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Pollution by metals and toxicity assessment using *Caenorhabditis elegans* in sediments from the Magdalena River, Colombia☆

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ABSTRACT

The Magdalena River is the most important river in Colombia, supplying over 70% of the population of fish and drinking water, and it also is the main river transportation way of the country. It receives effluents from multiple sources along its course such as contaminant agricultural and industrial discharges. To evaluate the toxicity profile of Magdalena River sediments through endpoints such as survival, locomotion, and growth, wild type strains of *Caenorhabditis elegans* were exposed to aqueous extracts of the sediments. To identify changes in gene expression, GFP transgenic strains were used as reporter genes. Physiological and biochemical data were correlated with metal concentration in the sediments, identifying patterns of toxicity along the course of the river. Levels of some metals such as Cd, Cu, and Ni were above TEC and PEC limits. Effects in survival, growth, and locomotion were observed in most of the samples, and changes in gene expression were evident in the genes *mtl-2, sod-4,* and *gst-1* using fluorescence expression. Cadmium and lead were the metals which were primarily associated with sediment toxicity, and the sampling sites with the highest increased expression of stress response genes were Barrancabermeja and Girardot. However, the diverse nature of toxic profiles observed in *C. elegans* in the study area showed the pervasiveness of different types of discharges throughout the river system.

1. Introduction

The Magdalena River with a length of 1612 km and drainage area of 257,400 km^2 is considered a world-class river and the most important Andean river system in South America in terms of sediment yield (Gottschalk et al., 2015; Restrepo and Kjerfve, 2000), representing the largest contributing source of sediment and freshwater to the Caribbean Sea (Higgins et al., 2015; Restrepo et al., 2014). It discharges 560 t km^{-2} each year, being one with the highest rates of sediment transport in the world, and the highest in South America (Restrepo et al., 2006a). Thus, the impact of sediment pollutants from the Magdalena River in the Caribbean is of special concern, as the disappearance of coral reefs and sea grass in this marine ecosystem has been attributed to sediment input from this waterbody (Moreno et al., 2015; Restrepo and Syvitski, 2006).

The pollution derived from the Magdalena River is a clear consequence of its river basin management. The land-use practices and the deforestation associated with increasing rates of urbanization, expansion of agriculture, pasture, mining activities and illicit crops in Colombia have disrupted the natural hydrographs in this basin, leading to deterioration of critical habitats and biodiversity (Caballero et al., 2015; Restrepo and Syvitski, 2006; Restrepo et al., 2006b; Restrepo, 2008). Seventy-nine percent of the population of Colombia, including its main cities, Bogota, Medellin, Cali, and Barranquilla, are located in the Magdalena watershed, corresponding approximately to 38 million inhabitants (Restrepo et al., 2006b). Most of the country's population depends on it as a source of water and food. Several towns in Colombia lack municipal wastewater treatment plants, affecting ecosystems and biodiversity (Baron et al., 2013; Blackman, 2009) by direct discharge of pollutants into the river, including municipal and industrial sewage, contaminated wastes from mining, petroleum-related activities, and agriculture runoff. The Bogota River, one of its major pollutant contributors has heavy metal concentrations above permitted levels (Mancera and Alvarez, 2006; Olivero-Verbel, 2012). Pollutant discharges from mining and other

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sources have led to metal bioaccumulation in fishes and turtles (Zapata et al., 2014) and decline of fishery production (Granado-Lorencio et al., 2012; Marrugo et al., 2008; Olivero-Verbel et al., 2015). Habitually, the Magdalena River produces over 60% of fish consumed in Colombia. However, from a production of nearly 80,000 ton annually in the 1970s, it dropped by 10% in the last decade (Galvis and Mojica, 2007).

The relationship between river sediment contaminants and toxicity has been reported using different biological models, including HepG2 cells (Pinto et al., 2015), *Danio rerio* (Bluhm et al., 2014), *Daphnia magna* (De Castro-Catala et al., 2015), and the nematode *Caenorhabditis elegans* (Menzel et al., 2009; Turner et al., 2013). The later has been used as a model to assess toxicity of soils (Baderna et al., 2014; Harmon and Wyatt, 2008; Höss et al., 2009; Huguier et al., 2013), water (Ju et al., 2014), wastewater (Wang et al., 2008, 2010), particulate matter (Sun et al., 2015; Zhao et al., 2014), sewage sludge (McLaggan et al., 2012), and river sediments (Höss et al., 2010; Tuikka et al., 2011; Wolfram et al., 2012). The transparency of its body, short life span, ability to self-fertilize and ease of being cultivated are advantages that make it ideal for use as a model in toxicology. Because some of their biochemical pathways are similar to humans, it has been used in research in various fields. Its use as a biological model in environmental assessments toxic allows the determination of several endpoints including survival, growth, reproduction, fertility, locomotion, survival, protein expression, gene expression and DNA damage, among other (Anbalagan et al., 2012; Megalou and Tavernarakis, 2009; Tejeda-Benitez and Olivero-Verbel, 2016). Transgenic nematodes carrying the GFP gene are also used in studies of biochemical pathways, including response to heavy metals (Du and Wang, 2009; Helmcke et al., 2009), cellular and oxidative stress (Bianchi et al., 2015; Salgueiro et al., 2014; Wang et al., 2014a), and xenobiotic detoxification (Anbalagan et al., 2012, 2013).

C. elegans is highly responsive to metals by expressing metallothioneins (MTs) *mtl-1* and *mtl-2*, small cysteine-rich proteins with strong affinity to metals, involved in metal sequestration, transportation, detoxification, and protection against oxidant agents (Höckner et al., 2011; Monteiro and Brinke, 2014; Wang et al., 2010). Another group is heat shock proteins (HSP) that work as molecular chaperones, supporting refolding and restoring of denatured proteins and aiding protein synthesis (Anbalagan et al., 2013; Helmcke and Aschner, 2010).

The nematode arsenal for detoxification includes glutathione peroxidases (GPx), superoxide dismutase (SODs), cytochrome P450 (CYP), and glutathione-S-transferase (GSTs), among others. GPxs belong to the first line of defense against peroxides, superoxide anion and hydrogen peroxide. Those could be Se-dependent or Se-independent (Doyen et al., 2008). SODs protect cells from oxidative damage. *C. elegans* has five genes that code for SODs: *sod-1* to *sod-5*, being Cu/Zn SODs *sod-1* and *sod-4* used in assays with *C. elegans* (Back et al., 2010). CYPs are proteins involved in xenobiotic stress response, being *cyp-29A2*, *cyp-34A9,* and *cyp-35A2*, representative members of this family (Anbalagan et al., 2013). GSTs consists of ubiquitous, dimeric proteins involved in the detoxification of endogenous and xenobiotic compounds by glutathione conjugation, peroxidase activity or passive binding, supporting the conversion of GSH to electrophilic GS^-, which binds to toxic compounds and leads their excretion (Campbell et al., 2001). Among them, *gst-1* and *gst-4* are strongly inducible by organic compounds (Hasegawa et al., 2008).

These families of genes can be altered by exposure to heavy metal; for this reason transgenic strains containing these genes were used to analyze changes in gene expression for exposure to the river sediment extracts.

As an aqueous extract was obtained from sediments to perform the toxicity studies with *C. elegans*, we focused on hydrophilic pollutants that can be found in this matrix, specifically metals. Thus, Co, Ni, Cu, Zn, Cd, Hg, and Pb concentrations were measured in sediment samples from 20 different sites in the Magdalena River, extending from its origin to its mouth. Sediment metal concentrations were compared with the threshold effect concentrations (TEC) and the probable effect concentration (PEC) for freshwater sediments. In addition, several pollution indexes such as contamination factor, pollution load index, and potential ecological risk index were calculated. Aqueous extracts from dried sediments were assayed by measuring physiological and molecular endpoints in *C. elegans*. Those results were then correlated with the pollutant levels in the sediments.

2. Materials and methods

2.1. Sediment sampling

Sampling sites were selected in 20 locations on the Magdalena River, mostly downstream urban centers. These sites represent different altitude, climate, and regional economic activities along the river. At each site, a composite sample was prepared from four sub-samples taken 25 m away from a reference point in the direction of each cardinal point using a grab sampler approximately 50 m off the shore in sites shown in Fig. 1. These were put in polyethylene bags, transported to the laboratory at 4 °C, freeze dried, crushed, sieved to <63 μm (United Nations Environment Programme, 2006), and stored at −20 °C. A description of each sampling sites appears in Table 1.

