution gradient (Beyene et al., 2009; Pham, 2017). Diatom-based indices are essential tools for assessment of environmental conditions in aquatic systems, particularly in temperate and sub-tropical climate (Almeida et al., 2014; Lavoie et al., 2014; Chen et al., 2016). Several diatom-based indices have been developed and successfully applied worldwide, especially indicative of eutrophication and organic pollution in the temperate region (Potapova & Charles, 2007; Kireta et al., 2012; Pham, 2017). In tropical areas, when used epilithic diatoms as indicators in tropical African rivers, Triest et al. (2012) indicated that both diatom assemblages and metric values had relationship with ecological condition and water quality. The same authors confirmed that epilithic diatoms as suitable water quality indicators in equatorial rivers. Because of the global distribution of most diatom species, diatom indices have been widely used as biological indicators (Bere & Tundisi, 2011). However, the physical and chemical parameters are the most common means of monitoring water quality in developing countries like Vietnam. There are still limited studies conducted on ecological assessment using diatom assemblages as indicators in tropical rivers, especially in Southeast Asia.

The Sai Gon River is essential because it provides drinking water for most of the residents of HCMC and the surrounding provinces (Nguyen et al., 2011). Over the past of years, the industrial cluster and the urban population of HCMC have grown considerably. This fast growth generated an increase of pollutants dumped in many spots of the river, resulting in surface water quality degradation. Therefore, the objectives of this research were: (1) to investigate the variations in water quality and benthic diatom assemblage structure along the Sai Gon River and relate those changes to environmental factors; and (2) to examine whether the diatom assemblages can sensitively reflect the variation in water quality and urbanization. It is hoped that the results of the present study can promote the dissemination of biological methods for water quality monitoring in tropical areas.

MATERIALS AND METHODS

The Sai Gon River locates in southern Vietnam that rises near Phum Daung in southeastern Cambodia. The river then flows south and southeast downstream of HCMC and empties into the Dong Nai River, which in its turn empties into the East Sea some 20 kilometers. The Sai Gon River has a total length of about 280 km, its catchment area covers approximately 4,750 km^2 and its average flow rate is 85 m^3/s (Nguyen et al., 2011).

In Dau Tieng District of Tay Ninh Province, the river is dammed to create Dau Tieng Reservoir, whose functions are flood control and irrigation for agricultural production in HCMC region. The Dau Tieng–Sai Gon River system is the most extensive irrigation system in southern Vietnam and an important water supply source for Ho Chi Minh City and nearby provinces (Pham et al., 2017).

The Sai Gon river basin has three regions with distinctive characteristics of occupation: the upper course is forest associated with agriculture and farmers' residences; the middle course shows intensive farming, and the lower course presents urban and industrial uses (Nguyen, 2011).

The water samples were collected in dry and wet season of 2016 at ten sampling stations (S1–S10). The sites were divided into three groups responded with three region categories, including 2 upper course sites (S1, S2), 3 middle course sites (S3–S5) and 5 lower course sites (S6–S10) (Fig. 1).

