

 ISSN:
 KHOA HỌC TỰ NHIÊN VÀ CÔNG NGHỆ

 1859-3100
 Tập 14, Số 3 (2017): 149-161

HO CHI MINH CITY UNIVERSITY OF EDUCATION JOURNAL OF SCIENCE

NATURAL SCIENCES AND TECHNOLOGY Vol. 14, No. 3 (2017): 149-161

Email: tapchikhoahoc@hcmue.edu.vn; Website: http://tckh.hcmue.edu.vn

THE SEASONAL AND SPATIAL VARIATIONS OF PHYTOPLANKTON COMMUNITIES IN CORRELATIONS WITH ENVIRONMENTAL FACTORS IN THE DONG NAI RIVER, VIETNAM

Pham Thanh Luu^{*}

Vietnam Academy of Science and Technology (VAST), Institute of Tropical Biology Received: 25/11/2016; Revised: 02/3/2017; Accepted: 24/3/2017

ABSTRACT

Phytoplankton community and their correlation with environmental factors were investigated in the Dong Nai River. Higher diversity was observed in dry season with the dominance of diatom. Environmental variables were different between upper and lower sections. Phytoplankton metrics and the nutrient concentration characterized a pollution gradient along the river. Nutrient levels and turbidity governed the distribution of phytoplankton structure in the river.

Keywords: bio-indicator, Dong Nai River, phytoplankton, water quality.

TÓM TẮT

Sự thay đổi theo không gian và thời gian của khu hệ thực vật phiêu sinh trong mối tương quan với các thông số môi trường ở sông Đồng Nai, Việt Nam

Nghiên cứu này khảo sát khu hệ thực vật phiêu sinh (TVPS) trong mối tương quan với các thông số môi trường ở sông Đồng Nai. Kết quả cho thấy khu hệ TVPS đa dạng hơn vào mùa khô, trong đó tảo silic chiếm ưu thế. Tính chất hóa lí thay đổi đáng kể giữa hai vùng thượng nguồn và hạ nguồn. Khu hệ TVPS và các thông số về dinh dưỡng thay đổi theo gra-đi-ăng chất lượng nước từ thượng nguồn về hạ nguồn. Hàm lượng dinh dưỡng và độ đục chi phối phần lớn cấu trúc quần xã TVPD ở sông Đồng Nai.

Từ khóa: chỉ thị sinh học, chất lượng nước, sông Đồng Nai, thực vật phù du.

1. Introduction

Phytoplankton plays an important role in aquatic ecosystems as they produce the foundation for aquatic food chains and has attracted great attention worldwide. To adequately understand the life cycle of phytoplankton communities and how they responds to ecological change, researchers have investigated the distribution of phytoplankton, both temporally and spatially, in various water bodies for years. In different types of inland water, changes in the phytoplankton community have long been recognized as providing a

Email: thanhluupham@gmail.com

good indicator of the trophic status and environmental quality. Phytoplankton with high species richness, high reproduction rate and very short life cycle enable the examination of both short-term and long-term effects. Therefore, the alterations in phytoplankton species composition and biomass in water body reflect a changing environment and indicate the trophic status [1].

Phytoplankton community has long being used for water quality evaluation and phytoplankton indices are the most common tool to summarize the information provided by the phytoplankton assemblages. However, phytoplankton is regulated by various environmental variables. The main environmental factors recognized as controlling community structure of phytoplankton are physical, (mixing of water masses, light, temperature, turbulence and salinity), chemical (nutrients) and biological variables (grazing by zooplankton and fishes). Previous phytoplankton studies have shown that nitrogen and phosphorus are the most important nutrients for maintaining the growth and reproduction of phytoplankton. Actually, various physico-chemical parameters are responsible for controlling phytoplankton growth and reproduction. These factors could include the impact of both environmental conditions and human stressors, such as variations in nutrients concentration, the combined effect of land use/land management and urbanization [1, 2].

The primary objective of this study was to illustrate the temporal and spatial distribution of the phytoplankton composition and bio-mass in the Dong Nai River (DNR). Additionally, the critical environmental factors that strongly influence the distribution of phytoplankton were identified with Canonical Correspondence Analysis (CCA) of phytoplankton community composition and aquatic environmental factors. In addition, the effect land-use change and urbanization on the DNR's phytoplankton populations are indicated and discussed. The case study in the DNR was chosen because of its high relevance for water supply to millions people in HCMC and nearby provinces and as a wastewater recipient from million inhabitants in Dong Nai, Binh Duong provinces and HCMC. It is hoped that the results of this study can accelerate the establishment of biological method for water quality monitoring in Vietnamese waters.

