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# Geochemical and ecotoxicological evaluation of sediments of a semiarid estuary on the northeast of Brazil (Natal / RN)

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ABSTRACT

The Jundiaí-Potengi Estuary (JPE), northeast of Brazil, suffers from intense degradation due to anthropogenic pollution from different sources, including several punctual treated and untreated domestic sewage discharges. To evaluate the estuary surface sediment quality near the outlet of a wastewater treatment plant (WWTP), five sampling points were selected. Four campaigns were performed on dry and rainy seasons of the years 2015 and 2016. The sediment samples were submitted to chronic ecotoxicological tests with *Nitocra* sp., and to metals, granulometry, and total organic matter analysis. The geochemical data were analyzed through enrichment indexes and, multivariate analysis, was performed integrating the ecotoxicological data. The sediment did not display toxicity to *Nitocra* sp. however, some metals' concentration (Mn, Pb, Cu, Ni, and Cd) exceeded the limits established by Brazilian legislation. In general, the concentration of the analyzed metals doubled from rainy season to dry season. Considering our findings, we suggest quantifying other classes of contaminants and conducting studies on the bioavailability of metals in JPE, as well as using other organisms of different trophic levels in ecotoxicity tests.



## 1. Introduction

Estuaries are nutrient-rich environments, responsible for their high primary productivity. Together with the mangrove forest, estuaries export biomass to coastal and marine ecosystems (MMA, 2010; Giri et al., 2011), being areas of residence and reproduction of various marine and pelagic organisms, nursery of their larvae and sustenance guaranty and protection of juvenile forms (Beck et al., 2001).

The Jundiaí–Potengi estuary (JPE) complex, where the Potengi river flows it is an important site of fishing and leisure. Currently, shrimp farms, effluent discharges from both wastewater treatment plants (WWTP) and untreated sewage, as well as textile and beverage industries effluents are, jointly with port activity, the main causes of JPE' pollution. Due to these multiple sources of anthropogenic pollution, Natal city area has served as a characteristic site to study the environmental impact caused by the rapid growth of Brazilian cities (Sindern et al., 2007).

Among the various types of contaminants that affect estuaries, metals are noteworthy because they accumulate at different trophic levels (bioaccumulation), and their persistence in the environment constitute a high ecological risk (Marengoni et al., 2013; Yousafzai et al., 2017). Studies have shown that sanitary sewage, domestic and industrial, as well as the flow from agriculture and waste disposal areas are the main anthropogenic sources of these elements in aquatic environments (Kalloul et al., 2012; Gurgel et al., 2016). The discharge of sanitary effluents into estuaries contributes to the degradation of their water and sediments quality (Camargo et al., 2015; Nilin et al., 2019) even when treated in traditional WWTP, because metals are not sufficiently removed and so, these discharges can be harmful to aquatic communities (Singh et al., 2004).

Sediments are considered sinks of some chemical elements, including the metals that may report the pollution history (Lesage et al., 2007). Ecotoxicological tests constitute an integrated evaluation tool for the effects of effluent discharges on organisms, being very useful to identify environmental impacts (Chapman, 2000; Mendonça et al., 2009; Garcia et al., 2017). Marine copepods, including *Nitocra* sp. (Marangoni et al., 2011; Krull et al., 2014; Souza and Silva, 2016) have been widely used to assess sediment toxicity (Souza-Santos et al., 2015).

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Within this context, this work aimed to evaluate the surface sediment quality of an estuarine strip on JPE located near a WWTP constructed in 2011, through ecotoxicological monitoring using *Nitocra* sp., along with geochemical analyses. It is crucial to determine pollution sources that have a potential impact on Potengi river sediments in order to correct or to improve two other treatment systems currently under construction in this region.

## 2. Materials and methods

### 2.1. Study area

The Potengi River with approximately 30 km length is located between latitudes 05° 52'00" and 05° 41'57", longitudes 35° 19'16" and 35° 08'24", including partially the municipalities of Macaíba, São Gonçalo and Natal, the state capital of Rio Grande do Norte — RN (Fernandes and Petta, 2008). The region climate is classified as tropical humid and semi-arid (Silva et al., 2007). During the rainy season, monthly rainfall average was 316.5 mm in 2015 and 156.6 mm in 2016, whereas in dry period it was 6.6 mm in 2015 and 22.7 mm in 2016 (EMPARN, 2016).

In 2016, the estimated population in the Metropolitan area of Natal (which include the above cities) was about 1.6 million residents (IBGE, 2016). Only 20 to 30% of the sewage produced in these municipalities are collected for a treatment system, where only about 70% of the sewage is effectively treated (SEHARPE, 2015). Currently, the JPE area studied receives effluent from a WWTP with tertiary treatment, by ultraviolet (UV) rays disinfection, and untreated or inadequately treated domestic wastewater. The sewage network of the region is being expanded with the addition of two more WWTP under construction. The effluents to be treated in these WWTP will also be discharged in JPE complex.

### 2.2. Water sampling collection

Four campaigns were carried out in the JPE during rainy seasons (RS) and dry seasons (DS) — July 2015 and June 2016, and October 2015 and 2016, respectively. Sediments were collected at five points in the vicinity of a WWTP (Fig. 1): P01 and P02 downstream the WWTP, P03 at the WWTP outlet (Baldo canal) and P04 and P05 upstream the WWTP. As reference for the ecotoxicological analysis, sediment samples were collected in the Galinhos estuary (RN northern coast, Brazil), located about 150 km from the JPE, an area that has been recognized as poorly polluted.

Sampling was carried out on the right bank of the estuary (where the WWTP is located), during low tide at a water depth of about 1 m. In July 2015, sediment samples were collected with a 3-liter Van Veen dredge and a 4-liter plastic beaker. From the sediment samples, only the surface layer (0–5 cm) were homogenized, identified and stored (≈4 °C) no more than 15 days until use.

### 2.3. Analyses of sediment samples

#### 2.3.1. Geochemical analysis

Granulometric analysis were performed by mechanical agitation of 50 g of sediment previously homogenized and dried in an oven at 60 °C for 18 h. The fraction of sediment recovered above 0.075 mm was considered sand, while that recovered below this opening was considered mud (fine sediment — silt/clay). Total organic matter (OM) content of sediment samples was determined by calcination (Kralik, 1999).

