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Toxicity Assessment of River Sediments Impacted by Open-Pit Coal Mining in Colombia Using *Caenorhabditis elegans*

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Abstract Coal mining is a critical economic for Colombia. However, mineral extraction is usually carried out near rivers that provide ecosystem services to riverside populations. Cesar River receives discharges from several open-pit coal mines, as well as from other anthropogenic sources. The aim of this work was to assess the chemical and the toxicity profile of the sediments from this river. Bottom sediment samples were collected from 12 points along the river, including tributaries and a Ramsar site, the Zapatosa Marsh. Trace elements were quantified employing ICP-MS, and mercury (Hg) was measured using a direct Hg analyzer. Aqueous extracts (K-medium) were obtained from dried sediments (1:3 ratio) and tested using *Caenorhabditis elegans*, assessing mortality, locomotion and growth as end points. Transcriptional effects associated with various toxicity mechanisms were evaluated using GFP-related transgenic strains (*mtl-2*, *sod-4* and *gst-1*). Some trace metals enriched along the course of the river, especially Hg and V. Sediment extract-induced lethality was low (1.5–6.4%); however, nematode growth and locomotion decreased downstream the river, showing inhibition rates up to 23.3 and 35.4%, respectively. Extracts from downstream points increased the mRNA expression of tested genes compared to that elicited by the most upstream site, with greater values on stations receiving domestic sewage and mining outputs. Cobalt and lead were positively associated with metallothioneins and *gst-1* expression. In short, coal mining areas should be closely monitored for trace-element release and their impact on biota. The Colombian government should implement laws and programs to protect key ecosystems from mining activities, as a commitment to sustainable development goals.

Keywords GFP reporter gene · Toxicity · Risk assessment · Coal mines · Pollutants

1 Introduction

Countries with emerging economies are experiencing rapid urban and population growth, a process usually linked to accelerated exploitation of subsoil natural resources (Fernando et al., 2018). Unfortunately, in many countries these activities are generators of

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social conflicts, economic inequality, and negative health indictors and environmental impacts, contributing to the destruction of agriculture, changes in the livelihoods of local communities, and generation of large volumes of mining waste and tailings (Hindersmann & Achten, 2018). Colombia is not the exception, and currently it is one of South America's largest coal producers, reaching productions of up to 91 060 million tons (MT) in 2016, with coal reserves greater than 4881 million tons of anthracite and bituminous coal, being the world's fifth largest coal exporter (BP, 2021).

Coal mining in Colombia is mostly carried out in the state of La Guajira and Cesar (Estrada et al., 2020), one of the largest open-pit mining regions in the world (affecting 298.391 ha) (de Paula Gutiérrez et al., 2020). The process occurs through large open pit mining, representing 93% of total operations, and the remaining 7.3% through underground mining (UPME, 2018). It is known that opencast coal mining operations are a source of pollutants that exerts different consequences on human and environmental health as a result of occupying large land areas that cannot be later recovered, forest degradation, change in soil uses, ecological, physical and chemical impacts on ecosystem function (Feng et al., 2019), disruption of hydrological regimes (Tiwary, 2001), air pollution, release acid mine drainages, and socio-environmental liabilities (Cardoso, 2015).

Large volumes of tailings from coal mining contain potential toxic chemicals (PTEs), such as heavy metals and other trace elements, that reach the surrounding water bodies and their sediments (Zhang et al., 2019). Mine-related trace elements have been reported to be highly hazardous to all organisms (Wright et al., 2017) in particular those present in the water column and sediments. Thus, the physicochemical evaluation of sediments is an adequate indicator of the state of contamination in aquatic systems, especially considering that bottom sediments are extensively known as natural geoabsorbents (Szara et al., 2020), with diverse ecological, and geochemical functions. Quantitative measurements of PTE, when accompanied by bioassay evaluations, are considered important tools to characterize the current environmental status of ecosystems in a comprehensive manner (Tarnawski and Baran, 2018). Different parameters, such as the geoaccumulation index, the ecological risk, the enrichment factor, the health risk, among others, make it possible to evaluate the ecological risk due to exposure to trace elements present in sediments (Baran et al., 2016). There are also different *in vitro* and *in vivo* model organisms that are used to evaluate the effects of toxic elements present in sediments. Among those, unicellular models (*Vibrio fischeri*), microalgae (*Isochrysis galbana*); insects, nematodes, bivalves, and fish, are common organisms used to evaluate sediment quality (Frouz et al., 2005; Simpson et al., 2016).