2.2. Metals analysis

Seven metals were analyzed: Co, Ni, Cu, Zn, Cd, Hg, and Pb. Labile trace metal concentrations of the sediments were measured by high resolution inductively coupled plasma—mass spectrometer (HR ICP-MS) following aqua regia digestion of freeze dried sediment samples, using established procedures (Odigie and Flegal, 2014; Soto-Jimenez et al., 2006). Total mercury concentrations were determined as previously published (Olivero-Verbel et al., 2015).

2.3. Pollution index calculations

Sediment metal concentrations were compared with the threshold effect concentrations (TEC) and the probable effect concentration (PEC) for freshwater sediments (MacDonald et al., 2000). The TEC and PEC values are listed in Table SM-1 in Supplementary Material. In addition, we calculated several pollution indexes:

2.3.1. Contamination factor (f_i) and pollution load index (PLI)

The level of metal contamination was determined by its f_i, calculated as the quotient between C_i, the concentration of each metal in the sediments, and C_b, the reference value of the metal in the Earth crust (Table SM-2) (Lide, 2008). The PLI was obtained to determine the number of times that metal concentrations in the sediments exceeded their background (Hakanson, 1980). PLI was derived from the equation:

$$\mathrm{PLI} = \sqrt[n]{\prod \mathrm{f_i}}$$

where n is the number of metals (Harikumar et al., 2009).

2.3.2. Potential ecological risk index

The potential ecological risk index (RI) was calculated to further

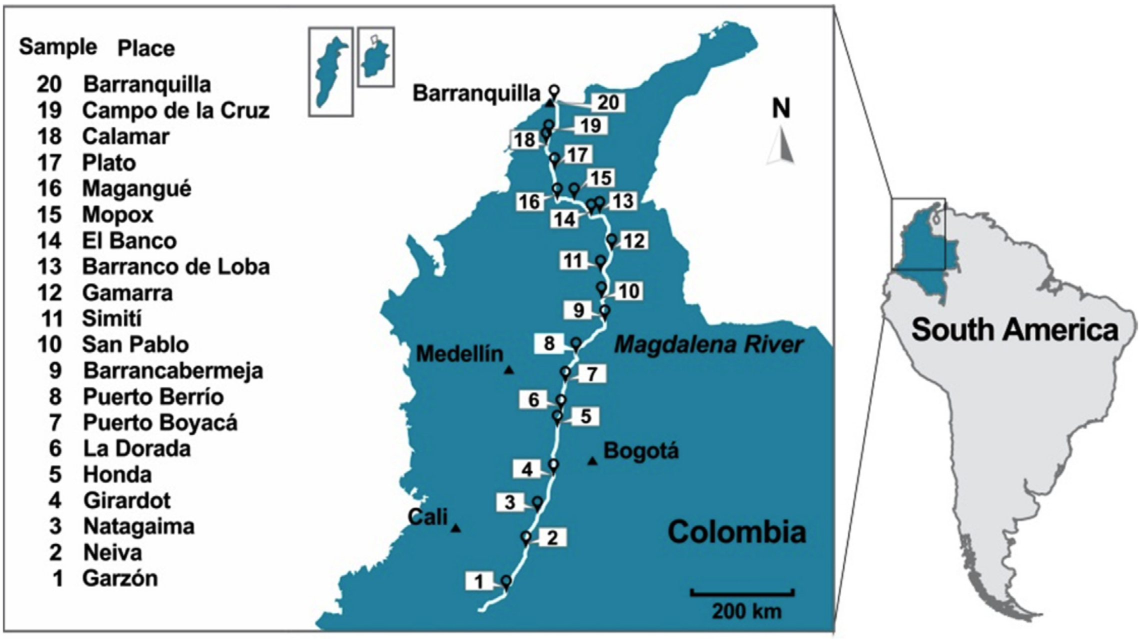


Fig. 1. Sampling sites in the Magdalena River.

Table 1
Description of sampling sites.

Site	Coordinates for sampling location		Anthropogenic activity
Garzon	N 2°11′57″	W 75°38′59″	Coffee crops
Neiva	N 2°59′55″	W 75°18′16″	Oil and agricultural activity, fishing, major city of high population and lack of municipal wastewater treatment plant.
Natagaima	N 3°37′23″	W 75°05′37″	Agricultural and livestock activity.
Girardot	N 4°18′04″	W 74°48′10″	Tourism, fishing, river transportation, contributions of contaminated water from the Bogota River and road traffic.
Honda	N 5°11′06″	W 74°44′08″	Small industry, tourism, fishing, livestock, river transportation, and road traffic.
La Dorada	N 5°27′20″	W 74°40′20″	Livestock, agriculture, fishing, gold mining, river transportation, small industry, fishing, and road traffic.
Puerto Boyaca	N 5°58′09″	W 74°35′06″	Livestock, agriculture, fishing, and oil transportation.
Puerto Berrio	N 6°29′06″	W 74°24′30″	Agriculture, livestock, gold mining, wood industry, fishing, river transportation, and oil activity.
Barrancabermeja	N 7°4′10″	W 73°52′09″	The second largest oil refinery in Colombia, and river transportation.
San Pablo	N 7°29′05″	W 73°56′10″	Agriculture, livestock, gold mining, fishing, river transportation, and oil activity. Contribution of steelmaker-related contaminated water from the Sogamoso River.
Simiti	N 7°58′05″	W 73°57′04″	Livestock, agriculture, fishing, gold mining, river transportation, and oil activity.
Gamarra	N 8°20′10″	W 73°45′20″	Livestock, agriculture, fishing, sand, gravel and clay mining, and river transportation. Contribution of contaminated water from gold and coal mining areas.
Barranco de Loba	N 8°57′07″	W 74°7′06″	Livestock, agriculture, fishing, gold mining, wood industry, and river transportation.
El Banco	N 9°0′13″	W 73°58′10″	Livestock, agriculture, fishing, and river transportation. Contribution of coal and gold mining-related contaminated water from the Cesar River.
Mompox	N 9°14′22″	W 74°25′30″	Livestock, agriculture, fishing, tourism, goldsmithing, ceramics, pottery, woodwork, and river transportation.
Magangue	N 9°14′03″	W 74°44′09″	Small industry, fishing, livestock, agriculture, and river transportation. Contribution of nickel mining-related contaminated water from the San Jorge River.
Plato	N 9°47′06″	W 74°47′07″	Fishing, livestock, agriculture, and road traffic.
Calamar	N 10° 15′ 07″	W 74° 55′ 10″	Fishing, livestock, agriculture, and river transportation.
Campo de la Cruz	N 10° 23′ 10″	W 74° 53′ 30″	Fishing, livestock, agriculture, and road traffic.
Barranquilla	N 10° 59′ 16″	W 74° 47′ 20″	The mouth of the Magdalena River. Diverse industrial activity as the production of vegetable fats and oils, pharmaceuticals, chemicals, footwear, dairy products, food, drinks, soaps, building materials, furniture, plastics, cement, metalworking, clothing and boats. Port activity, major city of high population.

assess the level of metal pollution in the sediments, based on the toxicity of heavy metals and the response of the environment (Chandrasekaran et al., 2015). The equation used was:

$$\mathrm{RI} = \sum \mathrm{E_i} = \sum \mathrm{T_i f_i} = \sum \mathrm{T_i}\frac{\mathrm{C_i}}{\mathrm{C_b}}$$

where E_i is the coefficient of the potential ecological hazard of each heavy metal; T_i is the toxicity coefficient of each heavy metal (Chandrasekaran et al., 2015) (Table SM-3). The classification of sediments according to the values of f_i (Chandrasekaran et al., 2015), PLI (Priju and Narayana, 2006), E_i (Jiao et al., 2015), and RI (Song et al., 2015) appears in Table SM-4.

2.4. Aqueous extracts

Approximately 30 g of dried sediment were mixed with 30 mL of ultrapure water (UW) and left overnight at 4 °C (Anbalagan et al., 2012, 2013; Power and De Pomerai, 1999). The wet sediment was then divided among four 20 mL syringe cylinders plugged with a stainless steel mesh disc and glass wool (Hasegawa et al., 2008). The cylinders were placed in 50 mL plastic tubes and centrifuged at 4,500 g for ten min. Primary extracts from each sample were mixed together and re-centrifuged at 10,000 g to eliminate any remaining sediment. The resultant liquid extracts were stored at −20 °C.