2. Materials and methods

2.1. Study area

The Dong Nai River originates in the Central Highlands region of the southern portion of Vietnam, northwest of Da Lat. It flows west and southwest for about 300 miles (480 km), joining the Saigon River southwest of Bien Hoa and empties into the East Sea (Fig. 1). At the rapids of Tri An, west of Dinh Quan, it is joined by the Be River. In Vinh

Cuu district of the Dong Nai Province, the river is dammed to create Tri An Reservoir, whose functions are flood control and irrigation for agricultural production. Currently, the river basin is experiencing rapid urbanization, and includes the rapid growing cities of Ho Chi Minh City (HCMC), Bien Hoa, and Thu Dau Mot. Continued urbanization and an expanding economy have been increasing stresses on water quality of the river. The river basin has two regions with distinctive characteristics of occupation: the upper course shows intensive farming and the lower course presents urban and industrial uses.



Figure 1. Map of the Dong Nai River and of the 15 sampling locations

2.2. Field sampling and nutrient analyses

Two surveys were conducted at 15 stations in the Dong Nai River in March (dry season) and September 2010 (rain season) (Fig. 1). DN1–DN6 stand for the upper course sites with intensive farming; and DN7–DN15 stand for the lower course sites present urban and industrial uses. Water samples were collected at a depth of 0.5 m, 3 replicated were collected at each station. Water temperature, pH, DO and turbidity were measured in situ using a multi-parameter (Hach 156, Co, USA). For measuring inorganic nutrient parameters, surface water sample was collected using plastic containers (2-L capacity). The plastic containers were rinsed thoroughly with sampling water before use. After filling the containers, they were sealed, kept in ice-box and transferred to the laboratory for the

physico-chemical analysis. Dissolved nutrients: nitrate $(N-NO_3)$, nitrite $(N-NO_2)$, ammonium $(N-NH_4)$ and phosphate $(P-PO_4)^{3-}$ were measured according to the methods of APHA (2005) [3].

Phytoplankton samples were collected from the surface waters by towing a plankton net (mouth diameter 0.5 m) made of bolting silk (No. mesh size 25 μ m). Subsequently, samples were kept in 150 mL plastic bottle and preserved in 4% neutralized formalin and used for qualitative analysis. For quantitative analysis, 10L of surface waters was filtered through the plankton net and concentrated to 50 mL then preserved in 4% neutralized formalin.

2.3. Phytoplankton identification

Phytoplankton samples were analyzed according to morphological observation and identified using standard works of Desikachary [4], Duong and Vo [5], Shirota [6]. The abundances of all taxa were expressed as relative counts. Quantitative analysis was carried out using Sedgewick Rafter counting sedimentation technique. Samples were allowed to settle in the counting chamber for 3–5 min prior to enumeration [7]. Counting of plankton was done with the help of hand counter.

2.4. Data analysis

One-way analysis of variance (ANOVA) was used to test the significance of the differences among the urban upstream and downstream sites based on the transformed water physical and chemical variables and the phytoplankton species structure metrics. The data was checked if it is fulfilled assumptions of homogeneity by Levene's test. In case of Levene's test showed homogeneity of variances was not fulfilled, data will be transformed for re-test. The analysis was completed using Tukey's HSD test significant difference. The Pearson correlation analysis was used to determined correlation among phytoplankton metrics and environmental variables. All statistical analysis was performed using SPSS v.16.0 (IBM Corp., Armonk, NY, USA).

The planktonic diatom community structural attributes of species richness Margalef's index (S), Shannon–Weiner diversity index (H'), Simpson's diversity index (D) and Pielou's evenness index (J) that are commonly used in water quality bio-assessment were used to characterize the phytoplankton community at each site. These metrics were calculated by using the PRIMER VI analytical package developed by Plymouth Marine Laboratory, U.K.