The nematode *Caenorhabditis elegans* has been shown to possess several advantages as a multicellular model, including short life cycle, simple and inexpensive propagation in the laboratory, ability to self-fertilize, transparent body, and quite important, its genes display high homology with human ones (Rai et al., 2019). In fact, there is a vast list of relevant transgenic strains that have incorporated Green Fluorescent Protein (GFP) to monitor gene expression in vivo in response to environmental stresses (Queirós et al., 2019).

Although the environmental damage generated by coal mining in Colombia has received some research attention, emphasizing on disputes over water-related issues (Fierro, 2014), socio-environmental and health liabilities (Cardoso, 2015), community displacement and changes in the economic dynamics of the region (Hernández, 2018), research on environmental and human health impacts are scarce, with some information on air, sediment and fish pollution (Arias-Vanegas et al., 2020; Espitia-Pérez et al., 2018), as well as on DNA-damage in mammals surrounding mining operations (Cabarcas-Montalvo et al., 2012; Guerrero-Castilla et al., 2014). The purpose of this study was to evaluate the content of trace elements in sediments collected from the Cesar River, together with the evaluation of the ecotoxicity of aqueous sediment extracts using *C. elegans*, integrating chemical analyses and bioassays in order to assess the environmental risk.

2 Materials and Methods

2.1 Study area

The Cesar River Basin (Fig. 1) has been subjected to extensive open-pit coal mining activities since

