

Phosphorus and Nitrogen in Waters of the Ocoí River Sub-basin, Itaipu Reservoir Tributary

Gilmar S. da Silva,^{*,a} Gilberto S. da Silva,^b Eliane R. de Sousa,^c Cristiane Konrad,^c
Carla C. Bem,^c Janaína Pauli^c and Adilson Pereira^c

^aInstituto Federal de Educação, Ciência e Tecnologia do Maranhão, Departamento Acadêmico de Química,
65025-000 São Luís-MA, Brazil

^bInstituto Federal de Educação, Ciência e Tecnologia Goiano, CP. 92, 75650-00 Morrinhos-GO, Brazil

^cUniversidade Tecnológica Federal do Paraná, 85884-000 Medianeira-PR, Brazil

Através da distribuição espacial-temporal das espécies do nitrogênio e do fósforo total na Sub-bacia do Rio Ocoí, tributário do Reservatório de Itaipu, este trabalho avaliou o impacto ambiental nas águas superficiais. Após as chuvas, as fontes difusas predominam, onde o fósforo total e nitrogênio são mais altos. Para o fósforo, os estados tróficos variaram de ultraoligotrófico a mesotrófico. As concentrações atendem à legislação nacional, tanto para águas superficiais quanto potável. Todavia, o nitrogênio total apresenta-se acima dos valores basais, e o nitrito, 0.06 mg L⁻¹ NO₂⁻-N, ficou acima do recomendado para proteção da vida aquática, segundo a Canadian Water Quality Guidelines. O controle das fontes difusas e pontuais é recomendado para minimizar o impacto dos poluentes, ampliando a capacidade de suporte das águas, reduzindo o seu aporte para o Reservatório de Itaipu.

Starting from spatial-temporal distribution of nitrogen and phosphorus species in Ocoí river Sub-basin, Itaipu's tributary, this work evaluated the environmental impact in surface waters. After rain, non-point sources are predominant, presenting higher levels of total phosphorus and nitrogen. In the case of phosphorus, trophic states varied from ultraoligotrophic to mesotrophic. Even though Brazilian legislation is obeyed for surface and drinking water, total nitrogen showed values above background levels, and nitrite concentration, 0.06 mg L⁻¹ NO₂⁻-N, lies above the recommended value meant to protect aquatic life, according to the Canadian Water Quality Guidelines. Non-point and point sources control is advisable to minimize environmental phosphorus and nitrogen species impact, increasing the water support capacity and minimizing its discharge into Itaipu Reservoir.

Keyword: Ocoí River, Itaipu Reservoir, phosphorus and nitrogen, principal component analysis, trophic index

Introduction

Nitrogen and phosphorus species in aquatic systems are known pollutants,^{1,2} but not less important since they are strongly linked with the lifestyle of our society. The European Community, for instance, established a directive that limits phosphorus emission varying from 1 to 2 mg L⁻¹ P and nitrogen emission from 10 to 15 mg L⁻¹ N for wastewater treatment located in risky areas.³ Recent studies have discussed the ecotoxicological aspects of NH₃ such as water quality index for aquatic life protection, developed by Silva and Jardim.⁴

Nitrogen and phosphorus come from urban-industrial and rural activities such as agriculture and livestock.^{5,6,7} Runoff is the principal transportation means responsible

for the discharge into rivers, lakes and reservoirs of great quantities of pollutants. Continuous and reversible reactions along terrestrial and aquatic compartments permit that nitrogen take different toxic forms that affect human and aquatic biota, as nitrite, toxic for aquatic life.¹ Together with phosphorus, both are responsible for nutrient enrichment of aquatic systems, and its negative consequences like eutrophication.⁸⁻¹¹ Eutrophication is a process that leads lakes, rivers and reservoirs to impairment, since excessive biomass production can result in alga bloom⁷ and other negative effects, for instance, a cyanobacterium bloom¹² being responsible for lethal toxins and proliferation of *Pfiesteria piscicida*, which is a dinoflagellate organism that produces toxins capable of damaging fishes and human beings health.¹³

The Ocoí River's Sub-basin aquatic systems have low flow, inserted in an environment characterized by

*e-mail: gilmarsilverio@gmail.com

agricultural, pig and poultry farms and urban-industrial activities. The environmental water quality of the Ocoí River has its importance increased by the fact that the Ocoí River is tributary of the Itaipu Reservoir. In order to carry out this work, several field campaigns took place during dry periods and after rainfalls, aiming at assessing nitrogen and phosphorus species and its environmental impact through their spatial-temporal distribution. In this direction, tools as multi variable analysis^{14,15} and the trophic status¹⁶⁻¹⁸ were used.