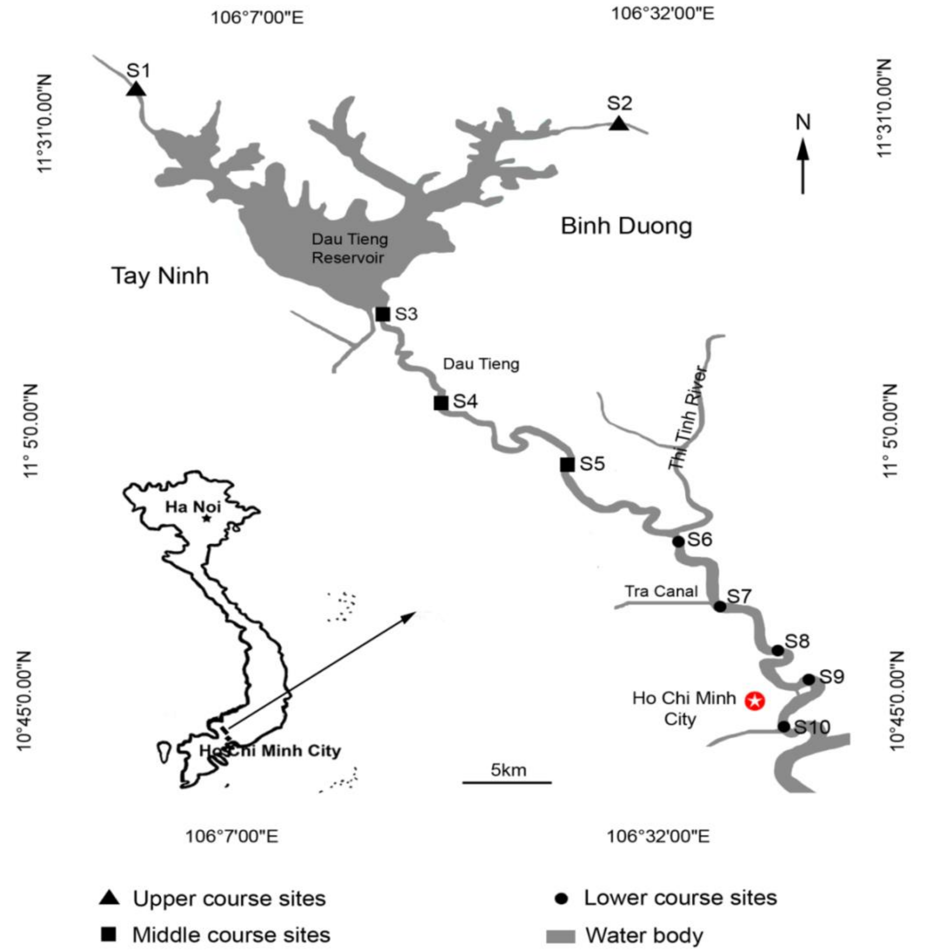

Fig. 1. Map of the sampling stations along the Sai Gon River. (S1-S2 stand for the upper course shows forest associated with agriculture and farmers' residences; S3-S5 stand for the middle course with intensive farming; and S6-S10 stand for the lower course present urban and industrial uses).

The conventional parameters like water temperature, water pH and dissolved oxygen were determined on-site by using a portable multi-meter (Hach Portable Meter–Sension 156, Hach, USA). The total suspended solids (TSS), biochemical oxygen demand after 5 days (BOD_5), chemical oxygen demand (COD), and water nutrients, total nitrogen (TN) and total phosphorus (TP) were measured in the laboratory according to Standard methods for the examination of water and wastewater (APHA, 2005).

Benthic diatoms were collected on hard substrates in quintuplicate by scraping five stones in natural streams or five areas on the cement or hard surfaces in an urban area with a hard toothbrush over a surface area of 10 cm^2 according to the method of Chen et al. (2016). Samples were preserved in 100 ml plastic bottles and fixed in Lugol solution. In the laboratory, about 20 ml samples of the diatom suspensions were cleaned of organic materials with concentrated nitric acid, and the treated samples were washed with distilled water until they reached a circumneutral pH according to the methods of Walker & Pan (2006). An aliquot (1 ml) of the cleared sample was deposited and counted on the Sedgewick rafter counting chamber. Diatom valves were scanned with a light microscope (Olympus BX51, Olympus, Tokyo, Japan) at 400 × magnification. A minimum of 500 diatom valves was counted on each slide; fewer valves were counted on some slides when diatoms were scarce. Valves were identified to the species or sub-species level. For diatom identification, the books of Krammer & Lange-Bertalot (1986; 1988; 1991a, b), Krammer (2000), Metzeltin & Lange-Bertalot (1998; 2002; 2007) and Rumrich, Lange-Bertalot & Rumrich (2000) were used.

One-way analysis of variance (ANOVA) was used to test the significance of the differences among the upper course sites, the middle course sites and the lower course sites based on the transformed water physical and chemical variables and the diatom species structure metrics. The analysis was completed using Tukey's HSD test a significant difference. The abundances of all taxa were expressed as relative counts before analysis. The diatom community structural attributes of species richness (S), Shannon–Weiner index (H), species evenness (J) and Simpson's diversity index (D), that are commonly used in water quality bioassessment (Stevenson et al., 2010), were used to characterize each site to understand the species changes during dry and wet seasons. Samples were classified based on diatom species relative abundance, using clustering analysis. Diatom metrics and cluster analysis were calculated by using the PRIMER V.5 analytical package developed by Plymouth Marine Laboratory, U.K.