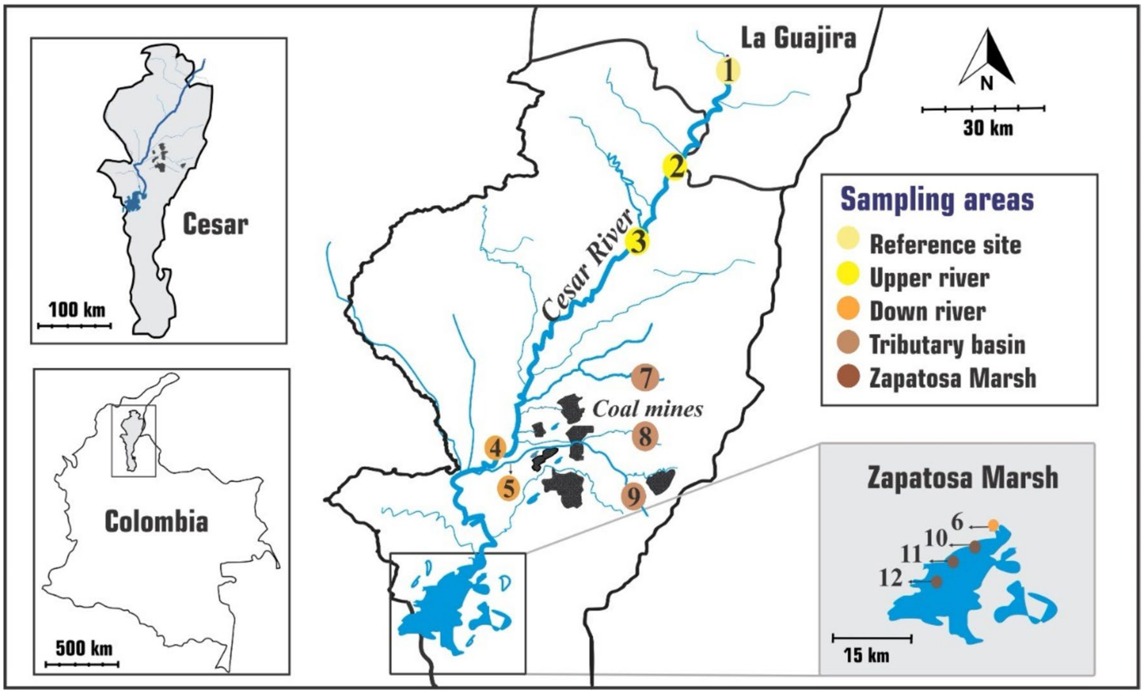

Fig. 1 Sampling sites in the Cesar River, Colombia

mid-1985. It originates in the southwest area of La Guajira state, flowing south through Cesar state, where 12 municipalities depend directly on the river course, extending approximately 280 km, finally flowing into the Zapatosa Marsh, the largest freshwater wetland in Colombia, a Ramsar site with high biodiversity that provides numerous ecosystem services to the region. The drainage area of the basin is approximately 22.931 km^2, with an average annual discharge of 396 m^3/s (Guzman-Finol, 2013). The study zone comprises a tropical forest with a subtropical monsoon climate, and an average annual temperature and rainfall of 30 °C and 1900 mm, respectively. The major threats for the Cesar River are intensive agriculture, mainly African palm and coal mining, among others.

2.2 Sediment sampling

A total of 12 bottom sediment samples were collected from different locations along the Cesar river basin (Fig. 1) in March 2019. After *in situ* measurements of physicochemical parameters, samples were obtained from the top layer of the sediment (0–10 cm), either manually, or using an Ekman sediment sampler. At each station four subsamples were collected and a composite sample of approximately 500 g was produced by homogenization. The geographical position of sampling sites was determined by a GPS (GARMIN GPS MAP 60C model). Samples were stored in polyethylene bags, and kept under refrigeration until transport to the laboratory, where they were stored at −20 °C. Subsequently, sediments were freeze-dried (Labconco Freezone 2.5) at −50 °C for 48 h. Next, the dried samples were homogenized and sieved to obtain a material with a particle size of 75 µm or less, and stored at −20 °C until analysis (Palacios-Torres, et al., 2020).

The hydric system of the Cesar River was divided into five areas: Reference station (S1), upper basin (S2 and S3), lower basin (S4, S5, S6); tributaries (S7, S8, S9), and Zapatosa Marsh (S10, S11, S12) (Fig. 1). A description of sampling stations is shown in Table S1.

2.3 Sediments chemical analysis

Concentrations of trace elements in the sediments were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Freeze-dried sediment samples (0.1 g) were digested using a mixture of HF and HNO_3 (8:3) solution at 90 °C for 24 h. This solution was dried at 180 °C, and the residue reconstituted in a mixture of HNO_3 and HCl (90 °C, 24 h). Once digested, 3 mL of HCl were added, digested again, the final residue recovered in 2% HNO_3, and completed to 100 mL with Milli-Q H_2O. In all cases high purity acids (Suprapur®, Merck) were used. Quantitative analysis was carried out using external calibration with standard solutions prepared from multi-element solutions (1: rare-earth elements, 2: alkalis, earth alkalis, and metals, and 3: Nb), and the quality of the results for trace elements was controlled by repeated analysis of certified reference standards SARM-1 (Granite) and SARM-4 (Norite) (Valdelamar-Villegas et al., 2021). The average precision and the accuracy were within normal analytical errors (5–10%), and limits of detection for trace-elements are shown in Table S2.

Total Hg concentrations were determined with a direct Hg analyzer (PYRO-915 +, RA915M, Lumex) on 180–200 mg of freeze-dried sediments. Calibration curves were obtained by using certified reference material IAEA 433 (Marine Sediment) following the USEPA 7473 method (USEPA, 1998). Accuracy was verified by the analysis of IAEA 158 (Marine Sediment). The LOD was calculated as three times the standard deviation of the blanks, consisting of empty preheated combustion boats (LOD, 0.003 µg/g). The precision was generally $< 10\%$. All samples were analyzed in duplicate (Osorio-Martinez et al., 2021).

2.4 Biological assays

Toxicity assessment of bottom sediments was conducted using aqueous extracts of the samples and the nematode *C. elegans* as a model system.