The following metals — Cd, Cu, Pb, Mn, Ni and Zn, were determined in 2 g of fine sediment fraction of each sample digested in 15 mL of *aqua regia*. Samples were previously diluted (1:10 dilution) to reduce high salinities interference on spectrophotometric analysis, performed on an atomic absorption spectrophotometer, AAS-220-FS. Metals quantification (mg kg $^{-1}$) was estimated through calibration curves using standard solutions with known concentrations of the different metals.

The presence and intensity of anthropogenic contaminants in sediments were evaluated by Enrichment Factor (EF) and Geoaccumulation Index (Igeo). These indexes considered different contamination levels calculated according to the equations proposed by several authors (Table 1). The Igeo use as geogenic background, a value provided by Sindern et al. (2007), multiplied by 1.5 — constant factor to neutralize variations due to lithogenic actions.

These indexes were only determined in sediment samples collected during 2015 the dry season (DS15) and the dry and rainy seasons of 2016 (DS16 and RS16, respectively). Ni and Zn contents were not determined on the rainy season of 2015 (RS15) due to equipment problems.

#### 2.3.2. Ecotoxicological analyses

Chronic tests with whole-sediment use the estuarine copepod *Nitocra* sp. following the methodology described by Lotufo and Abessa (2002). Polyethylene test chambers were filled with 2 g of sediment samples and 20 mL of seawater (17 psu) previously filtered with activated charcoal and disinfected with UV. Ten healthy ovate females were inserted into test chambers (15 test chambers), fed with two drops of fish meal and Fleischmann® biological yeast suspension (*Saccharomyces cerevisiae*) and exposed to a photoperiod of 12 h:12 h (light–dark), at 24 °C ± 2 °C for 10 days. After the incubation period, the organisms (from each triplicate) were fixed and stained with 2 mL strong lugol (4%) and approximately 0.01 g Rose-Bengal dye. Finally, the adult females and their offspring (*nauplii* and copepodites) were counted using a stereomicroscope. The estimated average lethal concentration (LC50 — 96h) for the reference substance, potassium dichromate ($K_2Cr_2O_7$), was 9.12 mg $L^{-1}$ ranging from 6.70 to 15.09 mg $L^{-1}$ (n = 3).

### 2.4. Statistical analyses

Normality of ecotoxicological data was verified by Chi-square test, whereas the homogeneity of variance was assessed by Fisher's exact test. Statistical' differences between the negative control and sediment samples tested were analyzed by one-way ANOVA and Student's t-test, post-hoc. Sediment samples were considered to be toxic when reproduction rate of *Nitocra* sp. females, was significantly different than the control ($p \leq 0.05$).

Geochemical and ecotoxicological data were integrated by means of multivariate analysis techniques, namely factor analysis (FA) and principal components analysis (PCA). As FA-PCA allows relationship evaluation between all environmental data measured and estimated chronic toxicity values, it may indicate possible causes of toxicity.

PCA was done by a graphical scree plot and performed considering the rotation of the axes by normalized Varimax approximation. In order to establish the most significant variables associations, a loading factor of 0.40 was considered, which is in agreement with the overlap percentage of the variances established by Comrey and Lee (1992) and is equal or higher than the cut-off values used by Choueri et al. (2009), Rodrigues et al. (2013), Krull et al. (2014) & Souza et al. (2016). To reduce magnitude differences between variables to treat them with equal importance, the data were auto-adjusted (standardized) by STATISTICA 7 software.

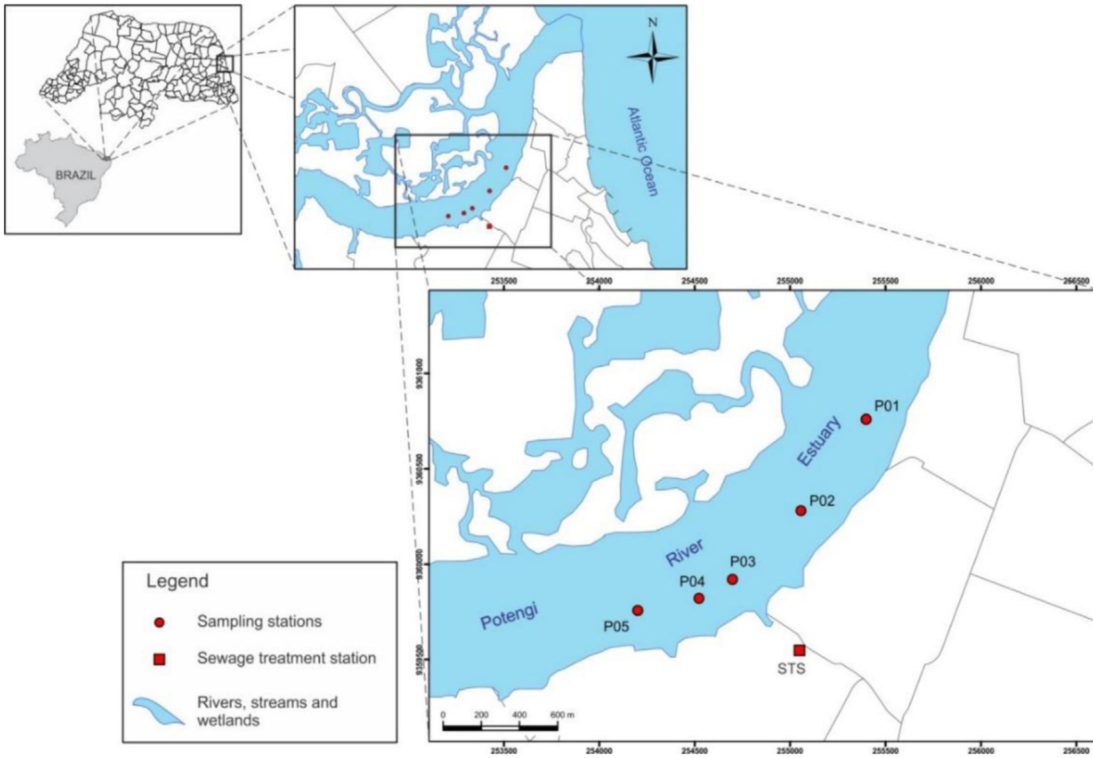


**Fig. 1.** Studied area and sampling points location: upstream the WWTP (P05 and P04), at the effluent discharge site (P03) and downstream the WWTP (P02 and P01).