2.5. Strains

The wild type strain N2 was used for survival, growth, and locomotion assay. Transgenic strains *hsp-6::GFP, hsp-16.2::GFP, hsp-70::GFP, sod-1::GFP, sod-4::GFP, gpx-6::GFP, mtl-2::GFP, mtl-1::GFP, gst-1::GFP; cyp-34A9::GFP* strains were used to identify changes in gene expression, GFP transgenic.

2.6. Toxicity assessment

Worms were subjected to age-synchronization via bleach solution (NaOH 0.5 M; HClO 0.8%). Strains were kept at 20 °C in Petri dishes with K agar, prepared with KCl (0.03 M), NaCl (0.05 M), agar (17 g L^{-1}), peptone (2.5 g L^{-1}), cholesterol (25.8 μM), $CaCl_2$ (1 mM) and $MgSO_4$ (1 mM). Larvae were seeded with *Escherichia coli* OP50 as suggested in the literature (Williams and Dusenbery, 1990). Experiments were carried out using whole extract and a 1:1 solution in UW in order to analyze dose dependence.

2.6.1. Survival

Nematodes in larval age L4 were exposed for 24 h to whole extract and a 1:1 solution (53 mM NaCl, 32 mM KCl). 10 ± 1 nematodes for treatment were used. Four replicates for treatment were made and each experiment was done three (Williams and Dusenbery, 1990; Helmcke and Aschner, 2010; Shen et al., 2009).

2.6.2. Growth

The body length of nematodes was measured in larval age L1 after 48 h of exposure to whole extract and a 1:1 solution. *E. coli* OP50 was inoculated as source of food. Body length was measured by using a dissecting microscope Nikon smz 745T and the software ImageJ. About 30 nematodes were examined per treatment. Each treatment was carried out for three times (Höss et al., 2009; Shen et al., 2009; Roh et al., 2010).

2.6.3. Locomotion

After 24 h of exposure to whole extract and 1:1 dilution, every examined nematode was transferred to a plate with K agar and scored for the number of body bends in 20 s. A body bend was counted as a change in the direction of the posterior bulb of the pharynx along the Y axis, assuming that the nematode was traveling along the X axis. About 30 nematodes were examined per treatment. The average number of movements was obtained for treatment and each experiment was done three times (Roh et al., 2010).

2.7. Quantification of GFP reporters on C. elegans

The GFP transgenic strains were grown on NGM agar plates and washed off using ice-cold K-medium. Equal aliquots of worms on all stages were placed into black non-fluorescent U-bottomed 96-well microplates with the samples and UW as control. Plates were incubated at 20 °C, and readings were done after 4 and 24 h. Fluorescence was quantified by using a Perkin–Elmer Victor 1420 multilevel plate reader using 485/525 nm as excitation/emission wavelengths, respectively (Anbalagan et al., 2012, 2013; Power and De Pomerai, 1999).

2.8. Statistical analysis

Data are presented as mean ± standard error. Normality and variance homogeneity were verified using the Kolmogorov–Smirnov and the Bartlett tests, respectively. Significant differences between two groups were performed with T-test. To test if the means were equal for more than two groups, ANOVA was employed. When normality was not achieved, non-parametric tests were conducted. After ANOVA, the Dunnett test was applied to compare each sample with the control. Multivariate methods were utilized to evaluate relationships between variables (metal concentrations and toxicological responses) and sampling stations. Spearman's rank correlations were used to assess the association between presence of metals and toxicity endpoints, as the data did not follow a normal distribution. Principal components analysis (PCA) was carried out to detect possible meaningful patterns and relationships between metals. Single linkage cluster analysis was done using the constrained Ward's method to identify interrelationships between the sites depending upon metal sediment content. The linkage distance was reported as D_{link}/D_{max}, a ratio between the linkage distance for a specific case (D_{link}) divided by the maximum distance (D_{max}) multiplied by 100, standardizing the D_{link}. For all statistical purposes, the criterion of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Metals

3.1.1. Metals analysis

The concentrations of Co, Ni, Cu, Zn, Cd, Hg, and Pb from Magdalena River sediments are listed in Table 2. Mean heavy metal concentrations (μg/g) were: Zn (81.3) > Cu (35.6) > Ni (17.0) > Pb (12.1) > Co (6.83) > Cd (1.35) > Hg (0.04). Relative concentrations of the metals differed by location. The highest Cd concentrations were found in San Pablo and Gamarra; those of Co were in Neiva and Campo de la Cruz; those of Cu were in Puerto Boyaca; those of Hg were in Barranquilla; those of Ni were in Gamarra and Campo de la Cruz; those of Pb were in Neiva and Gamarra; and those of Zn were in Neiva and San Pablo. As that listing indicates, sediments from Gamarra had the highest levels of Cd, Pb, and Zn. In this area, the Magdalena River receives contaminants from gold and coal mining and oil transportation sources.

Table 2
Metal concentration in sediments (μg/g).

	Co	Ni	Cu	Zn	Cd	Hg	Pb
Garzon	5.97	9.02	11.7	54.6	0.27	0.01	15.9
Neiva	[a]**13.2**	22.0	64.4	118	0.34	0.05	**18.1**
Natagaima	7.16	10.5	14.6	48.3	0.22	0.02	10.2
Girardot	5.13	8.94	9.28	43.7	0.29	0.01	6.28
Honda	4.43	8.89	10.5	36.0	0.11	0.01	4.26
La Dorada	5.56	9.79	11.7	43.2	0.26	0.03	5.42
Puerto Boyaca	6.35	11.8	**211**	94.2	0.17	0.04	12.1
Puerto Berrio	5.44	12.8	10.4	71.0	1.01	0.01	7.83
Barrancabermeja	5.09	19.1	8.50	99.4	2.23	0.01	14.6
San Pablo	6.92	21.7	19.5	108	**3.33**	0.01	16.1
Simiti	5.63	16.3	10.3	90.0	1.89	0.03	10.5
Gamarra	7.03	23.9	18.26	**132**	**3.95**	0.06	**18.0**
Barranco de Loba	4.73	12.6	11.9	80.2	1.25	0.02	11.6
El Banco	6.22	19.0	13.2	106	2.18	0.06	11.5
Mompox	6.25	19.2	13.6	103	2.39	0.02	14.2
Magangue	7.29	19.2	15.0	61.7	0.88	0.04	10.6
Plato	7.30	17.8	16.3	71.5	0.57	0.04	12.9
Calamar	7.31	21.4	16.3	79.2	1.44	0.05	11.3
Campo de la cruz	**10.2**	**28.8**	30.7	88.0	1.46	0.07	13.1
Barranquilla	9.48	**26.8**	26.9	99.0	2.02	**0.12**	16.8
Minimum value	4.43	8.89	8.50	36.0	0.11	0.01	4.26
Maximum value	13.2	28.8	211	132	3.95	0.12	18.1
Average	6.83	17.0	27.2	81.3	1.31	0.04	12.1

[a] Values higher than the average plus 1.5 standard deviations are in bold faced.

3.1.2. Pollution index of sediments according heavy metal concentration

Metal levels in sediments were compared to TEC and PEC values as it is displayed in Fig. 2. Cadmium concentrations were above their TEC in Puerto Berrio, Barrancabermeja, San Pablo, Simiti, Gamarra, Barranco de Loba, El Banco, Mompox, Calamar, Campo de la Cruz, and Barranquilla are above TEC; and also above their PEC in San Pablo and Gamarra, indicating that harmful effects are likely to occur. Copper concentrations were above their TEC in Neiva, Girardot, Puerto Boyaca, San Pablo, Calamar, Campo de la Cruz, and Barranquilla; and also above their PEC in Puerto Boyaca. Nickel concentrations were above their TEC in Gamarra, Campo de la Cruz, and Barranquilla; and also above their PEC in Campo de la Cruz and Barranquilla. Zinc concentrations were above both their TEC and PEC in Gamarra. In contrast, Pb and Hg levels were below their TEC and PEC at all sites, indicating their toxic effects on riverine biota may be minimal. In summary, Gamarra had the most metals exceeding their TEC (Cd, Ni, Zn) and PEC (Cd, Zn); and several sites (Baranquilla, Campo de la Cruz, Gamarra, Puerto Boyaca, and San Pablo) had sediments with at least one metal (Cd, Cu, Ni, and/or Zn) above its PEC, indicating the probability of adverse biological effects to living organisms in those areas (Mac Donald et al., 2000).

