Science of the Total Environment 660 (2019) 826–833

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Assessment of the toxicity toward *Vibrio fischeri* in sediments of a mining impacted estuary in the north of Spain

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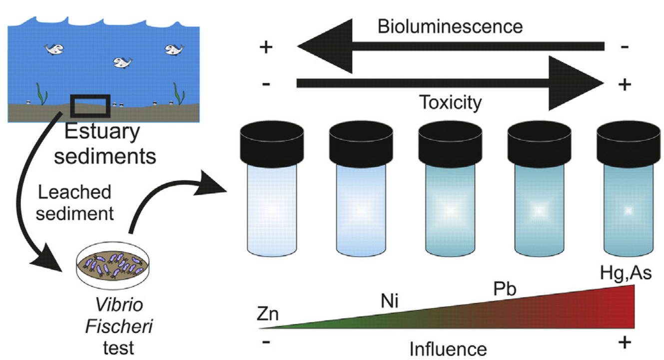
## HIGHLIGHTS

- Estuary sediments showed high levels of As, Hg and Pb as a consequence of mining.
- Bioassay toxicity test by means of *Vibrio fischeri* was accomplished on sediments.
- Bioavailable concentrations of metal (oids) are the main cause of toxicity.
- Sediments present important toxicity, reaching 1470 TU $g^{-1}$.
- Hg and As appear to be the main toxic elements in the sediments.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 4 October 2018
Received in revised form 6 January 2019
Accepted 8 January 2019
Available online 9 January 2019

Editor: Julian Blasco

*Keywords:*
Estuary
Sediments
Metals(oids)
Bioavailability
Toxicity
*Vibrio fischeri*

## ABSTRACT

This study has been carried out on the Nalón estuary, a mining impacted estuarine contaminated by metals(oid), to evaluate how the metals(oids) concentrations in the sediments contributes to the toxicity and, therefore, supposes a potential risk for the biota. For this purpose, a total of 14 surface sediment samples were collected and analysed by different techniques. Estuary sediments showed a maximum high concentration of As (68.10 μg $g^{-1}$), Hg (1.33 μg $g^{-1}$) and Pb (189.60 μg $g^{-1}$), exceeding the NOAA Effects Range Low. Likewise, these three elements were one of the most bioavailable in the sediments according to the toxicity characteristic leaching procedure performed, reaching average values of 14.28% for As, 12.81% for Hg and 9.23% for Pb. The bioavailable concentrations of As and Hg significantly correlated with toxicity ($R > 0.92$), suggesting that both were the main contributors to the toxicity of the sediments. Toxicity values detected (avg. 499 TU $g^{-1}$) were similar to those showed by other sites considered contaminated in the Cantabrian coastline, confirming its status as a contaminated area. The location of the highest toxicity values in the estuary was restricted to the port areas where the fine sediments that act of sink of metals(oids) are mainly deposited. This result is very important if re-mobilization of sediments take place in these areas related to dredging or other human activities.



## 1. Introduction

Among coastal ecosystems, estuaries are considered important productive areas, but very sensitive to human pressure (Covelli et al., 2012). They represent dynamic and complex environments, frequently

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https://doi.org/10.1016/j.scitotenv.2019.01.086

impacted by anthropogenic activities such as fishing, mining, industry, wastewaters, transport and/or recreational activities (Lei et al., 2016). Therefore, these transitional areas are constantly exposed to a wide variety of pollutants, such as inorganic metals and metalloids (Chiesa et al., 2018), organic (Anim et al., 2017) and new contaminants, such as pharmaceuticals (Liu et al., 2013) and micro plastics (Peng et al., 2017).

Within the variety of potential contaminants, metals(oids) have demonstrated to have hazardous effects because of their special characteristics and behaviour in the environment (Förstner and Wittmann, 2012). Sediments of coastal ecosystems can play a key role as sink or source for metals(oids), trapping concentrations many times higher than those in the water column (Li et al., 2017). Contaminated sediments pose an important risk for benthic fauna which bioabsorbed the contaminants initiating biomagnification trough the food chain (Sijm et al., 2007) whose last link are humans. Contaminants enter to the trophic chain by diverse ways, but from sediments, chemical species play a significant role in its bioavailability. However, given the wide range of different chemical forms of elements, it is difficult and comprehensive a proper analysis of the risk of all of them. To solve this fact, toxicity analyses have been adopted as a powerful tool to assess the damage effects on organisms.

In the scientific bibliography, there is a wide variety of toxicity tests depending on the nature of the contaminant and the environment. However, for metals(oids) in coastal environments the application of luminescent bacteria to toxicity assays has shown great interest for its satisfactory result (Rosado et al., 2016). One of the most used bacteria is the *Vibrio fischeri* (*Photobacterium phosphoreum*) due to its adaptation to the saline medium, sensitivity and ecological relevance (Ghirardini et al., 2009; Pérez et al., 2012). Toxicity test performed with this bacterium have shown comparable results to other more complex tests with the advantage of its speed and reproducibility (Serafim et al., 2013). Thus, this test is widely used and accepted to assess the potential risk from sediments to biota (Abbas et al., 2018).

Comparisons between the results of toxicity tests and chemical analysis of the leached used allow to assess possible relationships between the observed toxicity and bioavailable forms of contaminants, discriminating in some cases the contaminants that may have the greatest influence on toxicity. The information reported only by toxicity tests is valuable but, in many cases, limited since they report the effects on biota but without pointing the causes of the same. The approach made in this article combining analysis of toxicity test and leached chemical analysis provides a useful tool for a better understand of the environmental risk analysis from the sediments.