2.4.1 *Preparation of sediment extracts*

Aqueous extracts were prepared by vigorous mixing (300 rpm) 10 g of lyophilized sediments (≤ 75 um) with 30 mL of K-medium (KCl, NaCl, Milli-Q Water), a liquid medium developed for conducting toxicity tests using *C. elegans* (Williams & Dusenbery, 1990), at 30 °C for 24 h. The supernatant was removed by centrifugation, filtered using 0.45 µm membranes (Millipore, Millex-HP) and then used in the bioassays as whole extracts (Acosta-Coley et al., 2020). Each sediment extract was utilized to perform three independent experiments for the different assays described below.

2.4.2 *Worm culture and handling*

Wild type strain (Bristol strain N2) and promoter tagged GFP transgenic strains BC20333 [*sod-4*::GFP]; BC20316 [*gst-1*::GFP] and BC20342 [*mtl-2*::GFP] of *C. elegans* were employed in this study. All strains and *Escherichia coli* OP50 strain were obtained from the Caenorhabditis Genetics Center (CGC, University of Minnesota, USA). Nematodes were maintained at 20 ± 2 °C and cultured under standard conditions on nematode growth medium plates (NGM), seeded with *E. coli* OP50 as food source with optical density (OD = 0.6) (Sana et al., 2021). Experiments were performed employing bleach synchronization of worm populations, lysing gravid nematodes using alkaline lysis solution (NaOH and HOCl).

2.4.3 *Lethality*

Approximately (10 ± 2) worms in L4 larval stage were exposed to the total sediment extract for 24 h, and K-medium served as a negative control in a 96-well microtiter plate. Following exposure, the live and dead nematodes were scored, and percent mortality was determined. The worms were counted and classified as alive or dead based on the lack of movement, touch response, or pharyngeal pumping (Tejeda-Benitez, 2018).

2.4.4 *Growth*

Synchronized nematodes in the L1 larval stage were exposed to the total extract for 72 h in 24-well microtiter plate at 15 °C in the presence of food. After treatment worms were killed by heat. The length of the worms was measured the flat surface area of nematodes using an Image-Pro® Express software. 30 worms each were scored for each concentration

(De la Parra-Guerra et al., 2020). Growth of worm is given as mean length/worm.

2.4.5 *Locomotion behavior*

Nematodes in the L4 larval stage were exposed to the total extract of the sediments, at 20 °C in the absence of food. After treatment, each worm was manually scored for body bend in an interval of 20 s under a stereomicroscope (Nikon TMZ) (Nagar et al., 2020). Thirty worms were examined per treatment.

2.4.6 *Quantification of GFP expression*

Synchronized worms in L4 larval stage from transgenic strains that carry a GFP reporter (*sod-4*::GFP; *gst-1*::GFP; and *mtl-2*::GFP) were exposed to total sediment extract using K medium as a control in non-fluorescent 96-well microtiter plate at 20 °C in the absence of food. After exposure fluorescence was measured using a Perkin-Elmer Victor 1420 multilevel plate reader at excitation/emission of 485/535 nm wavelengths, respectively (Acosta-Coley et al., 2020). The results are represented relative to control (%).

2.5 Statistical analysis

All data are presented as mean ± standard error. Normality and variance homogeneity were verified using Shapiro–Wilk and Bartlett tests, respectively. Differences between group means were assessed using ANOVA. The Dunnett test was applied to compare treated groups against the control group. Correlation analysis was utilized to evaluate relationships between variables (metal concentrations and toxicological responses). The criterion of significance was set at $p < 0.05$.

3 Results

3.1 Trace element concentration in sediments

The summary of main statistics for analyzed trace element concentrations found in bottom sediment samples from Cesar River is presented in Table 1. The analysis included a total of 27 elements. The median concentration for the ten more abundant elements followed the order Sr > V > Rb > Zn > Ce > La > Cu > Ni > Ga > Sc. The greatest differences between the concentrations of trace elements obtained at the reference site (Table 1) and the other evaluated stations occurred with the following elements: Vanadium, levels in tributary rivers located in the mining area were between 3.7- and 4.2-fold greater than that obtained at the reference site. Similar data were obtained in the same sampling area for Cr (between 2.5- and 3.2-fold) and Zn (2- and 2.9-fold).

