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Heavy Metals Content and Ecotoxicity of Sediments from the Congo River

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Abstract: This research aimed to quantify the contamination of Congo River sediments by heavy metals and to evaluate their ecotoxicological effects in the municipalities of Maluku, Gombe, and Kinsuka, Kinshasa Province. Metal concentrations in sediments reached 340 mg kg^{-1} for Cr, 310 mg kg^{-1} for Cu, 826 mg kg^{-1} for Zn, 63 mg kg^{-1} for as, 1.9 mg kg^{-1} for Cd, 262 mg kg^{-1} for Pb and 2.9 mg kg^{-1} for total Hg. Contrasting with this, the metal concentrations in sediments from a control river with minor anthropogenic activities were one to three orders of magnitude lower. The Geoaccumulation Index indicated that sediment samples from the Congo River were "heavily polluted" to "extremely polluted" by Cu, Zn, As, Cd, Pb, and Hg, and the Enrichment Factor pointed out from "severe" to extremely "severe" enrichment of sediments in Cu, Zn, As, Cd, Pb and Hg. The high metal concentrations in the Congo River exceeded the recommended limits set up in the Sediment Quality Guidelines for the protection of aquatic life. Experimental toxicity tests carried out with ostracods (*Heterocypris incongruens*) exposed to sediments from the Congo River resulted in mortality percentages ranging from 15.9-72.6% for Maluku sediments and mortality percentages generally at 100% for sediments from Gombe and Kinsuka areas. Positive and also negative Spearman order correlation coefficients were observed between the analyzed metals, suggesting multiple sources and different environmental transport pathways for those metallic elements in the Congo River sediments. Globally, the results showed that sediments from the Congo River are heavily polluted by metals, representing a major toxicological risk to the aquatic ecosystem and a threat to public health through the consumption of contaminated water.

Keywords: Sediment Pollution, Heavy Metals, Ecotoxicological Risk, Toxicity Tests, Ostracods

Introduction

The quality of surface waters, groundwater, and soils can be deteriorated with pollution by organic and inorganic chemical compounds such as Persistent Organic Pollutants (POPs), pharmaceutical drugs, radionuclides, and heavy metals (Atibu *et al*., 2022; Ngweme *et al*., 2020; Kilunga *et al*., 2017; Laffite *et al*., 2016; Mwanamoki *et al*., 2014; Atibu *et al*., 2021; Carvalho, 2017a-b). In particular, the pollution by metals is of global concern because of their persistence in the environment and toxic effects on biota.

In sub-Saharan Africa, freshwater quality is often jeopardized because of intensive and uncontrolled waste discharges from anthropogenic sources such as industries, mines, hospitals, and untreated urban sewage containing toxic metals. This represents a serious threat to environmental and human health (Atibu *et al*., 2023;

Banze *et al*., 2022; Niane *et al*., 2019; Kayembe *et al*., 2018; Manna and Maiti, 2018). Action is needed to reduce the contamination of fresh waters and abate potential ecotoxicological risks posed by heavy metals. However, such action requires, as a starting point, scientific data from environmental monitoring and risk assessment studies in order to underpin the identification of pollutant sources, prevention of waste discharges, and implementation of measures to remediate the polluted sites (Atibu *et al*., 2023; Devarajan *et al*., 2016; 2015).

The main water body in sub-Saharan Africa is the Congo River. Its basin spreads over a total of 3.7 million square kilometers shared by ten countries. The Congo River is the second-longest river in Africa and the third-largest river in the world by water discharge volume. The Congo River harbors many activities, such as fishing, navigation, trade, hydroelectricity production, irrigation, and recreational activities. It is also a main source of drinking water for populations of the Congo Basin and it supports the livelihood of millions of human beings. In particular, in the lower Congo Basin, the river is a very important asset for the socio-economic development of the Democratic Republic of the Congo (DRC), the Congo-Brazzaville, and the Central African Republic.