Nalón estuary acts as natural outlet for the most important hydrosystem of the north of Spain. During the last 150 years, the estuary has been subject to continuous contaminated contributions from the basin whose main source has been Hg mining. Because of these contributions, recent sediments have stored important concentrations of metals and metalloids that can be transferred to the food chain, causing an environmental concern in the area (Garcia-Ordiales et al., 2018). Previous studies on the area (Garcia-Ordiales et al., 2015, 2016, 2017) investigated the geochemical association of toxic metals in the estuary sediments, but their potential impact to the biota has not been studied in detail. Thus, the assessment of the potential toxicity derived from the sediments is a priority in the estuary to know the risk for biota. The use of easy, speed and reproducibility toxicity test combined with leachate procedures allows a quick assessment of the potential risk in the environment to select measures or actions aimed at mitigating the impact on the ecosystem. In this context, this work is focused on investigating the toxicity of estuarine sediments by means of bioassay test, with emphasis put in the possible relationship between the observed toxicity and bioavailability of measured metals(oids).

## 2. Materials and methods

### 2.1. Study area and sediment sampling

The estuary of the Nalón River is located in the continental shelf of the Cantabrian coastline in the northern coast of the Iberian Peninsula (Fig. 1). At the basin level, the different rivers of the Nalón hydrosystem flow on Paleozoic materials, covering in their water course materials from the Cambrian to the Upper Carboniferous periods. The western sector of the drainage basin is characterised by the presence of low-grade metamorphic siliciclastic rocks interbedded with calcareous series of grey limestones and dolomites; in the eastern part, the calcareous and siliciclastic series predominate (Julivert et al., 1972). The Nalón river presents an average annual flow of 55 $m^3\ s^{-1}$, 7.3 of pH and Eh levels >100 mV. The estuary is over 6 km long and its upper limit is established according to the salt wedge extension which moves upstream following the tidal range. The main estuarine channel has retained its natural river banks with the exception of the lower section close to the outlet, where two small regional ports (San Juan de la Arena and San Esteban de Pravia) altered the natural morphology and sedimentation dynamics of the estuary (Flor-Blanco et al., 2015). The main channel is >2 m deep and is affected by an annual meso-tidal range between 1.0 and 4.2 m; in general, the tides are over 2 m for >70% of the year (Flor et al., 1998).

Since the beginning of the XIXth century, mine activities in the basin experienced a dramatic increase with the discovery of important mercury and coal deposits. All the mining runoffs were directly poured into the fluvial channel, reaching the contamination to the estuary (Garcia-Ordiales et al., 2017). However, the situation of the estuary improved significantly since the 1970s due to the closure of the mercury mining whose contributions have been identified as the most important sources in terms of contaminants (Garcia-Ordiales et al., 2015, 2017). The estuary still suffers nowadays, the pressure coming from decommissioned tailings and residual deposits (Loredo et al., 2003; Ordóñez et al., 2013).

Fourteen sampling stations were selected, covering the channel and the port areas of the estuary. The position of each station has been represented in Fig. 1. Surface sediments (0–5 cm) were collected by means of a stainless-steel Van Veen grab. Approximately 1 kg of sediment was collected from each sampling point, homogenised in the field, stored in double zip-lock bags, and transported to the laboratory in a portable fridge. Each sample was divided into different representative subsamples for analyses, using a riffle-type sample splitter with a removable hopper.

### 2.2. Physico-chemical characterisation

#### 2.2.1. Granulometry

Dried samples were treated with a solution of 3% (v/v) $H_2O_2$ for 48 h to remove most of the organic matter. The samples were then wet sieved at 2 mm and analysed with a Fritsch ANALYSETTE MicroTec Plus 22. Grain-size data were synthesised using the classical sand-silt-clay notation according to the Udden-Wentworth scale.

#### 2.2.2. pH

Dried sediments will be analysed for pH using standard soil analytical techniques; instruments will be calibrated, and quality assurances practices employed as described within primary reference for the method used (SW846 Method 9045D). After calibration at pH 10 and 4, a check measurement will be made in a buffer solution with a pH of 7. The check measurement must be ±0.1 pH units of 7.0 (6.9–7.1).

#### 2.2.3. Chemical concentrations determination

Metals(oids) concentrations (As, Cr, Cu, Hg, Ni, Pb and Zn) were determined on the <2 mm fraction. Samples were digested in a microwave using a multi-acid solution (HCl-$HNO_3$-HF) and analysed by inductively