Experimental

Study area

The study area is the Ocoí River Sub-basin (Figure 1), which discharges into the Itaipu Reservoir, and into two of its tributaries, the Alegria River and the Ouro Verde River. These rivers belong to class 2 according to the Conama Directive 357/05¹⁹ (Brazilian National Council for the Environment). The Ocoí River Sub-basin is characterized by pig and chicken industrial processes; agriculture, especially soy bean and corn, in which a large quantity of synthetic fertilizer and manure from pig and poultry is applied, following that treated and non-treated municipal wastewater from the cities of Medianeira and Matelândia is discharged into the rivers. Most of the riparian forest area does not obey legal specification,²⁰ such as the 30 meters of forest for rivers with less than 10 meters of width, fact that contributes to the pollutants transportation along the drainage area by runoff. The Ocoí River Sub-basin soils

are classified as Red Latosol, where degradation of natural vegetation covering takes place, increasing the soil's susceptibility to erosion and creating favorable conditions to carry large loads of sediment.⁵

The study area has subtropical climate with high humidity, hot summers characterized by rainfall accumulation and winter with an average temperature below 18 °C, without a defined dry period. The cities of Medianeira and Matelândia have about 40,000 and 14,000 inhabitants, respectively. In these municipalities, approximately 8% of the wastewater is treated, while the rest, more than 90%, is discharged into septic or rudimentary tanks²¹ or directly into the rivers.

Collecting samples

Water samples were collected in six different points, along the three different rivers (Figure 1). The P.1 point is placed in the Ocoí River (S 25° 14' 24"; W 54° 05' 21"), being the one nearest to its headwater. Upstream this point is located one of the Ocoí River's tributaries, the Ocozinho Stream that receives treated and non-treated wastewater from the city of Matelândia. The P.2 point is located in the Ouro Verde River (S 25° 12' 47"; W 54° 06' 28"), another Ocoí River's tributary, which is characterized by typical activities described above, except, recently, for urban-industrial wastewater. The P.3 point is located in the Ocoí River (S 25° 13' 10"; W 54° 06' 28"), downstream the Ouro Verde River. The P.4 point is located in the Ocoí River (S 25° 11' 08"; W 54° 10' 42"), upstream the Alegria River mouth. The P.5 point is placed in the Alegria River

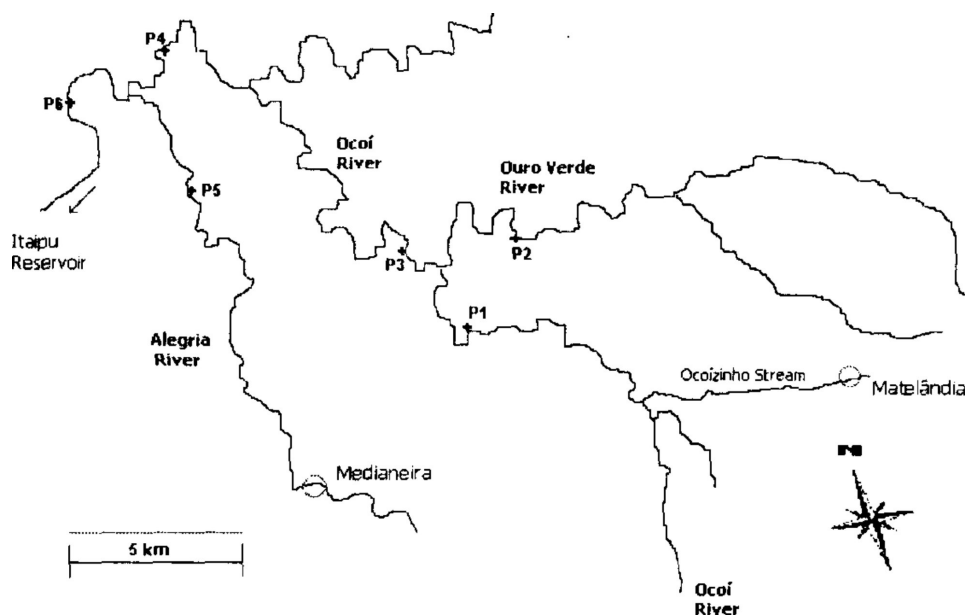


Figure 1. Ocoí River hydrography: the Ocoí, Ouro Verde, Alegria and the Ocozinho aquatic system.

(S 25° 12' 59"; W 54° 10' 00"), whose waters cross the Medianeira city. At this point, industrial and treated and nontreated municipal wastewaters are discharged into the Alegria River. Finally, the P.6 point is the last point in the Ocoí River and is located close to its mouth that flows directly into the Itaipu Reservoir (S 25° 11' 40"; W 54° 12' 12"). Samples were collected on the water surface, conserved according to NBR 9898 Protocol²² and sent to UTFPR laboratory for following analysis. In total, six field campaigns were carried out between 2005 and 2006.

Table 1 presents dates and runoff conditions during water sampling. Samples with the largest monthly average of rainfall intensity are the 3rd and 4th Campaigns, but only along the 3rd and 6th Campaigns the water samples were collected immediately after the end of the rainfall event, still under influence of surface runoff. Runoff presence is of paramount importance because is responsible for pollutants transportation from the drainage area, and is one kind of non-point source. The 2nd Campaign was carried out after one dry month and possibly was better to represent the Ocoí River Sub-basin behavior without non-point sources in dry period, without runoff.

