Acid Phosphatase Activity in Freshwater Ecosystems of Western Cuba and its Relationship with Water Quality

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Abstract

Enzyme activity plays an important role in the functioning of aquatic ecosystems. It is sensitive to changes in environmental conditions such as pH, temperature, and nutrient concentration. The objective of this work was to determine the acid phosphatase activity (AcPA) in the Almendares and San Juan rivers (western Cuba) and its relationship with physicochemical and microbiological indicators. For this purpose, AcPA, temperature, pH, total dissolved solids, electrical conductivity, dissolved oxygen, concentration of nitrates, nitrites, ammonium, phosphates, total heterotrophs, enterococci, *Escherichia coli*, thermotolerant coliforms, chlorophyll *a* and chemical oxygen demand (COD) were determined at three sampling stations on the Almendares River and at three sampling stations on the San Juan River. In addition, the nutrient pollution index (NPI) and the N:P ratio were calculated. In both ecosystems, spatio-temporal variability was observed in the enzymatic activity. In the Almendares River (polluted ecosystem), AcPA was positively correlated with nitrate concentration and COD. While in the San Juan River (slightly contaminated ecosystem) the AcPA correlated negatively with the pH and NPI and positively with the concentrations of total heterotrophs, *Escherichia coli*, chlorophyll *a* and the N:P ratio. These results show the impact of anthropogenic pollution on AcPA in freshwater ecosystems with a tropical climate.

1. Introduction

The extracellular enzymes produced by microorganisms play an important role in the biogeochemical cycles of nutrients in aquatic ecosystems, contributing to the remineralization of organic matter, specifically macromolecular debris (Arnosti et al. 2014; Egli and Janssen 2018). For the degradation of macromolecular debris into assimilable substrates, heterotrophic microorganisms release enzymes into the environment through active secretion or cell lysis (Arnosti et al. 2011; Sinsabaugh and Shah 2012). Among the enzymes produced by microorganisms are phosphatases, which release soluble inorganic phosphate from organophosphates, making it available to most organisms (Jackson et al. 2013).

There are three groups of hydrolytic enzymes responsible for the hydrolysis of organic phosphorus, phosphosterases (mono and diesterase), nucleotidases, and nucleases (exo and endonuclease) (Chróst and Siuda 2002; Torres et al. 2017). Phosphomonoesterases (PMEase) are non-specific enzymes that hydrolyze simple phosphomonoesters and can be produced by different microorganisms. Furthermore, depending on the pH at which they exhibit their maximum activity, they can be divided into two groups: acidic (pH 2.6–6.8) and alkaline (7.6–10) (Torres et al. 2017). Although it is suggested that both enzymes are regulated by the availability of orthophosphate, acid PMEase (hereinafter acid phosphatases) are usually considered constitutive enzymes whose synthesis is related to phosphorus concentration and demand within the cell (Siuda 1984; Jasson et al. 1988). In freshwater ecosystems, acid phosphatases (AcP) have been less studied compared to alkaline phosphatases, possibly due to the high number of systems with neutral pH where the preservation of AcP enzyme activity could be affected (Siuda 1984). Due to this, the activity of AcP has hardly been investigated in freshwater ecosystems with different trophic states where the pH is neutral or slightly alkaline. Studies carried out in freshwater lakes with different pH in Japan (Tabata et al. 1988), showed that in systems with neutral pH the activities of acid and alkaline phosphatases were similar. Therefore, both enzymes show activity in these systems although they do not exhibit their maximum activity (Jasson et al. 1988).

On the other hand, research on the factors that affect phosphatase activity in freshwater ecosystems have focused more on the analysis of the factors that affect the activity of alkaline phosphatase in the water column and sediment and the activity of acid phosphatase (AcPA) in sediments of acidotrophic freshwater ecosystems (Huang and Morris 2003; Torres et al. 2017) and little attention has been paid to AcPA in the water column of ecosystems with neutral or slightly alkaline pH. Taking these aspects into account, we hypothesize that in contaminated freshwater ecosystems where the pH is neutral or slightly alkaline, AcPA will be more influenced by polluting factors (eg, phosphate concentration, organic matter) compared to less impacted systems where AcPA will depend on natural conditions (eg: temperature, pH, phytoplankton activity).

The objective of this study is to determine the acid phosphatase activity (AcPA) in the Almendares and San Juan rivers (western Cuba) and its relationship with physicochemical and microbiological indicators.

