

RESEARCH ARTICLE

Evaluation of the metallic contamination degree in green turtle's feeding area Lanzanillo-Pajonal-Fragoso, Cuba.

Evaluación del grado de contaminación metálica en el área de alimentación de tortugas verdes Lanzanillo-Pajonal-Fragoso, Cuba

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Abstract

The present work evaluates the contamination level by metallic elements in the marine sediments of Lanzanillo-Pajonal-Fragoso Wildlife Refuge, Villa Clara province, Cuba. Eight sampling stations were selected and the levels of Cr were quantified by X-Ray Fluorescence and Zn, Cu, Cd, Fe, Pb, Ni, Mn and Co by Flame Atomic Absorption Spectrophotometry. The chemical pre-treatment of the samples was carried out following the modified ISO 11466 standard method and some validation parameters of the analytical technique were evaluated, showed the accuracy and feasibility of the method. To evaluate the study area contamination the International Sediment Quality Guide of the National Oceanic and Atmospheric Administration and four environmental contamination indexes were used: two enrichment indexes (the enrichment factor and the geoaccumulation index), an ecological risk index (the average ratio of moderate risk effects), and a contamination index (the modified degree of contamination). The results showed that the elements representing the greatest risk are Ni and Cr, since they exceed the concentration that causes Moderate Biological Effects. According to the evaluated indexes, it can be concluded that there is a presence of anthropogenic contamination in the protected area that may be affecting the turtle's health due to the stress that they cause in them. The site of highest risk was Jácate and the element that more contribute to the metallic contamination is Cr, although significant values were obtained for Ni, Co and Fe.

Keywords: *Chelonia mydas*, marine sediments, environmental contamination indexes, X-Ray Fluorescence, Atomic Absorption Spectrophotometry.

Resumen

En el presente trabajo se evalúa la contaminación por elementos metálicos en los sedimentos marinos del Refugio de fauna Lanzanillo-Pajonal-Fragoso, provincia Villa Cla-

ra, Cuba. Se seleccionaron ocho estaciones de muestreo y se cuantificaron en las mismas los niveles de Cr mediante Fluorescencia de Rayos X y de Zn, Cu, Cd, Fe, Pb, Ni, Mn y Co por Espectrofotometría de Absorción Atómica con llama. El pre-tratamiento químico de las muestras se realizó empleando la norma modificada ISO 11466 y se evaluaron parámetros de validación de la técnica analítica, los cuales mostraron la exactitud y factibilidad del método empleado. Para evaluar la contaminación de la zona de estudio se empleó la Guía Internacional de Calidad de Sedimentos de la Administración Nacional del Océano y la Atmósfera y cuatro índices de contaminación: dos índices de enriquecimiento (el factor de enriquecimiento y el índice de geoacumulación), un índice de riesgo ecológico (el cociente medio de los efectos de riesgo moderado), y un índice de contaminación (el grado modificado de contaminación). Los resultados obtenidos mostraron que los elementos de mayor riesgo son el Ni y el Cr, pues superan la concentración que provoca Efectos Biológicos Moderados. Los índices evaluados permiten concluir que hay presencia de contaminación antropogénica en el área protegida que puede estar afectando la salud de las tortugas debido al estrés que provocan en estas. El sitio de mayor riesgo fue Jácate y el elemento que aporta la mayor contaminación es el Cr, aunque el Ni, Co y el Fe mostraron valores significativos.

Palabras clave: *Chelonia mydas*, sedimentos marinos, índices de contaminación ambiental, Fluorescencia de Rayos X y Espectrofotometría de Absorción Atómica.

Introduction

Currently, contamination by metallic elements represents one of the most serious environmental problems (Mishra *et al.*, 2019). Metals and metalloids are considered as potentially harmful substances released by anthropogenic activity, demonstrating risk to the environment and the human being (Rezania *et al.*, 2016, Ali & Khan, 2019). The increasing contamination by these elements, in the various environmental

components, leads to an increase in the global risk for human and ecological health (Resongles *et al.*, 2015, Cervantes-Guerra *et al.*, 2017, D'Costa *et al.*, 2017, Green, 2019).

Metallic elements are substances that do not degrade in the environment, they intervene in mechanisms and process such as chemisorption, precipitation, adsorption and ion exchange (Varela, 2010). They also participate in bioaccumulation and biomagnification processes in biota (Ali & Khan, 2019). Through the aforementioned mechanisms, these pollutants can pass to the biotic and abiotic components of aquatic systems. Within the abiotic components, sediments are very vulnerable to contamination, due to the particular affinity of their fine particles for metals and metalloids (Mugica *et al.*, 1996). These fine particles are easily transported by the river and deposited in the sea. For this reason, marine sediments constitute sinks of substances incorporated into water bodies by natural or anthropic processes; therefore, they can affect both the ecosystem and the food web. Hence, the importance of determining the levels, origin and risks for the aquatic system of metallic elements in this environmental matrix (Halli *et al.*, 2014).

These pollutants can cause negative effects on the health of marine animals. In the case of the green turtle (*Chelonia mydas* Linnaeus 1758), it was declared endangered according to the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) since 2004, and its international trade regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 2007. Among the threats they face, the effects of environmental pollution is one of the least studied despite the fact that it is one of the main causes of diseases and decline of these species (Jones *et al.*, 2016). Among the effects that these pollutants can have on sea turtles are: damage to the endocrine system, which causes harmful effects on

growth, development and reproduction; the immune system, promoting the appearance of other diseases, such as fibropapillomatosis; in the case of embryos and offspring: alteration of the sex ratio, malformations, increased early mortality and low hatching success (D'ilio *et al.*, 2011).

In Cuba, few studies have been carried out on the quality of the environment in green turtle's key habitats (Russet-Rodríguez *et al.*, 2021, Russet-Rodríguez *et al.*, 2019; Ricardo *et al.*, 2018). In particular, in Lanzanillo-Pajonal-Fragoso protected area, at north of Villa Clara province, it was found the presence of metallic elements in green turtles juvenile's main food source, seagrasses (*Thalassia testudinum* and *Syringodium filiforme*), when they were conducting a study on their feeding (Russet *et al.*, 2020) and health (Russet-Rodríguez *et al.*, 2021). However, the levels of these pollutants to which turtles may be exposed are currently unknown, since an exhaustive environmental assessment of the area in question has not been carried out yet.

Due to the damage caused by these pollutants, a more rigorous study is necessary on the concentration levels of metallic elements in the environment where these species live, both in coastal waters and in the open sea, due to their possible relationship with the appearance of tumors in turtles. Some metals and metalloids, along with other contaminants, immunologically compromise green turtles, facilitate herpesvirus infection, and with it, the development of the disease known as fibropapillomatosis (Adnyana *et al.*, 1997). However, it is still not clear whether the incidence of this disease is related to the presence of metallic elements, as some authors suggest, until it is known whether these are at toxic levels or not.

The objective of this work is to evaluate the level of contamination by metallic elements of the marine sediments of the Lanzanillo-Pajonal-Fragoso Wildlife Refuge, north of Villa Clara province, Cuba.

Methods and materials

Sampling collection and analysis

Eight sampling stations were located across the area to determine spatial variation of metallic contamination and, in addition, the possible circulation of water was taken into account to see possible external sources (Fig. 1).

The sediment samples were collected in October 2017 and were taken manually at a depth of approximately 5 cm. After, about 1 kg of each samples were introduced into plastic bags for better handling and transportation. Samples were transported to the laboratory and preserved frozen at -20 °C until further chemical processing and analysis (ISO-5667-15, 2009). Subsequently, they were placed in an oven (Boxun Series BGZ II) at a temperature of 105 °C for drying. A particle size reduction process was carried out using a sieve with a mesh size of 63.0 µm.