Table 1. The weather conditions during the water sampling

Campaign	Date	Monthly average of the rainfall intensity (mm)	Runoff presence/absence
1st	07/07/05	87.7	absence
2nd	08/18/05	105.4	absence
3rd	09/01/05	261.8	presence
4th	10/20/05	413.5	absence
5th	11/24/05	81.5	absence
6th	03/23/06	137.5	presence

Source: UTFPR-Medianeira weather station

Analysis

Water samples were analyzed according to APHA.²³ Nitrate (NO₃⁻), nitrite (NO₂⁻), ammonium (NH₃) and Total Phosphorus (TP) were measured using UV-Vis spectrophotometer (Fentom, Plus model). The concentrations of NO₃⁻ and NO₂⁻ were expressed as nitrogen, mg L⁻¹ NO₃⁻-N and mg L⁻¹ NO₂⁻-N, respectively. Ammonium (NH₃) was expressed as nitrogen, mg L⁻¹ NH₃-N. The total Kjeldahl nitrogen is the combination of organically bound nitrogen and NH₃. The combination of the organic nitrogen and the inorganic nitrogen (NH₃, NO₃⁻, NO₂⁻) corresponds to the total nitrogen. TP concentration was expressed in mg L⁻¹ P.

The following parameters were also determined: pH, turbidity, conductivity, ion sodium (Na⁺) and ion chloride

(Cl⁻). The sodium (Na⁺) concentration was measured by spectrometry of atomic emission using a flame photometer (CELM, model FC-180). The chloride concentration was determined by Mohr method. Each water sample was measured in triplicate and the coefficients of variation are, on average, 8% for NO₃⁻ and NO₂⁻, 12% for TP, 5% for NH₃ and 6% for Cl⁻.

Statistical analysis

Principal Component Analysis (PCA)^{14,24} was applied to evaluate the similarity of water samples considering the value of the measured variable. PCA creates a graph of scores that permits to check the relationship using the Euclidean distance, where the new axes, that are orthogonal to each other, correspond to the principal component (PC). Besides a graph of scores, PCA provides a loading graph able to determine the extent of a variable that contributes to each PC axis. PCA was applied to the matrix composed of 6 sampling points (P.1 to P.6) in 5 different campaigns (2nd to 6th), and 6 variables (NH₃, TP, conductivity, Na⁺, Cl⁻ and pH), which were previously centered on average due to different orders of magnitude. The matrix submitted to PCA corresponds to 30 water samples (columns) and six variables (lines).

Trophic status index (TSI)

In order to evaluate the trophic status of the water body, an index created by Carlson,^{16,17} and recently adapted for the rivers of the State of São Paulo by Lamparelli,¹⁸ was applied. This index can be applied using TP and Chlorophyll *a*, but this work we used the former parameter, known as the trophic status index based on TP (TSIp),²⁵ according to equation 1. TSIp acts as the potential capacity of the aquatic systems to produce biomass in excess, while Chlorophyll *a* is an effective measure of that biomass.⁸

$$TSIp = 10(6 - (1.77 - 0.42 \ln TP / \ln 2)) \quad (1)$$

where TP = total phosphorus concentration, in µg L⁻¹ P.

Results and Discussion

Total phosphorus (TP) behavior

Table 2 shows Cl⁻, conductivity, pH, turbidity and Na⁺ values of water samples from the last 5 campaigns. In the 1st Campaign, only the TP concentration and NH₃ were determined. TP and NH₃ were shown separately from Table 5 in order to emphasize spatial temporal distribution

in the Ocoí River waters. In Figure 2 it is possible to observe the temporal-spatial distribution of TP in waters of the Ocoí River.

Table 2. Chemical and physical-chemical parameters measured in the Ocoí River Sub-basin

Parameters ¹	Campaigns				
	2nd	3rd	4th	5th	6th
P.1 Point					
Cl	71.6	14.9	27.8	9.2	9.9
Conductivity	84	94	91	124	386
pH	7.19	7.17	7.58	7.38	7.2
Turbidity	-	-	11	7	766
<i>Na</i>	12.2	10.3	8.4	8.8	11.3
P.2 Point					
Cl	64.6	12.9	25.8	8.5	10.7
Conductivity	102	91	95	237	168
pH	6.9	7.3	7.4	7.3	7.3
Turbidity	-	-	8	3	351
<i>Na</i>	15	10.3	9.3	9.6	10.8
P.3 Point					
Cl	81.5	18.9	27.8	6.4	9.2
Conductivity	94	86	89	141	195
pH	7.4	7.31	7.47	7.65	7.3
Turbidity	-	-	8	7	701
<i>Na</i>	14.1	10.3	9.3	10.1	10.3
P.4 Point					
Cl ⁻	70.6	18.9	24.9	7.8	9.9
Conductivity	94	89	93	79	137
pH	7.3	7.4	7.7	7.7	7.4
Turbidity	-	-	10	5	512
<i>Na</i>	13.2	11.3	10.3	10.5	11
P.5 Point					
Cl ⁻	118.3	52.7	34.8	9.2	16.3
Conductivity	237	143	104	70	137
pH	7.09	7.16	7.62	7.53	7.2
Turbidity	-	-	21	16	497
<i>Na</i>	37.6	25.4	15.9	18.3	23.4
P.6 Point					
Cl ⁻	73.6	23.9	29.8	7.8	9.9
Conductivity	87	93	105	75	117
pH	7.28	7.33	6.62	7.57	7.4
Turbidity	-	-	10	7	633
<i>Na</i>	16	13.2	10.3	11	12.2

¹ Cl⁻ and *Na* unities are mg L⁻¹. Conductivity is mS cm⁻¹ and turbidity is NTU.