In general, the maximum average concentrations of most trace elements were found in the tributary basin area, influenced mainly by mining activities. Particularly, V showed its highest concentration in the mining area, followed by Ni, both displaying enrichment behavior as the river moves towards the marsh. The other areas that showed this same behavior were the lower river basin and the Grand Marsh of the Zapatosa for the elements Hg, As, and Cs.

3.2 Physiological responses induced by sediment extracts on *C. elegans*

The lethal effects associated with exposure of *C. elegans* to aqueous extracts of the sediment samples are shown in Fig. 2A. In general, lethality was low (1.5–6.4%). The sampling point with the highest lethality was located in the up-per basin area of the Cesar River (S3), 13.5 km downstream the discharge of the sewage treatment plant of Valledupar, reaching a maximum of 6.4%, followed by stations at tributary basins with an average of 3.8%. The lowest lethality was observed at the reference site (S1).

Despite low lethality, nematode growth (Fig. 2B) showed significant differences between the reference station and those downstream S2 (S3 → S12), including tributaries and Zapatosa Marsh. However, greatest growth inhibition (23.4%) was observed for samples located in the marsh (S11).

The locomotion of the nematode (Figs. 2C) displayed significant differences along the river, with greatest inhibition at S6 (35.5%). Interestingly, all tributary inputs into the river abrogated the locomotion inhibition along the natural course, although the stations at the entrance of the Zapatosa Marsh was similar to that at S6, the movement of the worm improved when exposed to sediments collected at the south of the marsh.

Table 1 Main descriptive statistics of trace element levels (µg/g, d.w) analyzed in Cesar River sediments

	Sampling zone							
Elements	Reference site	Upper Basin	Lower Basin	Tributary Basin	Zapatosa Marsh	**Average**	**Median**	**Error**
Be	2.25	1.58	1.67	1.97	1.33	1.76	1.67	0.16
Sc	4.45	10.4	10.8	15.40	11.1	10.4	10.8	1.75
V	35.1	95.8	94.85	140	95.4	92.2	95.4	16.7
Cr	24.6	46.5	50.7	66.8	42.8	46.3	46.5	6.79
Co	4.31	10.6	9.89	13.8	11.5	10.0	10.6	1.57
Ni	11.3	19.9	21.9	31.1	21.1	21.0	21.1	3.14
Cu	17.5	36.5	25.0	36.5	44.1	31.9	36.5	4.71
Zn	49.5	72.7	78.7	121.6	91.6	82.8	78.7	11.9
Ga	21.4	19.9	19.0	24.9	16.8	20.4	19.9	1.35
As	1.58	4.01	6.83	4.42	3.19	4.01	4.01	0.86
Se	1.68	3.26	3.32	4.27	3.60	3.23	3.32	0.43
Rb	143	95.1	103	111	69.5	104	103	11.8
Sr	246	260	118	171	85.2	176	170.51	34.21
Cs	1.24	2.03	5.15	4.98	3.31	3.34	3.31	0.78
Hf	2.55	2.66	2.01	2.47	1.83	2.30	2.47	0.16
Hg	0.01	0.01	0.01	0.03	0.13	0.07	0.01	0.03
Pb	13.6	13.4	17.2	24.5	16.7	17.1	16.7	2.01
La	22.0	35.1	34.5	41.8	31.98	33.07	34.47	3.21
Ce	41.6	69.7	71.6	86.8	65.5	67.0	69.7	7.30
Nd	15.8	28.8	30.6	37.1	28.6	28.2	28.8	3.46
Sm	2.76	5.42	5.86	7.21	5.60	5.37	5.60	0.72
Eu	0.77	1.26	1.21	1.58	1.18	1.20	1.21	0.13
Tb	0.35	0.71	0.71	0.92	0.74	0.69	0.71	0.09
Dy	2.00	3.97	3.75	5.02	4.05	3.76	3.97	0.49
Yb	1.50	2.13	1.82	2.55	2.04	2.01	2.04	0.17
U	2.01	1.86	2.07	2.49	1.88	2.06	2.01	0.11
Th	7.99	7.23	9.46	10.0	8.04	8.55	8.04	0.52