Canonical correspondence analysis (CCA) was used to elucidate the main environmental driving force in the planktonic diatom community. All variables (except pH) were log(X+1) transformed to normalize their distributions before analysis. Monte Carlo permutation tests were used to reduce further the environmental variables to those correlated significantly with the derived axes. Only those taxa that were observed in more than 5% of the samples were included in analyses of taxa abundances to minimize the influence of rare taxa. All ordinations were performed using CANOCO version 4.5 for Windows.

3. Results

3.1. Environmental variable

The average physico-chemical variables concentrations from the surface waters of the DNR in dry and wet were showed in Table 1. The seasonally fluctuations in the pH varied from 6.2 to 7.3 with minimum during dry season and maximum during wet season. The surface water temperature varied between 27.3 and 31.8°C with minimum during wet season and maximum during dry season. The mean seasonally dissolved oxygen values ranged from 4.5 to 6.2 mg/L. Turbidity ranged from 10.7 to 179.7 NTU with minimum during dry season and maximum during rainy season. Nutrients such as nitrate varied between 0.16 and 0.48 mg/L with minimum and maximum values during dry season. Ammonium varied from 0.03 to 0.24 mg/L with minimum during rainy and maximum during dry seasons. Inorganic phosphate ranged between 0.01 and 0.08 mg/L with minimum during wet seasons.

In general, the lower course sites had higher nutrient, turbidity concentrations and lower water quality than the upper course sites. One-way ANOVA and Tukey's HSD test showed that the mean of turbidity, ammonium, nitric, nitrate and phosphate were significantly different (p<0.05) between lower course sites and upper course sites in both dry and wet seasons. The water quality generally tended to deteriorate down-stream as the river pass through the urban area due to discharge of treated and untreated domestic and industrial effluents as well as other diffuse sources of pollution from the cities and towns along the river. The pH decreased slightly down-stream; however, the difference was not statistically significant (ANOVA, p> 0.05) among the two site categories. On the other hand, nutrient concentrations such as NH₄⁺, NO₂⁻, NO₃⁻, PO₄³⁺ and turbidity increased significantly downstream (ANOVA, p<0.05).

		Dry season			Wet season		
	Variables	Ave±SD	Min	Max	Ave±SD	Min	Max
Upper course sites	pН	7.0±0.1	6.8	7.1	6.9±0.2	6.7	7.3
	Temperature	30.5±0.8	29.7	31.8	28.7 ± 1.1	27.3	29.9
	Turbidity (NTU)	12.9±1.9	10.7	16.3	34 <u>+</u> 4.2	27.5	39.8
	DO (mg/L)	5.8±0.2	5.6	6.2	5.5±0.2	5.3	5.8
	NH ₄ ⁺ (mg/L)	0.072±0.015	0.05	0.09	0.072±0.03	0.03	0.11
	NO2 ⁻ (mg/L)	0.006 ± 0.001	0.004	0.008	0.006 ± 0.001	0.005	0.006
	NO ₃ ⁻ (mg/L)	0.302±0.069	0.19	0.39	0.273 ± 0.062	0.22	0.38
	PO ₄ ³⁻ (mg/L)	0.020 ± 0.006	0.01	0.03	0.025±0.01	0.01	0.04
	pН	6.5±0.3	6.2	6.9	6.6±0.2	6.2	6.9
Lower course sites	Temperature	29.9±0.2	29.3	30	29.2±0.5	28.1	29.7
	Turbidity (NTU)	20.6±5.7	12	27.7	73.3±7.6	22.2	179.7
	DO (mg/L)	5.1±0.5	4.5	5.9	5.5±0.4	5.1	6
	NH4 ⁺ (mg/L)	0.186±0.053	0.07	0.24	0.108 ± 0.038	0.04	0.16
	NO2 ⁻ (mg/L)	0.013±0.004	0.005	0.018	0.011 ± 0.004	0.006	0.017
	NO ₃ ⁻ (mg/L)	0.278±0.107	0.16	0.48	0.328 ± 0.062	0.27	0.47
	PO ₄ ³⁻ (mg/L)	0.044±0.019	0.01	0.070	0.048 ± 0.019	0.02	0.08

Table 1. Temporal and spatial variation of physio-chemical variablesfrom the surface waters of the Dong Nai River