**Table 1**
Assessment of metals contamination level in sediments: equations to the determination of Enrichment Factor (EF) and Geoaccumulation Index (Igeo).

| Index | Equation | Pollution level | | | References |
|---|---|---|---|---|---|
| | | Value | Class | Metals Contamination | |
| EF | $EF\,(\%) = \left(\frac{C-Cmin}{Cmax-Cmin}\right) * 100$ | >50% | | Enriched | Zonta et al. (1994) [27] |
| | | <50% | | Not enriched | |
| Igeo | $Igeo = log2\,\frac{C_n}{1.5B_n}$ | <0 | 0 | uncontaminated | Muller (1969) [28] |
| | | 0–1 | 1 | uncontaminated to moderate | |
| | | 1–2 | 2 | moderate | |
| | | 2–3 | 3 | moderate to heavy | |
| | | 3–4 | 4 | Heavy | |
| | | 4–5 | 5 | heavy to extremely contaminated | |
| | | >5 | 6 | extremely contaminated | |

C = mean concentration of the metal; $C_{min}$ and $C_{max}$ = minimum and maximum concentrations of the metal, respectively, determined during the experimental period; Cn = concentration of metal; Bn = background concentration of the metal.

## 3. Results and discussion

### 3.1. Sediment characterization

Overall higher levels of OM (Table 2) were observed during the dry period (3,79–16,98% in DS15 and 7,64–22,45% in DS16); this may be related to lower turbidity due to the lower water bodies turbulence which increase deposition of suspended matter. The estimated OM contents in the analyzed samples were similar to those previously determined in sediment samples from estuaries of the northeast region of Brazil (Marins et al., 2007; Silva et al., 2017), including JPE (Buruaem et al., 2013; Souza et al., 2016).

Fine sediments (Mud %) did not present a clear gradient of spatial distribution along the sampling points. However higher mud contents were assessed on 2016 campaigns (Table 2), especially in the rainy season (RS16), being evident P03 as a site with low mud content.

### 3.2. Metals contents

Metals concentrations on sediments from sampling points were rarely below CONAMA resolution limits (Fig. 2) and Probable Effects Level (PEL) was exceeded in P01, for Cd on DS15 and for Cu, Pb, Ni and Zn, on DS16. As a conservative element Mn has no established limit, assisting in investigations about anthropogenic enrichment, as it come from lithogenic origin.

Generally, some of the analyzed metals increased significantly its concentrations from rainy season to dry season (Table 2–Fig. 2). In 2015, at P01, Cd concentration increased about 10 times from rainy to dry seasons. In 2016, at the same site the increased concentration from rainy to dry seasons were about 5x higher for Cu, 3x higher for Pb and Ni and 8x higher for Zn.

Considering metals natural supply, its increase in dry period may be associated with the reduction of Potengi river and their tributaries water flow. This tends to increase the residence time of water in flooded areas, intensifying materials deposition (Mud and MO), and favoring metals' imprisonment in the sediments of

**Table 2**
Jundiaí–Potengi estuary' sediment characterization considering the granulometry [Mud (%) and organic matter (OM) %], geochemical analysis (metals mg kg$^{-1}$) and toxicity test (copepod reproduction rate) on dry (DS) and rainy (RS) seasons of 2015 and 2016. The metal concentrations above the levels established by Brazilian law (CONAMA 454/12) are marked in bold and exceed PEL are marked in red.

| | | Mud (%) | OM (%) | Metals (mg kg$^{-1}$) | | | | | | Toxicity ((n+c)/♀) |
|---|---|---|---|---|---|---|---|---|---|---|
| Campaigns | Samples | | | Cd | Cu | Pb | Mn | Ni | Zn | Reproduction rate |
| RS15 | Control | | | | | | | | | 14,9 ± 0,4 |
| | P01 | 1.96 | 1.90 | 0.73 | 30.84 | **48.37** | 91.31 | – | – | 22,7 ± 2,4 |
| | P02 | 4.00 | 1.82 | 1.26 | 25.14 | 37.71 | 182.04 | – | – | 13 ± 2,4 |
| | P03 | 0.41 | 2.70 | **2.00** | **101.37** | **104.86** | 93.63 | – | – | 20,3 ± 6,1 |
| | P04 | 2.00 | 4.00 | **1.54** | **41.71** | **61.79** | 148.54 | – | – | 21,4 ± 4,5 |
| | P05 | 0.13 | 0.58 | 0.25 | 4.00 | 22.48 | 8.99 | – | – | 19,1 ± 4,8 |
| DS15 | Control | | | | | | | | | 14 ± 2,4 |
| | P01 | 1.90 | 10.50 | **7.50** | 22.25 | 34.62 | 27.25 | 18.12 | 67.50 | 7,4 ± 5,6 |
| | P02 | 7.89 | 9.13 | **2.50** | **37.00** | 44.87 | 40.00 | 3.37 | **178.12** | 13 ± 4,5 |
| | P03 | 4.71 | 4.82 | **1.50** | **89.50** | **65.37** | 39.75 | **37.87** | **334.37** | 23 ± 7,7 |
| | P04 | 7.50 | 16.98 | **1.25** | **35.87** | **64.12** | 322.87 | **43.87** | 143.75 | 18 ± 3,7 |
| | P05 | 8.00 | 3.79 | **1.75** | **43.12** | **49.00** | 100.82 | **33.37** | 148.12 | 12 ± 7,5 |
| RS16 | Control | | | | | | | | | 17,2 ± 8,4 |
| | P01 | 15.90 | 8.25 | **1.32** | **47.00** | **51.50** | 92.50 | 19.00 | 110.65 | 11,5 ± 1,2 |
| | P02 | 13.37 | 20.30 | 0.82 | 24.25 | 35.25 | 107.90 | 17.92 | 73.15 | 16,73 ± 5,3 |
| | P03 | 1.31 | 11.43 | 0.80 | **48.90** | 44.82 | 70.82 | 18.75 | **160.22** | 18,73 ± 5,1 |
| | P04 | 21.32 | 14.98 | 0.67 | **45.75** | **111.50** | 70.82 | 13.92 | 144.05 | 21,53 ± 6,1 |
| | P05 | 21.40 | 17.03 | 0.76 | 22.00 | 43.35 | 76.07 | 12.65 | 46.50 | 15,43 ± 4,4 |
| DS16 | Control | | | | | | | | | 33 ± 4,1 |
| | P01 | 10.35 | 10.95 | **1.44** | **228.12** | **143.50** | 60.25 | **50,87** | **934.37** | 53 ± 9,7 |
| | P02 | 18.50 | 22.45 | **2.50** | 25.62 | 39.75 | 174.97 | **24,12** | 99.37 | 49 ± 3,9 |
| | P03 | 9.37 | 12.34 | **2.50** | **55.75** | **56.62** | 57.32 | **27,50** | 130.50 | 61 ± 1 |
| | P04 | 6.40 | 7.64 | **1.75** | **46.00** | **62.87** | 125.00 | **29,00** | **177.50** | 32 ± 6,4 |
| | P05 | 12.00 | 15.48 | **1.75** | 30.75 | **89.75** | 149.97 | **26.37** | 101.87 | 39 ± 8,5 |
| CONAMA[a] | | | | 1.20 | 34.00 | 46.70 | – | 20.90 | 150 | |
| *Background*[b] | | | | 0.01 | 2.65 | 1.72 | – | 6.05 | 9.35 | |
| PEL[c] | | | | 4.20 | 108 | 112 | – | 42.80 | 271 | |