Recently, some studies were carried out in this region namely on the concentration, distribution, and toxicity of heavy metals in soils, sediments, surface waters, and edible fish samples (e.g., at the DRC west coast at the Atlantic Ocean and on the Congo River at Maluku Kinsuka section) (Ngweme *et al*., 2020; Lundemi *et al*., 2022; Mata *et al*., 2020a-b; Suami *et al*., 2018; Mees *et al*., 2013; Alghamdi *et al*., 2019; Al-Robai, 2023). However, despite the relevance of the Congo River, few studies have been conducted to assess the status of this large freshwater ecosystem (Dupré *et al*., 1996; Verhaert *et al*., 2013 Mwanamoki *et al*., 2015). In particular, it is notorious for the total lack of information on the concentration and distribution of heavy metals and ecotoxicity of the Congo River sediments in the heavily populated municipalities of Maluku, Gombe, and Kinsuka, in the Kinshasa Province of the Democratic Republic of the Congo. Notwithstanding, the urgency of this information is crucial because the Congo River is the main source of water for the population of the Kinshasa province.

In aquatic systems, sediments are considered as pollutant reservoirs because they can accumulate both the organic and inorganic pollutants discharged into the environment (IAEA, 2004). The analysis of sediments enables the assessment of ecological and human health risks and also the possibility of retracing the pollution history of a watercourse (Thevenon and Poté, 2012; Wildi *et al*., 2004).

This study is an assessment of the contamination of Congo River sediments by heavy metals in the Kinshasa province. Furthermore, it is also an assessment of sediment toxicity to aquatic biota and a preliminary assessment of the implications to public health in the above-mentioned areas of the Democratic Republic of the Congo.

Materials and Methods

Description of Study Sites and Sampling Procedure

Three areas located along the Congo River in the DRC were selected. These were the areas of Maluku municipality, Gombe municipality, and Kinsuka town, all located in the Kinshasa province Fig. (1). These areas are port areas of the Congo River and were chosen based on the dense human population and intensive anthropogenic activities that take place therein. In these areas, the Congo River receives waste discharges from several anthropogenic sources located on the river banks. These waste discharges include untreated urban sewage, industrial effluents, and debris from activities such as fishing, markets, and operation of barges and whaleboats Fig. (2).

The Maluku sampling area is located in the municipality of Maluku, at the Northeast and upstream Kinshasa metropolitan area (Figs. 1-2). Maluku is a rural municipality with agricultural activities on the Plateau des Batékéǁ. It was once known for its steel factory which closed its doors in the 1980s. Maluku supplies the city of Kinshasa with most of the foodstuffs, such as vegetables, tomatoes, cassava, celery, eggplant, onions, potatoes, peppers, and corn. It also supplies Kinshasa with charcoal. In this area it is implanted the important fluvial port of Mazere where the activities related to trade, fluvial transportation, and fishing are based Fig. (2B). The sampling area at Maluku was located around the central coordinates: E 015° 32’ 51.5’’/S 04° 03’ 40.0’’.

The sampling area of Gombe is located in the Kinshasa metropolitan area (Figs. 1-2). Gombe is situated near the crossroads of the Limete municipality, by the industrial district of Kingabwa, which houses the Cité du Fleuveǁ district, several industries, and the majority of the port facilities of Kinshasa city and of the municipality of Barumbu. Industries such as cosmetics manufacture, cardboard production, a sawmill, a shipyard, a brewery, and a lemonade factory, are installed in this area. The GPS coordinates at the center of the 110 sampling area were: E 015°20' 04.2"/S 04° 18' 45.6".

The Kinsuka sampling area, commonly called "Kinsuka-pécheurs", is located in the municipality of Ngaliema at the Southwest and downstream of Kinshasa city (Figs. 1-2). Kinsuka-pécheursǁ is also located downstream of Ngaliema municipality, which hosts a shipyard, a large hospital center, rubble extraction quarries, and fairground activities by the river. As the name Kinsuka-pécheursǁ suggests, artisanal fishing is practiced there. The coordinates of the center of this sampling area were: E 015° 13' 59.7"/S 04°19' 43.9’’.