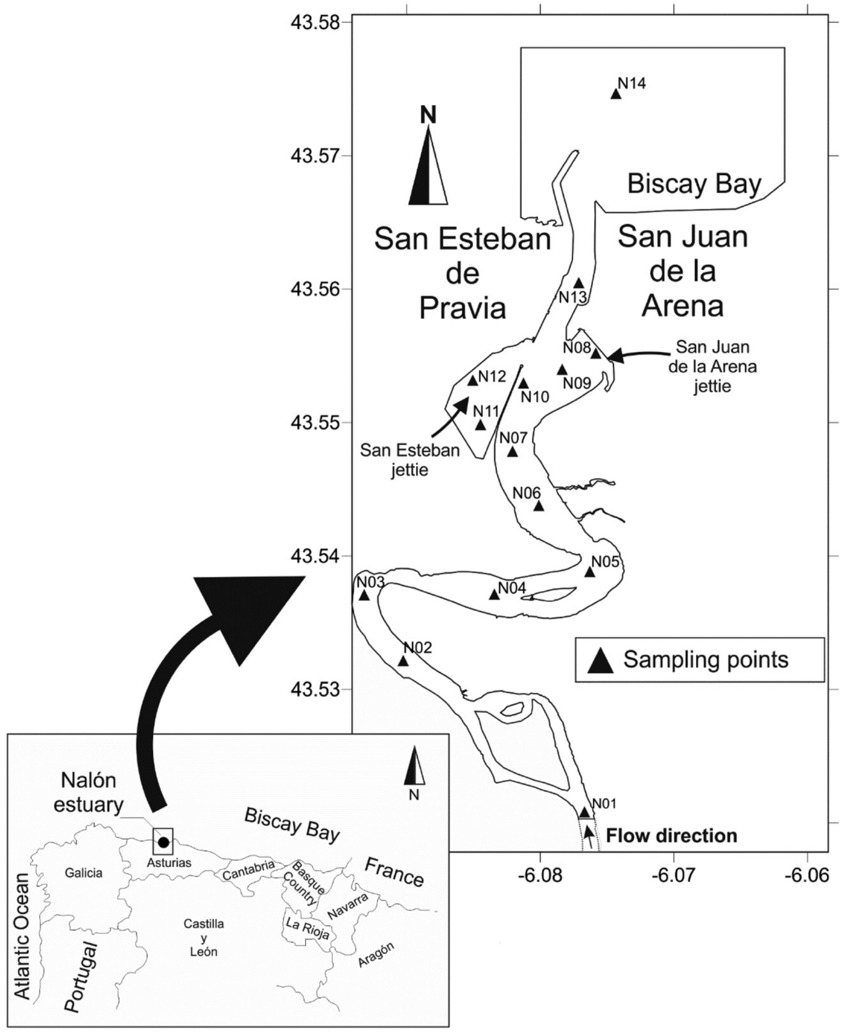


**Fig. 1.** Index map of the Nalón River estuary along with location of the sediment sampling points.

coupled emission spectroscopy (ICP-ES) and inductively coupled plasma mass spectroscopy (ICP-MS; Bureau Veritas, Vancouver, Canada). The accuracy of the results was verified by comparison against analysis of Standard Reference materials PACS-2 (marine sediment), RTCCRM026-050 (sandy loam soil) and CRM042-056 (loam soil). Percentage recoveries for all elements considered ranged from 93% to 106%.

### 2.3. Trace elements bioaccessibility based on Toxicity Characteristic Leaching Procedure (TCLP)

Metals(oids) concentrations potential bioavailable for organism in sediments were studied following the standard Toxicity Characteristic Leaching Procedure (TCLP), according to EPA Method 1311 (USEPA, 1992) because in previous studies in the area, the sediments had shown certain characteristics of potential toxicity (Garcia-Ordiales et al., 2018). In detail, bioavailable concentrations were estimated by mean of an extraction of the aqueous soluble trace elements by using a 1:16 sediment/water ratio, adjusting pH to 4.5 ± 0.1 with acetic acid. After 24 h of extraction, the final volume (V) was adjusted following the Eq. (1):

$$V = 20V_i - 16W – A \qquad (1)$$

where $V_i$ is the volume of deionised water, W is the weight of the sediment used for the extraction and A is the volume of acetic acid used during the extraction. After the extraction step, samples were centrifuged at 3600 rpm during 15 min and the supernatant vacuum filtered by 0.45 μm. Three subsamples from each sediment sample were leached individually and the bioavailable trace elements concentrations (BM) in the extracts were determined by ICP-MS.

### 2.4. Inhibition of light production of *Vibrio fischeri*

To investigate the potential ecotoxicity of sediment samples, a toxicity test using *Vibrio fischeri* was carried out with each TCLP extract previously neutralized to pH > 6. The analyses were run in a Microtox® system. Bacteria were exposed to concentrations of 45, 22.5, 11.25 and 5.625% (v/v) elutriate diluted with Microtox® test medium (Azur Environmental Ltd.). The inhibition of luminescence was measured after 30 min of incubation. Inhibition was initially calculated as the concentration of sediment (mg $L^{-1}$) that caused a 50% reduction of the bacteria bioluminescence ($EC_{50}$).

For the determination of dose-response curve and the $EC_{50}$ value the following Eq. (2) was used:

$$\Gamma = \frac{I_c}{I_s} - 1 \qquad (2)$$

Being Γ the light lost at the end of exposure time for a given sample concentration; $I_c$ and $I_s$ the intensity of bioluminescence of the control and a given sample concentration, respectively. Data of concentrations and their associated Γ values of the two-dilution series are transformed in log form and used for linear regression analysis. $EC_{50}$ value is calculated from the intercept and the test result was accepted when $R^2 > 0.95$.

Inhibition was expressed as toxicity units per g dry soil ($TU_{50}$) follow the recommendations of Araujo et al. (2005) because the use of $TU_{50}$ are a more intuitive comparative scale than $EC_{50}$ values which decrease when toxicity increases (Rosado et al., 2016). The toxicity units are calculated by means of the following Eq. (3) in which the units of $EC_{50}$ (mg $L^{-1}$) have been converted to g $L^{-1}$ for its introduction in the equation:

$$TU_{50} = \frac{1}{EC_{50}} \times 100 \tag{3}$$

### 2.5. Statistical data treatment

Data statistics for all parameters was performed with Minitab 15 statistical software and for the cluster analysis normalized data with similarity as the distance between groups and Ward's linkage was used.