In Figure 2 results of the TP concentration for the P.1, P.3, P.4 and P.6 points, that belong to Ocoí River, show values below the limit-values established by the Conama Directive 357/05,¹⁹ 0.05 mg L⁻¹ P, except for the 3rd Campaign, when all points had its TP concentrations exceeding the limit-value. In addition, during the 3rd campaign, P.5 point, located in the Alegria River, showed the TP concentration of 0.11 mg L⁻¹ P, close to the limit-value required by legislation, 0.10 mg L⁻¹ P. The reason for the high TP values observed in the 3rd campaign can be attributed to rainfall few hours before water sampling, when runoff still was present, suggesting a significant presence of phosphorus input from non-point sources. One kind of non-point source is surface waters that drain the Ocoí River Sub-basin. They can load different phosphorus species, especially phosphorus adsorbed on mineral particles. The soil of the area is rich in clay minerals and oxides like gibbsite and goethite, known for their capacity of adsorbing phosphate.^{26, 27}

The P.5 point shows high TP concentration compared with the other sampling points during rain or dry periods. During the dry period, urban-industrial wastewater from the Medianeira city predominates and, during the rainfall, there is likely the contribution of unidentified non-point sources too. It is important to emphasize that all points in the Ocoí River, P.1, P.3, P.4 and P.6, independently of the season, show a relatively similar TP value. In addition, if the only source of phosphorus was runoff, in the dry period and under absence of point source, the TP concentration values should be very small compared with dry period with point source, for instance, Alegria River (P.5 point) in the 2nd campaign. This behavior did not occur. As it can be seen, the P.3 point during 2nd campaign shows 0.038 mg L⁻¹ P, higher than P.5 point, which was 0.031 mg L⁻¹ P. The same occurs between the points P.1 and P.5 in the 4th campaign. The results suggest that there should be a non-point source acting independently of runoff. In this direction, it is important to mention that the Ocoízinho Stream is a non-point source that provides TP during the entire year. Finally, in order to understand why the P.3 point, in the Ocoí River, showed TP concentration higher than the P.5 point, although it did not rain during the 2nd Campaign, it is necessary to consider non-point sources that act independently of rainfall and, therefore, runoff. Those non-point sources may be an animal waste discharge from farms, which exist in a large number along the banks.¹⁰

Trophic status index

In order to evaluate the trophic status of the Ocoí River and its tributaries, Alegria and Ouro Verde rivers,

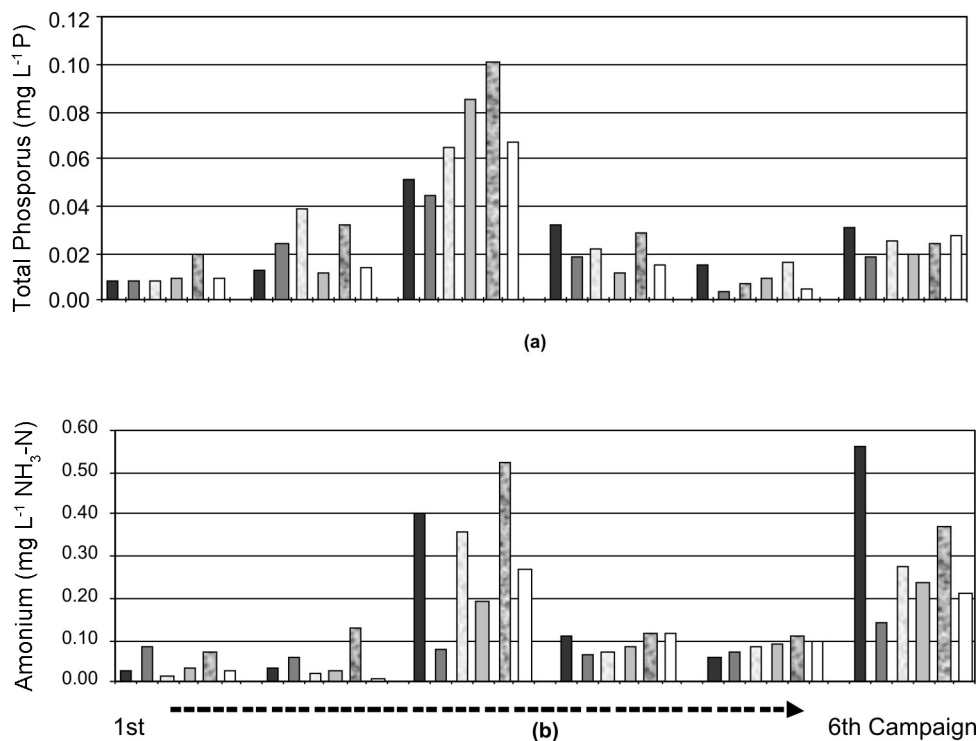


Figure 2. The spatial-temporal distribution of the TP and NH_3 from P1 to P6 point at Ocoí River Sub-basin. Water samples have influence of runoff during the 3rd and 6th Campaigns. Legend: P1 (dark column) until P6 (white column).

the trophic status index (TSIp) was applied based on the TP concentration.²⁵ In Table 3, the TSIp results from six sampling points during all campaigns are presented, which categories varied from ultraoligotrophic ($\text{TSIp} \leq 47$; $P \leq 13 \mu\text{g L}^{-1} \text{P}$) to mesotrophic ($52 < \text{TSIp} \leq 59$; $35 < P \leq 137 \mu\text{g L}^{-1} \text{P}$) classes. In the 3rd Campaign the TSIp was the most critical, presenting mesotrophic waters, being the P.5 point, the Alegria River, responsible for the highest TSIp, 57.9. The mesotrophic status indicates a water body with intermediate biomass productivity, and still does not represent water impairment in most of situations. In the other campaigns, the trophic status remained between ultraoligotrophic and oligotrophic