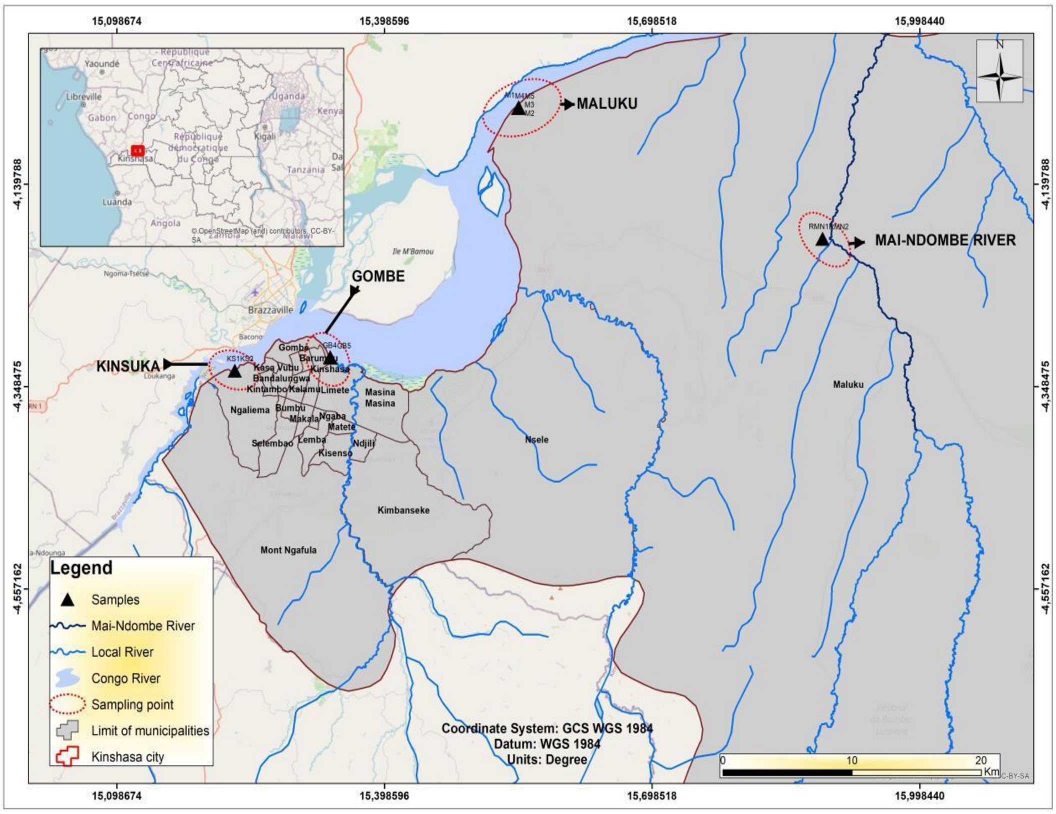

Fig. 1: Map of DRC in Africa and Congo River basin by Kinshasa region. Sampling points are indicated in the Congo River by the study areas of Maluku, Gombe, and Kinsuka and in the control area of the Mai-Ndombe River

Fig. 2: Human activities taking place in the areas of (A) Gombe, (B) Maluku, (C) Kinsuka, (D) Mai-Ndombe River (reference site with no human activity in the region) (Photos taken by D. MABIDI)

The Mai-Ndombe River, a tributary to the Congo River, was chosen as a control river for comparison (Figs. 1-2). The Mai-Ndombe River flows throughout the rural municipality of Maluku, Northeast of Kinshasa. In this river, the sampling area was located outside the Kinshasa metropolitan area, in a territory with forest cover and no significant human activity Fig. (2). Two sampling points were chosen therein: RMN1 (Coordinates: E 015° 58' 19.4" S 04° 16' 45.4") and RMN2 (Coordinates: E 015° 57' 59.2" S 04° 16' 40.3").

Surface sediment samples from both riverbeds were collected in April 2022 in the described areas. The sediment samples were collected at a distance of about 2 m from the shoreline and at about 1 m water depth. Samples were collected manually, using a plastic shovel, and transferred into 1.5 L clean plastic bottles, as described by Lundemi *et al*. (2022). Five samples were collected in each sampling area, and the distance from each other was approximately 50 m. The sediment samples, each with 250-300 g of surface sediment, were labeled as follows: M1, M2, M3, M4, M5 for the Maluku site; Gb1, Gb2, Gb3, Gb4, Gb5 for the Gombe site; Ks1, Ks2, Ks3, Ks4, Ks5 for the Kinsuka site; RMN1 and RMN2 for the Mai-Ndombe River.

All samples were stored at 4°C and transported in ice chest boxes to the analytical platform of the University of Geneva, Switzerland, for analysis.

Sediment Physicochemical Characteristics

The sediment samples were homogenized and aliquots were taken for analytical determinations, as needed. Sediment particle grain size, including mean grain size and percentages of clay, silt, and sand were determined using a Laser Coulter® LS-100 diffractometer (Beckman Coulter, Fullerton, CA, USA) as described in Banze *et al*. (2022).