The diatom community analysis was performed using canonical correspondence analysis (CCA), a multivariate analysis direct of gradients and developed by Ter-Braak (1986). CCA was applied to reveal the main gradients of change in the species composition. This analysis related the gradients of change to the eutrophication processes to identify which environmental variables best explained the distribution of the diatoms at the sampling stations. Abundant species in two or fewer samples were omitted from the review due to the low representation of their scores. Environmental and biological matrices were log10(X+1) transformed to normalize their distribution before analysis. Analyses were processed using the software PAST 3.11.

RESULTS AND DISCUSSION

Physico-chemical variables were given in Table 1. A wide range of physico-chemical was observed. In general, the downstream sites had higher nutrient concentrations and lower water quality than the upper- and middle course sites. One-way ANOVA showed that water physico-chemical variables were significantly different (p<0.05) between lower course sites and both top- and middle course sites. The water quality generally tended to deteriorate down-stream as the river pass through the urban area due to the discharge of treated and untreated domestic and industrial effluent as well as other diffuse sources of pollution from the city. The water temperature increased slightly down-stream; however, the difference was not statistically significant (ANOVA, p>0.05) among the three site categories. On the other hand, electrical conductivity, turbidity (TB), BOD_5, COD, TSS, TN and TP increased significantly downstream (ANOVA, p<0.05) while DO decrease significantly down-stream (ANOVA, p<0.05).

Table 1. Median water quality variable (range in parentheses) from three categories of sampling sites in dry and wet seasons. Results in boldface type are significantly different from other sites. The value of *n* was the number of water samples measured in the sites.

Parameters	Unit	Upper sites (n=4)	Middle sites (n=6)	Lower sites (n=10)
WT	°C	28.8 (27.0-30.0)	30.0 (29.0-31.0)	29.7 (28.0-31.0)
pH		7.1 (6.7-7.4)	7.1 (7.0-7.3)	6.7 (6.1-7.2)
DO	mg/l	6.1 (5.6-6.5)	4.2 (3.9-4.5)	3.2 (2.3-3.8)
EC	µS/cm	26.5 (19.0-38.0)	47.7 (28.3-90.5)	213.5 (83.8-534.0)
Turbidity	NTU	9.3 (4.0-15.0)	73.7 (13.3-132.8)	131.9 (83.7-173.3)
COD	mg/l	5.5 (4.0-7.0)	14.4 (11.8-19.2)	18.4 (13.0-28.2)
BOD_5	mg/l	3.9 (3.0-5.0)	7.5 (5.8-10)	10.2 (6.5-15.2)
TN	mg/l	1.4 (1.1-1.7)	2.4 (2.0-2.9)	2.5 (2.0-2.8)
TP	mg/l	0.1 (0.1-0.2)	0.3 (0.1-0.7)	0.43 (0.04-1.25)
TSS	mg/l	12.5 (8.0-17.0)	34.6 (5.7-61.5)	51.2 (18.9-99.0)

A total of 103 diatom species, belonging to 32 genera, were identified. *Achnanthidium*, *Coscinodiscus*, *Cymbella*, *Eunotia*, *Gomphonema*, *Gyrosigma*, *Navicula*, *Nitzschia*, *Pinnularia*, and *Surirella* were the dominant genera (Fig. 2). The relative abundance of each dominant genus among sites was quite different. In the upper course sites, *Achnanthidium*, *Cymbella*, *Eunotia*, *Gomphonema* and *Pinnularia* reached the highest relative abundance. Diatoms *Achnanthidium minutissimum* and *A. exigua* were the most dominant species (a relative abundance of 10% in at least once) in the upper course sites. In the middle course sites, the genera *Melosira*, *Eunotia*, *Navicula*, *Nizschia* and *Pinnularia* registered the highest relative abundance. *M. granulata* and *Navicula viridula* were the dominant species in the middle course sites. In the lower course sites, the genera *Coscinodiscus*, *Navicula*, *Nitzschia*, *Pinnularia* and *Surirella* had the highest relative abundance. In particular, the *Nitzschia* relative abundance reached 43.5%. The most abundant species were *Navicula cryptotenella* and *Nitzschia palea* (Fig. 2).