2. Materials And Methods

2.1. Study area and sampling

To analyze the AcPA at Almendares and San Juan rivers, sampling was done on February, April, June and October during the year 2017 at three sampling stations in each river. The sampling stations in the Almendares River were Río Cristal (23º01'59.99" N, 82º24'03.77" E), Paila (23º03'23.94" N, 82º24'09.75" E) and Puente de Hierro (23º07'36.55" N, 82º24'40.22" E) (Online Resource 1)(Larrea et al. 2014). In the San Juan River the sampling stations were Presa El Palmar (22º50'42.35" N, 82º56'23.33" E), Presa San Juan (22º50'46.01" N, 82º56'27.09" E) and Baños del San Juan (22º49'24.02" N, 82º55'35.08" E) (Online Resource 2)(Romeu et al. 2015a). The samples were taken in the morning at a distance of one meter from the shore and 15 cm deep. The samples were transferred to the laboratory in sterile plastic bottles of 500 mL in a refrigerator (4°C) and were processed in a period of less than 12 h (AFNOR 2009).

2.2. Determination of physicochemical and microbiological indicators

Determination of temperature, pH, electrical conductivity, total dissolved solids (TDS), dissolved oxygen, nutrients (PO_4^{3-}-P, NH_4^{+}-N, NO_2^{-}-N, NO_3^{-}-N), chemical oxygen demand (COD) and bacterial indicators (*Escherichia coli*, enterococci and thermotolerant coliforms) are described in Izquierdo et al. (2020).

2.3. Calculation of the nutrient pollution index

The nutrient pollution index (NPI) was calculated according to Isiuku and Enyoh (2020), using the formula:

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$$NPI = \frac{C_N}{MAC_N} + \frac{C_P}{MAC_P}$$

where $C_{N/P}$ is the average concentration of nitrate and phosphate in the water body, $MAC_{N/P}$ is the maximum allowable concentration taken from W.H.O. (2003) which is 10 $mg.L^{-1}$ and 0.1 $mg.L^{-1}$ for nitrate and phosphate in recreational waters respectively. According to the NPI values, the waters can be classified as NPI < 1 (no pollution), NPI de 1 ≤ 3 (moderated polluted), NPI > 3 ≤ 6 (considerable polluted) and NPI > 6 (very high polluted)

2.4. Calculation of N:P ratio

The nitrate to phosphate ratio was used to determine the limiting nutrient pattern in the water bodies evaluated according to Wemedo et al. (2021). It was determined by dividing the nitrate concentration by the phosphate concentration. The results were interpreted based on the Redfield ratio (Redfield 1958), as N: P ratio < 16 = Nitrate limitation, while N: P ratio > 16 = Phosphate limitation.

2.5. Determination of acid phosphatase activity

Total acid phosphatase activity was determined colorimetrically using p-nitrophenyl phosphate as substrate according to the methodology of Asaduzzaman et al. (2011) with modifications. Assays were performed in triplicate and a negative control was used for each sample to assess the development of enzymatic non-color. The reaction mixture was prepared in 15 mL corning tubes and consisted of 1.5 mL of citrate buffer (0.05 M pH 4.8), 1.5 mL of 1mM p-nitrophenyl phosphate and 1.5 mL of water sample. The reaction mixture was incubated at 37 ° C for 10 minutes. To stop the reaction, 0.5 mL of reaction mixture was added to a tube containing 4.5 mL of NaOH (0.02M) and the absorbance at 410 nm was read in a spectrophotometer. The units of AcPA were expressed as µmol of p-nitrophenol produced per milliliters of sample per minute

2.6. Statistical analysis

To determine the existence of significant differences among sampling stations and sampling months in each river related to AcPA, the normal distribution and homogeneity of variance of the data were verified using the Kolmogorov-Smirnov and Cochran-Bartlett tests, respectively. Non-parametric Mann-Whitney U test ($p < 0.05$) was subsequently applied. To evaluate the correlation among the physicochemical indicators, the indicators of faecal contamination and AcPA, the Spearman non-parametric linear correlation test was performed. Statistical analyzes were carried out in the Statistica version 8.0 program (StatSoft 2007).

3. Results

3.1. NPI in Almendares and San Juan rivers

The nutrient pollution index for each sampling station of the Almendares and San Juan rivers, as well as the annual mean in each river is presented in Table 1. All the sampling stations of the Almendares River are considered very high polluted. The Paila station presented the highest pollution values followed by Puente de Río and Río Cristal.