The organic matter content was determined by ignition losses procedure. The mass of each individual sample obtained in the moisture determination process, was considered as initial mass in this procedure. After heat treatment in the oven, the samples were placed in a muffle (HERAEUS model) at 550 °C for 16 h. After this period of time, the samples were placed in a desiccator for 4 h and weighed in an analytical balance. This procedure was repeat up to constant weight.

For the chemical pre-treatment of the samples, a modified standard analytical method 11466 was taken into account (ISO 11466, 1995; Herrera *et al.*, 2015). In a Kjeldhal type digestion tube, 1.0 g of sample was placed and 3.0 mL of HCl (37 %) and 9.0 mL of HNO₃ (65 %) were added. The sample was left in contact with the acid mixture for 16 hours at room temperature, with the condenser placed on top to avoid losses of analytes due to volatilization. After this time interval, the Gerhardt GmbH & Co heating system was activated for 2 hours at a temperature of 150 °C. The resulting solution was cooled to room temperature, then it was filtered with

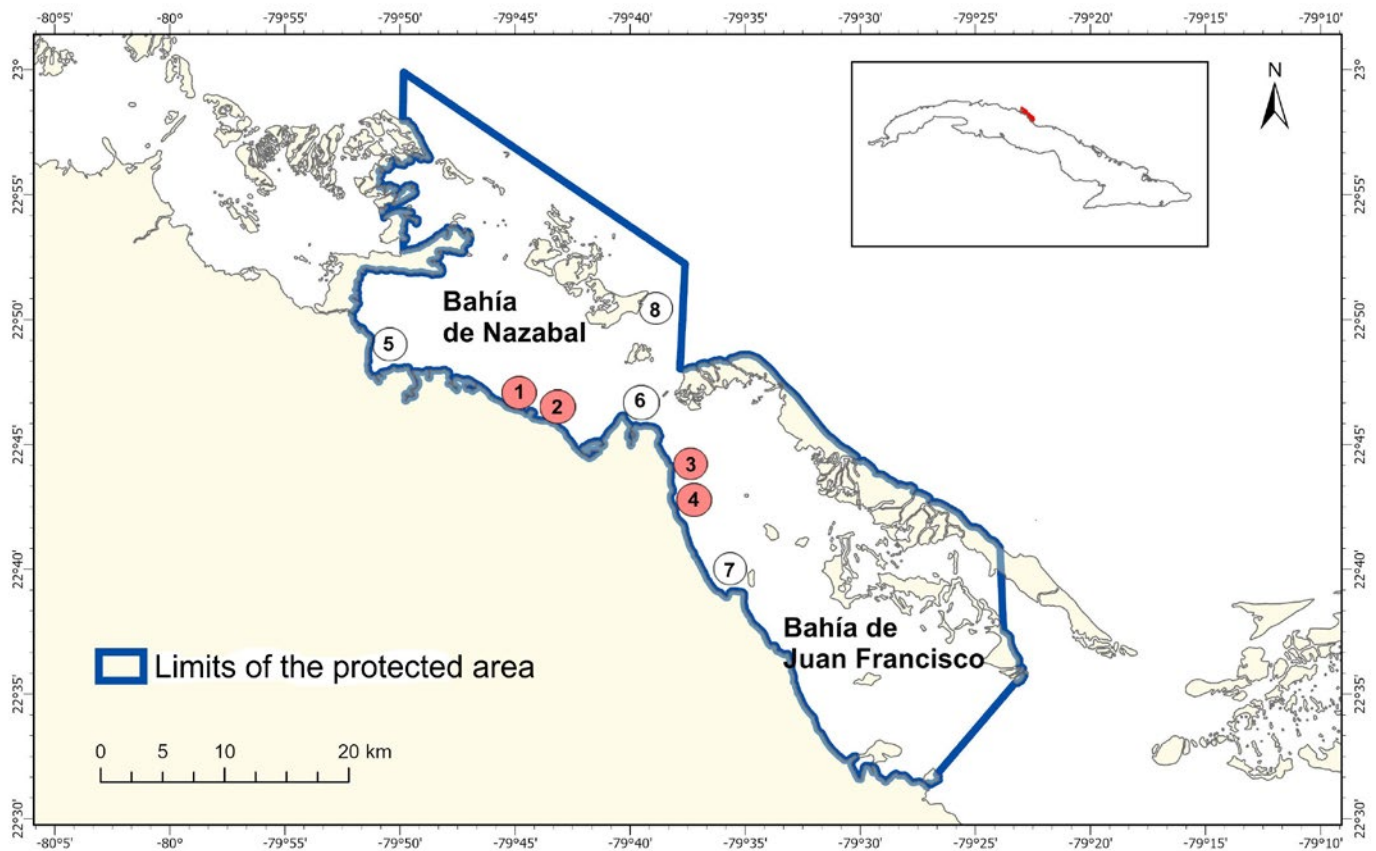


Fig. 1. Study area and location of sampling stations at Lanzasillo-Pajonal-Fragoso Wildlife Refuge, Sabana-Camagüey Archipelago. In orange color the stations previously evaluated by Russet-Rodríguez *et al.* (2019) and in white, the new stations incorporated in the present work. 1- Canal de Córdoba (22.778025; -79.745230), 2- Jácate (22.760954; -79.717489), 3- Cubereta (22.734580; -79.635796), 4- Juan Francisco (22.709700; -79.635060), 5- Estero de Granadillo (22.805754; -79.852374), 6- Río Sagua la Chica (22.762504; -79.650977), 7- Guaisy (22.656318; -79.599644), 8- Punta El Pino (22.851168; -79.647211).

filter paper (medium filtration) and transferred to a volumetric with 50.0 mL of capacity with distilled water. Finally, the solution was transferred to amber bottles for subsequent analysis by Flame Atomic Absorption Spectrophotometry (FAAS).

The quantification of the metal elements Cd, Cu, Ni, Pb, Zn, Co, Fe and Mn in the sediment samples by FAAS, was carried out according to the Standard Operating Procedures of the Atomic Absorption Laboratory at Higher Institute of Technologies and Applied Sciences (InSTEC): GAA.PNO.02 and

GAA.PNO.14. For this, a spectrophotometer model 4530F, INESA brand was used.

For the determination of the Cr content in the sediment samples, ED-XRF was used. For this, 4.0 g of each sample (dry and particle size less than 63.0 μm) was weighed and 1.0 g of cellulose was added, mixed well and pressed with a pressure between 15-20 tons, to obtain cylindrical tablets with a diameter of 2.5 cm and a height of approximately 0.4 cm. The thickness obtained is adequate to use the infinite thickness analytical method.

Pellets obtained were analyzed by ED-XRF, using a radioisotopic source of direct excitation in a central

configuration of plutonium (^{238}Pu) with ring geometry supplied by AMERSHAN, with an activity of 1.1 GBq. The spectrometer used was a Si (Li) detector model ESLX 30-150 No. 319 from CANBERRA, with an energy resolution of 150 eV for the $K_{\alpha 1,2}$ line of Mn (5.8 KeV) and a window thickness 12 μm Beryllium, supplied at -500 V by an NHQ105M source from ISEG. The rest of the measurement chain consists of a PSC 954 preamplifier, a model 2026 spectroscopic amplifier, (12 μs pulse shaping time) and a model 8075 ADC operating at 2048 channels, all from CANBERRA. The spectrum acquisition program is winTMCA32 and all the spectra were processed with the WinAxil version 2.5 code. The measurement time was 4 hours of live time and the dead time was kept below 3 %.