[a]Resolution CONAMA (Conselho Nacional do Meio Ambiente–National Council for the Environment) 454/2012 [37].
[b]Sindern et al. (2007) [4].
[c]Probable Effects Level (PEL) defined by National Oceanic and Atmospheric Administration (NOAA) and Canadian Council of Ministers of The Environment (CCME, 1995) [38].

this estuarine region (Unda-Calvo et al., 2019). Widely discussed (Lacerda et al., 2007, 2012) metals' imprisonment in Brazilian semiarid region estuaries sediments was reported in the JPE (Lopes, 2012; Souza et al., 2016). Associated with high tidal prism, the low water flow on Brazilian semiarid northeast estuaries, can result in an increase of the residence time of water bodies, ten times greater on drought periods (Dias et al., 2011).

Conversely, water balance inversion during rainy seasons directs a positive net flow from the river to the estuary, and contaminants associated to sediments are export to the sea. According to data from the EMPARN pluviometric bulletin (EMPARN, 2016), in the RS15 rainfall (316.5 mm) was twice as high as in the RS16 (156.6 mm).

Normally, runoff contributes to a large pollution load in urban stretches (Singh et al., 1997; Walker et al., 1999). So, the high metals concentrations assessed at P01 during the rainiest period (RS15) can be justified by a permanent source of surface drainage and untreated effluent.

Regarding the anthropic contribution, the dry seasons' high metals levels may have resulted, at least partially, from the additional supply of domestic sewage due to the greater annual tourist flow in RN. Some 2.5 million passers-by abide on JPE margins, in summer months (SEPLAN, 2014). The temporary rise in population may intensify WWTP activity, increasing OM and metals emissions, possibly accelerating the deposition of these materials on sediments during drought.

Additional contributions of metals on sediments were showed by geochemical indices at least on one sampling point, during the performed campaigns . The metals' enrichment in the sediments assessed by EF (Table 3) were > 50% near the WWTP (P03) at DS15 and RS16, on P01 (RS16 and DS16) and on P04 (DS15). The estimated values for Igeo (Table 3) were ≥ 3 in all estuarine

**Table 3**
Metal enrichment factor (EF %) and geoaccumulation index (Igeo) estimated on sediment simple samples of Jundiaí–Potengi estuary during dry (DS15 and DS16) and rainy (RS16) seasons.

| Campaign | Sample | EF — Total (%) | Igeo — Total | Class Igeo |
|---|---|---|---|---|
| DS15 | P01 | 23 | 3.7 | 4 |
| | P02 | 20 | 3.4 | 4 |
| | P03 | 66 | 4.4 | 5 |
| | P04 | 57 | 3.9 | 4 |
| | P05 | 36 | 3.9 | 4 |
| RS16 | P01 | 72 | 3.6 | 4 |
| | P02 | 40 | 3.1 | 4 |
| | P03 | 55 | 3.6 | 4 |
| | P04 | 49 | 3.6 | 4 |
| | P05 | 6 | 2.8 | 3 |
| DS16 | P01 | 67 | 5.3 | 6 |
| | P02 | 33 | 3.6 | 4 |
| | P03 | 25 | 4.1 | 5 |
| | P04 | 24 | 4.0 | 4 |
| | P05 | 28 | 3.8 | 4 |

section analyzed, being somewhat in agreement with the Igeo data obtained in the Jundiaí River, the main tributary of the Potengi River (Guedes, 2012), highlighting the maintenance of high contamination of sediments.