Table 1
Nutrient pollution indices (NPI) in the sampling stations of the Almendares and San Juan rivers in the period February-October 2017

Sample Stations		2017				
		February	**April**	**June**	**October**	**Anual**
Almendares River stations	**Río Cristal (RC)**	6.83	10.12	9.63	9.68	9.07
	Paila (P)	59.48	62.35	45.57	44.04	52.86
	Puente de Hierro (PH)	38.05	40.51	23.65	39.37	35.40
San Juan River stations	**Presa El Palmar (S1)**	2.19	1.75	0.11	2.82	1.72
	Presa San Juan (S2)	0.95	7.70	0.95	0.62	2.56
	Baños del San Juan (S3)	2.44	3.98	0.92	1.39	2.18

The values in red represent NP1 > 6 (very high polluted), values NPI < 1 (no polluted), NPI de 1 ≤ 3 (moderately polluted), NPI > 3 ≤ 6 (considerable polluted) according to [16].

In the case of the San Juan River, the Presa El San Juan and Baños del San Juan sampling stations only in the month of April were considered very high polluted and considerable polluted, respectively. Presa El Palmar station in June, Presa San Juan in February, June and October and the Baños del San Juan station in June were considered no polluted. The rest of the time, the Presa El Palmar and Baños del San Juan stations were considered moderately polluted. In a general sense, the Almendares River is classified as a very high polluted ecosystem and the San Juan River as a moderately polluted ecosystem.

3.2. N:P ratio in Almendares and San Juan rivers

Table 2 shows the nitrate to phosphate ratios in the Almendares and San Juan rivers stations, as well as the annual mean in each ecosystem. In the Almendares River, with the exception of the Río Cristal station in February, which presented a phosphate limitation (N: P ratio > 16); all stations were limited by nitrate (N: P ratio < 16). The lowest values were obtained at the Paila station, followed by Puente de Hierro and Río Cristal. In the San Juan River, with the exception of the Presa El San Juan station in April, which presented a limitation due to nitrate (N: P ratio < 16); all stations were limited by phosphate (N: P > 16). The highest values were obtained at the Presa El San Juan station in February (9444.44) and Presa El Palmar station in October (1061.84). In general, the Almendares River presented a nitrate limitation during the study period and the San Juan River presented a phosphate limitation.

Table 2

N: P ratio in the sampling stations of the Almendares and San Juan rivers in the period February-October 2017

Sample Stations		2017				
		February	**April**	**June**	**October**	**Anual**
Almendares River stations	**Río Cristal (RC)**	24.70	10.07	2.77	2.55	10.02
	Paila (P)	2.07	0.06	0.45	0.17	0.69
	Puente de Hierro (PH)	5.28	4.59	8.87	6.14	6.22
San Juan River stations	**Presa El Palmar (S1)**	42.15	18.85	52.78	1061.84	293.91
	Presa San Juan (S2)	9444.44	13.06	455.65	533.64	2611.70
	Baños del San Juan (S3)	36.38	30.33	554.17	148.44	192.33
N: P ratio < 16 = Nitrate limitation, while N: P ratio > 16 = Phosphate limitation.						

3.3. Acid phosphatase activity (AcPA) and its relationship with physicochemical and microbiological indicators of water quality

In Almendares River, AcPA showed significant differences between the sampling months in each sampling station and between the river sampling stations in each of the sampled months (Fig. 1). AcPA values were found between 0 and 0.025 $\mu mol.mL^{-1}min^{-1}$. Enzyme activity was detected in all the sampling stations with the exception of the Río Cristal station in April. In the month of October, the highest AcPA values were presented in the three sampling stations ($p < 0.05$). Meanwhile, in June the lowest values were obtained in all stations ($p < 0.05$). The Puente de Hierro sampling station showed the highest enzyme activity values in the month of October ($p = 0.043$) and February ($p = 0.037$) compared to the rest of the sampling stations.

AcPA positively correlated with the COD ($p < 0.01$) and with the concentration of nitrates ($p < 0.05$) in the Almendares River, but it did not significantly correlate with the rest of the physicochemical and microbiological indicators of water quality, the NPI and the N: P ratio (Table 3).

Table 3

Spearman`s correlations among acid phosphatase activity, the physicochemical and microbiological indicators, the NPI and the N:P ratio determined in Alm period February-October 2017.