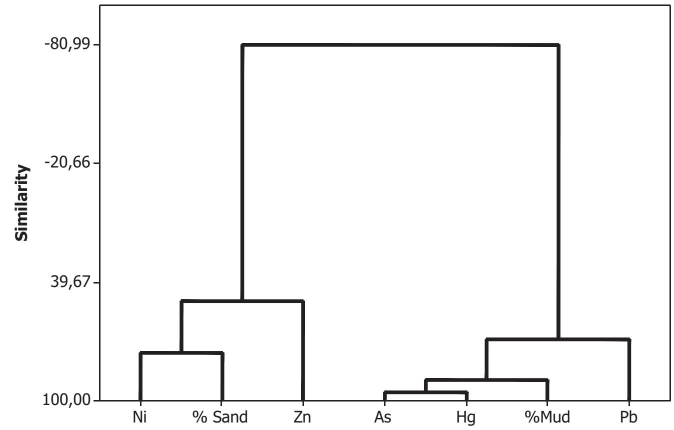


**Fig. 2.** Clustering analysis of the physico-chemical parameters of the Nalón estuary sediments.

## 3. Results and discussion

### 3.1. General characteristics

General results of the sediments are shown in Table 1. Overall, sediments are dominated by sandy component (38.00–71.20%) whose proportion decreases along the canal toward to the sea (samples N06, N07, N10, N13 and N14). Major proportions of finest component were detected in the port areas, especially in the San Esteban port (samples N11 and N12) in which the mud (silt + clay) proportion reached its maximum (56.50%). Considering the single elements, Pb was the most abundant element in the estuary sediment, ranging from 14.10 to 189.60 μg $g^{-1}$ DW, followed by Zn (42.00–109.00 μg $g^{-1}$ DW), As (31.80–68.1 μg $g^{-1}$ DW), Ni (12.05–19.39 μg $g^{-1}$ DW) and Hg (0.29–1.33 μg $g^{-1}$ DW). The highest concentrations of As, Hg and Pb were in the lower part of the estuary, especially in the San Esteban port where the three elemental concentrations reached their maximum (As: 68.10 μg $g^{-1}$ DW, Hg: 1.33 μg $g^{-1}$ DW, Pb: 189.60 μg $g^{-1}$ DW) as consequence of their association to the mud fraction (Fig. 2). Conversely, the highest concentrations of Ni (19.39 μg $g^{-1}$ DW) and Zn (109.00 μg $g^{-1}$ DW) were in the upper-middle part of the estuary where the sandy component with which they associate (Fig. 2), reaches its maximum values.

When concentrations measured in sediments are compared to the marine standards reported in the Screening Quick Reference Tables by the US NOAA, concentrations of Ni and Zn were lower than those corresponding to the Effects Range Low (ERL). Thus, the concentrations of these elements seem to be natural and in consequence, it is expected a low environmental risk. In case of Pb, major part of the sediment samples had concentrations below the ERL but five samples grouped in two different estuary areas exceeded the ERL. This sectorization suggests local enrichment sources of Pb in the sediments. In case of the stations N02 and N03, both are downstream of a sewage discharge point located between the stations N01 and N02, which would be the most reasonable point source of Pb enrichment in this area. On the other hand, in case of stations N11, N12 and N13, their vicinity to San Esteban suggest than port activities would be the cause of Pb anomaly. Finally, for the As and Hg concentrations, all the sediment samples were above the ERL and in case of Hg, four samples surpassed the Effects Range Median (ERM). The presence of high concentration of both elements in the estuary sediments has already been reported by Garcia-Ordiales et al. (2016, 2017, 2018) who linked thought sediment dating these enrichments with the contributions of the historic mercury mining in the region.

### 3.2. Trace elements bioaccessibility

Bioavailable metal(oids) concentrations detected in sediments and percentage of bioavailable concentrations respect to the total metal (oid) concentration (%BM) are shown in the Table 2 and Fig. 3. Overall, As was the most bioavailable element (avg. 14.28%) follow by Zn (avg. 13.11%), Hg (avg. 12.81%), Pb (avg. 9.23%) and Ni (avg. 9.11%).

**Table 1**
Physico-chemical parameters of the sediments in the Nalón estuary. Sediments granulometry (2 mm > sand > 63 μm > mud). Elemental concentrations expressed in μg $g^{-1}$ dry weight (DW).