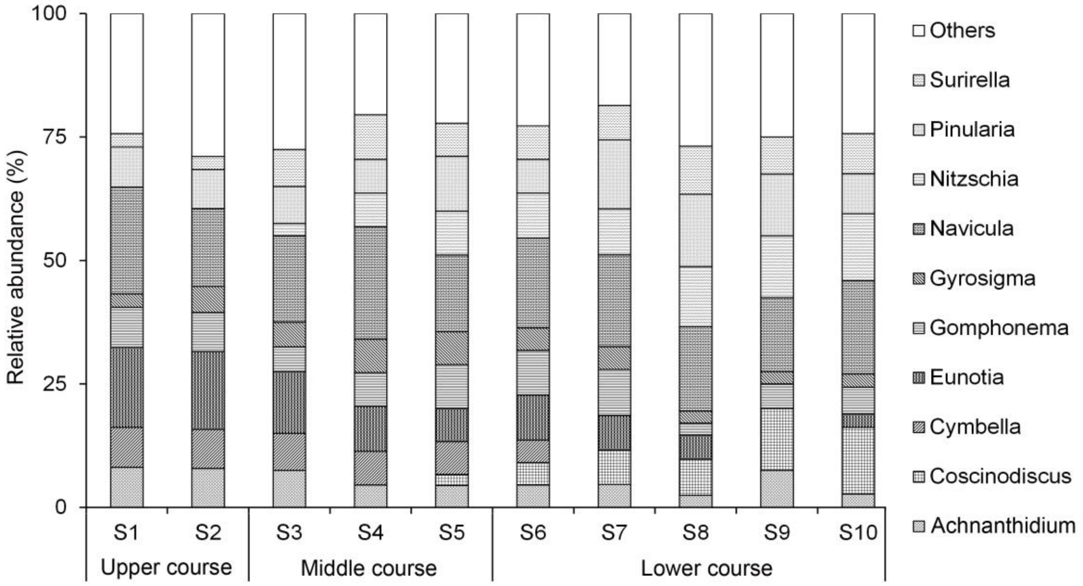

Fig. 2. Composition of 10 dominant genera at the sampling sites by combining samples of dry and wet season.

Analysis of diatom assemblage species diversity metrics, including species richness (S), Shannon diversity (H'), Pielou's evenness (J') and Simpson diversity (D), showed that there were significant differences between upper course sites and both middle- and lower course sites (ANOVA, $p<0.05$). However, no significant differences (ANOVA, $p>0.05$) in S, H', J' and D ware observed between middle course sites and lower course sites. The lower course sites scored the lowest of all groups in S, H' and J' and had the greatest percent relative abundance of dominant taxa (Fig. 3).

The values of the Shannon–Weiner index calculated for each site, with correspondent judgment and class of quality was presented in Table 2. The ecological quality in the Sai Gon River varied from good, moderate to poor status (strongly polluted in SG9 and SG10) based on the classification systems of Sven et al. (2010). However, based on BOD_5, COD, total nitrogen and total phosphorus according to QCVN 08:2008, the water quality was classified into A_1 and B_1 class, which could be used for drinking, irrigation and transportation purposes (Table 2).

The species composition and relative abundance of species clearly showed that all samples were classified into three groups according to the cluster analysis (Fig. 4). The first group was composed of 10 samples, belonging to the 2 sites from the upper course of the river (S1 – S2). The second group contained 15 samples belonging to 3 sites in the middle zone (S3–S5) were members of the second group. The third group was mainly composed of lower course sites consisting of the last 25 samples. The shared characteristic of S6, S7, S8, S9 and S10 was that they had one or two species with higher relative abundance than others (Appendix 1).