Slightly higher Igeo values were recorded for the dry seasons (DS15 and DS16), being the highest contamination record on P01 (DS16) and on P03 (DS15 and DS16). These indices are mainly due to the high nominal concentrations of Cu, Pb and Zn (Supplementary material, Table 01). Similarly, Sindern et al. (2007) recorded anthropogenic enrichment on the JPE sediments on sites near to sewage discharges (with increased concentrations of Cd, Cu, Pb and Zn), an evidence attributed to the input of

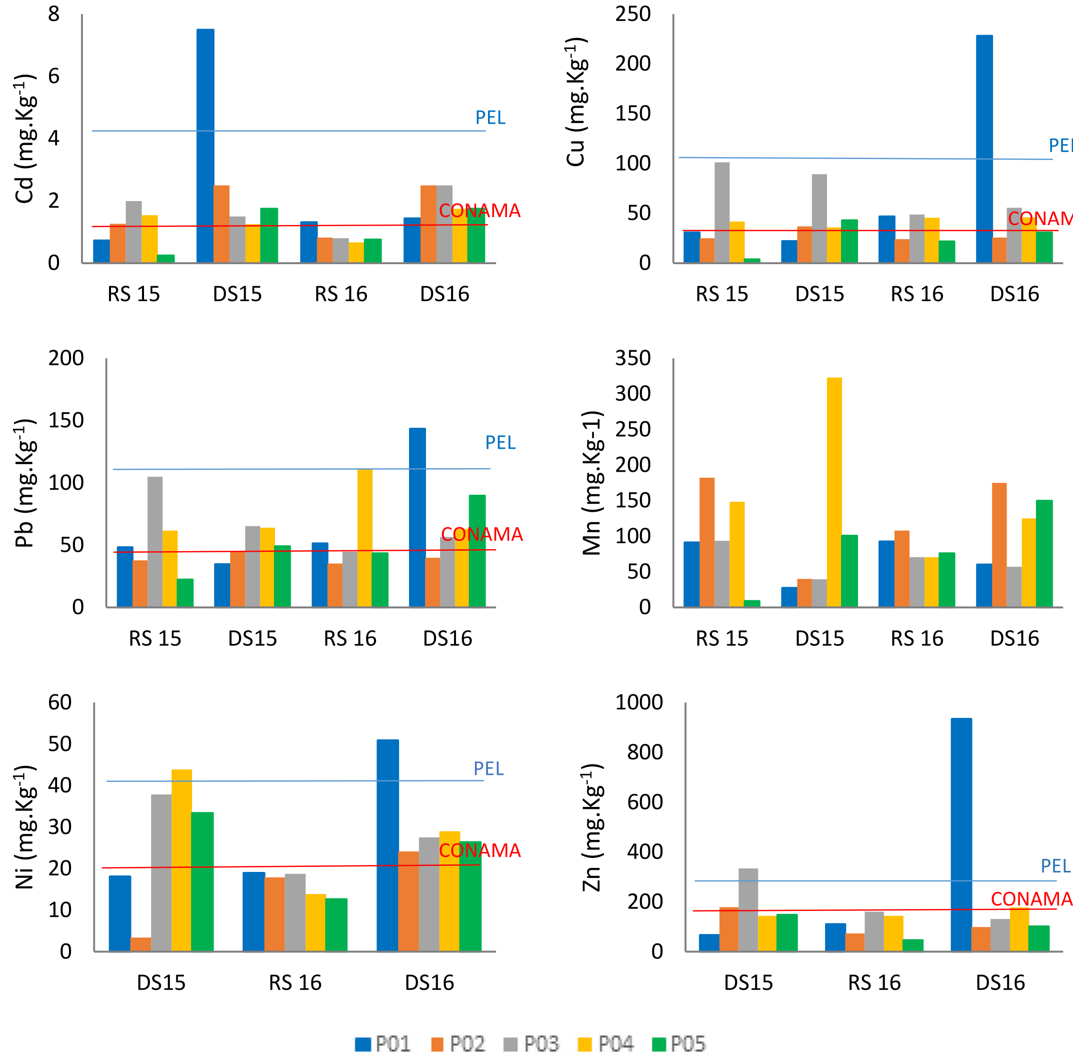


**Fig. 2.** Potengi River metal concentrations (mg kg$^{-1}$) at the five sampling points on the dry (DS) and rainy (RN) campaigns of 2015 and 2016. Metal concentrations allowed by Resolution N° 454/2012 (CONAMA) are marked by the red line and the blue one indicate Probable Effects Level — PEL (CCME).

domestic wastewater, animal waste, combustion products and hydrocarbons. Such metals are predominantly bound to organic matter. The increase in Cu, Pb, and Zn, was also reported in places near ports (Buruaem et al., 2013; Delshab et al., 2017).

Overall, the EF and Igeo data determined on the sampling points revealed to be consistent during the experimental period exhibiting a similar pattern of metal accumulation with more expressive values on P01 (RS16 and DS16) and P03 (DS15 and RS16). These results show a relative enrichment of metals at surface layers of the sediments, reflecting an additional contribution from anthropogenic sources on the outermost part of the estuary (P01). This is influenced by sources of contamination originating on: the port area of Natal (traffic, supply, maintenance and boats' tankage), effluents from shrimp farms (IDEMA, 2009; Souza, 2017) and discharge of raw effluents, and nearness exit from WWTP (P03).

In fact, the highest concentrations of metals occurred in sediments located near potentially, anthropogenic sources, corroborating the results previously obtained in JPE (Silva et al., 2001, 2003). These authors pointed out as the main sources of metals for this estuary the use of fertilizers and pesticides, discharge of untreated domestic and hospital sewage, the use of textiles dyes and discharge of tannery effluents and from shrimp farms. This justifies the need for permanent monitoring the of the Potengi river estuary and for greater control and regulation of waste discharges.

Additional contribution of metals in sediments has also been associated to discharge of domestic effluents (Poté et al., 2008; Unda-Calvo et al., 2019) with high organic load (Silva et al., 2017). Urban wastewaters are collected from homes, commercial establishments, industries, and also, from rainwater runoff from roads, which are probably, another source of metals (Unda-Calvo et al., 2019). Although the metals content of these domestic wastewaters does not represent an immediate risk, it can persist and accumulate on sediments, constituting a long-term problem (Lesage et al., 2007).

Determination of metals' concentrations in sanitary effluent discharged on the fraction of the JPE studied were not the scope of this work and this subject was not found in the researched literature. However, as P01 and P03 are the closest sampling points to discharges of raw (illegal dumping) and treated domestic sewage (evident sources of anthropic pollution), the highest concentrations of metals determined in these sediment samples, suggested that the permanent input of raw sewage and WWTP discharge, actively contribute for enrichment in metals at these points.