According to Blott and Pie (2001); and IAEA (2004) when the average grain size is less than 2 µm the clay predominates in the sediment; when the average grain size is between 2 and 63 µm the silt predominates, while an average grain size between 63 µm and 2 mm indicates that the sample contains sand mainly.

The Water Content (WC) of samples was determined as the sediment weight loss following oven drying at 100°C until constant weight. The sediment Organic Matter (OM) and carbonate ($CaCO_3$) content were determined based on the weight loss of dry sediment aliquots following ignition during 1 h at 550 and 1000°C, respectively, in a muffle furnace (Salvis AG, Luzern, Switzerland) (Atibu *et al*., 2023; Poté *et al*., 2008).

Analysis of Heavy Metals in Sediments

Portions of the homogenized sediment samples were lyophilized, sieved, and dissolved in acids according to the protocols previously described in Atibu *et al*. (2023); Banze *et al*. (2022); Adler *et al*. (2016). In short, from the sieved grain size fraction <2 mm, nearly 1 g of an accurately weighed sediment aliquot was mixed with 10 mL of 2 m HNO_3 in "Teflon bombs" and heated at 105°C for 16 h for full dissolution of samples. The sample solution was diluted 200 times with 1% HNO_3 solution (Suprapur®, Merck KGaA, Darmstadt Germany). An aliquot of 10 mL of the diluted solution of metals extract was used for the determination of trace elements (Sc, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd, and Pb) with an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, Agilent 7700× series, Santa Clara, CA, USA).

For the purpose of analytical quality control, the lake sediment Certified Reference Material (CRM) LKSD-4 and the stream sediment certified reference material STSD-2, both from the National Research Council, Canada, and with matrices similar to those of the Congo River samples, were used to check the sample digestion procedure, the instrument responsiveness and the reliability of the results. The recovery yield values for both LKSD-4 and STSD-2 were higher than 95% for all metals targeted in this study.

The detection limits (µg kg^{-1}) for digested samples measured by ICP-MS, calculated as 5 times the standard deviation of the blank, were the following: Ti (0.098±0.033), V (0.024±0.008), Cr (0.000±0.000), Mn (0.150±0.050), Fe (0.681±0.227), Co (0.025±0.008), Ni (0.000±0.000), Cu (1.516±0.505), Zn (2.033±0.678), As (0.049±0.016), Cd (0.063±0.021), 170 Pb (1.019±0.364).

The determination of each trace element was conducted in triplicate and the results averaged. Standard deviations of the three replicate measurements were below 2.5% relative standard error and the chemical blanks were always less than 1.5% of the sample signal. The bias of metal determinations (coefficient of variation) was lower than 5% of the CRM metal concentrations. The results were expressed in mg kg^{-1} sediment dry weight (ppm).

In the analysis of total Hg, the mercury-specific Atomic Absorption Spectrometer Advanced Mercury Analyser (AMA, Altecs.r.l., Czech Rep.) was used, and the method described by Atibu *et al*. (2023); Bravo *et al*. (2011) was applied. This method consists of sample combustion and mercury amalgamation in a gold trap, followed by the measurement of gaseous mercury by the AMA.

For analytical quality control to check the performance of the entire analytical procedure for Hg and to determine the accuracy of measurements, the marine sediment-certified reference material MESS-4 from the National Research Council, Canada, was used. The relative uncertainty of these measurements was approximately 4%. The detection limit (value of the blank +3 SD) was 0.003 µg g^{-1} and the reproducibility was better than 5%. The analytical results for Hg concentrations were expressed in mg kg^{-1} dry weight (ppm).

Evaluation of Sediment Pollution

The sediment pollution level for trace elements was evaluated through the use of two well-known parameters, the Enrichment Factor (EF) and the Geoaccumulation Index (Igeo) (Maanan *et al*., 2004; Mubedi *et al*., 2013).

The EF was calculated as:

$$Ef = (Metal/Sc)_{sample}/(Metal/Sc)_{Background}$$

Where *Metal* represents the concentration of any heavy metal in the analyzed samples and in the geochemical background and "*Sc*" is the concentration of scandium in analyzed samples and in the geochemical background. Therefore, the EF is a geochemical normalization performed using scandium (*Sc*) as a reference element. The Upper Continental Crust (UCC) average values were used as geochemical background values for metals, as described by McLennan (2001).