($47 < \text{TSIp} \leq 52$; $13 < P \leq 35 \mu\text{g L}^{-1} \text{P}$), demonstrating satisfactory water conditions. It is necessary to emphasize that, in general, the P.5 point in the Alegria River, shows the worst TSIp, and probably because is doubly affected by both non-point and point sources, being the latter from urban-industrial wastewater. Another important point is the P.3, in the 2nd Campaign, whose water was classified as mesotrophic. The reason for this high TSIp was mentioned above. In general, it is worth to mention that point and non-point polluting sources, independent of the rainfall, which discharge into water bodies when flow and water velocity are low, are more favorable for biomass production.^{9,28,29}

Table 3. The Trophic Status Index (TSIp) values of the Ocoí River and its tributaries

Point	1st Campaign		2nd Campaign		3rd Campaign		4th Campaign		5th Campaign		6th Campaign	
	TSIp	Class	TSIp	Class	TSIp	Class	TSIp	Class	TSIp	Class	TSIp	Class
P1	45.0	Ultra*	47.0	Ultra	54.4	Meso ^b	52.1	meso	47.7	Oligo	51.7	Oligo
P2	44.7	Ultra	50.5	Oligo	53.8	meso	49.2	Oligo	40.7	Ultra	49.0	Ultra
P3	44.9	Ultra	52.9	meso	55.6	meso	50.0	Oligo	44.6	Oligo	50.7	Ultra
P4	45.4	Ultra	46.3	Oligo	57.0	meso	46.7	Ultra	45.4	Oligo	49.5	Ultra
P5	49.5	Oligo ^a	51.9	Oligo	57.9	meso	51.4	Oligo	48.5	Oligo	50.5	Oligo
P6	45.5	Ultra	47.6	Oligo	55.8	meso	48.1	Oligo	41.7	Ultra	51.1	Ultra

* ultraoligotrophic, ^a oligotrophic, ^b mesotrophic

NH₃, NO₃⁻ and NO₂⁻ behavior

Due to NH₃ ecotoxicity, this substance plays an important role for the aquatic life protection.⁵ According to the Conama Directive 375/05,¹⁹ the limited values of NH₃ in water bodies class 2 are 3.7 mg L⁻¹ NH₃-N to pH < 7.5 and 2 mg L⁻¹ NH₃-N to pH > 7.5. Figure 2 presents the NH₃ concentration in the river water. The maximum values did not exceed 0.7 mg L⁻¹ NH₃-N, below the limit-value. On the other hand, the lowest NH₃ concentration was observed in the water samples collected without rainfall, during the 1st, 2nd, 4th and 5th Campaigns, where runoff was no longer present in the Ocoí River Sub-basin. Among the high value observed in the presence of runoff, 3rd and 6th campaigns, the value of P.1 point, the nearest to the Ocoí River's headwaters, was higher than the ones of other points, except the P.5 point. This result suggests significant non-point sources activated by runoff. The transport of NH₃ in the soil where pig and poultry manure has been spread out depends on the interval between the application and the first rains. In general, on the first days NH₃ prevailed. With time, a decrease in the initial NH₃ concentration takes place, firstly because of the nitrification process,³⁰ increasing the NO₃⁻ production, and secondly because volatilization of ammonia to atmosphere. The transport of the nitrogen species has different characteristics. As NO₃⁻ is very soluble in the water and due to its negative charge it is not adsorbed by the mineral particles,³¹ on the other hand, NH₃ can be converted into NH₄⁺ in the soil, where positive charge contribute to its adsorption by the particles.

The P.5 point stands out, presenting higher NH₃ concentration than the other points practically in all campaigns, with observed for TP. This behavior agrees with the fact that Alegria River receives both non-point and point sources, what explains not only high NH₃ concentration, but the high level of TP as well.

To give initial information about distribution of nitrogen species in these water bodies, in Table 4 are presented different concentrations of them for only the 6th Campaign: NH₃, NO₃⁻, NO₂⁻ and TN.³² The NO₂⁻ concentration values varied from 0.024 to 0.240 mg L⁻¹ NO₂⁻-N, and NO₃⁻ varied from 0.099 to 0.167 mg L⁻¹ NO₃⁻-N. At the P.5 point, the NO₂⁻ concentration was 0.240 mg L⁻¹ NO₂⁻-N, higher than NO₃⁻, 0.133 mg L⁻¹ NO₃⁻-N, corroborating the influence of urban-industrial wastewater. Urban-industrial wastewater can discharge a large quantity of NH₃ into the rivers, which can be rapidly converted into NO₂⁻ and, later, NO₃⁻. In a broad aspect, the NH₃ concentration was higher than nitrogen oxidized forms for all points, suggesting recent pollutant discharge.