Yet, we cannot neglect the additional contributions of wastes from the two industrial parks located on areas adjacent to the JPE.

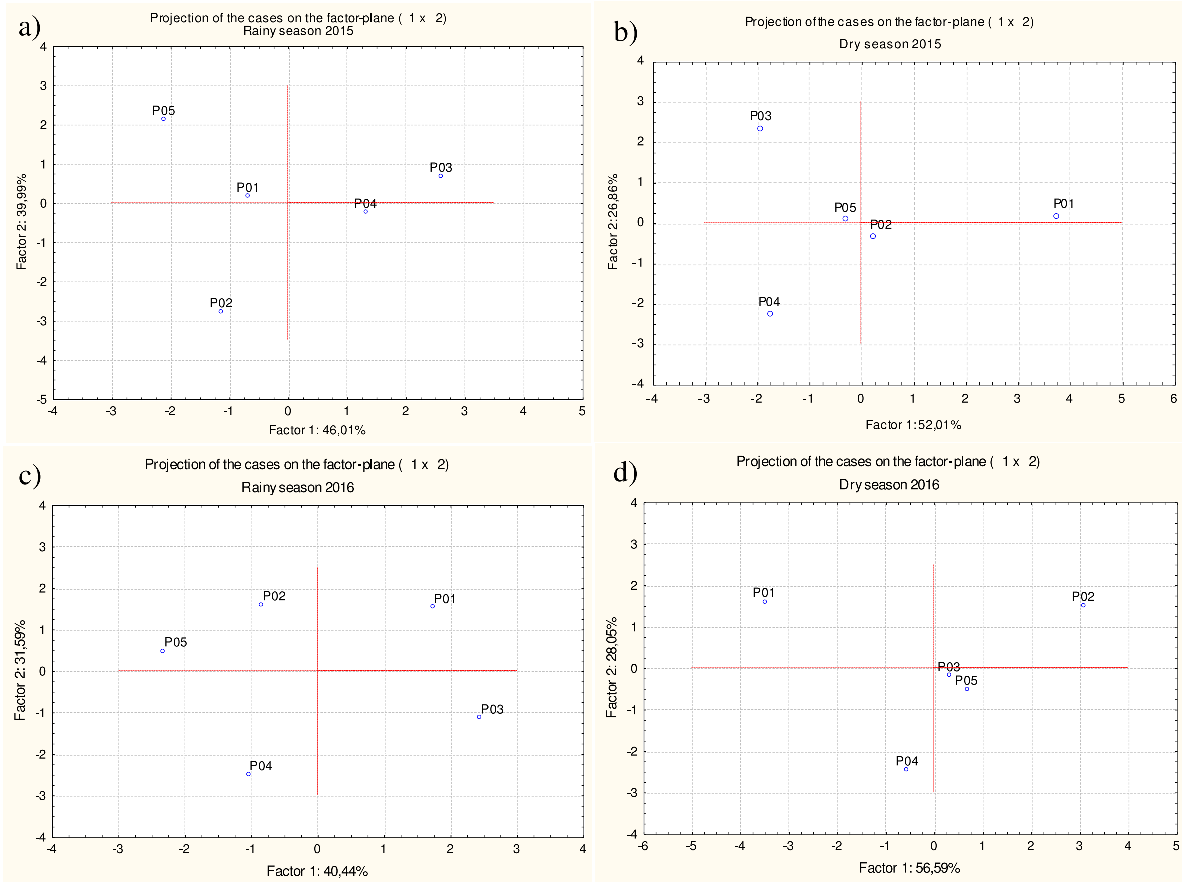


**Fig. 3.** Factor analysis using geochemical and ecotoxicological variables of the Jundiaí–Potengi estuary (JPE) for the rainy and dry season of the years 2015 (a e b) e 2016 (c e d).

The majority of the 26 industries, operate on textile, beverage, dairy, paper and leather industries (IDEMA, 2008a,b; SEHARPE, 2015). These industries discharge their final effluents into water bodies that converge with the JPE's main channel at P03 site (Souza and Silva, 2011). High levels of metals in sediments from this location were also found by Ruiz (2001) and Buruaem et al. (2013).

### 3.3. Sediment toxicity

None of the sediment samples collected caused chronic toxicity in *Nitocra* sp ($p > 0.05$). Unlike our findings, chronic toxicity with inhibition of *Nitocra* sp. fecundity (Buruaem et al., 2013; Souza et al., 2016) embryonic development anomaly of the echinoderm *Lytechinus variegatus* (Souza et al., 2016), and acute toxicity in the amphipods *Tiburonella viscana* (Buruaem et al., 2013) and *Leptocheirus plumulosus* (Lopes, 2012) have been previously recorded in JPE sediments in locations close to those considered in the present study (Table 4).

Despite the non-toxicity of sediments show in this study on tests with *Nitocra* sp., the metal contents were higher than in studies that showed chronic toxicity to *Nitroca* sp., even with lesser OM and mud.

The non-toxicity of the sediment even with the presence of large concentrations of metals can be due to its limited bioavailability, a complex subject, as it is affected by several factors: sediment components, such as concentration of ferrous, manganese and aluminum oxides, organic carbon, acid-volatile sulfide (Ankley et al., 1996; Mountouris et al., 2002); characteristics of water (e.g. pH) and mangrove vegetation type (Marchand, 2011). As reported by Chapman and Wang (2001), enrichment in relation to the background does not necessarily imply toxicity, since the biological effects of the investigated substances are not considered.

Moreover, the metals concentrations found in our samples generally did not exceed the values of Probable Effects Level (PEL), which, when exceeded, often produces adverse biological effects. PEL are defined by Sediment Quality Guidelines (SQG's) of United States — National Oceanic and Atmospheric Administration (NOAA) and Canada - Canadian Council of Ministers of The Environment (CCME, 1995).