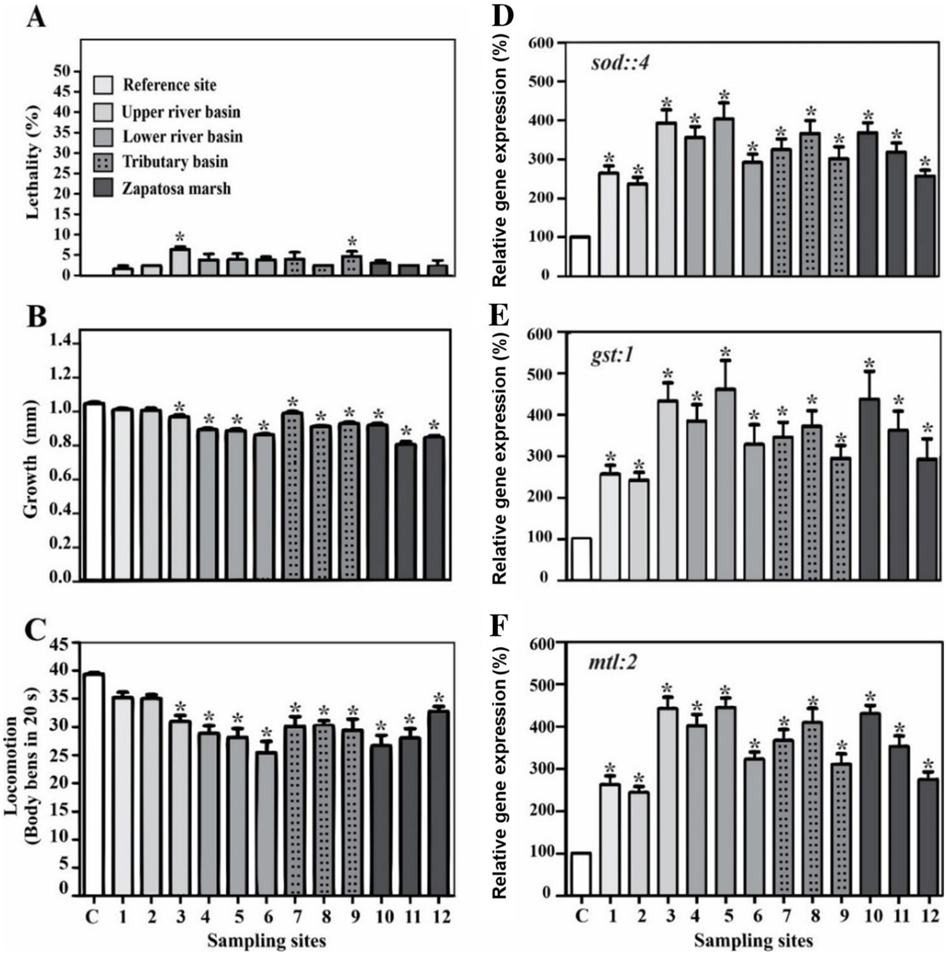

Fig. 2 Response of *C. elegans* exposed to sediment extracts. **A**: Lethality. **B**: Growth. **C**: Locomotion. **D, E, F**: Relative gene expression for *sod-4, gst-1,* and *mtl-2*, respectively. * Significant difference compared to the control (C). $p < 0.05$. Dunnet's test

The gene expression result obtained after *C. elegans* was exposed to sediment extracts is shown in Figs. 2D–F. Extracts induced over expression of all tested genes: *sod-4*::GFP (Fig. 2D), *gst-1*::GFP (Fig. 2E) and *mtl-2*::GFP (Fig. 2F). The mRNA expression pattern was quite similar among all tested genes, with tributaries decreasing the toxicity and stations in the marsh displaying lower gene inductions as the distance from the input of the river (S10) increases. In stations along the river, gene expression was lower in those sites located before the main course received sewage waters from the treatment plant of Valledupar (S2 and S3) enters the river connection with greater values for sites receiving domestic sewage (Upper river). Down river and tributary basin.