To guarantee the correct performance of the analytical procedures used, the following analytical parameters were evaluated: detection limit, quantification limit, accuracy and precision, according to the criteria contained in the document Analytical Methods Adequate for Your Purpose. Laboratory Guide for the Validation of Methods and Related Topics (Eurachem, 2005). For this purpose, a marine sediment reference material for trace metals (MAT-SS-0105) were used in order to evaluate the quality of the analytical analysis. In addition, blank samples, check samples, and duplicate samples were used. The detection limits of FAAS analytical procedure were estimated from three times standard deviation from repeated analysis of Cd, Cu, Ni, Pb Zn, Co, Fe and Mn, and the quantification limits were estimated from five times standard deviation from repeated analysis of those metals as suggest the Eurachem (2005).

Quality control of the ED-XRF analysis

The infinite thickness condition made possible to calculate the concentrations without the need of knowing the efficiency of the system and to make use of the relative method of external standard. For the calibration by external standards, the Certified

Reference Materials (CRM) were used as standards: IAEA Soil-5 "Soil Sample", IAEA Soil-7 "Soil Sample", IAEA-356 "Polluted Marine Sediment", IAEA SL-1 "Lake Sediment" (all supplied by the IAEA), BCSS-1 "Marine Sediment" from the National Research Council of Canada, BCR-2 "Basalt Columbia River", SGR-1 "Green River Shale", MAG-1 "Marine Sediment".

To evaluate the quality control of the analysis, the MRC IES-951 was used applying the McFarrel criterion (Geigert, 2002), which provides a measure of the quality of the analysis. The analytical magnitude Reference Service (RS) is then calculated:

$$RS = \frac{|C_{exp} - C_{rep}| + 2\sigma}{C_{rep}} * 100 \%$$

Where: C_{exp} is the experimentally determined concentration of the element, C_{rep} is the concentration reported for the reference sample and σ is the standard deviation of C_{exp} . A value of $RS \leq 25 \%$ indicates an optimal analysis, if it is $25 \% < RS \leq 50 \%$ the analysis is considered acceptable and for $RS > 50 \%$ the method is considered unacceptable for quantification. According to the concentration values detected and reported for Cr in the MRC IES-95 of 90.1 (± 11.0) and 89.6 (± 10.9) respectively, it is determined that the quantification method is optimal for the analysis ($SR = 25 \%$).

Environmental quality of the marine sediment samples

For the environmental evaluation of the sediments, the Sediment Quality Guide of the National Oceanological and Atmospheric Agency of the United States of America (NOAA) were used (NOAA, 2008). In this case, it was considered four levels of toxicity in marine sediments: Threshold Effects Level (TEL), under this level the sediment is considered not toxic; Low Range Effects (LRE) at which toxic effects are expected at low probability; Probable Effects Levels (PEL)

for concentration above this level, there is a high probability of toxic effects on organism living in those sediments; Medium Range Effect (MRE) concentration above which adverse environment effects will frequently occur.

Also, two enrichment indexes were selected to attribute a contamination value (geoaccumulation index and enrichment factor), an ecological risk index (average ratio of moderate risk effects) and a contamination index (modified degree of contamination).

Background enrichment index

Geoaccumulation index (I_{geo})

The degree of contamination can be calculated using the so-called geoaccumulation index, which is calculated using the Müller equation (Müller, 1979):

$$I_{geo} = \log_2 \frac{C_n}{(1.5B_n)}$$

Where: C_n is the measured concentration of the metal "n" in the sediment to be investigated, B_n is the concentration of the metal "n" in the bottom sediment. The factor 1.5 is introduced to minimize the effects of possible variations in the background values. According to the Müller geoaccumulation index, the levels of contamination of the sediments are classified as: Not contaminated ($I_{geo} \leq 0$); not contaminated to moderately contaminated ($0 \leq I_{geo} \leq 1$); moderately contaminated ($1 \leq I_{geo} \leq 2$); moderately contaminated to heavily contaminated ($2 \leq I_{geo} \leq 3$); heavily contaminated ($3 \leq I_{geo} \leq 4$); heavily contaminated to extremely contaminated ($4 \leq I_{geo} \leq 5$); extremely contaminated ($I_{geo} \geq 5$) (Müller, 1979).

Enrichment factor (EF)

The enrichment factor was normalized with respect to Fe, used as reference metal for the determination of this index, using the outermost point (Point 8, see Fig. 1) as background of the study zone, which contains the lowest concentrations of the metallic elements studied. The EF was calculated according to the following equation:

$$EF = \frac{\left(\frac{[X]}{[Y]}\right)_{sample}}{\left(\frac{[X]}{[Y]}\right)_{background}}$$

Where: $[X]_{sample}$ is the metal concentration in the sample, $[Y]_{sample}$ is the concentration of the conservative element in the sample, $[X]_{background}$ is the background metal concentration and $[Y]_{background}$ shows the concentration of the background conservative element based on the lowest value found in the study area as proposed by Birch (2003). According to the same author, it indicates that there is no enrichment when ($EF < 1$), small ($1 < EF < 3$), moderate ($3 < EF < 5$), moderately severe ($5 < EF < 10$), severe ($10 < EF < 25$), very severe ($25 < EF < 50$) and extremely severe ($EF > 50$) (Schropp *et al.*, 1990).

Ecological risk index

Mean ratio of moderate risk effects (m-MRE-Q)

The m-MRE-Q considers in a combined way the adverse potential of pollutant groups, thus offering a more realistic assessment of the toxicity of sediments.

$$m - MRE - Q = \frac{\sum_{x=1}^n C_x / MRE_x}{n}$$

Where: C_x is the concentration of X element in the sediment, MRE_x is the concentration value of the X element that causes moderate effect in the biota according to what it is established in the Sediment Quality Guidelines (NOAA, 2008), and n is the number of elements. The classification of the sediment contamination degree according to this index is: not contaminated for m-MRE-Q < 0.1 ; slightly contaminated when $0.1 < m-MRE-Q < 0.5$; moderately contaminated if $0.5 < m-MRE-Q < 1.5$; and highly contaminated for m-MRE-Q values > 1.5 (Quevedo *et al.*, 2014).

Contamination index

Modified degree of contamination (mCd)

This index evaluates the sediment contamination degree for a given sample according to the following equation:

$$mCd = \frac{\sum_{i=1}^n C_i}{n}$$

Where: C_i is the metal measured concentration in the sample under study; B_i is the background metal concentration, and n is the numbers of metal. Depending on the mCd values obtained, the sediment can be classified according to its contamination as: zero to low contamination: ($mCd < 1.5$); low contamination: ($1.5 \leq mCd \leq 2.0$); moderate contamination: ($2.0 \leq mCd \leq 4.0$); high contamination: ($4.0 \leq mCd \leq 8.0$); very high contamination: ($8.0 \leq mCd \leq 16.0$); extremely high contamination: ($16.0 \leq mCd \leq 32.0$) and ultra-high contamination: ($mCd \geq 32.0$).