The Geoaccumulation Index (Igeo) was calculated using the following equation:

$$Igeo = Log_2(Ci)/1.5(Bi)$$

where ,"*Ci*" represents the concentration of the metal "*i*" in the sediment samples; "*Bi*" is the concentration of the metal "*i*" in the geochemical background and 1.5 is a safety factor to account for the natural variation of metal concentrations in the lithospheric background (Lundemi *et al*., 2022; Atibu *et al*., 2018; 2016).

The evaluation of toxicological risks posed by Congo River sediments to the aquatic fauna was performed through a comparison of metal concentrations determined in the sediments against the limits recommended in Sediments Quality Guidelines for the protection of aquatic life (SQG) and the Probable Effect Levels (PEL) adopted by Canada Bilgin (2018).

Experimental Assessment of Sediment Ecotoxicity

A thorough and complete assessment of sediment toxicity must combine chemical analysis, such as those of toxic metals, with ecotoxicological tests (Lundemi *et al*., 2022; Mantis *et al*., 2005; Pablos *et al*., 2011). Ostracods and various other animal species, such as the brine shrimp (*Artemia franciscana*) and the cladocera (*Daphnia magna*), have been often used as test organisms or bioindicators of toxicity of soils and sediments (Devarajan *et al*., 2015; Anadón *et al*., 2002; Boomer and Eisenhauer, 2002). In this study, the toxicity of river sediments was assessed using the F TK36-Ostracodetox kit (MicroBioTests Inc., Belgium) based on the use of the freshwater benthic ostracod crustacean *Heterocypris incongruens* as a test organism. In this ecotoxicity assessment, the manufacturer's protocol and standard operation procedures were followed (Johnson, 2000). In brief, 54 h before the toxicity tests, ostracod specimens hatched in standard freshwater at 25°C and under permanent lighting (approximately 3000-4000 lux) were collected from a laboratory culture. The length of the hatchlings was measured before placing them in test wells. Each toxicity test consisted of exposing 10 live ostracods in test wells containing 2 mL of clean fresh water, 1g of sediment from the Congo River, and 2 mL of microalgae food suspension (supplied with the kit). Assay plates containing six wells (replicates) were sealed with Parafilm®, covered with a lid, and incubated at 25°C in the dark for 6 days.

At the end of the incubation period, the percent (%) mortality of the organisms in test wells was determined using the following formula:

$$\%mortality = B/A \times 100$$

where, "*A*" is the total number of ostracods added to the test plate and "*B*" is the total number of dead ostracods at the end of the exposure time.

The body length of the surviving ostracods was measured using a micrometer strip placed at the bottom of the microscope glass plate. Ostracod growth inhibition was calculated using the following formula (Kilunga *et al*., 2017; Mata *et al*., 2020a):

$$Growth\ inhibition\ (\%) = 100 - [(growthin\ test\ sediment / growth\ in\ reference\ sediment) \times 100]$$

The reference sediment was provided with the ostracod test kit. Growth inhibition was not calculated when the mortality rate was greater than 30%, according to the ostracod test protocol.

Data Analysis

Basic statistical analysis of results and the Spearman's rank order correlation were performed using XLSTAT (New York USA) (XLSTAT, 2020).

Results and Discussion

Physico-Chemical Characteristics of Sediment Samples

The characteristics of the Congo River sediment samples collected at Maluku, Gombe, and Kinsuka areas and at the Mai-Ndombe River control sites were determined and the results for Water Content (WC), total Organic Matter (OM), Carbonate ($CaCO_3$), and particle mean grain size are shown in Table (1).

The composition of sediments in clay, silt, and sand is graphically shown in Fig. (3). The five sediment samples from within each area in the Congo River were similar in composition while sediments from the three areas were distinct for all parameters in Table (1).