3.3 Correlation analysis

The results of correlation analysis between the trace element level in sediments and the biological responses observed in *C. elegans* are presented in Table 2. Overall, this analysis showed that all studied elements displayed negative correlations with nematode locomotion, with greater associations for Sc, Co, Zn, Se and Pb. Growth was negatively impacted by Hg-T. On the other hand, Co and Pb were positively associated with both metallothionein and glutathione transferase mRNA expression.

Table 2 Correlation between element concentrations in sediments and biological responses in *C. elegans*

		Trace element											
Bioindicator		**Sc**	**V**	**Cr**	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**Se**	**Cs**	**Pb**	**Hg**
Lethality	r	−0.046	−0.093	−0.054	−0.221	−0.018	−0.027	−0.007	−0.239	−0.257	0.339	−0.296	−0.136
	p	(0.886)	(0.774)	(0.869)	(0.489)	(0.956)	(0.429)	(0.982)	(0.454)	(0.420)	(0.281)	(0.349)	(0.673)
Growth	r	−0.343	−0.218	−0.182	−0.329	−0.224	−0.645	−0.392	−0.0139	−0.343	−0.343	−0.294	−0.716
	p	(0.276)	(0.499)	(0.572)	(0.297)	(0.484)	**(0.032)***	(0.200)	(0.966)	(0.276)	(0.914)	(0.354)	**(0.009)**
Locomotion	r	−0.860	−0.664	−0.629	−0.741	−0.629	−0.655	−0.776	−0.538	−0.832	−0.594	−0.741	−0.681
	p	**(<0.001)**	**(0.018)**	**(0.028)**	**(0.006)**	**(0.028)**	**(0.029)**	**(0.003)**	(0.071)	**(<0.001)**	**(0.042)**	**(0.006)**	**(0.015)**
sod-4	r	0.336	0.252	0.273	0.517	0.266	0.343	0.064	0.147	0.399	0.294	0.517	0.196
	p	(0.286)	(0.430)	(0.391)	(0.085)	(0.404)	(0.853)	(0.276)	(0.649)	(0.199)	(0.354)	(0.085)	(0.540)
gst-1	r	0.483	0.399	0.406	0.657	0.392	0.227	0.490	0.266	0.531	0.315	0.608	0.396
	p	(0.112)	(0.199)	(0.191)	**(0.020)**	(0.208)	(0.502)	(0.106)	(0.404)	(0.075)	(0.319)	**(0.036)**	(0.202)
mtl-2	r	0.441	0.343	0.364	0.601	0.357	0.182	0.427	0.231	0.497	0.315	0.588	0.344
	p	(0.152)	(0.276)	(0.245)	**(0.039)**	(0.255)	(0.593)	(0.167)	(0.471)	(0.101)	(0.319)	**(0.045)**	(0.274)

*. Significant values ($p < 0.05$) appear in bold

4 Discussion

Coal extraction has been linked to a large number of environmental problems in rivers within the influence area of mining operations (Jabbar-Khan et al., 2020; Ping et al., 2017). Due to their importance as sinkers for many types of pollutants, sediments always offer a window to characterize the potential impacts of human activities on river basins (Mora et al., 2021; Tejeda-Benitez, 2018). In this work, trace element concentrations were measured in sediments of Cesar River, a stream that flows through the most extensive area of coal mining at Northern Colombia, evaluating the toxicity of sediment extracts on the soil nematode *C. elegans*. The study found that although several toxicologically relevant elements are present in sediments, their concentrations do not vary extensively across all sampling locations, although the toxicity exerted on the worm has a clear dependence on geographical location of the sediment source.