Similarly to our data, in the sediments of the port of Santos, Fenili et al. (2011) assessed metal concentrations above those indicated by CONAMA 344/2004, current resolution CONAMA 454/2012 (2012), without toxicity in ecotoxicological tests with *T. viscana* and *L. variegatus*. In the present study we performed the ecotoxicological tests only with *Nitocra* sp. so, we do not know if other test organisms can reveal different degrees of toxicity to JPE sediments. Indeed, biomonitoring bivalve molluscs for human consumption shows alarming values of Zn and Cd contents, in the low estuary region of the JPE (Silva et al., 2001). Also above the limit allowed for human consumption are Cu contents (Senez-Mello et al., 2020). The contaminants concentration accumulated in a biomonitor denote its bioavailability in the organism, at the place and time of exposure (Silva et al., 2006).

### 3.4. Integration of geochemical and ecotoxicological data

The multivariate analysis (FA-PCA) performed with the geochemical and ecotoxicological data (Table 5 and Fig. 2) created

**Table 4**
Geochemical data (min–max) and ecotoxicological tests carried out on sediments of the Potengi River estuary, near the Baldo ETE, from different studies.

| | Lopes (2012) n=4 | Buruaem et al. (2013) n=1 | Souza et al. (2016) n=2 | This study (P03) n=4 |
|---|---|---|---|---|
| Coletas | Jul/11, ago/11, set/11 e out/11 | Jul/2011 | Mai/13 e dez/13 | Jul/15, out/15, jul/16 e out/16 |
| Mud (%) | 4.12–8.70 | 40.00 | 17.90–69.90 | 0.41–9.37 |
| OM (%) | 2.52–10.40 | 6.03 | 3.12–6.39 | 2.70–12.39 |
| Cd | 0.60–0.60 | 0.27 | 0.26–0.49 | 0.80–2.50 |
| Cu | 5.20–5.20 | 46.35 | 0.65–6.63 | 48.9–101.37 |
| Pb | 24.00–28.00 | – | 2.45–4.74 | 44.82–104.86 |
| Mn | 10.60–20.40 | – | – | 39.75–93.68 |
| Ni | 6.00–12.00 | – | 1.29–1.51 | 18.75–37.87 |
| Zn | 34.00–118.00 | 167.74 | 2.21–26.22 | 130.50–334.37 |
| N | – | – | 950–2580 | – |
| P | – | – | 1.00–12.00 | – |
| *Nitocra* sp. Chronic toxicity | – | Toxic | NT/Toxic | NT/NT/NT/NT |
| *Tiburonella viscana* acute toxicity | – | NT | Toxic | – |
| Leptocheirus plumulosus acute toxicity | NT/NT/NT/Toxic | – | Toxic | – |
| L. variegatus Chronic toxicity | – | – | Toxic | – |

– No result.

two main factors that explain most of the total variance of the original data from each sampling campaign. The first two factors explain 85.99% and 78.86% of the total variance, respectively in RS15 and DS15, whereas for RS16 and DS16 the first two factors explained respectively, 72.03% and 84.64% of the total variance.

In RS15, the main factor F1 positively associated the metals Cd, Cu and Pb with OM. The projection on factor-plane 1 x 2 (Fig. 3a) allowed the identification of point P03 as the main responsible for F1 formation. This suggests common sources for the contents of variables (metals and OM): effluents from the WWTP located in the vicinity of P03, domestic and industrial sewage, in addition to urban surface drainage (more intense in rainy events), that contains unequivocally relatively high amounts of organic material and metals in their constitution.

In turn, factor 2 (F2) positively correlated mud with the conservative metal Mn and with inhibition of *Nitocra* sp. fecundity. This axis' formation has the major contribution of P02, although P05 has also some contribution. The positive correlation between these variables (mud, inhibition fecundity and Mn), indicates F2 as a lithogenic component associated with chemical compounds (not measured in this study), capable of causing damage to reproduction, since Mn generally have their natural concentrations relatively high. Therefore, its presence in estuarine sediments should not be of anthropogenic sources (Niencheski et al., 1994).

These findings are consistent with EF and Igeo data, which indicate relatively low anthropogenic contribution in P02 (Table 3). Thus, the factorial analysis may be indicating that sediment toxicity in sites farther from the WWTP launch point (such as P02 and P05) is not directly influenced by the deposition of fine sediments neither by the presence of elements associated with suspended particles, such as Cd, Cu, Pb (trace metals listed for F1). Otherwise, F1 axis showed the closest point to WWTP (P03) as the most impacted by these metals at RS15 (Tables 2 and 4).

In DS15 (Fig. 3b), the predominant factor (F1) positively associated the metals Cu, Pb, Ni and Zn on the same orthogonal axis and showed an inverse relationship with Cd and with the fecundity inhibition of *Nitocra* sp. This suggests that most metals are not in a bioavailable fraction and therefore they are not responsible for the low fertility of copepods, biggest contributor to the formation of the F1 axis. During the DS15 campaign, the outermost part of the JPE seems to have been more susceptible to the influence of contaminants, whose carrying for this portion was very significant and continuous, and the dilution caused by the tidal variations was not sufficient to generate the effective dispersion of contaminants. These can come from multiple sources and are probably: hydrocarbons, estrogens, ammonia, sulfides, among others (we note that further studies need to be conducted).

As well as the data extracted for F2 factor in RS15, for DS15 there was also a positive association between mud and Mn. On the other hand, the negative correlation of these variables with Cd and inhibition of fertility of *Nitocra* sp. seems due to data from points P03 and P04. These samples greatly contribute to the formation of the F2 factor. The relatively high concentrations of Cd (Table 2) above the levels permitted by law (CONAMA, 2012), together with contaminants not measured in the present study, are possibly contributing to a change in the quality of sediments in P03 and P04.

In RS16 (Fig. 3c), F1 showed a positive correlation between Cu, Pb and Zn. Contrariwise, these metals presented a negative correlation with OM and Mn, conservative element indicative of natural accumulation of metals (Niencheski et al., 1994). The presence of these metals at P03, the main contributor to the F1 formation (Fig. 3c) seems to be primarily influenced by the proximity and intensity of anthropic sources (pseudo-persistent concentrations) rather than by the physical–chemical parameters of the sediments (Mud and OM). In turn, F2 showed a strong positive correlation between Cd and (to a lesser extent) Mn, and fecundity inhibition of *Nitocra* sp. The negative association between these variables and OM (Table 4), suggests that the OM input at P04 (main contributor to the formation of the axis) may also have anthropogenic origin, either by the eventual increase in surface runoff, which tends to be more intense in rainy periods, or by the increased volume of domestic effluents released from WWTP or from those discharged *in natura* into channels that converge there.