3.1.3. Contamination factor

The f_i values for Magdalena River sediments are presented in Table 3. The values of Cd indicate a “very high pollution” at eleven sampling sites (Puerto Berrio, Barrancabermeja, San Pablo, Simiti, Gamarra, Barranco de Loba, El Banco, Mompox, Calamar, Campo de la Cruz, and Barranquilla); “considerable pollution” at Magangue and Plato, and “moderate pollution” at six sites (Garzon, Neiva, Natagaima, Girardot, La Dorada, and Puerto Boyaca). Similarly, Cu pollution was “strong” at Puerto Boyaca and “moderate” in Neiva; Hg pollution was “moderate” at Barranquilla; Pb pollution was “moderate” at seven of the sites (Garzon, Neiva, Barrancabermeja, San Pablo, Gamarra, Mompox, and Barranquilla); and Zn pollution was “moderate” at 14 of those sites. In contrast, the f_i values of Co and Ni indicated they were not enriched at any of the sampling sites. According to the PLI values depicted in Table 3, riverine sediments in both Gamarra and Barranquilla are polluted with metals.

3.1.4. Potential ecological risk index

Values of E_i and *RI* for sediments from Magdalena River are listed in Table 3. As expected, data suggest the sites with concentrated industrial activities had higher *RI* compared to the less developed areas. Cadmium was found to be the primary toxicological risk factor in ten sites located from Barrancabermeja to Barranquilla. This region is impacted by several anthropogenic activities associated with elevated Cd emissions, including gold mining and oil refining. Elsewhere (Campo de la Cruz and Barranquilla), Hg was determined to be moderately polluting. The toxicity profile of the sediments is illustrated in Fig. 3.

3.1.5. Principal components analysis

The rotated loading matrix and the rotated component biplot are showed in Figure SM-2 and Table SM-5. Two PCs are sufficient to cover 74% variance for seven metals. PCs were set according to their shares of variance. The first PC explains 52% of the variance in the sediment data, and it shows high loadings for Cd, Hg, Ni, Pb, and Zn. Based on the information provided on Table 1, this component may be associated to gold mining extraction, industrial activity, and oil refining. The second PC, with 22% of the variance, is loaded by Co, Cu, and Hg, most likely related to particulate matter from roads. The presence of Cu, Zn, Co, Cd and Pb has been associated to traffic-related pollutants (De Kok et al., 2006; Hinwood et al., 2014). In addition, Co, Cu, Ni, Cd, Pb, and Zn are linked to diesel combustion in highways (Wang et al., 2003). It should be emphasized that Hg is moderately shared by both PCs, suggesting that its likely source may be diverse in nature.

3.2. Biological responses

3.2.1. Survival

Reduced survival of the sediment extracts to the L4 larvae after 24 h-exposure was observed in most samples (Fig. 4a). Reduced survival greater than 20% occurred in seven sites: Neiva, Girardot, Puerto Boyaca, Barrancabermeja, Gamarra, and Barranquilla. In Neiva and Barranquilla, which are major cities, reduced survival was 31.3% and 30%, respectively. Puerto Boyaca and Barrancabermeja, located in oil industrial areas, had reduced survival of 21.2% and 24.3%, respectively. The reduced survival in Gamarra, located in a gold mining area, was 23.9%; and that in Girardot, near the industrial area of Bogota, was 23.5%. Significant differences ($p < 0.05$, T test) between extract metal concentrations used were observed in 12 of 20 samples, showing a possible dose-dependency.

3.2.2. Growth

The mean body length (μm) is shown in Fig. 4b, and the inhibition caused for each sample on growth is presented in Fig. 5. Among 20 sites, 17 had significant ($p < 0.05$, Dunnet test) differences with the control when whole extract was used, and six had significant ($p < 0.05$, Dunnet test) differences with 1:1 dilution. In addition, four sites, Puerto Boyaca, Gamarra, Barranco de Loba, and Campo de la Cruz, presented statistical differences between extract concentrations ($p < 0.05$, T test), showing a weak dose-dependency.

Sediment extracts from five sites, Garzon, Neiva, Simiti, El Banco, and Barranquilla, had the highest growth inhibition (34–38%). The Magdalena River is impacted by agricultural effluents, especially coffee farms, in Garzon; municipal and industrial discharges wastewaters from capital cities in Neiva and Barranquilla; and gold mining activities near Simiti and El Banco (Olivero-Verbel et al., 1995, 2004, 2008). Some studies have reported growth inhibition on *C. elegans* exposed to soil and river sediments. For instance,

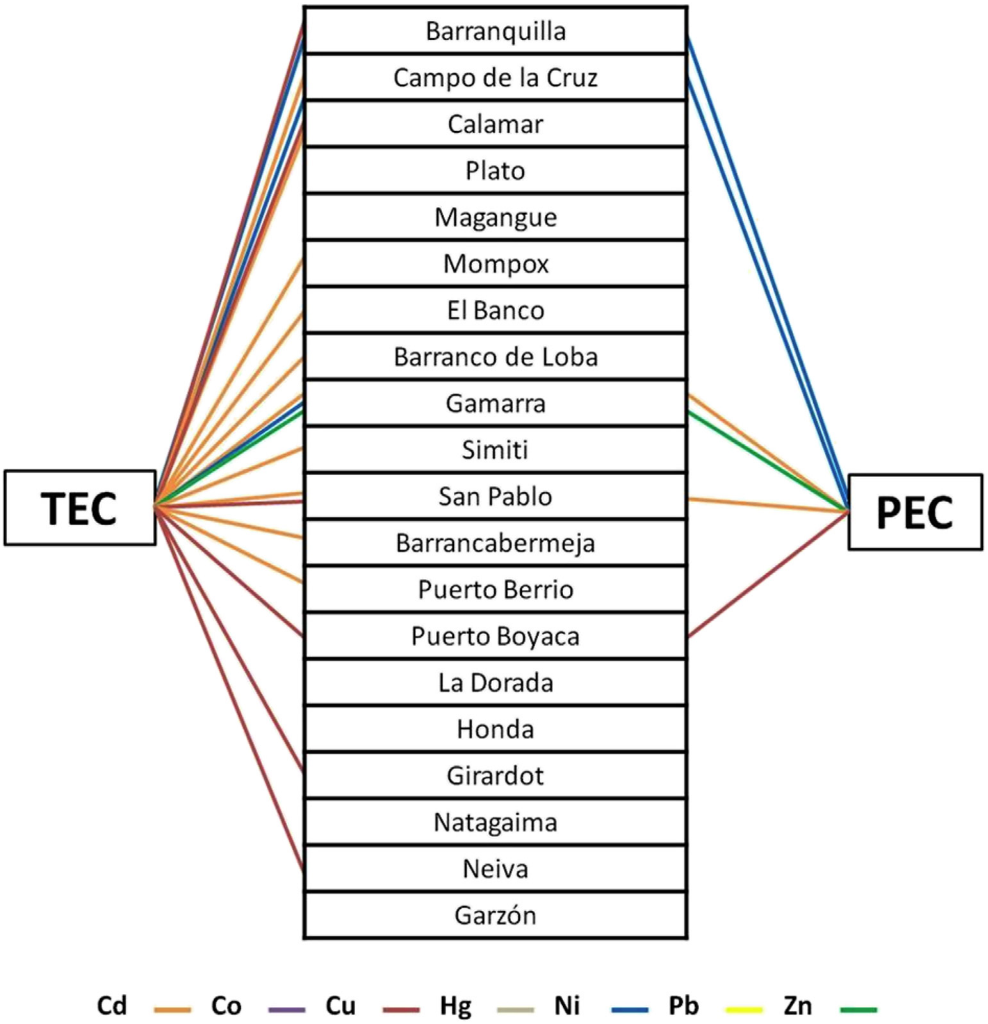


Fig. 2. Sediments above the reference values of metals.

C. elegans exposed to freshwater sediments with low-level anthropogenic contamination suffered a 25% growth inhibition (Höss et al., 2010). Higher growth inhibition was reported on *C. elegans* exposed to sediment extracts of three German rivers for 72 h. They found inhibition of 47 and 93% (Tuikka et al., 2011). *C. elegans* exposed to 22 different contaminated soils for 72 h had a growth inhibition between 10 and 60% (Höss et al., 2009).