	Temp.	**pH**	**EC**	**DO**	**TDS**	**Sal.**	**COD**	**NO_2^--N**	**NO_3^--N**	**PO_4^{3-}-P**	**NH_4^+-N**	**Clor-α**	**Ent.**	***E. coli***	**Th**
AcPA Almendares River	-0.118	0.188	0.322	0.295	0.322	0.322	0.538**	0.087	0.372*	0.014	-0.035	0.232	-0.112	-0.017	-0.
AcPA San Juan River	0.031	-0.478**	0.134	-0.219	0.060	0.018	-0.269	-0.163	-0.220	0.099	-0.328	0.358*	0.051	0.352*	-0.

Legend: AcPA: Acid phosphatase activity, Temp.: Temperature, EC: Electrical conductivity, DO: Dissolved Oxygen, TDS: Total Dissolved Solids, Sal.: Salinity, C NO_2^--N: Nitrite, NO_3^--N: Nitrate, NH_4^+-N: Ammonium, PO_4^{3-}-P: Phosphate, Ent.: Enterococci, *E. coli*: *Escherichia coli*, ThC.: Thermotolerant coliformes, TH: Tota pollution index, N:P: ratio nitrate/phosphate. Significant correlations are indicating in red. *($p < 0.05$), **($p < 0.01$), n = 36 for both rivers.

In San Juan River, AcPA showed significant differences between the sampling months in each sampling station and between the river sampling stations in the months of February, June and October (Fig. 2). AcPA values were found between 0 and 0.018 $\mu mol.mL^{-1}min^{-1}$. In a general sense, enzymatic activity was detected in all sampling stations with the exception of the Presa San Juan and Baños del San Juan stations in the month of February and in the month of April for the three stations. In the month of February, the highest AcPA value was obtained at the Presa El Palmar station ($p = 0.037$), followed by the Presa San Juan station in the month of October ($p = 0.034$) and the Baños del San Juan station in the months of June and October ($p = 0.043$). Meanwhile, in April the lowest values were obtained in all stations ($p < 0.05$).

AcPA positively correlated with chlorophyll *a* ($p < 0.05$), the N: P ratio ($p < 0.05$), the concentration of *E. coli* ($p < 0.05$) and the concentration of total heterotrophs ($p < 0.01$) and negatively correlated with the pH ($p < 0.01$) and the NPI ($p < 0.05$) (Table 3).

When the AcPA values obtained in the Almendares and San Juan rivers during the dry and rainy periods were compared (Fig. 3), it was observed that the highest values were obtained in the Almendares River in both periods ($p < 0.05$). However, the existence of significant differences between the dry period and the rainy period was only evidenced in the San Juan River ($p = 0.000329$).

4. Discussion

4.1. NPI and N:P ratio in Almendares and San Juan rivers

The Nutrient Pollution Index (NPI) takes into account the possible summative effects of nitrate and phosphate concentration on environmental health, allowing the estimation of water quality (Isiuku and Enyoh 2020). According to the NPI values obtained in the present study, the Almendares River is ratified as a very high polluted ecosystem (Table 1), which is in accordance with previous research carried out in this ecosystem using different physicochemical and microbiological indicators and the water quality index (Arpajón et al. 2011; Romeu et al. 2015b; Izquierdo et al. 2020) (Online Resource 3). The values obtained

ined by Isiuku and Enyoh (2020) in water bodies of southeastern Nigeria. The Almendares River is located in the province of Havana and receives little or no treatment wastewater of domestic and industrial origin from the city that contributes to the pollution of the ecosystem (Romeu et al. 2015b). The high phosphate concentrations determined in the Almendares River [see Izquierdo et al. (2020) and Online Resource 3 for values of physicochemical and microbiological indicators] contributed to the high NPI values obtained in this study.

In the case of the San Juan River, the NPI indicated that this is a moderately polluted ecosystem (Table 1), which is in correspondence with previous studies carried out in this river (Izquierdo et al. 2020; Larrea et al. 2021). The San Juan River is located in the province of Artemisa, in the "Sierra del Rosario" Biosphere Reserve, where it does not receive the same anthropogenic impact as the Almendares River because it is in a rural area where there is no presence of industries. In April, the highest NPI values were obtained at the Presa el San Juan and Baños del San Juan stations, classifying them as very high polluted and considerably polluted respectively. This result is in correspondence with the water quality index values reported by Izquierdo et al. (2020) for these two stations. The month of April constitutes one of the months that are within the dry season in Cuba. In particular, the province of Artemisa (western Cuba) was one of the most affected areas with the greatest deficit in accumulated rainfall according to the spatial analysis carried out by the Institute of Meteorology (INSMET 2018) in the dry season. Drought and increased temperature are factors that can cause the concentration of nutrients in water bodies (Roy et al. 2014) and thus affect water quality.