Results and discussion

In order to evaluate the quality and performance of the analytical procedures in the quantification of Cd, Cu, Ni, Pb Zn, Co, Fe and Mn by FAAS and using the modified normative ISO 11466 for the chemical treatment of the sediment samples, different quality analytical parameters were evaluated. As results, the detection limits (DLs) and quantification limits (QLs) values ($\mu\text{g/g}$ dry weight) of each metal were as follows: Cd (0.4; 0.6), Cu (0.03; 2.5), Ni (1.5; 6.7), Pb (2.2; 20.6), Zn (0.1; 1.7), Co (0.5; 2.9), Fe (3.5; 4.5) y Mn (0.1; 1.7). In order to determine whether the analytical method selected in this work was adequate to subsequently evaluate the environmental quality of the sediments under study, in terms of metallic contamination, the Threshold Effects Level (TEL) reported by the Sediment Quality Guide (NOAA, 2008) for each metal was taken as a reference, since there are no sediment guides in our country. In all cases, the estimated DLs and QLs are lower than the TEL reference concentrations established by NOAA (Buchman, 1999), as report Table S1 (Supporting information) which shows that it is possible to quantify the metallic elements studied, even below the concentration limits established in the Sediment Quality Guide (NOAA,

2008). In this sense, it is important to notice that Co, Mn and Fe do not present reference values in the guide used, for that reason the establish quality reference values (QRVs) by Alfaro (Alfaro *et al.*, 2015) was used as reference. The QRVs values were as follows ($\mu\text{g/g}$): Co (25), Mn (1947) and Fe (54.055), this suggests that the analytical methods employed will allow to quantify those metallic elements.

The analytical performance parameters obtained for accuracy evaluation of the analysis method for the quantification of the metallic elements under study are reported in Table S2 (Supporting information 1). Results indicated that the difference between the analyzed values and reference values was lower than 5 %, compared with the certified values in MAT-SS-0105; this indicate the veracity of the method. On the other hand, the recoveries of the check samples ranged from 91.5 % to 108.3 %, this demonstrates that there are no losses in the sample handling, acidic digestion and measurement process. For that reason, the method is precise and accurate, therefore exactly, which guarantees the reliability of the results and it can be used for the marine sediments analysis under study.

The values obtained for the organic matter (OM) content in the studied sediments, ranged between 4.8 and 32.1 % of the total weight of the individual sample (Fig. 2). The sample with the highest OM content was the one collected in station number 8, although stations 6 and 7 also showed OM values above 20 %, while stations 3, 4 and 5 yielded values between 10-20 %. Only stations 1 and 2 had OM content below 10 %. As can be seen, in general the OM values obtained in the present work has high values taking into account that the OM in marine sediments oscillates between 0.1 and 10.0 % (Establier, 1984).

In the study area, there are stations with OM content higher than what it is established by the ISO 11466 standard. The implementation of this standard use for sediments chemical digestion *aqua regia*, however, one of the limitations of this standard is that it cannot be

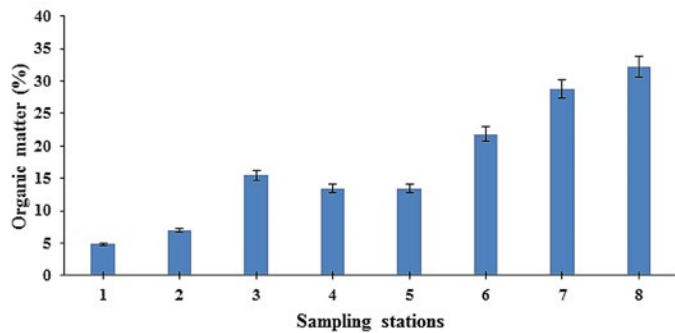


Fig. 2. Organic matter content in sediments per sampling station. Numbers 1-8 represent samplings stations (for name reference see Fig. 1).

applied to samples containing organic carbon levels above 20 %. That is why the HNO_3 content must be higher than that of HCl (inverted *aqua regia*), to achieve the total dissolution of the organic matter present. Taking into consideration the above, the modified ISO 11466 standard was used to carry out the partial acid digestion of the sediments under study. This modified regulation was implemented and validated at the Environmental Analysis Laboratory of the Higher Institute of Technologies and Applied Sciences for the determination of Hg, Cd, Cu, Ni, Pb, Zn, Co, Fe, Mn and Cr in sediment and soil samples. (González *et al.*, 2014; Herrera *et al.*, 2015).

The range of variations (maximum and minimum values), mean concentrations, and relative standard deviations (RED) for each element analyzed are reported in Table 1. As results, a wide dispersion of pollutant's levels can be observed in the study area. Only for Cd, in 50 % of the total samples analyzed, the values were

lower than the quantification limit of this element even though we worked with a sample mass of 3.0 g, which is the maximum allowed by the acidic digestion method used. Likewise, there was a high variability of metals levels among sampling stations, since the coefficients of variation expressed as the relative standard deviation, varied between 34.6 % for Zn and 72.6 % for Co.

Levels of metallic elements obtained in this study are compared with other sites located in the northern part of Cuba and with other protected areas worldwide (Table 2). In general, the concentration levels of the metallic elements quantified in the present study are in the range of those obtained by Cubans and foreign researchers. But, comparing our results with Sagua La Grande, Cayo Coco, Cayo Guillermo and Guardalavaca areas in Cuba, it can be observed that these values are higher than the reported for those places. However, in the specific case of Cu, Pb and Zn the concentration levels are lower than Atarés, Bahía de La Habana, Bahía de Matanzas and Moa, Holguín. With respect to other protected areas worldwide, we can find tenors both higher and lower than those of our area of interest. The point with the lowest concentration of Cu and Zn turned out to be Punta El Pino (Point 8) and the one with the highest levels was Juan Francisco (Point 4) and Cubereta (Point 3), respectively. In the case of Pb, the highest value was obtained in Guaisy (Point 7).

In the case of Cd, as can be seen, the levels obtained in our work was similar to those reported for the Szeroki Bór Peat Bog National Park, Poland (Borgulat *et al.*, 2018) and the Caohai Plateau Wetland National

Table 1. Variation of metal levels in collected sediments.

Metal	Cd	Cu	Ni	Pb	Zn	Co	Fe	Mn	Cr
Maximum ($\mu\text{g/g}$)	1.4	39.5	600.3	11.4	66.9	94.2	46432.9	1441.1	13397.9
Minimum ($\mu\text{g/g}$)	< 0.4*	9.2	108.5	< 2.2*	11.6	< 0.5*	3891.8	52.9	5.8
Mean ($\mu\text{g/g}$)	0.8	23.7	336.6	6.8	45.8	37.3	24808.1	728.1	3563.0
RED (%)	54.0	44.2	44.5	36.2	34.6	72.6	46.6	66.8	71.8

*Values under quantification limit

Table 2. Comparison of the concentration of metallic elements with the marine sediments of other sites in the north of Cuba and other protected areas worldwide.