In DS16 (Fig. 3d), F1 explain a positive correlation between Cu, Pb, Ni and Zn. However, the elements Cd and Mn are negatively correlated with this factor suggesting that in P01 and P02 the trace metals (Cu, Pb, Ni and Zn) have an anthropogenic common source. Finally, F2 is positively associated with the inorganic (fine, *i.e.* mud) and organic fractions of the sediment. This axis is essentially due to P04 data. In this period (DS16) the sedimentological characteristics (fine content and OM) in P04 configure as an inefficient geosorbent in the retention of trace metals.

In general, the multivariate analysis showed that a clear pollution gradient (spatial or seasonal) was not identified along the studied estuarine strip. This suggests that the JPE suffers

**Table 5**
Eigenvalues and eigenvectors obtained by FA-PCA using geochemical and ecotoxicological data from Potengi river sediments (cut-off value > 0.40). Bold values indicate stronger correlations.

| Variable | RS15 | | DS15 | | RS16 | | DS16 | |
|---|---|---|---|---|---|---|---|---|
| | Factor 1 | Factor 2 | Factor 1 | Factor 2 | Factor 1 | Factor 2 | Factor 1 | Factor 2 |
| Mud | −0.17 | **0.89** | 0.08 | **0.97** | −0.15 | 0.04 | −0.12 | **−0.99** |
| OM | **0.46** | −0.10 | −0.28 | 0.07 | **−0.70** | **−0.63** | −0.28 | **−0.95** |
| Cd | **0.88** | 0.14 | **−0.64** | **−0.75** | 0.04 | **0.99** | **−0.77** | −0.36 |
| Cu | **0.98** | −0.15 | **0.93** | −0.01 | **0.92** | 0.24 | **0.94** | 0.13 |
| Pb | **0.95** | −0.22 | **0.88** | 0.31 | **0.70** | −0.30 | **0.95** | 0.17 |
| Mn | 0.25 | **0.72** | 0.15 | **0.40** | **−0.70** | **0.43** | **−0.43** | −0.59 |
| Ni | – | – | **0.71** | −0.11 | 0.06 | 0.52 | **0.96** | 0.17 |
| Zn | – | - | **0.88** | 0.16 | **0.88** | −0.10 | **0.96** | 0.09 |
| Fecundity inhibition | −0.062 | **0.89** | **−0.81** | **−0.55** | −0.21 | **0.96** | −0.18 | 0.35 |
| Eigenvalues | 3.680 | 3.20 | 5.20 | 2.68 | 4.044 | 3.16 | 5.650 | 2.80 |
| Total variance (%) | 46.00 | 39.99 | 52.0 | 26.80 | 40.44 | 31.59 | 56.59 | 28.05 |
| Cumulative variance (%) | 46.00 | 85.99 | 52.01 | 78.86 | 40.44 | 72.03 | 56.59 | 84.64 |

influences from different contaminant sources besides the WWTP effluents, creating a heterogeneous scenario from the geochemical and ecotoxicological point of view. A similar scenario was also verified by Rodrigues et al. (2013) in Guaratuba Bay (southern Brazil), as well as in other places around the world (Du et al., 2012; Araujo et al., 2013; Roig et al., 2015). Watts et al. (2017) suggests that the use of geochemical indices coupled with multivariate statistical methods contributes to a more comprehensive assessment of human-induced environmental risk assisting decisions in coastal management.

## 4. Conclusions and recommendations

Estuarine sediments are degraded by the presence of metals and this situation is caused by a combination of natural and anthropogenic sources. The outermost portion of the Jundiaí–Potengi estuary (P01) and the one most directly influenced by WWTP dumping (P03) are considered, regardless of the season, to be the most deteriorated seeming to suffer most, from the continuous and intense input of materials. At these points, the results of the metals suggest the occurrence of anthropogenic sources (in addition to the lithogenic ones) resulting from domestic sewage and various effluents (sanitary, industrial and shrimp waste).

The worst conditions were observed during the dry season, when the water flow and the local hydrodynamic energy tended to be reduced and increase the adsorption of metals in the geoadsorbent particles of the sediments, causing their accumulation and contamination.

The application of multivariate methods proved to be particularly useful for evaluating and interpreting the data set in an integrated and descriptive manner, in particular, due to the univariate statistical analyses conducted for ecotoxicological tests that did not detect sediment toxicity. Although the toxicity tests have not been positive, the high concentrations of metals cannot be neglected.

In order to complement our findings, we suggest that additional experiments be carried out. Assessing other classes of contaminants and incorporating other lines of evidence, such as the use of biomarkers, bioaccumulation and bioavailability of metals, may allow to obtain broader data to achieve greater inferential capacity answers regarding sources of contamination and their impacts on JPE. Another important study is the quantification of metals directly in the effluent of the WWTP. These will complement the evaluation of the estuarine sediments' quality, in order to collect more and better information to enhance the wastewater treatment systems under construction.

## CRediT authorship contribution statement

**Jaísa Marília dos Santos Mendonça:** Conceptualization, Methodology, Investigation, Data collection and analysis, Writing - original draft, Writing - review & editing, Visualization. **Ivanildo Surini de Souza:** Data collection and analysis, Writing - original draft, Visualization. **Guilherme Fulgêncio de Medeiros:** Conceptualization, Methodology, Investigation, Visualization. **Isabel Maria Cravo Aguiar Pinto Mina:** Conceptualization, Methodology, Investigation, Writing - review & editing, Visualization, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2021.101676. The following is available online at www.mdpi.com/2076-3298/7/6/42/s1, Table S1: Metal enrichment factor (% EF) and geoaccumulation index (Igeo) estimated in sediment simple samples of Potengi river during dry (DS15 and DS16) and rainy (RS16) seasons of 2015 and 2016.

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