3.2. Seasonal and spatial distributions of phytoplankton compositions and abundance

A total of 139 species of phytoplankton belonging to 6 phyla and 68 genera were identified. Among these species, 26 species belonging to 14 genera in Cyanophyceae represented approximately 19% of the total species, 58 species belonging to 26 genera in Bacillariophyceae represented 42% and 42 species belonging to 20 genera in Chlorophyta represented 30%. In addition, the samples included 9 species belonging to 4 genera in Euglenophyceae, 2 species belonging to 2 genera in Chrysophyceae and 2 species belonging to 2 genera in Dinophyceae. The number of phytoplankton species was greater in dry season. An increase in Bacillariophyta species occurred in dry season, when 11 species were found, in contrast a decrease in Chlorophyta was found, when 9 species disappeared. The phytoplankton composition in the DNR was showed in Fig. 2.



Figure 2. The seasonal distributions of phytoplankton composition in the Dong Nai River in dry (A), wet (B) season and (C) both seasons

The temporal and seasonal distributions of the most dominance genera and phytoplankton abundance in the Dong Nai River were showed in Fig. 3.



Figure 3. The temporal and seasonal distributions of the most dominance genera and phytoplankton abundance in the Dong Nai River in dry (left columns) and wet (right columns)

The highest average algal cell density (up to 210×10^3 cells/L) occurred in DN1 station in dry season, whereas the lowest density (13×10^3 cells/L) was recorded in DN15 station in dry season (Fig. 3). The average algal cell densities for DNR were $133 \pm 54 \times 10^3$ cells/L in dry and $45 \pm 25 \times 10^3$ cells/L in rainy season (Fig. 3). The temporal and

spatial distribution of the most important algae and the average cell density varied substantially among the seasons. In both dry and rainy season, the average algal cell density in the upper part of the river was higher than lower section. The abundances of *Coscinodiscus* (Bacillariophyceae) increased accompanied with the decreasing abundance of *Microcystis* (Cyanophyceae) and *Cosmarium*, *Staurastrum* (Chlorophyceae) was observed along the downstream of the DNR. At almost stations especially in upper section, blue-green algae such as *Microcystis*, *Oscillatoria* and diatom *Aulacoseira* contributed the most to phytoplankton abundance, whereas centric diatoms such as *Aulacoseira* and *Coscinodiscus* provided the greatest contributions to algal abundance at the lower section especially in dry season at DN14 and DN15 (Fig. 3). In all station the 3 most important phyla were cyanobacteria, diatom and green algae, while the abundances of the other phyla (Chrysophya, Eughylenophyta and Dinophyta) were not as large as the abundances of the three main phyla. They all most had some sporadic disappearances at different stations.

3.3. Phytoplankton metrics

Temporal and spatial variation of phytoplankton metrics including species richness (S), Shannon diversity (H'), species evenness (J) and Simpson diversity (D), in the DNR was showed in Fig. 4. Results showed that there were significant differences in species richness between upper course sites and lower course sites (Fig. 4A, p<0.05). The mean values of species richness in dry and wet seasons were 37.7 and 30.5, respectively. The mean values of H' index were 2.0 in dry and 2.3 in wet season. The mean values of species evenness were 0.6 in dry and 0.5 in wet season. The mean values of Simpson diversity index were 0.45 in dry and 0.42 in wet season (Fig. 4B). The lower upper sites scored the lowest of all groups in S, H' and J, but had the greatest percent relative abundance of dominant taxa (Fig. 4A).



Figure 4. Change of phytoplankton metrics (S, H', J and D) in the upper and lower course sites (A) and in dry and wet seasons (B).

The values of Shannon-Weiner index calculated for each site, with correspondent judgments and class of quality were presented in Table 2. The water quality in the DNR was classified in to moderate (DN1 to DN7) to poor (DN to DN15) status based on the H' index value [8]. However, according to QCVN 08:2011/BTNMT, the water quality was classified in to B1 class, which could be only acceptable for irrigation and transportations (Table 2).

with correspondent judgment of ecological status and water quality class.								
Sampling site	H' value	Ecological status	Quality class					
DN1–DN7	2.0 - 2.7	Moderate status	B1					
DN8–DN15	1.6 – 1.9	Poor status	B1					

Table 2. Results of Shannon–Weiner index (H')

3.4. Canonical Component Analysis

Of the 139 phytoplankton taxa identified in dry season, 28 taxa with relative abundance \geq 10%, were included in data analysis using CCA (Fig. 5A). The CCA was done for the species richness and phytoplankton abundance, in relation to environmental variables and nutrients. The first two axes exhibited 74.3% variability of the total with 56.4% for axis 1 and 17.9 for axis 2 of the total variance in dry season (Fig. 5A). The first axis was positive correlated with nutrients and negative correlate with DO, pH and temperature to a lesser extent. It may represent an upper to lower water quality gradient. In wet season, 24 taxa with relative abundance \geq 10%, were included in CCA analysis (Fig. 5B).