Sample site	Metallic element concentrations ($\mu\text{g/g}$)									Reference	
	Mn	Fe	Co	Zn	Pb	Cu	Ni	Cd	Cr		
Canal de Córdoba	1358	24588	42	38	Nd	13	269	Nd	10691	Current study	
Jácate	842	25045	45	61	3	31	410	Nd	13398		
Cubereta	1441	46432	94	66	6	37	600	0.44	1245		
Juan Francisco	647	34954	35	54	6	40	436	Nd	1326		
Estero de Granadillo	762	17728	8	46	Nd	16	218	Nd	436		
Río Sagua la Chica	341	24435	27	42	6	20	435	0.36	1216		
Guaisy	379	21387	8	44	11	24	216	0.99	188		
Punta El Pino	52	3891	Nd	11	8	9	109	1.42	6		
Atarés. Bahía de La Habana. Cuba	373	3 %	12	922	93	135	76	-	156		(Díaz-Arado <i>et al.</i> , 2009)
Bahía de Matanzas. Cuba	190	1.3%	-	9-294	6-114	9-200	7-124	-	-		(Quevedo <i>et al.</i> , 2014)
Sagua La Grande. Cuba	0.4%	2.17 %	7	38	11	21	117	Nd	228	(Varela, 2010)	
Cayo Coco. Cuba	4	0.5%	-	4	7	11	13	-	43	(Vaillant, 2019)	
Cayo Guillermo. Cuba	35	0.5%	-	6	8	22	17	-	4		
Guardalavaca. Cuba	64	0.5%	-	5	8	6	16	-	38		
Moa. Cuba	19700	12940	388	286	29	79	280	-	8200		
Szeroki Bór Peat Bog. Poland	7	2000	-	15	49	-	-	0.5	-	(Borgulat <i>et al.</i> , 2018)	
Caohai Plateau Wetland. China	-	-	-	458	54.3	23.3	-	1.74	58.6	(Hu <i>et al.</i> , 2017)	
Kizilirmark Delta. Turkey	315	724	-	72.3	38.5	-	318	-	-	(Engin <i>et al.</i> , 2017)	
Roztocze. Poland	22.4	-	-	65.2	1.83	11.3	1.52	-	0.6	(Mazurek <i>et al.</i> , 2017)	
Kanwarjheel. India	349	48673	-	150	-	202	76.8	-	312	(Singh & Jayakumar, 2017)	

(Nd): Under quantification limit

(-): Undetermined

Reserve, China (Hu *et al.*, 2017). According to the consulted literature, it was found that the quantification of this element in sediment samples is scarce and in many study cases this analyte is below to the quantification limits. This behavior is shown by the works reported for Cuba (Cervantes-Guerra *et al.*, 2017; Díaz-Arado *et*

al., 2009; Quevedo-Álvarez *et al.*, 2014; Varela, 2010; Vaillant, 2019) where this element was not detected. However, our results are in correspondence with the reported range for Cuban soils (0 - 2.30 $\mu\text{g/g}$) by Muñiz (2008). The point where the highest levels of Cd were found in our study turned out to be Punta del Pino

(Point 8) while in half of the other stations it was not possible to quantify this metal.

On the other hand, the levels of Cu, Pb and Zn also reported in the northern zone of Villa Clara show similar values to those obtained in this work, but they are lower than other studies within Cuba. With respect to other protected areas worldwide, we can find tenors both higher and lower than those of our area of interest. The point with the lowest concentration of Cu and Zn turned out to be Punta El Pino (Point 8) and the one with the highest levels was Juan Francisco (Point 4) and Cubereta (Point 3), respectively. In the case of Pb, the highest value was obtained in Guaisy (Point 7).

Regarding Ni, the levels obtained in our study generally exceed those reported for studies carried out, both in Cuba and in the world. Stations of Jácate (Point 2), Cubereta (Point 3), Juan Francisco (Point 4) and Río Sagua (Point 6), showed higher values than those reported for Moa, also known as Capital of Nickel in Cuba (Cervantes-Guerra *et al.*, 2017). However, the level of this element in station 8 (109 µg/g), although under those reported for Moa (Cervantes-Guerra *et al.*, 2017), are close to the average (122.3 µg/g) reported by Muñiz (Muñiz, 2008). This could indicate an accumulation of this metal may be occurring in our study area and it could be a possible trigger of fibropapillomatosis found in juvenile turtles since it is known that it can cause immunosuppression (Adnyana *et al.*, 1997). The point with the maximum contamination by this element was Cubereta (Point 3) and the minimum was obtained in Punta del Pino (Point 8). High levels found near Cubereta (Point 3) could be the result of the presence of biological and physical contamination near this station and, in addition, previous studies affirm that this point presents high contamination with Ni (Russet-Rodríguez *et al.*, 2019). So it can be considered that there is a risk of contamination with respect to this metal.

The metals Mn and Co reached higher values, if we compare them with other studies from Cuba, with the exception of the study carried out by Cervantes-Guerra

et al. in Moa (2017), where values were higher. As for the studies carried out in other countries, they presented levels lower than those determined in the present work for the case of Mn or not reported as occurs for Co. Again, the point with the maximum contamination for these elements was Cubereta (Point 3) and the minimum was obtained in Punta del Pino (Point 8).

Iron is an element that is found in high concentrations in the sediments and soils of Cuba, due to the geological characteristics of our country (Obregon *et al.*, 1986). In this study, levels of this analyte were higher than those reported for other regions of the Cuban archipelago and other areas of the world. Only in the Kanwarjheel Sanctuary, India (SINGH & Jayakumar, 2017), values were higher than those obtained in the present work. Maximum concentration value for this element that was found in Cubereta (46 432 µg/g), was similar to the one obtained by Herrera-Martínez (2015), in 2010 (44 023 µg/g) at Puente de Husillo sampling station, on the Almendares river, La Habana, Cuba. In that station, according to Herrera-Martínez (Herrera-Martínez, 2015), the main source is sewage waters. Punta El Pino (Point 8) was the only site with concentrations lower than those found in Moa.

When comparing Cr concentration values obtained in the present work, with those reported for other regions of our country and the world, they are much higher. Stations 1 (Canal de Córdoba) and 2 (Jácate) showed the highest levels with 10 691 µg/g and 13 398 µg/g, respectively; values exceeding those obtained by Cervantes-Guerra *et al.* (2017) in Moa. Only three stations (5, 7 and 8) are in the range values for Cr reported by Muñiz (2008) in Cuban soils, which was 2.0 - 896.0 µg/g. Our study area is very close to the province of Camagüey, where mineral deposits of this metal are found (Simba-Wanga, 2021). However, taking into account the harmful effects of this element, even in low concentrations, for the environment and living organisms, it is suggested to further evaluate which could be the sources of its incorporation into the environment

in the study area and carry out studies of speciation of this element. The point with the maximum contamination by this element was Jácate (Point 2) and the minimum was obtained in Punta del Pino (Point 8).

Stations with the highest levels of metallic elements in our study were located in Nazábal Bay where the largest number of turtles with fibropapilomas was found (Russet *et al.*, 2021). Therefore, these high levels of contamination could be related to the presence of the disease in green turtles in the Protected Area.

Sediment Quality Guides

Fig. 3, shows the concentration of the nine metallic elements under study and their comparison with the Sediment Quality Guide (NOAA, 2008), when available. As it can be observed, Zn and Pb are only the elements below the Threshold Effects Level, therefore they do not represent an environmental concern in the study area. However, in the case of Cr, Cd and Cu, one (8), two (3 and 6) and three (1, 5 and 8) sampling stations did not exceed this guide criterion respecting to TEL, respectively. Therefore, beside two stations (7 and 8) for Cd and four stations (2, 3, 4 and 7) stations for Cu exceed the Low Range Effects (LRE) these values are close to this LRE. On the other hand, Ni at all sampling stations exceeded the maximum values of the Medium Range Effect (MRE) concentration above which adverse environment effects will frequently occur. This behavior is more remarkable in station 3. Similar behavior to Ni was obtained for Cr, Punta El Pino station (Point 8), as was mentioned above, was the only area that did not exceed the TEL value, however, the most affected sites by Cr with the most highest levels of concentration for this element are Canal de Córdoba (Point 1) and Jácate (Point 2).