In order to evaluate nitrogen species impact in aquatic life, it is necessary to regard, besides NH₃, the

Table 4. The Ocoí River's nitrogen species. The 6th Campaign was carried out after runoff

Point	NH ₃ -N	NO ₂ ⁻ -N	NO ₃ ⁻ -N	TN*
P1	0.569 ± 0.028	0.107 ± 0.013	0.210 ± 0.025	2.625
P2	0.140 ± 0.011	0.043 ± 0.004	0.099 ± 0.012	7.452
P3	0.272 ± 0.024	0.067 ± 0.008	0.131 ± 0.011	0.470
P4	0.239 ± 0.014	0.049 ± 0.003	0.167 ± 0.011	3.705
P5	0.371 ± 0.029	0.240 ± 0.028	0.133 ± 0.016	2.145
P6	0.206 ± 0.016	0.024 ± 0.002	0.122 ± 0.013	0.632

Source: *TN corresponds to all nitrogen species: organic and inorganic.

oxidized nitrogen form, NO₂⁻. High NO₂⁻ concentration can cause harmful effects on aquatic life. The Canadian Water Quality Guidelines (CCME, 2001)³³ establishes 0.06 mg L⁻¹ NO₂⁻-N for protection of sensitive living organism in the water bodies. According to Table 7, the points P.1, P.3 and P.5 showed NO₂⁻ concentration above of the 0.06 mg L⁻¹ NO₂⁻-N. Since NO₃⁻ can be reduced to NO₂⁻, the same guideline recommends maximum interim value of 13 mg L⁻¹ NO₃⁻-N.

In addition, the oxidized nitrogen, NO₂⁻ and NO₃⁻, are species that deserve rigorous control when the subject is human health. With this direction, NO₂⁻ and NO₃⁻ are limited by the Conama Directive 357/05¹⁹ in 1 mg L⁻¹ NO₂⁻-N and 10 mg L⁻¹ NO₃⁻-N, respectively. Those values are the same required for drink waters according to the Federal Law n°. 518/2004 of the Brazilian Health Ministry.³⁴ Therefore, all water samples obeyed the legislation for drinkable water.

The nitrogen species described above, NH₃, NO₂⁻ and NO₃⁻, not only are toxic for living beings, but they contribute to total nitrogen (TN) bulk in the body of water. TN can enrich aquatic ecosystem leading to excessive biomass production. In this case, the bulk of TN in the water body may function as trophic status index in aquatic systems, similar to TP, since both are essential macronutrients. Table 4 shows the TN concentration values, which varied from 2.63 mg L⁻¹ N at the P.1 point (the nearest to Ocoí River's headwaters) to 7.5 mg L⁻¹ N at the P.2 point, the Ouro Verde River. Those values are considered high compared with other aquatic systems, where it is considered a basal level³⁵ of 1 mg L⁻¹ N. If the limited value of TN proposed by Dodds *et al.*,³⁶ are adopted for streams in temperate environment, *i.e.* 0.7 mg L⁻¹ N for oligotrophic water bodies, and 1.5 mg L⁻¹ N for eutrophic/mesotrophic limit, the results showed in Table 4 indicate that the water can be classified as eutrophic for P.1, P.2, P.4 and P.5 points.

Principal Component Analyses

According to the eigenvalues showed in Table 5, PC1 and PC2 account for the total variance, 83.72% and 15.44%,

respectively, being 99.16% the cumulative eigenvalues for both components. The PC1 has great influence on group 1 separation, while PC2 on group 2. In this latter group, the water samples correspond to the 2nd Campaign, which better represents sources independent of rainfall (Figure 3).³² In this group 2, only the P.5 point remained separated, suggesting a different chemical behavior from the other points, probably because the Alegria River is a receiving water body of the urban-industrial wastewater from Medianeira city. In contrast, the P.5 point in the other campaigns is more similar to other points because urban-industrial wastewaters are no longer so predominant compared to non-point source activated by rainfall event.

Table 5. % Eigenvalue and Cumulative % eigenvalues to PCA analyses of water sample form Ocoí River Sub-basin

Principal Component	% Eigenvalue	Cumulative % eigenvalues
PC1	83.721	83.721
PC2	15.443	99.164
PC3	0.835	99.999
PC4	0.001	100
PC5	0	100
PC6	0	100

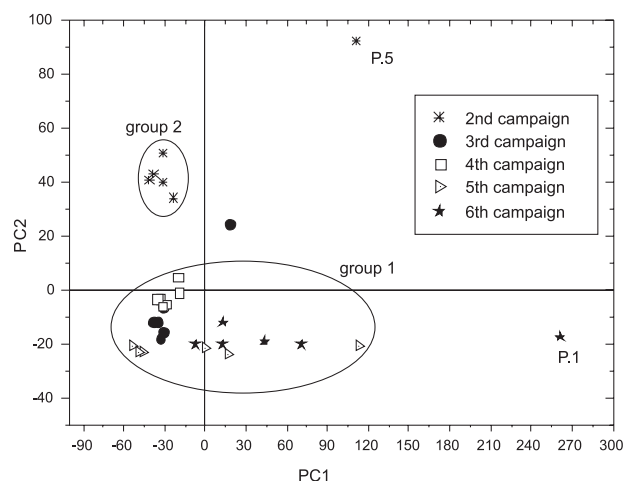


Figure 3. The Score graph for the first two principal components, PC1 x PC2.