In terms of trace elements, sampling sites located upstream Cesar River basin showed slightly lower trace element concentrations than those downstream, finding that changes after the effluent from Valledupar sewage treatment plant enters the river, a process usually linked to trace element enrichment (Khan et al., 2021; Soyol-Erdene et al., 2019). However, sediment pollution by trace metals does not always appears after inputs from urban effluents (Moyo & Rapatsa, 2019). In this case, the small size of the city of Valledupar, together with the fact that it has few industrial activities that usually incorporate large amounts of trace elements in the sewage, may support the lower loads of trace elements incorporated into Cesar River sediments.

Although these data are considered preliminary, as only one sampling campaign was carried out, the average value for most trace elements in the lower basin are greater than those in the upper basin, this last including the effect of the urban sewage from Valledupar. This indicates that other anthropogenic activities may be responsible for the increased trace element levels, likely coal mining over agroindustry, as the first is able to deposit mineral materials that leach trace elements into the environment (Shan et al., 2019), and is largely known to pollute surrounding areas (Feng et al., 2019). However, more detailed experiments from core sediments would be required to address the source of these elements.

It is noteworthy to mention that Cesar River tributaries play a major role diluting most trace elements in the main river, as also described for other waterbodies (Li et al., 2019). Unfortunately, the pollutants carried by the river are finally disposed at the Grand Marsh of Zapatosa, an extensive body of water with a large number of ecosystem services, such as fishing, natural landscapes, water supply, diversity of animal species and tourism (Castaño-Barreto et al., 2020).

The evaluation of toxicological endpoints (growth and reproduction) in *C. elegans* have been included in standard sediment toxicity tests (ASTM, 2002; ISO, 2010). Despite the lower concentrations observed for trace elements in sediments, biological assays conducted using *C. elegans* revealed the sediments are toxicologically active, as demonstrated by several biochemical and physiological alterations in the nematode, including growth inhibition, which occurred significatively at stations downstream the urban sewage treatment plant. Previous studies using macroinvertebrate organisms as bioindicators also reported critical pollution in sections of the river where point discharges occur (Oñate-Barraza & Cortéz-Henao, 2020). This also suggests the toxic chemicals present in sediments are bioavailable, and therefore they may be able to impact the local biota. Moreover, the correlations observed for Co and Pb concentrations in the sediments and the expression of metallothionein and glutathione S-transferase (GST) induced by the aqueous extract, may indicate these two metals are rapidly recognized by signaling pathways in the nematode, likely targeting oxidative-stress related mechanisms (Martinez-Finley & Aschner, 2011). Although the role of Co as a marker of environmental pollution is less common, it is known that *C. elegans* is highly sensitive to trace elements such as Pb (Fajardo et al., 2020). Similarly, our results show the negative impacts that lesser-known elements, for example, rare earth elements, such as Scandium, can have on worm growth, consistent with previous reports (Xu et al., 2017). It should also be considered that toxic effects associated with coal mining in the area have been observed under laboratory conditions (Caballero-Gallardo & Olivero-Verbel, 2016) as well as in wildlife (Cabarcas-Montalvo et al., 2012).

Environmental data obtained for Cesar River suggest this ecosystem requires better management practices and programs to promote environmental education. A revision of the efficiency of the sewage treatment plant should also be carried out as a critical factor for the quality of the river. It is absolutely clear that the input of polluted waters from this plant may masking the role of other pressures, in particular coal mining.

5 Conclusions

The present study quantified and evaluated the enrichment by anthropogenic sources of trace elements. the levels of contamination. and toxicity in the biological model *C. elegans* in sediment samples from the Rio Cesar water system mainly affected by coal mining. The load of trace elements along the water system shows variability with a tendency to enrichment in the sampling points near the mining areas and in the stations of the Zapatosa swamp. the evaluation with the nematode *C. elegans* showed different negative effects on physiological parameters and the expression of genes associated with oxidative stress and heavy metal binding proteins. especially in the station of the coal mining area. Correlation analysis showed that trace elements such as V, Co, Ni, Zn and Pb negatively affected worm locomotion, Cobalt also affected the positive expression of *mtl-1* and *gst-1* mRNA expression.

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Data Availability Data will be available on request.

Declarations

Conflicts of Interest The authors declare no conflict of interest.

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