| | As | Hg | Ni | Pb | Zn | pH | %Sand | %Mud |
|---|---|---|---|---|---|---|---|---|
| N01 | 32.30 | 0.41 | 17.00 | 19.80 | 57.00 | 7.21 | 69.30 | 30.70 |
| N02 | 35.20 | 0.52 | 15.50 | 59.80 | 108.00 | 6.51 | 65.10 | 34.90 |
| N03 | 37.70 | 0.39 | 18.06 | 51.30 | 96.00 | 7.03 | 71.20 | 28.80 |
| N04 | 50.10 | 0.70 | 15.56 | 22.40 | 72.00 | 6.73 | 56.20 | 43.80 |
| N05 | 31.80 | 0.43 | 19.39 | 28.10 | 72.00 | 7.12 | 64.50 | 35.50 |
| N06 | 40.80 | 0.62 | 17.39 | 33.20 | 109.00 | 7.03 | 51.20 | 48.80 |
| N07 | 43.50 | 0.69 | 15.56 | 29.70 | 77.00 | 6.76 | 48.50 | 51.50 |
| N08 | 63.50 | 1.07 | 12.06 | 14.10 | 59.00 | 6.83 | 48.40 | 51.60 |
| N09 | 34.50 | 0.29 | 15.17 | 26.70 | 70.00 | 7.22 | 71.20 | 28.80 |
| N10 | 55.40 | 0.69 | 15.00 | 29.80 | 89.00 | 6.82 | 50.20 | 49.80 |
| N11 | 68.10 | 1.33 | 14.56 | 189.60 | 97.00 | 6.03 | 43.50 | 56.50 |
| N12 | 66.80 | 0.94 | 14.56 | 103.70 | 42.00 | 6.08 | 54.20 | 45.80 |
| N13 | 49.70 | 0.83 | 12.33 | 55.30 | 55.00 | 6.91 | 52.70 | 47.30 |
| N14 | 41.70 | 0.70 | 19.17 | 43.50 | 62.00 | 7.42 | 58.90 | 41.10 |
| ERL | 8.20 | 0.15 | 20.90 | 46.00 | 150.00 | – | – | – |
| ERM | 70.00 | 0.71 | 51.60 | 218.00 | 410.00 | – | – | – |

**Table 2**
Bioavailable metal concentrations for each element in the sediments from the Nalón estuary (μg $g^{-1}$).

| | As | Hg | Ni | Pb | Zn |
|---|---|---|---|---|---|
| N01 | 2.89 | 0.01 | 0.50 | 1.36 | 6.56 |
| N02 | 4.66 | 0.10 | 1.83 | 6.07 | 8.32 |
| N03 | 3.62 | 0.03 | 0.88 | 2.80 | 7.49 |
| N04 | 2.07 | 0.01 | 0.63 | 2.43 | 9.65 |
| N05 | 1.93 | 0.02 | 1.04 | 2.33 | 7.94 |
| N06 | 4.38 | 0.07 | 1.28 | 1.42 | 7.33 |
| N07 | 4.51 | 0.10 | 1.31 | 2.13 | 9.37 |
| N08 | 8.23 | 0.12 | 1.31 | 1.88 | 6.96 |
| N09 | 5.69 | 0.07 | 2.04 | 2.63 | 5.98 |
| N10 | 15.05 | 0.11 | 1.94 | 3.03 | 10.98 |
| N11 | 19.13 | 0.27 | 1.81 | 27.85 | 16.64 |
| N12 | 19.75 | 0.33 | 1.71 | 21.45 | 10.45 |
| N13 | 7.10 | 0.09 | 1.93 | 3.05 | 13.77 |
| N14 | 3.46 | 0.01 | 1.08 | 0.83 | 8.29 |

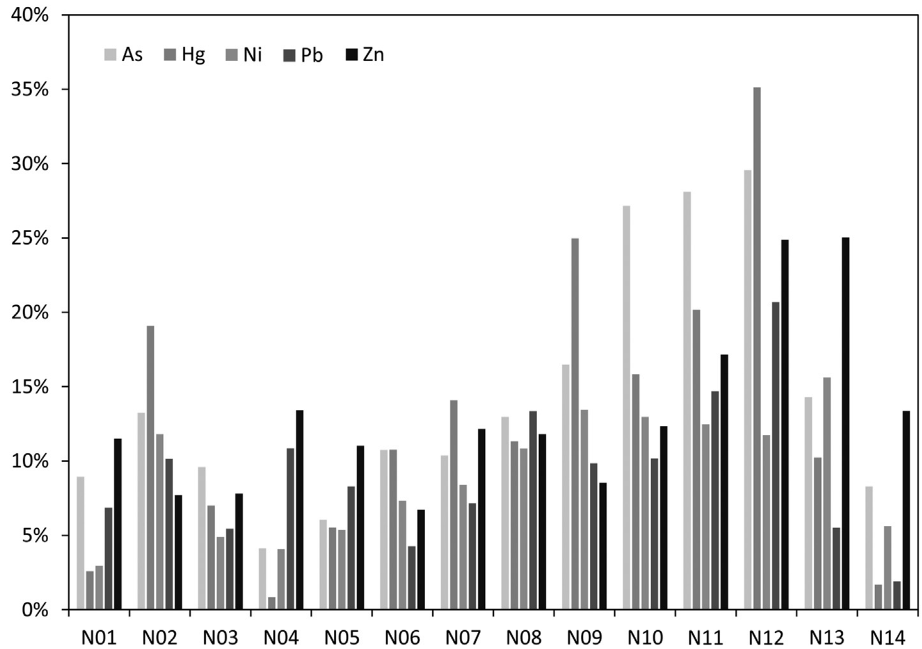


**Fig. 3.** Percentage of bioavailable concentrations respect to the total concentration (%BM) for each element in the sediments from the Nalón estuary (%).