Co, Mn and Fe do not present reference values in the Sediment Quality Guide used as was mentioned before, so it was consider appropriate to compare their levels with studies carried out for other marine sediments quantification of the Cuban north coast (See

Table 2). Those results which can give us information about the source of these metals, if they come from anthropogenic sources, or are normal values for the country sediments. According to the high levels of Cr, Ni and Fe found in the present study, anthropogenic effects can be established since their levels in the Protected Area differ highly from the average values found in the northern area of Cuba (Cervantes-Guerra *et al.*, 2017; Diaz-Arado *et al.*, 2009; Quevedo-Álvarez *et al.*, 2014; Varela, 2010; Vaillant, 2019).

Pollution indicators

Enrichment factor

For most of the elements, the contents of the analytes in the sediments are higher than the usual contents in carbonate sedimentary rocks, so it is possible to think about anthropogenization. Therefore, the enrichment factor (EF) was used to differentiate between the natural or anthropogenic contributions of the elements levels.

In Fig. 4, it is observed that the elements Cd, Co, Cu, Ni, Pb and Zn no show an enrichment in all the sampling points evaluated in the present study. In the case of Mn, it presents a slightly higher enrichment compared to the other elements in most of the sampling points, except in the Canal de Córdoba (Point 1) and Estero de Granadillo (Point 5) stations, where there is a higher value of this factor corresponding to a moderate enrichment.

For the case of Cr, the area is characterized by a severe enrichment by this metallic element, obtaining EF values > 10 in all the sampled points (Fig. 5), except for the sampling stations 7 and 8 (moderate to severe enrichment). The two maximum values of this index, 291.9 and 359.1, were obtained for Canal de Córdoba (Point 1) and Jácate (Point 2), respectively; where the EF obtained exceeds the lowest content to consider the area as extremely enriched by this element.

Therefore, the EF shows that there could be a greater probability that the Cr element represents a greater

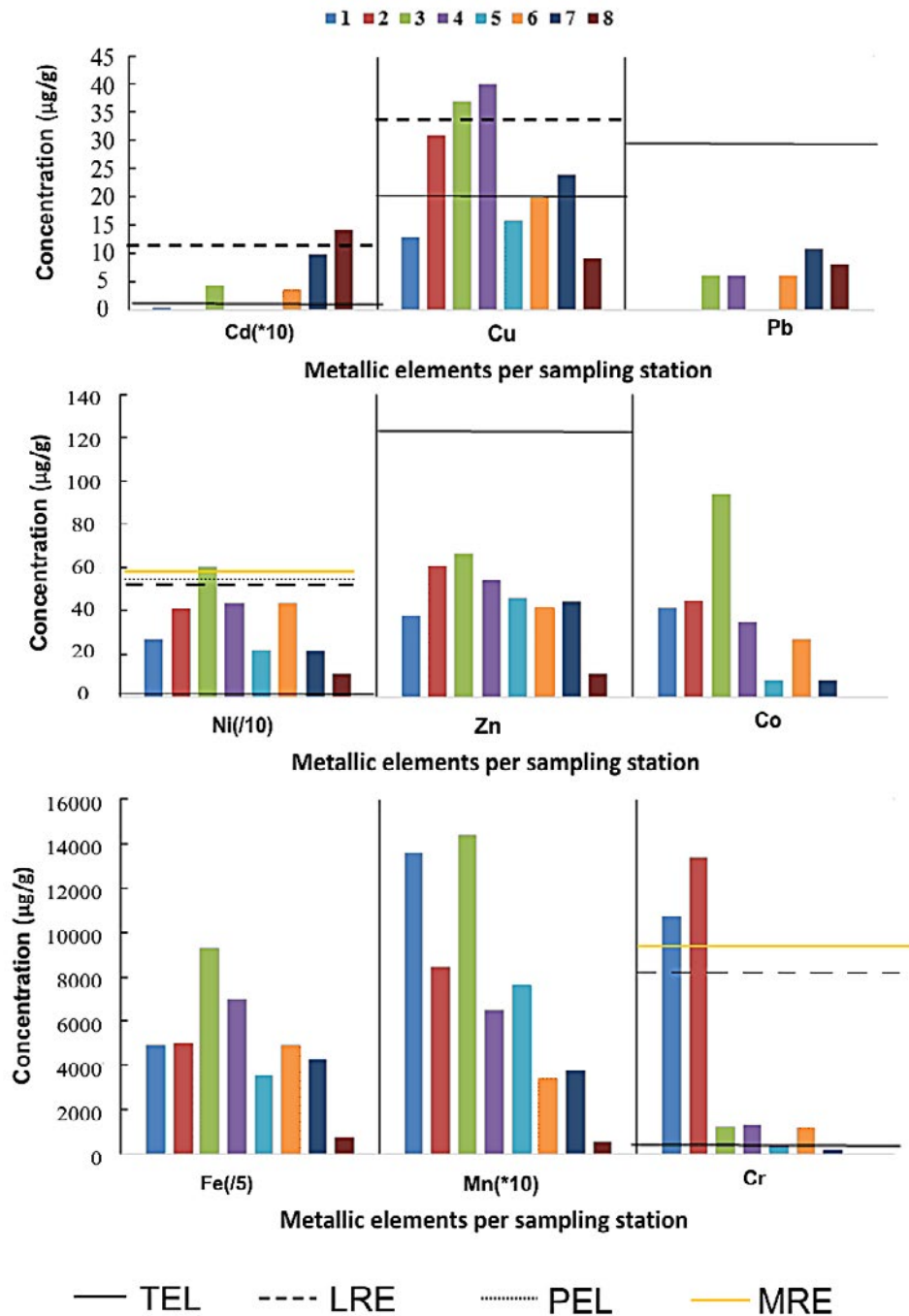


Fig. 3. Comparison of Cd, Cu, Pb, Ni, Zn y Cr concentrations in each sampling site with NOAA Reference Guide concentrations. Threshold Effects Level (TEL), under this level the sediment is considered not toxic; Low Range Effects (LRE) at which toxic effects are excepted at low probability; Probable Effects Levels (PEL) for concentration above this level, there is a high probability of toxic effects on organism living in those sediments; Medium Range Effect (MRE) concentration above which adverse environment effects will frequently occur. Risk level increase in the following direction: TEL < LRE < PEL < MRE. Numbers 1-8 represent samplings stations (for name reference see Figure 1). For the elements Co, Fe and Mn, no data was reported by the Sediment Quality Guide (NOAA, 2008)

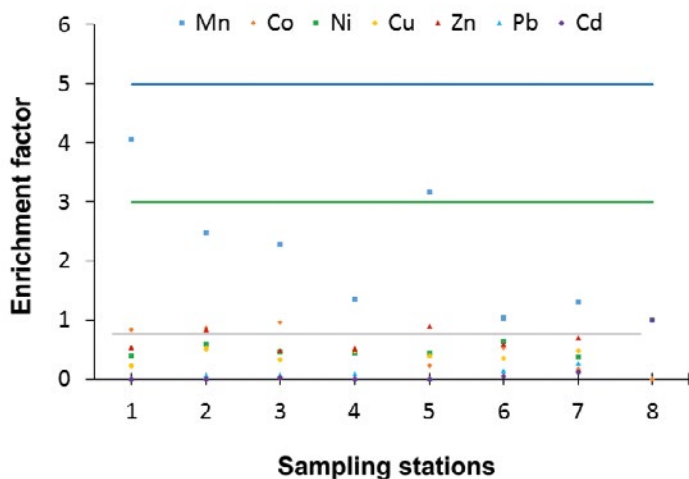


Fig. 4. Enrichment factor for each station for Mn, Co, Ni, Cu, Zn, Pb and Cd. The black line represents the limit of no enrichment; green line represents the value from which a moderate enrichment begins to appear and the blue line separates the limit for severe enrichment. Numbers 1-8 represent samplings stations (for name reference see Fig. 1)

risk for the ecosystem of Lanzanillo-Pajonal-Fragoso Wildlife Refuge. For this reason, it is recommended to monitor this element by the corresponding authorities, as well as, to verify the sources of its incorporation into the environment. The fact that the Cr contents in the