3.2.3. Locomotion

Larvae movement behavior after exposure to extracts from different sites are listed in Fig. 4c, and the inhibition caused for each sample on locomotion is presented in Figure SM-3. Effects on BBF were observed in all sites. With the exception of the 1:1 dilution of sample from Garzon, all samples showed significant differences ($p < 0.05$, Dunnet test) with the control. However, there were not significant differences between whole extract and 1:1 dilution. Sediments from Neiva, Puerto Boyaca, Gamarra, Barranco de Loba, Calamar, Campo de la Cruz, and Barranquilla, exerted the greatest inhibition (55–61%).

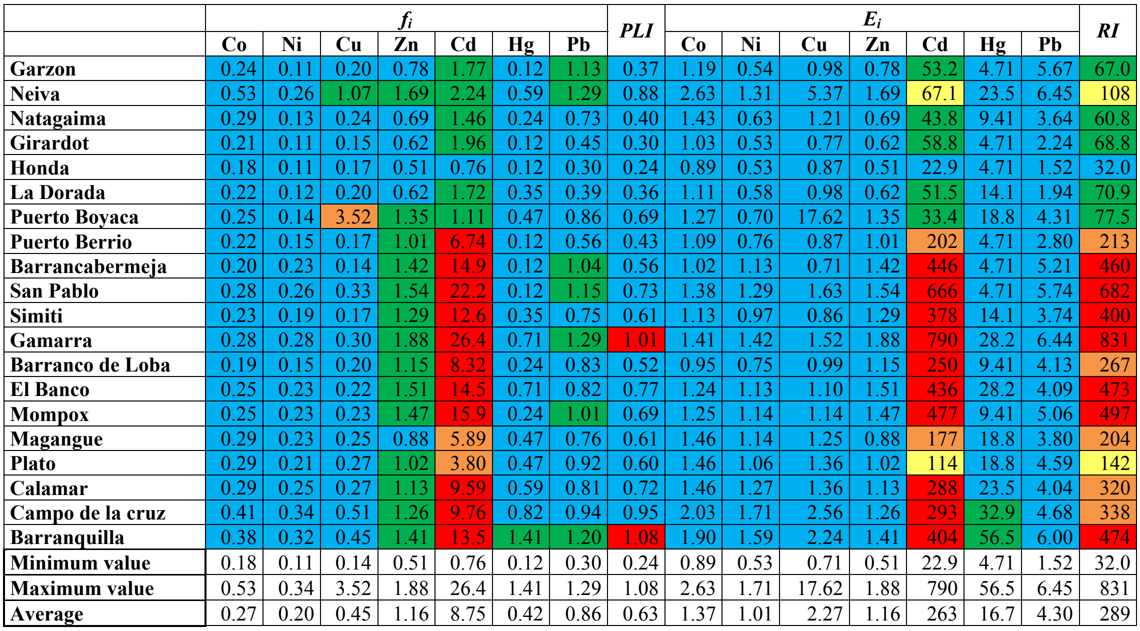
3.2.4. Effect of concentration extract in biological parameters

The increase in survival between the exposure to the 1:1 solution and the whole extract was of 28% in average. In contrast, the decreases of growth and locomotion were 11% and 13% in average, respectively. This likely occurs because the individuals, which survive to exposure, adapt themselves by creating tolerance to changes in terms of development and behavior (Table 4).

3.2.5. Quantification of GFP reporters

The gene expression profile obtained after exposure to sediment extracts are presented in Figures SM4 to SM-7. Four of these genes presented the greater expression (Fig. 5): *mtl-2*, *sod-4*, *cyp3-A9* and *gst-1.* The most sensitive gene was *mtl-2,* which had the highest ratio between fluorescence of the sample and the control. This strain showed a two-fold increase in fluorescence level compared to the control, with sediments from four sites, Neiva, Barranco de Loba, El Banco, and Barranquilla; and a three-fold increase in sediments from Girardot and Barrancabermeja. The binding of metals to MTs promotes the expression of *mtl-2* gene, which is related to metal content in these sites, in particular Cd, Pb, and Zn. The expression of MTs is induced when nematodes are exposed to excess metal ions, thereby preventing metal accumulation and further cytosolic damage (Polak et al., 2014); thus, MTs detoxify and protect cells against oxidant-producing properties of some metals (Höckner et al., 2011). In fact, in a soil from a Pb/Zn mine, the expression of *mtl-2* was two-fold the control (Anbalagan et al., 2012). MTs can also be overexpressed as a result of exposure to several organic pollutants, including pesticides (Lim et al., 2015;

Table 3
Pollution index according to heavy metals for Magdalena River sediments.



	f_i							*PLI*	E_i							*RI*
	Co	**Ni**	**Cu**	**Zn**	**Cd**	**Hg**	**Pb**		**Co**	**Ni**	**Cu**	**Zn**	**Cd**	**Hg**	**Pb**	
Garzon	0.24	0.11	0.20	0.78	1.77	0.12	1.13	0.37	1.19	0.54	0.98	0.78	53.2	4.71	5.67	67.0
Neiva	0.53	0.26	1.07	1.69	2.24	0.59	1.29	0.88	2.63	1.31	5.37	1.69	67.1	23.5	6.45	108
Natagaima	0.29	0.13	0.24	0.69	1.46	0.24	0.73	0.40	1.43	0.63	1.21	0.69	43.8	9.41	3.64	60.8
Girardot	0.21	0.11	0.15	0.62	1.96	0.12	0.45	0.30	1.03	0.53	0.77	0.62	58.8	4.71	2.24	68.8
Honda	0.18	0.11	0.17	0.51	0.76	0.12	0.30	0.24	0.89	0.53	0.87	0.51	22.9	4.71	1.52	32.0
La Dorada	0.22	0.12	0.20	0.62	1.72	0.35	0.39	0.36	1.11	0.58	0.98	0.62	51.5	14.1	1.94	70.9
Puerto Boyaca	0.25	0.14	3.52	1.35	1.11	0.47	0.86	0.69	1.27	0.70	17.62	1.35	33.4	18.8	4.31	77.5
Puerto Berrio	0.22	0.15	0.17	1.01	6.74	0.12	0.56	0.43	1.09	0.76	0.87	1.01	202	4.71	2.80	213
Barrancabermeja	0.20	0.23	0.14	1.42	14.9	0.12	1.04	0.56	1.02	1.13	0.71	1.42	446	4.71	5.21	460
San Pablo	0.28	0.26	0.33	1.54	22.2	0.12	1.15	0.73	1.38	1.29	1.63	1.54	666	4.71	5.74	682
Simiti	0.23	0.19	0.17	1.29	12.6	0.35	0.75	0.61	1.13	0.97	0.86	1.29	378	14.1	3.74	400
Gamarra	0.28	0.28	0.30	1.88	26.4	0.71	1.29	1.01	1.41	1.42	1.52	1.88	790	28.2	6.44	831
Barranco de Loba	0.19	0.15	0.20	1.15	8.32	0.24	0.83	0.52	0.95	0.75	0.99	1.15	250	9.41	4.13	267
El Banco	0.25	0.23	0.22	1.51	14.5	0.71	0.82	0.77	1.24	1.13	1.10	1.51	436	28.2	4.09	473
Mompox	0.25	0.23	0.23	1.47	15.9	0.24	1.01	0.69	1.25	1.14	1.14	1.47	477	9.41	5.06	497
Magangue	0.29	0.23	0.25	0.88	5.89	0.47	0.76	0.61	1.46	1.14	1.25	0.88	177	18.8	3.80	204
Plato	0.29	0.21	0.27	1.02	3.80	0.47	0.92	0.60	1.46	1.06	1.36	1.02	114	18.8	4.59	142
Calamar	0.29	0.25	0.27	1.13	9.59	0.59	0.81	0.72	1.46	1.27	1.36	1.13	288	23.5	4.04	320
Campo de la cruz	0.41	0.34	0.51	1.26	9.76	0.82	0.94	0.95	2.03	1.71	2.56	1.26	293	32.9	4.68	338
Barranquilla	0.38	0.32	0.45	1.41	13.5	1.41	1.20	1.08	1.90	1.59	2.24	1.41	404	56.5	6.00	474
Minimum value	0.18	0.11	0.14	0.51	0.76	0.12	0.30	0.24	0.89	0.53	0.71	0.51	22.9	4.71	1.52	32.0
Maximum value	0.53	0.34	3.52	1.88	26.4	1.41	1.29	1.08	2.63	1.71	17.62	1.88	790	56.5	6.45	831
Average	0.27	0.20	0.45	1.16	8.75	0.42	0.86	0.63	1.37	1.01	2.27	1.16	263	16.7	4.30	289

For f_i: blue = no pollution; green = moderate pollution; orange = considerable pollution; red= very high pollution (Hakanson, 1980). For *PLI*: blue = no pollution; red = pollution (Chandrasekaran et al., 2015). For E_i and *RI*: blue = no pollution; green = moderate pollution; yellow= strong pollution; orange = very strong pollution; red = extremely strong pollution (Jiao et al., 2015).