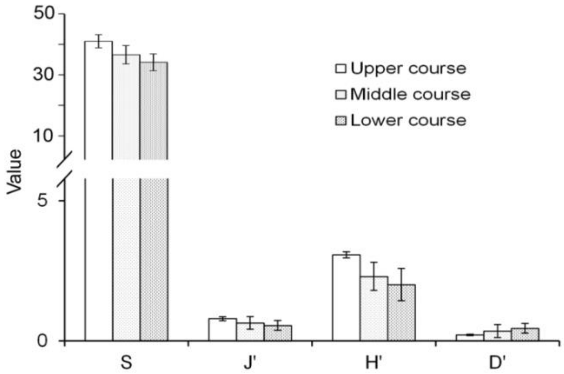

Fig. 3. Comparison of diversity indices (S, H', J', D') between upper course sites and lower course sites.

Table 2. Ecological quality classification. Water quality class was based on BOD_5, COD and total nitrogen according to QCVN 08:2008–the Vietnamese national technical regulations for surface water quality (A_1–for drinking water purposes, B_1 acceptable for irrigation and transportation or other activities that does not require a high quality standard).

Sampling site	Ecological quality	Quality class
S1	Good status	A_1
S2	Good status	A_1
S3	Moderate status	A_1
S4	Moderate status	A_1
S5	Moderate status	A_1
S6	Moderate status	A_1
S7	Moderate status	A_1
S8	Moderate status	A_1
S9	Poor status	B_1
S10	Poor status	B_1

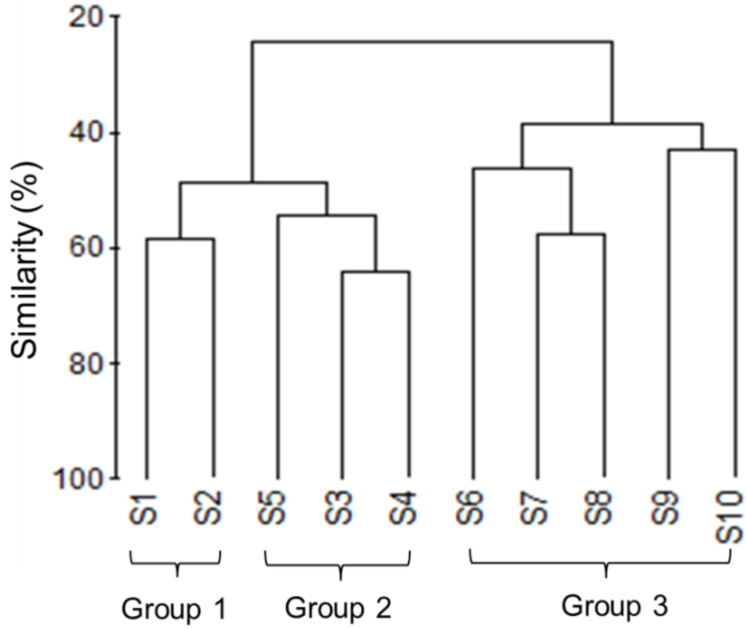

Fig. 4. Cluster analysis indicating the similarity of sites using diatom community structure and abundance at each site for dry and wet season.