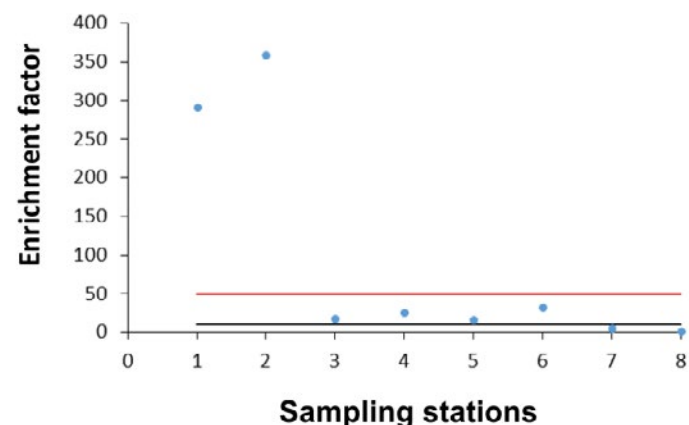


Fig. 5. Enrichment factor for Cr in each sampling station. The black line represents the value from which severe enrichment begins to appear and red line indicates extremely severe enrichment. Numbers 1-8 represent samplings stations (for name reference see Fig. 1)

evaluated sediments are higher than the rest of the evaluated analytes, leads us to think of their anthropogenization for this element.

Geoaccumulation index

In most of the stations, the elements presented an $I_{geo} < 2$, which indicates that the evaluated sediments are considered not contaminated or moderately contaminated by the metallic elements under study (Fig. 6). The Mn stands out with an I_{geo} between 3 and 5, practically in more than 50 % of the stations studied, which classifies the sediments from these stations as heavily to extremely contaminated with Mn.

Fe appears with a moderate to heavily level of contamination, presenting a correlation with respect to the areas of greatest contamination determined for Mn. Specifically, the Co is also highlighted in the Cubereta station (Point 3), which presents a heavily contamination by this element according to this index, although the levels found are lower than the studies reported in the vicinity of the sampling area, this station also brings coupled with a moderate deterioration by the element Ni where the highest level of this element was also obtained.

Meanwhile, Cr shows a tendency to extreme contamination in the entire evaluated area with I_{geo} values higher than 10 (Fig. 7). These results are in agreement with those obtained for EF, observing that points 1 and 2, which present a higher I_{geo} , also present a greater EF.

Average ratio index of moderate risk effects

The average ratio index of moderate risk effects (m-MRE-Q) for each sampling stations is shown in Fig. 8. According to this index, only Punta El Pino (Point 8) is slightly contaminated ($0.11 < m\text{-MRE-Q} < 0.5$) which represents a 30 % probability of causing toxic effects. Two of the stations, Estero de Granadillo (Point 5) and Guaisy (Point 7), are classified as moderately contaminated with a 40 % probability of causing adverse

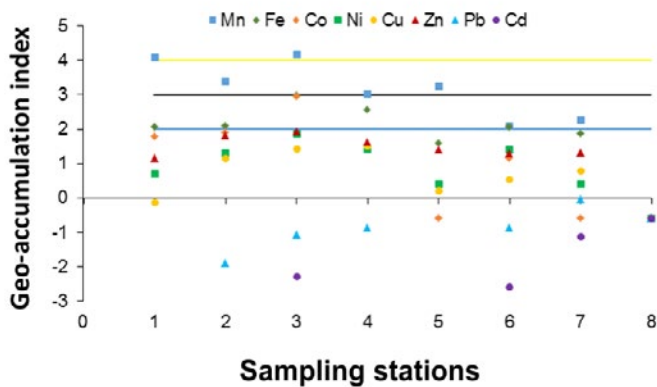


Fig. 6. Geo-accumulation index for Mn, Fe, Co, Ni, Cu, Zn, Pb and Cd. Blue line indicates the transit from moderate to strongly contaminated; black line, indicates strong contamination and yellow line separate strong contamination from extreme contamination. Numbers 1-8 represent samplings stations (for name reference see Fig. 1)

biological effects (Essien *et al.*, 2009). The rest of the sampling stations shows values respecting to this index greatest to 1.5, indicating a high contamination for metallic elements in this area. But this behavior is more remarkable in the sampling stations 1 and 2, which are in agreement with the EF and I_{geo} results, where Cr has

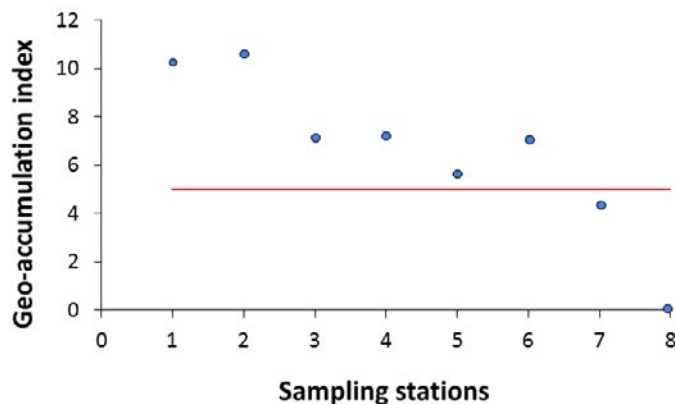


Fig. 7. Geo-accumulation index for Cr. Red line indicates extremely contaminated sites. Numbers 1-8 represent samplings stations (for name reference see Fig. 1)

the greatest contribution regarding metallic contamination in these points.

Similar results were obtained when comparing the total concentrations of the elements with the NOAA guidelines (NOAA, 2008). Canal de Córdoba (Point 1), Jácate (Point 2), Cubereta (Point 3), Juan Francisco (Point 4) and Río Sagua la Chica (Point 6) stations shows a high concentration levels of Ni and Cr (See Fig. 3) that represent a greatest ecological risk in this area. Therefore, the assessment of the contamination degree must be carried out on the basis of the complementation of the information provided by the different evaluated index.

Modified degree of contamination (mC_d)

The modified degree of contamination was calculated as an indicator of the ground contamination of the area in general. Values are between 1.0 and 261.3 for the elements under study. An average value of 72.2 was obtained, classifying the area with an ultra-high degree of contamination. In Fig. 9, the mC_d is observed for each of the sampling sites, where only in the outermost point, Punta El Pino (Point 8), the degree of contamination was zero or very low, so in the present work it was taken as a point of reference. Canal de Córdoba (Point 1) and Jácate (Point 2) by reaffirm the degree of contamination within the area under study ($mC_d > 32$), contributing to these high values the levels of Cr obtained in those points. The remaining stations are between high and extreme contamination degree.

Results obtained for this contamination index, agrees with those previously reported with the other index indicating that Cr provides the highest contamination to the stations where its value is extreme. It is also confirmed that the points where concentrations of metallic elements had been detected in previous studies correspond to the points where the modified degree of contamination presents the highest values.