Considering that the %BM is the easiest exchangeable fraction for each element associated with sediments and it may readily enter to the aquatic media, some considerations can be made based on the results obtained. The use of the percentage of bioavailable concentrations (%BM) together with the elements total contents has been widely applied in scientific literature as preliminary tool to assess the potential risk for the aquatic medium (Chen et al., 2010; Garcia-Ordiales et al., 2015). In this context, using the classification developed by Perin et al. (1985) for the %BM risk and considering the total contents respect to the standards previously discussed, in a first term, only As, Hg and Pb seem to exhibit a significant potential ecological risk. In the case of As and Hg, their total average concentrations were up to the ERL and the average %BM exceed the %BM limit of 10% established by Perin et al. (1985) to consider a low transference risk. Thus, both elements exhibit signs of a potential ecological risk. On the other hand, total average concentration of Pb was above the ERL and its average %BM is close to the limit considered. In this case, it cannot be discarded that Pb may exhibit a potential ecological risk.

### 3.3. Toxicity in Nalón estuary in the Cantabrian coastline

The results of the Microtox® test have been represented in Fig. 4. In general, the average toxicity of the sediments respect to the Microtox® test was 499 TU $g^{-1}$ that surpass the toxicity criteria of Environment Canada (2002) set in TU $g^{-1} > 100$, or the adopted toxicity threshold for some Spanish authors, set in TU $g^{-1} > 133$ (Morales-Caselles et al., 2008; Rosado et al., 2016). In the estuary, toxicity of the sediment is increased in the canal to the area close to the ports (from 18 TU $g^{-1}$ at station N01 to 748 TU $g^{-1}$ at station N10) to subsequently decrease along the canal toward to the sea (31 TU $g^{-1}$ at station N14). In the

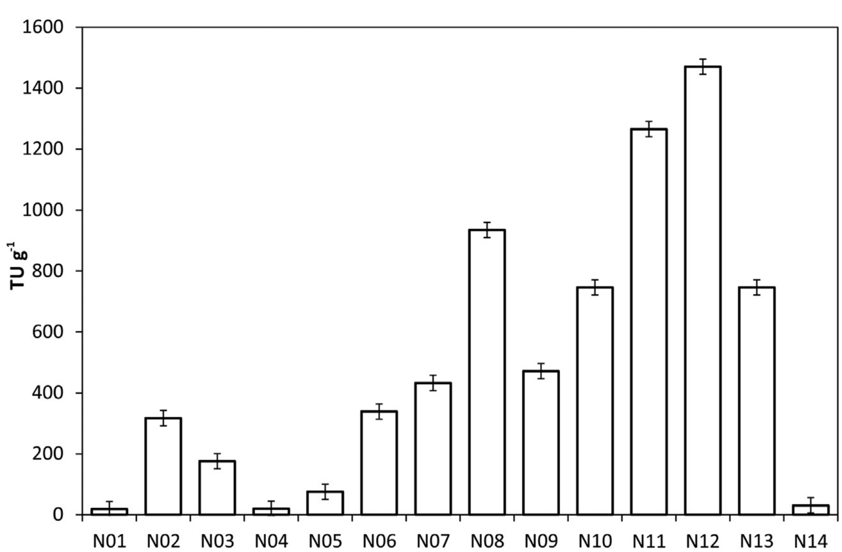


**Fig. 4.** Toxicity in sediments from the Nalón estuary (TU $g^{-1}$) and standard error.

**Table 3**
Toxicity in sediments from the Nalón estuary and other nearby and distant coastal areas. $EC_{50}$ results from the different studies have been transformed in TU $g^{-1}$ using Eq. (3).

| | Toxicity average (TU $g^{-1}$) | Toxicity range (TU $g^{-1}$) | |
|---|---|---|---|
| Nalón estuary (Asturias, Spain) | 499 | 18–1470 | This study |
| Pasajes estuary (Basque Country, Spain) | 186 | 17–495 | Montero et al. (2013) |
| Bilbao estuary (Basque Country, Spain) | 480 | 102–1961 | Montero et al. (2013) |
| Deba estuary (Basque Country, Spain) | 200 | 142–333 | Belzunce et al. (2004) |
| Lekeitio estuary (Basque Country, Spain) | 45 | 33–111 | Belzunce et al. (2004) |
| Bermeo estuary (Basque Country, Spain) | 400 | 286–667 | Belzunce et al. (2004) |
| Bay of Santander (Cantabria, Spain) | 18 | 1–38 | Coz et al. (2008) |
| A Coruña harbour (Galicia, Spain) | 496 | 153–714 | Riba et al. (2004) |
| Corme-Laxe estuary (Galicia, Spain) | 16 | 5–22 | Morales-Caselles et al. (2008) |
| Ons and Cie Islands (Galicia, Spain) | 178 | 60–279 | Morales-Caselles et al. (2007) |
| Vigo estuary (Galicia, Spain) | 187 | 46–625 | Montero et al. (2013) |
| Tinto Estuary (Huelva, Spain) | 5725 | 2500–8700 | Rosado et al. (2016) |
| Odiel Estuary (Huelva, Spain) | 5108 | 2540–8000 | Rosado et al. (2016) |
| Portmán Bay (Murcia, Spain) | – | 28–476 | Mestre et al. (2017) |
| Gulf of Trieste (Italy) | 551 | 415–732 | Cibic et al. (2008) |
| Mar Piccolo (Taranto, Italy) | 2194 | 1305–3693 | Calace et al. (2005) |

port areas (station N09, N11 and N12) toxicity reaches the maximum values, especially noticeable at the station N12 (1470 TU $g^{-1}$) which shows the highest toxicity.