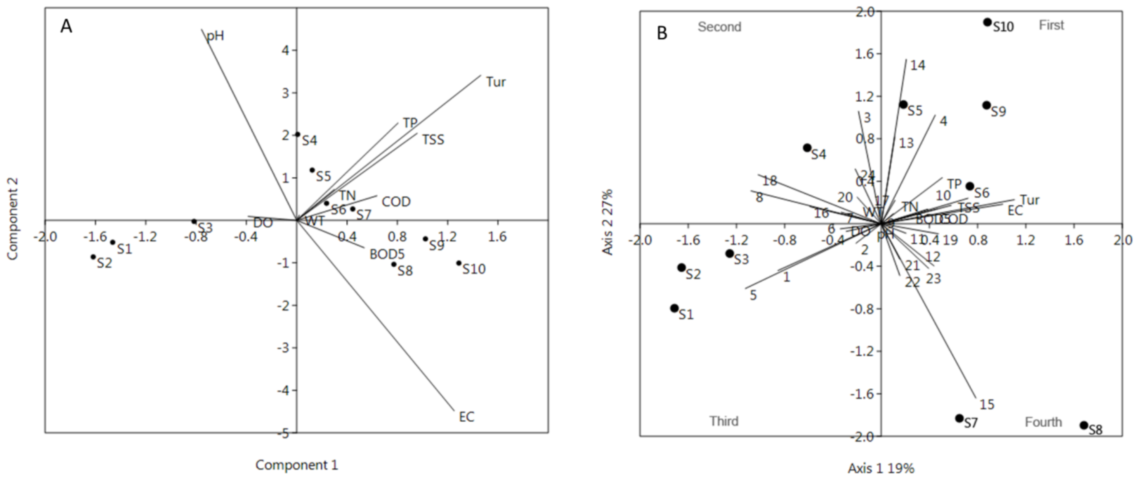

Fig. 5. Canonical correspondence analysis (CCA) diagram showing selected environmental variables and most frequently occurring diatom taxa in the ordination space of the first and second axes. Taxa codes correspond to those in Appendix 1.

CCA axis 1 and 2 account for 92% of the variance and ordination separated the samples into three groups along the gradient of the urban-to-rural site. Axis 2 represents an upper course to lower the course of water quality gradient. Most of the lower course sites were ranked in the fourth quadrant, the middle course sites were in the first quadrant, and the upper course sites were in the third quadrant (Fig. 5a). Axis 1 was positively correlated with TB ($r = 0.41$) and negatively with EC ($r = -0.78$). Axis 2 was positively correlated with EC and TB ($r = 0.59$; 0.49) and it may represent an urban impact or water quality degradation gradient.

Of the 103 diatom taxa identified in this investigation, 24 taxa, with relative abundance ≥ 10%, were included in data analysis using CCA (Fig. 5b). Results of CCA enable us to relate diatom distribution to diatom succession during the sampling period. The first axis account for 19% of the variance and ordination clearly separated the samples into two groups, characterizing two different diatoms community structures with the first group in the half negative axis side, and the second group in the positive side. The first group consisted of upper course sites (S1, S2) and two middle course sites (S3, S4) in the second and third quadrant. These samples were associated with high DO and low EC, TB, TSS compared to the lower course sites. These parameters were highly positively associated with diatom *Achnanthidium* minutissimum, *Cymbella lanceolata*, *Eunotia gracilis* and *Navicula veneta*) (Fig. 5b). The second group mainly composed of lower course sites in the first and fourth quadrant. Samples S5, S6, S7, S8, S9 and S10 were associated with high TB, EC, TSS, TP and low DO. These environmental conditions were highly positively correlated with diatom *Cyclotella meneghiniana*, *Melosira granulata*, *Navicula cryptotenella*, *N. gregaria*, *N. viridula* and *Nitzschia palea*.

In Vietnam and Ho Chi Minh City surface water quality monitoring program has been implemented and routinely conducted. However, the traditional method of water quality monitoring is mainly by inspection of physical and chemical parameters, or by calculation of a comprehensive water quality index based on physic-chemical variables (Trinh & Long, 2011). An example is the water quality index, which relies on normalizing or standardizing data parameters chosen according to expected concentrations and interpretation of "good status" versus "polluted status" conditions (Trinh & Long, 2011). Physical and chemical data are essential information on indicating the present status of water quality (Duong et al., 2007). However, whether water quality is good or bad is not only reflected in terms of physic-chemical parameters of the index. Quality also should be embodied in the response of all kinds of aquatic organisms, especially those that are considered to be sensitive to the changes of the water environment, such as the diatoms, zooplankton, macro-invertebrates (Chen et al., 2016). They all are very important in the aquatic food web and often used as monitoring indicator species.

The diatom species composition sensitively reflected the ecological status of the river and could be divided into three groups through the dominant genera and clustering analysis. Based on species responses to pollution and relation to the characteristic of the habitats, the indicator species in each group appear to have ecological significance. For example, A. minutissima, considered to be a low nutrient indicator species (Potapova & Charles, 2007; Chen et al., 2016), was one of the dominant species in the upper course sites; *Melosira granulata* and *Navicula viridula*, which were seemed to prefer moderately eutrophic water, were dominant in middle course sites; and *Nizschia palea* and *N. recta*, which were tolerant to very heavy pollution in many areas (Duong et al., 2007), were the dominant species in the lower course sites with high impact form urbanization. *N. palea* was also reported the most abundant species in all the stations and tended to increase downstream in several African rivers (Triest et al., 2012). *Navicula*, *Nitzschia*, *Cymbella*, *Eunotia*, *Pinnularia* and *Gomphonema* were among the most diverse genera in African rivers (Triest et al., 2012). *Achnanthes* (*Achnanthidium*), *Gomphonema* and *Navicula* were also reported as the most common genera in East Africa (Bellinger et al., 2006).