These high levels could be influencing the health and well-being of the animals that inhabit this protected area,

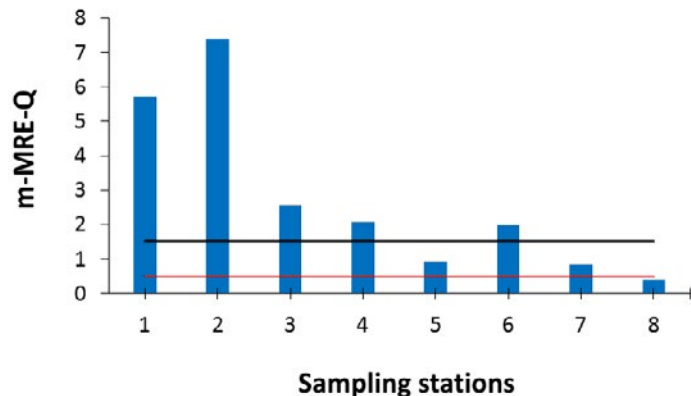


Fig. 8. Average ratio index of moderate risk effects (m-MRE-Q) by sampling stations. Red line indicates moderate contamination and black line, highly contaminated. Numbers 1-8 represent sampling stations (for name reference see Fig. 1)

mainly green turtles, in which the harmful effects that these metallic elements can cause fibropapillomatosis disease.

Conclusions

The concentration of Ni, Cr and Fe in the sediments collected in the study area were higher than the rest

of the analytes and Cd was the element with the lowest values in the entire area. The sampling stations where the highest levels of metallic concentration were found at Canal de Córdoba (Point 1), Jácate (Point 2) and Cubereta (Point 3), which coincides with the sites where the presence of these metallic elements had been qualitatively detected. Punta El Pino (Point 8) was the area with the lowest concentration values, which corroborates its use as a reference point for the area under study. According to the analysis of the NOAA quality sediment guide, in the Lanzanillo-Pajonal-Fragoso protected area, the elements that represent a greater risk are Ni and Cr because they exceed the concentration above which adverse effects will frequently occur. According to the evaluated index, there is anthropogenic contamination in the protected area that may be affecting the health of the turtles due to the stress it causes to them. The use of contamination indexes allowed determining Jácate (Point 2) as the site with greatest deterioration.

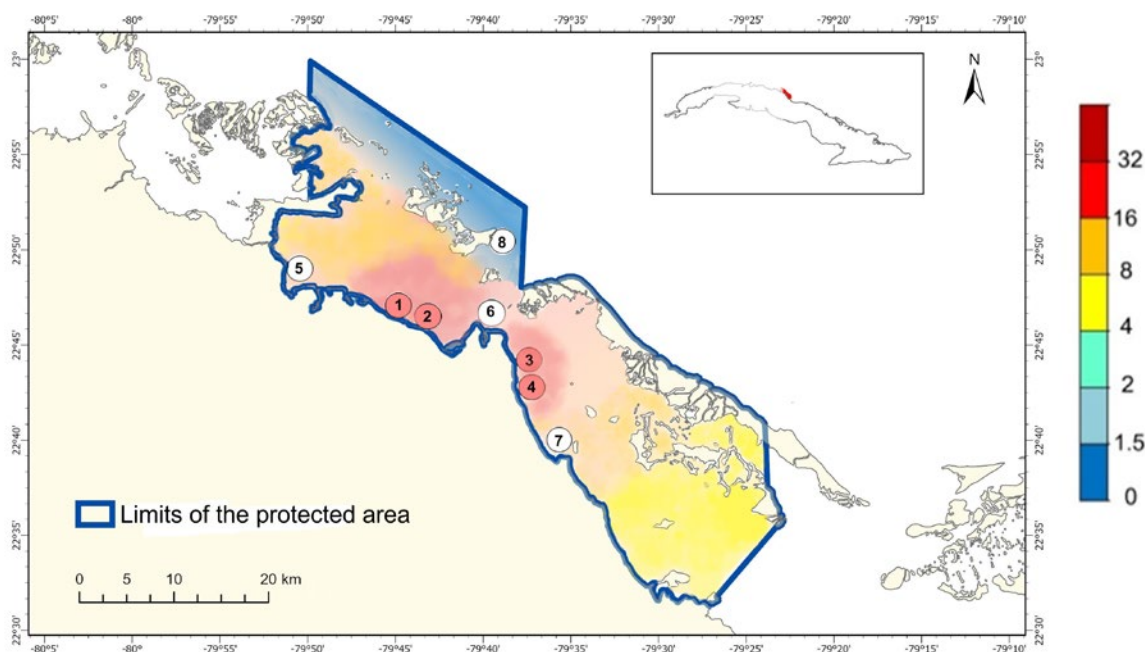


Fig. 9. Modified degree of contamination for the protected area "Lazanillo-Pajonal-Fragoso" at north of Villa Clara, Cuba. Numbers 1-8 represent sampling stations (for name reference see Fig. 1)

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Statements

Author Contributions

Conceptualization, J.A.R., K.M.R. E.G.A. and M.M.A.; Methodology, D.E.M., K.M.R. and J.A.R.; Software, D.E.M., K.M.R., M.M.A. and O.D.R.; Validation, K.M.R. and J.A.R.; Formal Analysis, D.E.M., K.M.R., M.M.A.; Investigation, D.E.M., E.G.A. and J.A.R.; Resources, K.M.R., O.D.R. and J.A.R.; Data Curation, D.E.M., K.M.R. and J.A.R.; Writing – Original Draft Preparation, D.E.M., K.M.R. and M.M.A.; Writing – Review & Editing, J.A.R.; Visualization, D.E.M. and J.A.R.; Supervision, K.M.R., M.M.A. and J.A.R.; Funding Acquisition, J.A.R.

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Conflict of interest

The authors have no financial or non-financial conflicts of interest to declare that are relevant to the content of the manuscript.

Ethical behaviour

The authors have followed all applicable international, national, and institutional recommendations related to the use and handling of animals for research.

Permits for sampling and other permits

No permits were required for the conduct of this research.

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Supporting information

Table S1: Values of DLs and QLs for each metallic elements.

Metals	Cd	Cu	Ni	Pb	Zn	Co	Fe	Mn
DLs ($\mu\text{g/g}$)	0,4	0,03	1,5	2,2	0,1	0,5	3,5	0,1
QLs ($\mu\text{g/g}$)	0,6	2,5	6,7	20,6	1,7	2,9	4,5	1,7
TEL ($\mu\text{g/g}$)*	0,7	18,7	15,98	30,2	124,0	nr	nr	nr

*Threshold Effect Level. nr: no data reported by the Sediment Quality Guide (NOAA, 2008)

Table S2: Analytical performance parameters obtained for accuracy evaluation of the method.

Metals	Metals ($\mu\text{g/g}$)	MR ($\mu\text{g/g}$)	t_{exp}	Repeatability (%)	Recovered (%)
Cd	34,5	26,7 - 36,0	3,2	0,4	98,9
Cu	25380,3	25407 - 30007	3,1	1,6	99,6
Ni	10268,4	7559 - 11228	3,1	1,3	95,5
Pb	977,6	759 - 1101	3,2	0,7	98,4
Zn	6961,3	6856 - 7759	3,1	1,1	108,3
Co	nd	nr	-	-	108,9
Fe	nd	nr	-	-	105,9
Mn	nd	nr	-	-	96,6

Note: t of Student value reported for $\alpha=0,005$ y $n=3$, $t_{\text{tab}}=3,3558$. nd: not determined. nr: no data reported for MAT-SS-0105 reference material passport.