The toxicity values of the Nalón estuary cannot be compared to those found in high contaminated coastal areas around the word (Table 3). These are the cases of Tinto and Odiel estuaries (Spain) where toxicity in sediment reaches 8700 TU $g^{-1}$ (Rosado et al., 2016), Mar Piccolo in Italy where toxicity ranges from 1305 TU $g^{-1}$ to 3693 TU $g^{-1}$ (Calace et al., 2005) or Venice Channels (Italy) where toxicity in sediment reaches 3067 TU $g^{-1}$ (Salizzato et al., 1998). However, toxicity values detected were similar to other areas affected by mining such as Portmán Bay (Spain) where toxicity reaches 476 TU $g^{-1}$, or the Gulf o Trieste (Italy), with a comparable Hg mining contamination as occur in the Nalón estuary, where toxicity ranges from 415 TU $g^{-1}$ to 732 TU $g^{-1}$.

When toxicity found in the Nalón estuary is compared to other nearby uncontaminated estuaries in northern Spain (Table 3), values detected were much higher than those found in these areas. This is the case of the estuaries of Deba and Lekeitio in the Basque Country (Belzunce et al., 2004), the estuaries of Pasajes (Basque Country) and Vigo (Galicia) (Montero et al., 2013), the bay of Santander (Cantabria) (Coz et al., 2008) and the Ons and Cie Islands (Galicia) and Corme-Laxe estuary (Galicia) (Morales-Caselles et al., 2007, 2008). But the toxicity levels found in the Nalón estuary are similar to other areas more affected by contamination. Toxicity values of the Nalón estuary were in the same order as those detected in the Bilbao estuary (Basque Country) where the average toxicity in sediment was 480 TU $g^{-1}$ (Montero et al., 2013), Bermeo estuary (Basque Country) where toxicity ranges from 286 TU $g^{-1}$ to 667 TU $g^{-1}$ (Belzunce et al., 2004) or the A Coruña harbour (Galicia) where toxicity ranges from 153 TU $g^{-1}$ to 714 TU $g^{-1}$ with an average of 496 TU $g^{-1}$ (Riba et al., 2004). This suggests that contributions from non-natural sources are the main causes of the toxicity in the Nalón estuary. In this comparative, it is noticeable the currently uses of the areas for a better understanding of the problem. While in Bilbao, Bermeo and A Coruña areas the industrial and port sources of contamination are still actives, in the case of the Nalón estuary this fact does not occur. Although San Esteban acted as industrial port until 1970, moment that coincides with the mercury mining stopped in the basin, it is not until the year 2000 when an important management plan for reclaimed the basin was accomplished, removing the controlled mining washing plant discharges upstream in the river. Nowadays, the main activity in the estuarine area is tourism, including recreational uses such as fishing and yachting. Thus, the currently significant toxicity detected in the sediments seems to be due to historical legacies rather than to new contributions, showing a significant toxicity of these sources.

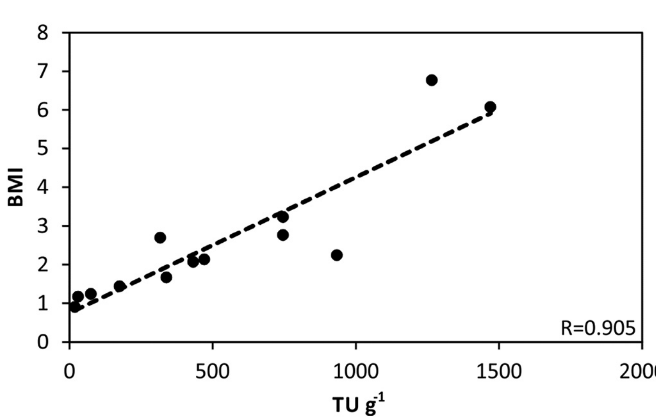


**Fig. 5.** Correlation between toxicity (TU $g^{-1}$) and BMI.

### 3.4. Role of the bioavailable trace elements in the toxicity

As the toxicity detected by the Microtox® test may be due to different organic and inorganic sources, it is necessary distinguish the influence of the BM have had on the toxicity. For this, BM concentrations were integrated in the bioavailable metals(oids) index (BMI; described in detail in Rosado et al., 2016) using the station N01 (18 TU $g^{-1}$) as reference station. Toxicity was correlated to the bioavailable metals(oids) index (Fig. 5), displaying a strong correlation ($R = 0.905$, $p < 0.05$). This result suggests that the concentration metals(odis) have a direct relationship with sediment toxicity in the Nalón estuary, which is in according to the negligible concentrations of organic contaminants measured on recent estuarine sediments by the Asturias Regional Port Authority in the period 2003–2015.

On the other hand, as each bioavailable metals(oids) may have a different weight in the bioavailable metals(oids) index, correlations between each bioavailable metals(oids) and TU $g^{-1}$ were performed to distinguish those of greater influence. As shown the Fig. 6, the greatest influence corresponds to Hg ($R = 0.943$, $p < 0.05$) and As ($R = 0.924$, $p < 0.05$), and to a lesser extent to Pb ($R = 0.766$, $p < 0.05$), Ni ($R = 0.659$, $p < 0.05$) and Zn ($R = 0.589$, $p < 0.05$). These results are in according to the discussion showed in the Section 3.2, supporting that in the estuary, As and Hg from abandoned Hg mining are the main contributors to the toxicity of the sediments and or from an environmental risk point of view, they are the main environmental problematic elements present in the sediments.