As to identify which variable had the highest influence on PC1 and PC2, loading values were used (Figure 4). According to loadings values, cond. is the almost exclusively variable that contributes to PC1, loading 1, as other parameters were much smaller: Cl^- (-1.45×10^{-2}), pH (-7.73×10^{-4}), Na^+ (1.99×10^{-2}), PT (-6.68×10^{-5}) and NH_3 (1.32×10^{-3}). These results can suggest that dissolved electrolytes from runoff increase conductivity in water bodies. Rainfall and specific features of each drain area

vary along the river (surface vegetation coverage, soil topography, riparian forest, etc.); therefore, it is expected that different electrolyte loads be discharged into the water bodies, which helps to explain dispersion of group 1. In this scenery, the P.1 point of the 2nd Campaign remained isolated (Figure 4), showing great susceptibility to non-point sources, what may help to explain TP and NH_3 high concentrations in the same point.

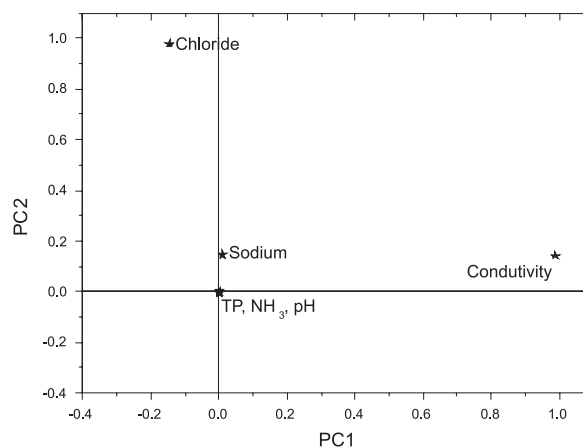


Figure 4. Loading graph for the two principal components, PC1 and PC2.

To PC2, Cl^- has a loading of 0.99 and it is almost responsible by group 2 separation. Other parameters were: Cond. (1.16×10^{-2}), pH (-2.83×10^{-3}), Na^+ (1.38×10^{-1}), PT (1.39×10^{-4}) and NH_3 (-1.54×10^{-3}). Cl^- from non-point source was not correlated with conductivity, as it can be seen in the loading graph, because they are orthogonal. The highest concentrations of this ion occur during low flow, without water contribution from runoff, especially in the 2nd Campaign, where the P.5 point in the Alegria River reached $118.29 \text{ mg L}^{-1} \text{ Cl}^-$; probably due to the presence of urban-industrial wastewater. It should be emphasized that all water samples from the 2nd Campaign presented the highest Cl^- concentration, suggesting that non-point and point sources independent of rainfall may act during the dry period, once the Cl^- is typically a species from the animal and urban-industrial wastewater.²⁴ Loadings to Na^+ , pH , PT and NH_3 are so low, much close to the axis origin, and contribute little to components. However, the absence of significant loading implies that there are no predominant PT or NH_3 sources in watershed, corroborating the idea that different pollutants' transportation mechanisms are acting along the ecosystem.

Conclusions

The results point out the presence of phosphorus and nitrogen from non-point and point sources acting in the Ocoí River Sub-basin. Non-point sources are more

predominant and identifiable after rainfall, due to runoff, and sometimes only the TP exceeds the Conama Directive 357/05 requirements, contrasting with NH_3 , which remained below the limit-value. In general, phosphorus and ammonium showed the highest concentration under runoff influence. Phosphorus impact in the water quality, according to modified Carlson Trophic Index, indicates trophic class varying from oligotrophic to mesotrophic. The latter class occurred in the presence of runoff. Nitrogen species are below the Brazilian law requirements for surface and drinkable water, as well as for aquatic protection life, although the bulk of total nitrogen can be considered high compared to basal level. However, nitrite concentration showed high values regarding the Canadian Water Quality Guidelines, above $0.06 \text{ mg L}^{-1} \text{ NO}_2\text{-N}$.

The Analysis of the Principal Component indicates two principal components, PC1 and PC2, which are able to separate two groups of water samples. Chloride (Cl^-) was mainly responsible for separating the water samples that corresponded to the dry period. High Chloride (Cl^-) concentration suggests also the presence of the non-point and point sources independent of runoff. Probably, non-point source independently of runoff can be the reason why during the dry period mesotrophic class was also observed in the Ouro Verde and the Ocoí rivers. The Alegria River is influenced by both non-point and point sources, being more susceptible in the future due to load increase from growing industrial and domestic activities.

Acknowledgements

The authors thank Fundação Araucária, Fundação Parque Tecnológico de Itaipu (FPTI) and UTFPR/Campus Medianeira for financial support.