Erdoğan et al., 2011) and pharmaceuticals (Quinn et al., 2011), among others.

The strain containing *sod-4* as a reporter gene also had a high (between two and three-fold the control) sensitivity to extracts of samples from five sites (Neiva, Girardot, Puerto Berrio, Barrancabermeja, and Barranquilla), possibly related to the presence of compounds that generate ROS, activating the expression of antioxidant genes such as SOD-4. This strain was also employed to evaluate the toxicity of a soil extract from an abandoned Pb/Zn mine in Spain, showing a four-fold increase in the expression of *sod-4*, compared to the control (Anbalagan et al., 2012). The Cu/Zn superoxide dismutase, *sod-4*, may also react to other xenobiotics, including PM2.5 (Sun et al., 2015).

The expression of metabolism-related genes, *gst-1* and *cyp-34A9*, was also noteworthy, as it has been reported that pesticides and other xenobiotics (Roh and Choi, 2011; Sun et al., 2016) can induce them. Moreover, agricultural soils can overexpress between three and four-fold the control mRNA for *gst-1* and two-fold for *cyp-34A9* (Anbalagan et al., 2013). This is in agreement with studies on Elbe and Rhine River sediments using DNA microarrays, which showed induction of genes related to the CYPs and GSTs families (Menzel et al., 2009).

Exposure to sediments from Barrancabermeja activated *mtl-2, sod-4, cyp-34A9, gst-1, mtl-1, hsp-70,* and *gpx-6* expression, between two and 3.5-fold greater than that of the control. Sediments from Girardot increased the expression of *mtl-2, gst-1, sod-4, cyp-34A9,* and *mtl-1* in two to 2.6-fold; those from Barranquilla increased the expression of *sod-4, mtl-2,* and *gst-1,* two to 2.5-fold; and those from Neiva increased the expression of *mtl-2* and *sod-4* two-fold. These data suggest that examined aqueous extracts contain several types of pollutants that activate different signaling stress responses on *C. elegans*. Further studies should characterize which chemicals are involved in this process.

Given the induction of genes related to oxidative stress, metabolism and xenobiotic responses by sediment extracts, it is appropriated to assume that other types of pollutants different from metals, such as PAHs, pesticides and chemicals present in wastewaters are present in some samples, and may exert toxicity through changes in the expression of related genes.

3.3. Multivariate analysis

3.3.1. Correlation between chemical and biological variables

The Spearman correlation matrix for studied variables is presented in Table SM-6. Based on the survival measurements, sediments were the most toxic at six sites (Neiva, Girardot, Puerto Boyaca, Barrancabermeja, Gamarra, and Barranquilla). Survival was strongly correlated with Pb ($R = 0.726$; $p < 0.01$), Zn ($R = 0.638$; $p < 0.01$), and Ni ($R = 0.509$; $p = 0.02$) concentrations of sediments at those locations. Several authors have reported the relationship between metals and *C. elegans* survival. Particles from coal combustion containing Pb, Zn, and Ni augmented survival in a dose-dependency manner (Sun et al., 2015). Metallothionein production induced by metals, measured as *mtl-2*, correlated with survival ($R = 0.712$; $p < 0.01$). Most likely, survival could also be affected by xenobiotic metabolism or maybe by glutathion depletion, as suggested by its association with *gst-1* ($R = 0.521$; $p = 0.02$) expression.

Locomotion was inversely correlated with Co ($R = -0.651$; $p < 0.01$), Ni ($R = -0.731$; $p < 0.01$), Pb ($R = -0.607$; $p < 0.01$), Zn ($R = -0.583$; $p = 0.01$), and Hg ($R = -0.755$; $p < 0.01$) as also it has been reported. For instance, after exposure to coal combustion related $PM_{2.5}$ containing metals, nematodes decreased their BBF (Sun et al., 2015). In another study, nematodes exposed to Cd, Cu, Pb, and Zn at environmentally relevant concentrations, experimented concentration-dependent locomotion inhibition (Yu et al., 2013). In general, locomotion has been observed as a rapid method of assessing acute toxicity from heavy metal exposure in *C. elegans*. The inhibition caused for each sample on growth and

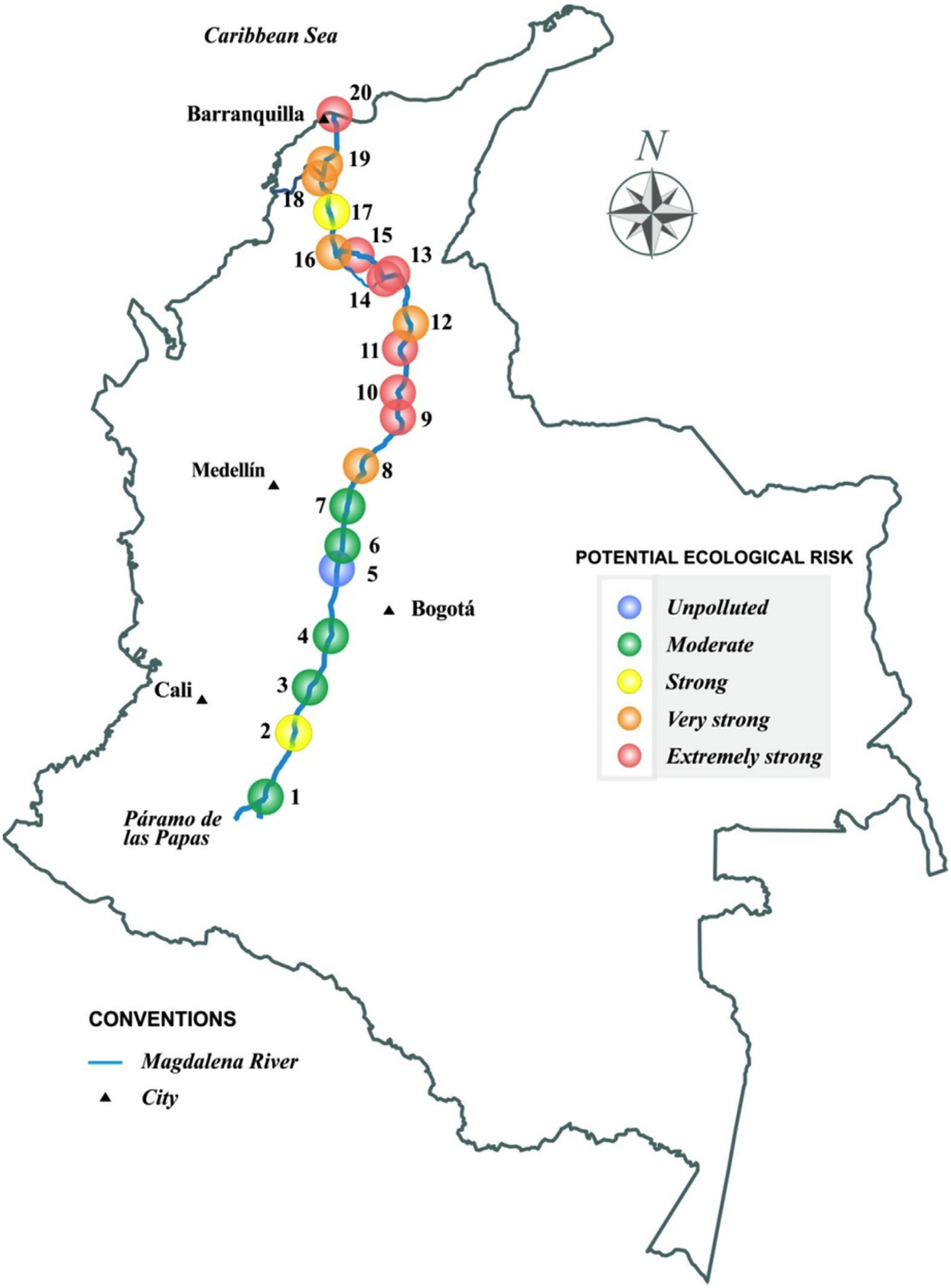


Fig. 3. Potential ecological risk index for Magdalena River sediments.

locomotion is presented in SI. BBF were also inversely correlated with the expression of *mtl-2* ($R = -0.661$; $p < 0.01$) and *gst-1* ($R = -0.516$; $p = 0.02$).