Figure 5. CCA ordination for environmental variables and total phytoplankton abundance of the Dong Nai River in dry season (A) and rainy season (B). Taxa codes correspond to those in Appendix 1

In total 67.8% of the relationship between selected species and environmental variables was explained by the first two axes of CCA (Fig. 5B). The first axis was correlated with TSS, COD and nutrients, and the second axis with TB, EC, TP and BOD₅. Abundance of phytoplankton shown correlated with TSS, COD, TN and TP, whereas species richness was positive correlated with ammonium and to a lesser extend nitric, but negative correlated with DO, pH and to a lesser extend nitrate. It may represent water quality gradient from upper to lower section. The second axis was positive correlated with phosphate and to a lesser extend nitric and nitrate. It may represent the nutrient gradient of the river. In both seasons, the diatom *Aulacoseira granulata* (Agra), *A. angustissima* and some cyanobacteria such as *Microcystis aeruginosa* (Maer) and *M. wesenbergii* (Mwes) were positive correlated with nutrient and may represent eutrophic condition in the lower section, whereas diatom such as *Synedra ulna* (Suln) and green algae *Scenedesmus acuminatus* (Sacu), *Staurastrum zoonatum* (Szoo), *Pediastrum duplex* (Pdup) postitive correlated with DO and may represent oligo–mesotrophic conditions in upper section.

4. Discussion

Water quality assessment for the DNR has been investigated [9]. However, long-term and short-term phytoplankton succession has been rarely investigated. Study on structure and function of phytoplankton communities are of utmost importance for studies of the river ecosystems [10]. The seasonal variation in the physical, chemical and biological characteristic of the DNR seems plays a regulatory role on phytoplankton dynamics, and annual variation in nutrient supply is an important determinant of phytoplankton variability. The identified phytoplankton assemblages included freshwater and estuarine species, which were dominated by Bacillariophyceae. Both environmental variables and phytoplankton metrics showed an upper–lower gradient along the DNR and characterized a pollution gradient along the river, where water quality differed significantly among the upper course- and lower course sites but no significant difference was found in dry and wet season. These observations were in line with the conclusion of Le et al. [9] that lower water quality was observed at downstream of the river.

Mixing of rural and urban land used creates the specific environmental gradients in the DNR, resulting in the complicated dynamics of phytoplankton community in both spatial and temporal scales. More spatial changes in environmental factors and higher abundance of phytoplankton were found in dry season. The limitation on phytoplankton growth only presented in wet season, probably high turbidity in wet season prevents light for phytoplankton growth. An increase of nutrients and turbidity going downstream could be attributed by land-derived runoffs; our results showed that diatom species increase while blue-green and green algae decrease when going downstream resulting in the significant decrease of algae abundance downstream. Compared to dry season, the rainfalls and associated runoffs may account for the higher turbidity in wet season; in dry season, less freshwater-runoff may give ways to the greater intrusions of saline water from open sea, leading to the higher in the number of estuarine species and increasing phytoplankton diversity.

Phytoplankton presented a clear functional response to the changes of the complex external aquatic environment from the river downstream. It is well known that changes in physico-chemical characteristics of any inland waters can lead to concomitant qualitative and quantitative changes in phytoplankton communities [10]. The physico-chemical factors of the DNR did not varied seasonally during the present study. However they changed spatially from upper to lower section. This result showed that the spatial decrease of the average algal cell density in the lower course sites was primarily related to environmental factors. Although species richness seemed to be related to DO, the most important factors driving algal abundance were turbidity and nutrients including total nitrogen and total phosphorus. The CCA ordination reflected the corresponding correlations between phytoplankton communities and major environmental variables. The ordination bi-plots showed that the environmental variables in turn influenced the dynamics of key species. Based on the results this study, the factor that determined the phytoplankton community structure was the temporal variation (weather periods), presenting higher or lower densities in relation to processes resulting from rainfall (turbidity) and spatial variation (increase nutrient input).