According to Redfield (1958), during phytoplankton growth, the N:P ratio should be equal to 16: 1. The N:P values obtained in the present study in the Almendares and San Juan rivers are not in correspondence with that observed by Redfield. In the Almendares River, phosphate concentrations are higher than nitrate concentrations, with a limitation due to nitrate. In the San Juan River, the opposite occurs, detecting a phosphate limitation (Table 2). The nitrate limitation conditions found in the Almendares River are associated with the anthropogenic activities carried out in this ecosystem, the low concentrations of dissolved oxygen and high concentrations of phosphate (Izquierdo et al. 2020) and possibly due to denitrification (Isiuku and Enyoh 2020; Wemedo et al. 2021). All of which leads to the eutrophication conditions that this river presents (Arpajón et al. 2011). In the San Juan River, the limitation by phosphate implies higher concentrations of nitrate, less addition of phosphates from polluting sources and therefore, less risk of eutrophication and better water quality (Izquierdo et al. 2020; Larrea et al. 2021).

These results show the usefulness of the NIP and the N: P ratio in evaluating water quality in aquatic ecosystems and should be used in conjunction with the water quality index to provide more complete information on the characteristics of the surface waters.

4.2. Acid phosphatase activity detected in the waters of Almendares and San Juan rivers

Acid phosphatase activity (AcPA) during the study period in the Almendares and San Juan rivers showed significant differences between the sampling sites and the sampling months (Figs. 1 and 2), showing spatial-temporal variability in both ecosystems. These results are in agreement with studies carried out in different aquatic systems, such as lakes (Tabata et al. 1988; Patel et al. 2018), rivers (Pandey and Yadav 2017; Patel et al. 2018), estuaries (Labry et al. 2016), mangroves (Luo et al. 2017) and seas (Ivancic et al. 2009), where the phosphatases activity (acid and/or alkaline) in the water column or sediment has been analyzed. In both, the Almendares River and the San Juan River, the highest AcPA values were obtained in the month of October in almost all the stations. This month marks the end of the rainy season in Cuba and was characterized by the presence of abundant rainfall (INSMET 2018). In addition, in the San Juan River the highest activities were found in the rainy season compared to the dry season (Fig. 3), which is in correspondence with the results reported by Giraldo et al. (2014) who obtained the highest phosphatase values in the rainy period in the Aburra-Medellín River in Colombia. Different researchers have suggested that during the rainy season the extracellular enzymatic activity increases as a result of the dilution of nutrients, which become less available to the microorganisms present in the waters. These microorganisms must synthesize enzymes to obtain nutrients from the organic matter available (Tiquia 2011; Giraldo et al. 2014; Jaramillo et al. 2016). On the other hand, in dry periods, there is an accumulation of nutrients that can be used by heterotrophic microorganisms, producing a decrease in the enzymatic activity (Cunha et al. 2010; Tiquia 2011).

In the Almendares River, despite obtaining the highest activity values in October, no significant differences were observed between the rainy season and the dry season, which may be due to the high levels of contamination by organic matter that reach the river from the discharges of domestic and industrial origin and from animal husbandry, throughout the year (Arpajón et al. 2011; Romeu et al. 2015b). In addition, AcPA positively correlated with COD (Table 3), which is an indicator of organic matter. This suggests that high levels of contamination maintain high levels of enzymatic activity, which is in correspondence with the results obtained by Jaramillo et al. (2016) who found that there was a correlation between COD and phosphatase activity in the Aburra-Medellín River in Colombia. Previously, it has been reported that contamination with sewage and sediments can constitute a source of alkaline phosphatase activity (Zhou et al. 2004; Nedoma et al. 2006) and could also constitute a source of AcPA considering that many microorganisms present in wastewater can produce both enzymes, as was demonstrated by Larrea et al. (2018) in isolates from Almendares River. The Puente de Hierro station on the Almedares River showed the highest AcPA values, which correlated with the COD and the nitrate concentration (Table 3). This station is located in the river estuary where a greater arrival of nutrients occurs with the entry of the tides and with the discharge of the freshwater ecosystem. In addition, the mixture of fresh river water with sea water causes the flocculation of fine suspended matter in large particles, and on the other hand, the action of the tides also causes the resuspension of the particles (Sigee 2005); contributing to the increase of organic matter and therefore to the increase in enzymatic activity. High values of alkaline phosphatase activity in the presence of high concentrations of organic matter and high concentrations of phosphates in estuaries have been fundamentally attributed to the presence of particles associated bacteria according to studies carried out in the Aulne and Elorn estuaries in the Northeast France (Labry et al. 2016); conditions that occurred in all the stations of the Almendares River.