Benthic diatom diversity (S, H', J', D) and similarity indices are routinely used in ecological health assessment and to assess the impacts of various human activities of freshwater ecosystems (Almeida et al., 2014; Lavoie et al., 2014). Diatom's species richness and abundance may depend on the degree and type of pollution (Walker & Pan, 2006). The results of this study were consistent with previous studies that diatom's species richness in the polluted city water bodies was lower than that in the unimpacted water bodies (Walker & Pan, 2006; Chen et al., 2016). Thus, species diversity can be used for the evaluation of the rural-urban gradient with increasing levels of pollution.

Results of CCA analysis showed that EC, TB, TSS, TN, TP, BOD_5 and COD were the critical factors in structuring benthic diatom communities in the study area. The diatom composition in lower course sites was greatly influenced by high EC, TB, TSS, and TP concentration, which may be due to the emissions of urban sewage and industrial wastewater, which is associated with urbanization (Nguyen et al., 2011). EC estimates the amount of total dissolved salts or the total amount of dissolved ions in the water (Walker & Pan, 2006). In this research, the average EC concentration in the lower course sites was 213 μs/cm, higher than the 27 μs/cm observed in the upper course sites and the 48 μs/cm observed in the middle course sites ($p < 0.01$). EC was also considered to be the determining factor of diatom species composition and distribution of the benthic diatoms in U.S. rivers (Stevenson et al., 2008). Results of this study were also in line with the previous observation that EC, TB and nutrients were the most important environmental factor to distinguish the diatom gradient from urban to suburban sites (Walker & Pan, 2006; Chen et al., 2016).

According to previous studies, the water quality of the Sai Gon River polluted mainly organic matter, heavy metal, micro organisms and toxic compounds (Le et al., 2016). Particularly, bacteria, EDCs, Fe, Cd and Mn had higher potential risks and may affect human health as well as safe water supply (Le et al., 2016). Results of the present study showed that the water quality of the Sai Gon River was severe eutrophication due to nutrient enrichment, particularly nitrogen and phosphorus. This may associate with storm water runoff, increase urban development and cause by other catchment activities such as agriculture and industrial wastewater discharge.

The use of more integrative indicators, such as diatoms together with the environmental parameters, achieves a more realistic approach for water quality and ecological health assessment (Chen et al., 2016). The combination of physico-chemical variables and biological indices provides a robust tool for reliably characterizing the river in terms of water quality. Physico-chemical variables indicate the water quality, while biological indices are practical tools for monitoring the ecological status of the river. Diatom metrics showed the ecological conditions in the Sai Gon River varied from good, moderate to poor status based on the classification systems of (Sven et al., 2010). Results of the present study agreed well with the observations of (Nguyen et al., 2011) that the downstream of the Sai Gon River had lower water quality than the upstream, always confirmed by the diatom metrics and also by the physicochemical parameters. This common trend is due to an increase in urbanization pressure and industrial activities on the surrounding lands in the lower course sites. The poor water quality in the downstream may also contribute by sewage discharge from several canals in the urban areas (Nguyen et al., 2011; Le et al., 2016). Although the physical-chemical monitoring program was already implemented in the study area, it was judged to lack sensitivity in ecological quality classification due to it only based on abiotic characteristics. Results of this study suggested whenever possible both abiotic and biotic parameters should be used to assess the overall condition of a river.

CONCLUSIONS

Changes in diatom assemblages reflected well on the rural-to-urban gradient. Biological metrics showed that diatom composition was more sensitive and accurate than the routine investigation of water physico-chemical parameters. It provides essential complimentary information for the evaluation of the water quality and ecological status in lotic systems. Therefore, it is crucial to use diatoms together with water physical and chemical parameters for surface water quality assessment.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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