References

1. Koo, B. K.; O'Connell, P. E.; *Sci. Total Environ.* **2006**, *359*, 1.
2. Lewis JR, W. M.; Donald, P. M.; *Transactions of the American Fisheries Society* **1986**, *115*, 183.
3. E.E.C. (European Economic Community); Directive 91/271/EEC, on *Urban waste water treatment*, 1991. <http://eur-lex.europa.eu/pt/index.htm>, accessed in July 2008.
4. Silva, G. S. ; Jardim, W. F.; *Quim. Nova* **2006**, *29*, 689.
5. Hooda, P. S.; Moynagh, M.; Svoboda, L. F.; Thurlow, M.; Stewart, M.; Thomson, M.; Anderson, H.A.; *Sci. Total Environ.* **1997**, *201*, 63.
6. Chapman, A S.; Foster, I. D.; Lees, J. A; Hodgkinson, R. A; Jackson, R. H.; *Sci. Total Environ.* **2001**, *66*, 95.
7. Kyllmar, K.; Carisson, C.; Gustafson, A; Ulén, B.; Johnsson, H. *Agr Ecosyst Environ.* **2006**, *115*, 15.
8. Spivakov, B. YA.; Maryutina T. A.; Muntau, H.; *Pure Appl. Chem.* **1999**, *71*, 2161.
9. United States Environmental Protection Agency, USEPA; *Nutrient Criteria-Technical Guidance Manual- Rivers and Streams* -EPA 822-BOO-002, Washington, DC, 2000, <http://www.epa.gov/waterscience/criteria/nutrient/guidance/rivers/index.html>, accessed in July 2008.
10. United States Environmental Protection Agency (USEPA); *Protocol for Developing Nutrient TMDLs* - EPA 841-B-99-007, Washington D.C., 1999, <http://www.epa.gov/owow/tmdl/nutrient/pdf/nutrient.pdf>, accessed in July 2008.
11. Bowes, M.J; House, W. A; Hodgkinson, R. A.; *Sci. Total Environ.* **2003**, *313*, 199.
12. Falconeri, I. R.; Humpage, A. R.; *Int. J. Environ. Res. Public Health* **2005**, *2*, 43
13. Burkholder, J. M.; Glasgow Jr, H. B.; *Limnol. Oceanogr.* **1997**, *42*, 1052.
14. Beebe, K. R.; Pell, R. J.; Seasholtz, M. B.; *Chemometrics: A Practical Guide*, 1st Ed. Wiley-Interscience:NewYork, 1998.
15. Sousa, E. R.; *PhD Thesis*, Instituto de Química da Universidade Estadual de Campinas, Brasil, 2005.
16. Carlson, R. E.; *Limnol. Oceanogr.* **1997**, *22*, 361.
17. Toledo Jr., A. P.; Agudo, E. G.; Talarico, M.; Chinez, S. J.; *XIX Congresso Interamericano de Engenharia Sanitária Ambiental-AIDIS*, Santiago do Chile, Chile, 1984.
18. Lamparelli, M. C.; *PhD Thesis*, Instituto de Biociências da Universidade de São Paulo, Brasil, 2004.
19. Portaria 365/05, <http://www.mma.gov.br/port/conama>, accessed in February de 2009.
20. Lei N° 7.803 de 18 de Julho de 1989. Diário Oficial da União. 20/07/1989. http://www.planalto.gov.br/ccivil_03/Leis/L7803.htm, accessed in July 2008
21. <http://www.cnm.org.br/infra/>, accessed in February 2009.
22. Associação Brasileira de Normas Técnicas, ABNT; NBR 9898: *Preservação e técnicas de amostragem de efluentes líquidos e corpos receptores*, Rio de Janeiro, Brasil, 1987.
23. American Public Health Association, APHA; *Standard Methods for the Examination of Water and Wastewater*. 16. ed., American Water Works Association and Water Pollution Control Federation: Washington, DC, 1985.
24. Sodr e, F. F.; Anjos, V. E.; Prestes, E. C.; Grassi, M. T.; *J. Environ. Monit.* **2005**, *7*, 581.
25. Companhia de Tecnologia de Saneamento Ambiental, CETESB; *Relat rio de Qualidade das  guas Interiores do Estado de S o Paulo*, S o Paulo, Brasil, 2004.
26. Bowes M. J.; House W. A.; Hodgkinson R. A.; *Sci. Total Environ.* **2003**, *313*, 199.
27. House, W. A.; Leach, D.; Warwick, M.S.; Whitton, B. A.; Pattison, S. N.; Ryland, G.; Pinder, A; Ingram, J.; Lishman, J. P.; Smith, J. M.; Rig, E.; Denison, F. H; *Sci. Total Environ.*, **1995**, *194*, 303.

28. Mainstone, C. P.; Parr, W.; *Sci. Total Environ.* **2002**, 25, 282.
29. Jarvie, H. P.; Neal, C.; Withers, P. J.; *Sc. Total Environ.* **2006**, 360, 246.
30. Ceretta, C. A. ; Basso, C. J.; Vieira, F. C. B.; Herbes, M. G.; Moreira, I. C. L.; Berwanger, A L.; *Cienc. Rural* **2005**, 35, 1296.
31. Jarvie, H. P.; Whitton, B. A; Neal. C.; *Sci. Total Environ.* **1998**, 210/211, 79.
32. Silva, G. S.; Konrad, C.; Pauli, J. ; Silva, G. S.; Sousa, E. R. *Abstract of the do XI Seminário de Iniciação Científica e Tecnológica*, Curitiba, Brasil, 2006.
33. <http://www.ccme.ca/> accessed in February 2009.
34. Ministério da Saúde; Portaria n°. 518 de 25 de março de 2004, Brasília DF, 2004. http://portal.saude.gov.br/portal/arquivos/pdf/portaria_518_2004.pdf, accessed in July 2008
35. United States Geological Survey, USGS; *The Quality of Our Nation's Waters - Nutrients and Pesticides: U.S Geological survey Circular* 1999, 1225, 82.
36. Dodds, W. K.; Jones, J. R.; Weich, E. B.; *Water Res.* **1998**, 32, 1455.

Received: October 15, 2008

Web Release Date: August 31, 2008