Changes in the expression of stress response-related gene were strongly associated with metals content. For instance, *mtl-2* was corresponded with the concentration of Cd ($R = 0.638$; $p < 0.01$), Ni ($R = 0.600$; $p < 0.01$), Pb ($R = 0.621$; $p < 0.01$), and Zn ($R = 0.688$; $p < 0.01$). This gene was also interconnected with survival ($R = 0.712$; $p < 0.01$) and locomotion ($R = 0.661$; $p < 0.01$). The expression of *sod-4* was slightly associated with Cd ($R = 0.600$; $p < 0.01$) and Zn ($R = 0.524$; $p = 0.01$). And, the expression of *hsp-6* was correlated to Cd ($R = 0.585$; $p = 0.01$), Ni ($R = 0.523$; $p = 0.01$), and Zn ($R = 0.521$; $p < 0.02$).

Cadmium concentrations had the highest ($R = 0.658$; $p < 0.01$; $R = 0.638$; $p < 0.01$; $R = 0.600$; $p < 0.01$; $R = 0.585$; $p = 0.02$; $R = 0.530$; $p = 0.01$; $R = 0.697$; $p < 0.01$) association with the expression of stress response related gene *mtl-1*, *mtl-2*, *sod-4*, *hsp-6*, *hsp-16.2*, and *hsp-70*, respectively.

It, therefore, could be concluded that in general, toxicity at both molecular and cellular levels, is associated with elevated levels of some metals, especially Cd, Ni, Zn, and Pb. The relatively high levels of those metals are likely the result of anthropogenic activities carried out in the river basin, including gold mining (Olivero-Verbel et al., 1995, 2004, 2008, 2015), petrochemical, and fluvial transportation, agriculture, and municipal and industrial wastewater across the Magdalena River.

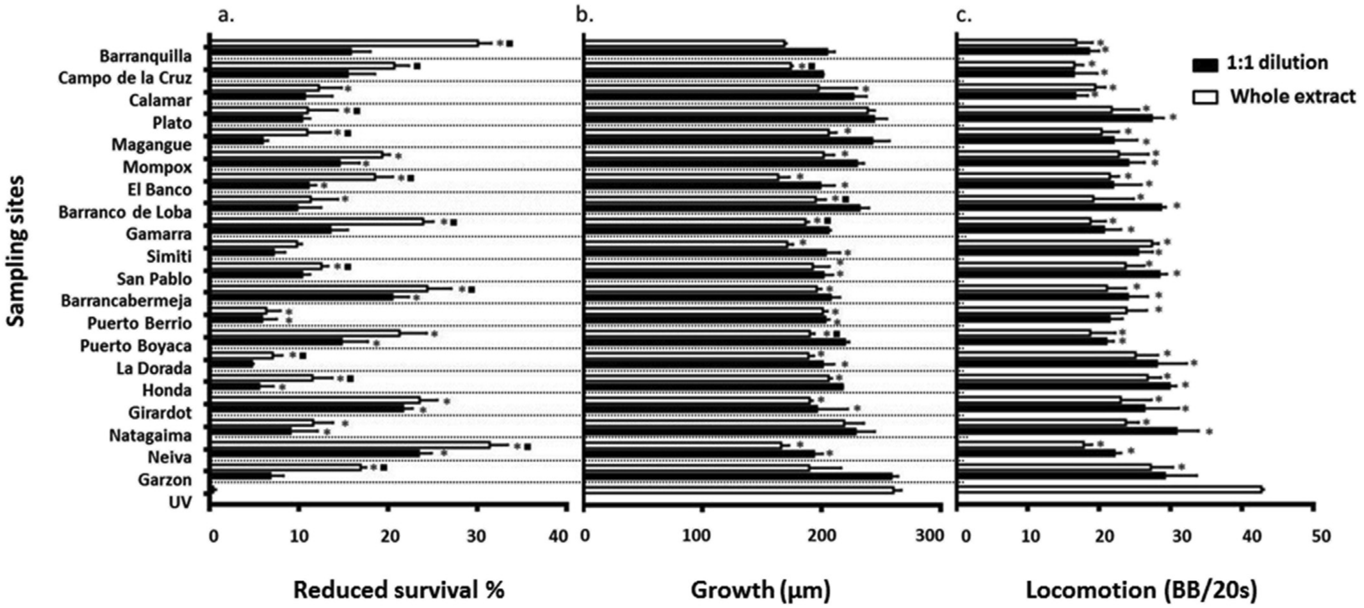


Fig. 4. Toxicological response of C. elegans exposed to sediment extracts. a. Reduced survival; b. Growth as body length; c. Locomotion. BB: body bends. *. Significant differences with control (UW) ($p < 0.05$, Dunnet test). ■. Significant differences between 1:1 dilution and whole extract ($p < 0.05$, T test).

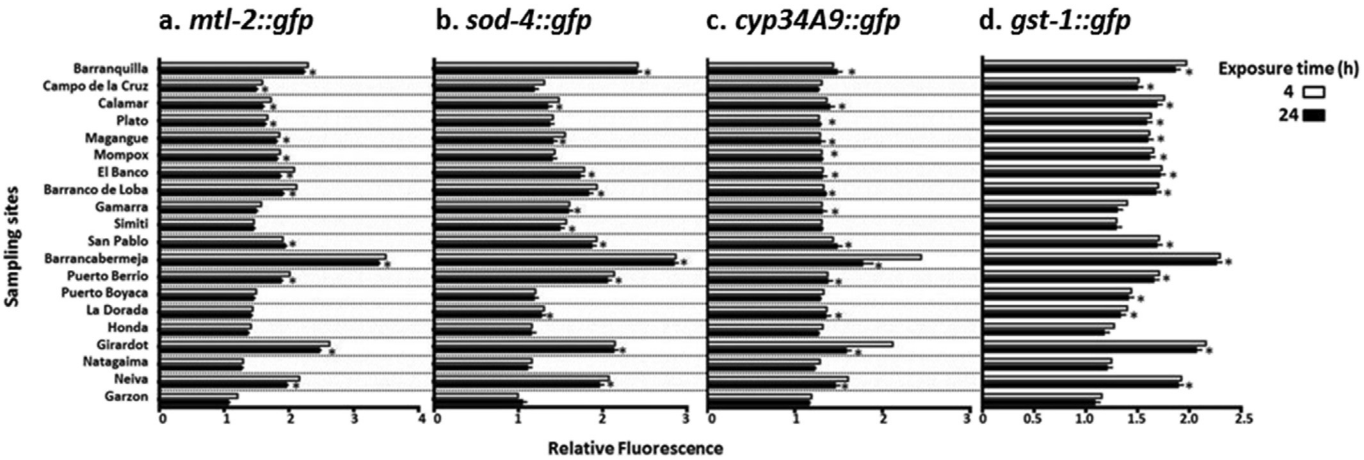


Fig. 5. Responsiveness of genes in C. elegans. a. mtl-2:gfp; b. sod-4:gfp; c. cyp-34A9:gfp; d. gst-1:gfp. Results are expressed as ratios between fluorescence expression at each sampling site and the control. *.Significant ($p < 0.05$, Dunnet test) differences with control.

The association between distance from the first sampling site and survival, growth, and locomotion inhibition is presented in Figure SM-8. The correlation analysis (R = 0.612, Spearman test) shows a strong association ($p < 0.01$) between locomotion inhibition and distance, verifying that the BBF decreased with the distance from the first site (Garzon), suggesting an accumulation process of metal contamination, and probably other pollutants, in sediments downstream. Moreover, the subsequent decreased toxicity observed in some sites could be attributed to the dilution from feeder rivers coming from watersheds with minimal intervention. For instance, the La Miel and Guali Rivers refresh the Magdalena River near Natagaima and Honda, and the Cauca and San Jorge Rivers do the same between Barranco de Loba and El Banco (Figure SM-1).