The fact that the highest AcPA values have been obtained in the Almendares River with respect to the San Juan River may be due to the high values of organic matter found in the Almendres River and the slightly alkaline pH observed in the San Juan River (Izquierdo et al. 2020) (Online Resource 3). Particularly, an increase in enzyme activity in the water column has been detected in streams with more intensive human land use (Williams et al. 2012) as is the case of Almendares River. In the San Juan River, pH correlated negatively with the AcPA, where fundamentally, in the months of February and April values of pH > 8 were obtained (Izquierdo et al. 2020). At these pH values, the activity of acid phosphatases is practically null because their activity occurs at pH 2.6–6.8

 negative correlation between the AcPA and the nutrient pollution index was also observed. The San Juan River is an ecosystem located in the Sierra del Rosario Biosphere Reserve, which is not exposed to the same pollutant load as the Almendares River because it is in a rural environment. In addition, this Reserve constitutes a mountainous ecosystem where abundant rainfall occurs that tends to dilute the pollutants present in the waters. Thus, the microorganisms present in the river waters, faced with a lower availability of nutrients, must synthesize extracellular enzymes to obtain the nutrients from the available organic matter (Cunha et al. 2010).

On the other hand, in the San Juan River, the AcPA positively correlated with chlorophyll *a*, the concentration of *Escherichia coli*, the concentration of total heterotrophs and the N: P ratio. This result suggests that both bacteria and phytoplankton present in this ecosystem are responsible for the production of acid phosphatase activity under phosphate-limited conditions, which is in correspondence with previous studies that have been carried out in different aquatic systems where the activity of alkaline or acid phosphatases has been determined (Yiyong et al. 2002; Nedoma et al. 2006; Labry et al. 2016; Li et al. 2019).

5. Conclusions

Our results show that in freshwater ecosystems with neutral or slightly alkaline pH, acid phosphatase activity is present and also plays an important role in recycling organic matter in these environments. Furthermore, in contaminated freshwater systems this activity is more influenced by polluting factors (eg, phosphate concentration, organic matter) compared to less impacted systems where it depends on natural conditions (eg: temperature, pH, phytoplankton activity); so acid phosphatase activity in water column could be a good indicator of the anthropogenic impacts on microbial functioning in freshwater ecosystems. Future research should be done to assess whether the observations in this article can be generalized to other aquatic systems. In addition, metaproteomics and metagenomics studies could make it possible to analyze the response of the microbial community to the degradation of organic matter and eutrophication in the freshwater ecosystems in western Cuba.

Declarations

Conflicts of Interest: The authors declare no conflicts of interest.

Data Availability Statement: Data related to physicochemical and microbiological indicators of water quality from February-June 2017 can be found as supplementary material in https://doi.org/10.1007/s11270-020-04909-z. The rest of the data is within the present article, including supplementary information of water quality on October 2017.

Code availability: Not applicable

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Figures

Figure 1

Acid phosphatase activity in the sampling stations of the Almendares River during the period February-October 2017. Capital letters indicate significant differences between the sampling months in the same sampling station. Lowercase letters indicate significant differences between the sampling stations in the same sampling month according to the Mann-Whitney U test ($p < 0.05$)

Figure 2

Acid phosphatase activity in the San Juan River sampling stations during the period February-October 2017. Capital letters indicate significant differences between the sampling months in the same sampling station. Lowercase letters indicate significant differences between the sampling stations in the same sampling month according to the Mann-Whitney U test ($p < 0.05$)

Figure 3

Acid phosphatase activity in the Almendares and San Juan rivers during the dry and rainy seasons in 2017. Capital letters indicate significant differences between the rivers in the dry period (A and B). Greek letters indicate significant differences between rivers in the rainy season (α and β). Lowercase prime letters indicate significant differences between dry and rainy seasons in the San Juan River. The same lowercase letters indicate the non-existence of significant differences in Almendares River according to the Mann-Whitney U test ($p < 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESM1.AlmendaresRiversamplingstations.pdf
- ESM2.SanJuanRiversamplingstations.pdf
- ESM3.Physicochemicalandmicrobiologicalindicators.pdf