5. Conclusions

In this study, the phytoplankton community together with physic-chemical variables in the DNR was investigated seasonally. Although ecological quality in the DNR varied between moderate to poor status, water quality was classified only into B_1 class based on physical and chemical variables. Changes in phytoplankton assemblages reflected well on the upper to lower gradient of the DNR. This implied that the species number and cell density of phytoplankton could serve as the biological water quality indicators, which would give overall descriptions of water quality by combining with the physical and chemical indicators. Results showed that the phytoplankton assemblage has been shown to be a precise indicator for surface water quality assessment. Therefore, it is better to establish and apply biological methods for water quality monitoring in Vietnamese water. Acknowledgments: Funding for this study was provided by the basic development foundation from Institute of Tropical Biology.

APPENDIX

List of key species collected in dry and wet seasons from the Dong Nai River.
The code number was used in the Canonical correspondence analysis (CCA).

Species name	Code	Dry	Wet
Coscinodiscus radiatus	Crad	+	+
Coscinodiscus subtilis	Csub	+	+
Cyclotella comta	Ccom		+
Aulacoseira granulata	Agra	+	+
Aulacoseira var.angustissima fo spiralis	Aang	+	+
Stephanodiscus sp.	Step		+
Surirella capronii	Scap	+	+
Surirella elegens	Sele	+	
Surirella linearis	Sline	+	
Synedra acus	Sacu	+	+
Synedra ulna	Suln	+	+
Ankistrodesmus gracilis	Angr	+	+
Ankistrodesmus fusiformis	Afus	+	
Arthrodesmus convergens	Athro	+	
Coelastrum microsporum	Cmic		+
cosmarium speciosum	Cosp	+	+
cosmarium sportella	Csp1	+	
Dictyosphaerium pulchellum	Dpul	+	
Eudorina elegans	Eele		+
Pandorina charkoviensis	Pcha	+	
Pediastrum duplex	Pdup	+	+
Scenedesmus acuminatus	Scea		+
Scenedesmus quadricauda	Squa	+	+
Stautrastrum connatum	Scon	+	
Staurastrum curvatum	Scur		+
Staurastrum paradoxum	Spar	+	+
Staurastrum tohopekaligense var insigne	Stoh	+	
Staurastrum zoonatum	Szoo	+	
Ceratium hirundinella	Chir	+	
Euglena acus	Eacu		+
Euglena spirogyra	Espi		+

REFERENCES

- [1] Reynolds C., *Ecology of Phytoplankton*. Cambridge University Press, Cambridge, 2006.
- [2] Edward, G.B. and David, C.S., *Freshwater Algae Identification and Use as Bioindicators*, Wiley-Blackwell, 2010.
- [3] American Public Health Association (APHA), *Standard methods for the examination of water and wastewater*. Washington DC, 2005.
- [4] Desikachary T.V., *Cyanophyta*, Indian Council of Agricultural Research New Delhi, 1959.
- [5] Dương Đức Tiến và Võ Hành, Tảo nước ngọt Việt Nam-Phân loại bộ tảo Lục. Hà Nội: Nxb Nông nghiệp, 1997.
- [6] Shirota A., *The plankton of South Vietnam Fresh Water and Marine Plankton*. Overseas Technical Cooperation Agency Japan, 1968.
- [7] Sukhanova Z.N., *Settling without inverted microscope*. Phytoplankton Manual, UNESCO, Page Brothers (Norwich) Ltd., UK, 1978.
- [8] Sven E.J., Liu X., and Robert C., *Handbook of ecological indicators for assessment of ecosystem health*. Second Edition, CRC Press, 2010.
- [9] Le T.M.T., Nguyen P.D., Dinh Q.T., Ngo H.H. and Do H.L.C., "Presence of e-EDCs in surface water and effluents of pollution sources in Sai Gon and Dong Nai river basin," *Sustainable Environment Research*, vol. 26, pp. 20–27, 2016.
- [10] Li G., Lin Q., Lin J., Song X., Tan Y. and Huang L., "Environmental gradients regulate the spatial variations of phytoplankton biomass and community structure in surface water of the Pearl River estuary," *Acta Ecologica Sinica*, vol. 34, pp. 129-133, 2014.