3.3.2. Cluster analysis

The dendrogram from cluster analysis including all measured variables is shown in Fig. 6. Samples are divided into seven groups with the first group containing two sub-clusters. The first sub-cluster is composed of Garzon, Natagaima, Honda, and La Dorada, which showed a low stress response. The second sub-cluster is comprised of several samples with moderate toxicity. The other five groups are independently formed by Neiva, Barranquilla, Girardot, Barrancabermeja, and Puerto Boyaca which had the greatest toxicity and mixture composition.

Samples from Neiva, Girardot, Barrancabermeja, and Barranquilla induced the highest response. These four sites are located in areas of high industrial activity. The sample from Barrancabermeja showed one of the highest gene responses, and high survival and locomotion inhibition. This is the site of the largest oil refinery and petrochemical complex in Colombia. In this region, the Magdalena River receives treated and untreated effluents from different industries related to oil activity, hydrocarbon transportation, deposition of compounds, and particles from industrial emissions. Although wastewaters from oil refineries are treated, several pollutants, such as heavy metals from crude oil or from catalysts used

Table 4
Variation in endpoints between exposure to whole extract and 1:1 dilution.

Sample	Survival	Growth	Locomotion
Garzon	0.60	0.37	0.07
Neiva	0.25	0.17	0.24
Natagaima	0.22	0.04	0.30
Girardot	0.08	0.03	0.14
Honda	0.52	0.06	0.12
La Dorada	0.33	0.06	0.12
Puerto Boyaca	0.31	0.15	0.12
Puerto Berrio	0.07	0.01	−0.10
Barrancabermeja	0.16	0.06	0.14
San Pablo	0.17	0.05	0.20
Simiti	0.27	0.19	−0.07
Gamarra	0.44	0.10	0.10
Barranco de Loba	0.13	0.19	0.49
El Banco	0.40	0.22	0.02
Mompox	0.25	0.14	0.06
Magangue	0.46	0.18	0.08
Plato	0.05	0.02	0.26
Calamar	0.13	0.15	−0.14
Campo de la Cruz	0.25	0.16	−0.00
Barranquilla	0.47	0.21	0.11
Average	**0.28**	**0.13**	**0.11**

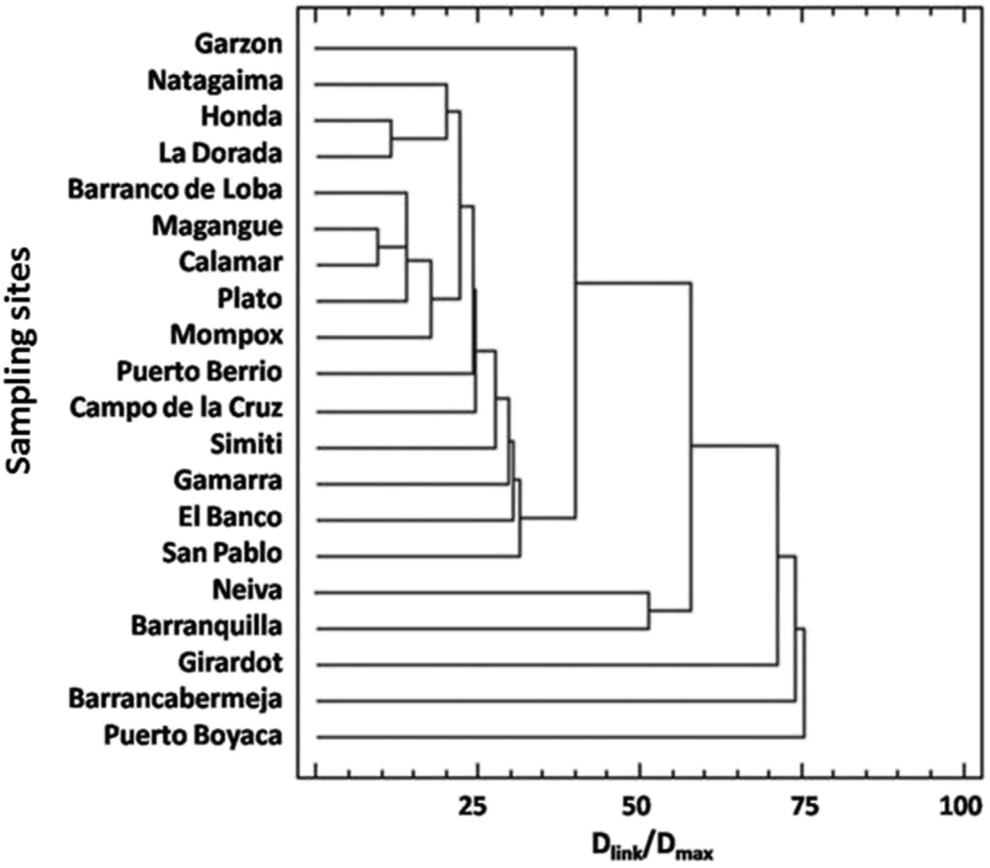


Fig. 6. Dendrogram for chemical and biological data on sediment samples.

in the refining process, flow into the river and are incorporated into the sediments (Wang et al., 2014b). Girardot, where the Magdalena River receives waters from the River, presented high stress gene response, survival and locomotion inhibition. The Bogota River collects pollution from artisanal tanneries, which dump their waste into the river. It also receives pollution from several industries, agriculture runoff, and untreated domestic water. About 28% of socio–economic activity in Colombia is generated near this region. The presence of metals such as Pb, Cd, and Cr in water and in organisms has been reported in the Bogota River (Lora and Bonilla, 2010; Rodriguez et al., 2009). Barranquilla, in the river mouth, is a city of great industrial and port activity (Baron et al., 2013). Several chemical, pharmaceutical, metal-mechanical, and agrochemical industries are located in this estuarine area, which receives pollution accumulated along the river, including discharges generated in the city itself (Taylor et al., 2002). This sample had the peak content of Hg, high gene response, high survival, and low locomotion.

Neiva is a major city lacking a wastewater treatment plant, located near active oil fields, where the crude oil has high Ni content (Mogollon et al., 1998; Vale et al., 2008). In addition, this is an agricultural production area where pesticides are released into the water. The sample from Neiva had the peak content of Co, Pb, and Zn; high stress gene response, high survival, low growth, and low locomotion. In addition, Puerto Boyaca, affected by oil activity, had the peak of Cu and low locomotion. Finally, Gamarra, influenced by gold mining and oil and gas pipelines, presented high contents of Cd, Ni, and Pb, and low locomotion.

The results from this study highlight the toxic effects of Magdalena River sediments, measured as the survival, growth and locomotion inhibition of *C. elegans*. The GFP transgenic *mtl-2* and *sod-4* showed significant changes in expression after extract exposure, suggesting that toxic responses in *C. elegans* were dependent upon metal concentration, probably through a mechanism that involves oxidative stress.

4. Conclusions

C. elegans was employed to generate the toxicity profile of sediments from twenty stations along the Magdalena River, Colombia, the major freshwater ecosystem in the country. Aqueous extracts from most sediment samples produced deleterious effects in survival, growth, and locomotion of the nematode. Transgenic strains of *C. elegans* carrying *gfp* reporters for *mtl-2* and *sod-4* showed significant changes in expression after extract exposure. Sampling sites that generated the greater expression of stress response genes were Barrancabermeja and Girardot. In the Magdalena River, the dumping of all types of waste from urban centers, mining, agriculture, and industrial activities carried out in its basin, sediment toxicity on *C. elegans* was dependent on metal content. Toxic pollutants, not only metals, but likely many other chemicals, are able to generate a response in the *C. elegans* model. The study therefore highlights the threat that release of potentially toxic effluents pose to aquatic ecosystem health, which is a concern, especially in developing countries where discharge of untreated domestic, industrial, and agricultural wastes to water bodies is prevalent and relatively unregulated.

This is the first study about the toxicity of Magdalena River sediments. Although *C. elegans* showed to be a sensitive model for this type of assessment, it is clear that future research should include native organisms, as well as sampling periods that could cover temporal variability, and quantification of input loads for specific discharges in the river. Moreover, there is a need to address the toxicological impact of other chemicals such as pesticides, PAHs and emerging pollutants, among others.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.01.057.

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