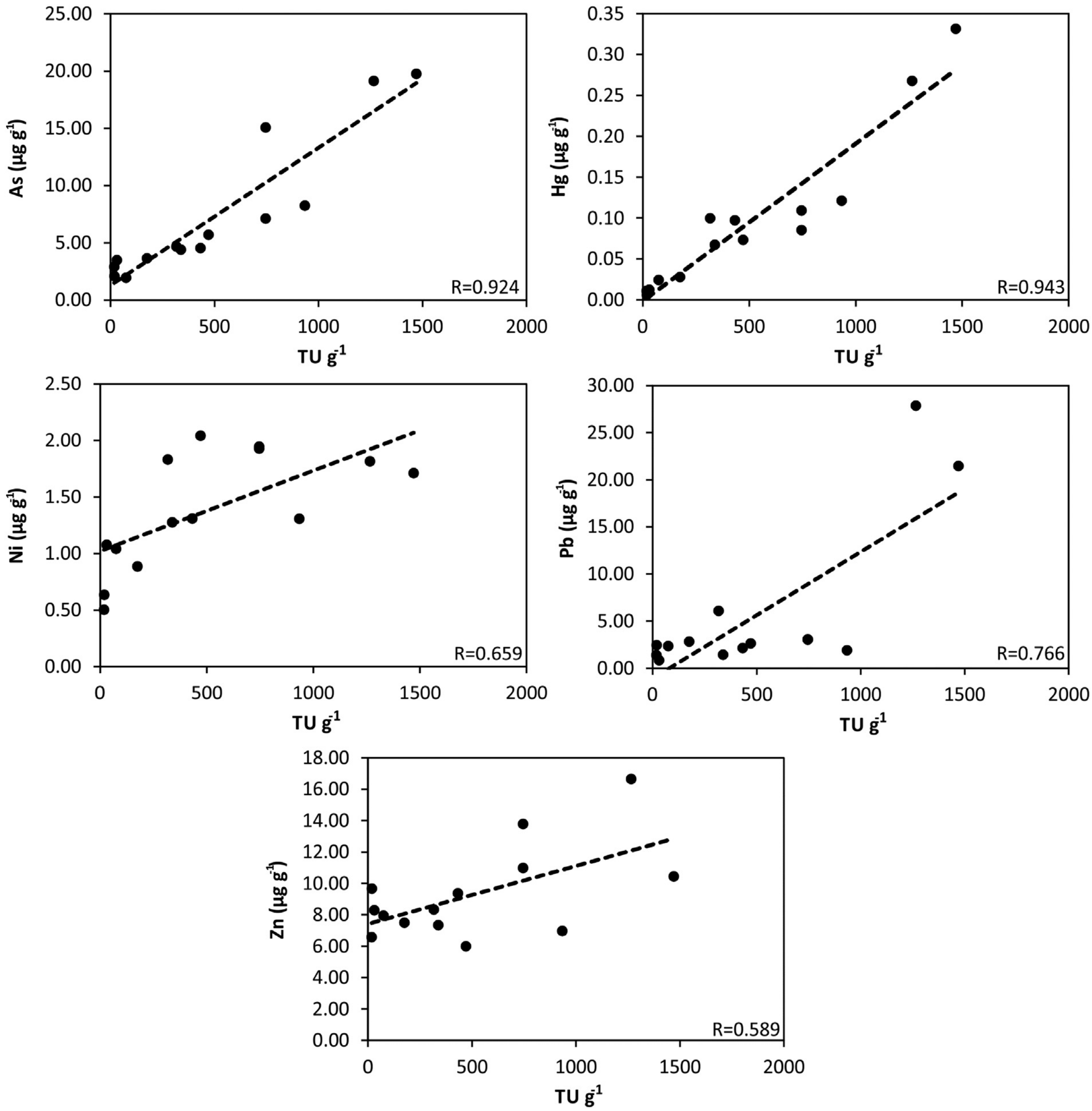


**Fig. 6.** Correlation between toxicity (TU $g^{-1}$) and bioavailable metals extracted.

## 4. Conclusions

MICROTOX tests have been performed on sediment samples from the Nalón estuary and the assessment of their results has shown that toxicity levels of sediments have a potential negative impact in the estuary environment. The average toxicity found exceeds toxicity standards like those suggested by Environment Canada (2002) or by different Spanish authors for uncontaminated areas, being the detected values only comparable with the other coastal areas in the north of Spain considered contaminated. Bioavailable metals(oids) obtained from the TCLP method were almost the only cause for sediment toxicity, and among them, bioavailable concentrations of As and Hg linked to Hg historical mining, contributed significantly to toxicity in sediments. The highest toxicity values are mainly located in the port areas of San Esteban and San Juan de la Arena where the highest metals(oids) concentrations as a consequence of the fine-sized particles predominance occurred. As the toxicity of the sediment is very important in actions that involve the re-mobilization of sediments in port areas such as dredging, future actions on the estuary should incorporate methodologies that consider the environmental impact of toxicity transferences from sediments to water in order to take correction action to prevent the potential risk in bioorganisms of the estuary.

## Acknowledgments

This study was co-supported by the Spanish Ministry of Economy, Industry and Competitiveness through the Research Projects METRAMER [grant number MINECO-13-CGL2013-44980-R] and ECOMER [grant number MINECO-18-CGL2017-84268-R], and by Asturias Ministry of Education and Science [grant number FC-15-GRUPIN14-067].

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