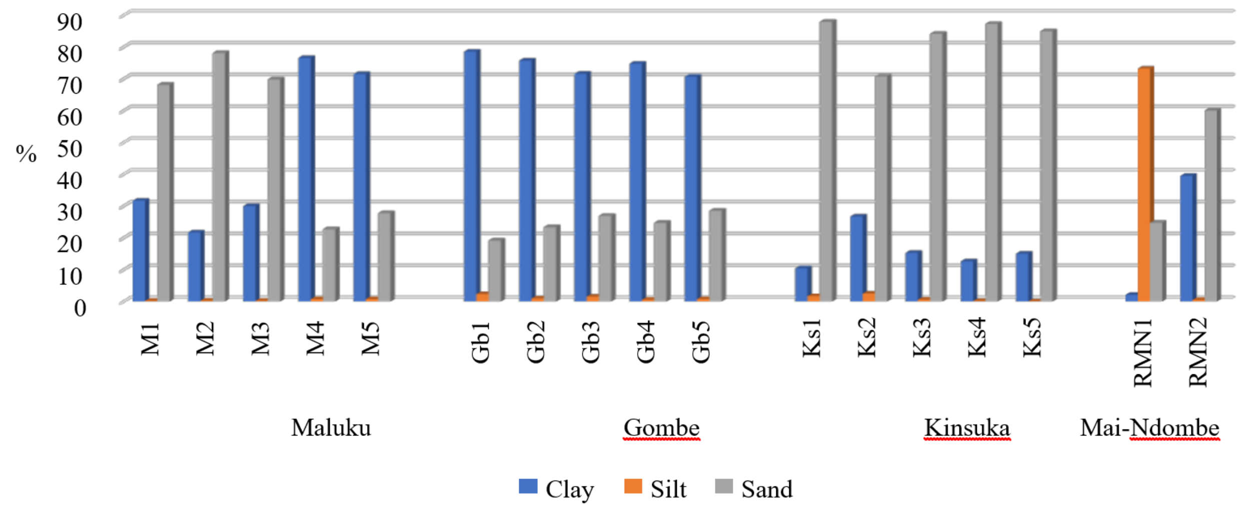

Fig. 3: Particle size distribution (%) in sediment samples from the Congo River and Mai-Ndombe River

Table 1: Physicochemical parameters of sediment samples

Site	Sample	WC (%)	OM (%)	$CaCO_3$ (%)	Mean grain size (µm)
	M1	31.33	6.25	2.23	14.50
	M2	28.33	7.35	2.88	15.21
Maluku	M3	36.34	11.35	3.21	13.60
	M4	38.72	7.43	3.22	12.33
	M5	31.34	12.65	3.81	15.20
	Gb1	57.39	14.32	5.47	35.34
	Gb2	52.23	15.60	5.31	36.72
Gombe	Gb3	47.27	13.22	5.33	32.81
	Gb4	51.82	16.32	4.76	30.26
	Gb5	45.36	14.88	4.17	35.17
	Ks1	56.34	2.12	0.33	116.22
	Ks2	60.26	5.23	1.26	124.66
Kinsuka	Ks3	54.81	7.25	0.74	129.31
	Ks4	32.29	2.32	1.81	136.73
	Ks5	41.53	3.49	1.52	125.65
Mai-Ndombe	RMN1	30.63	12.01	-	23.37
	RMN2	62.88	24.45	-	54.42

Based on the mean grain size Table (1) and sediment composition Fig. (3), the sediments from Maluku and Gombe were silty while the sediment from Kinsuka can be classified as sandy. The sediments from the control river Mai-Ndombe were silty and sandy. Therefore, the texture of sediments and their composition varied among the investigated areas.

The water content (WC) of sediment samples was variable. For the fine grain sediments (all but Kinsuka sediments), the water content correlated positively with the grain size, i.e., the larger the grain size the higher the water content of sediments (r = 0.91, n = 12).The Organic Matter (OM) content of sediments from the Congo River varied from 2.12-16.32% and the OM values from the control site were in the range and even above OM values for Congo River sediments. This indicated that OM in sediments may be also from a natural origin (decaying vegetation for example) and not only from anthropogenic discharges. Globally, the OM content was correlated with the $CaCO_3$ content of river sediments (r = 0.90, n = 15).

Heavy Metals Content of Sediment Samples

The results of trace metal analysis in the Certified Reference Materials (CRM) LKSD-4 and STSD-2 are shown in Table (2) and demonstrated good agreement with the certified values. The value obtained by repeated total Hg analyses of the CRM MESS-4 was 0.081±0.009 µg g^{-1} which was comparable to the CRM certified value of 0.09±0.04 µg $g-^{1}$.

The heavy metal concentrations in sediment samples are shown in Table (2). Metal concentrations in the five samples from each area of the Congo River displayed small inter-sample variations and, globally, these five samples provided a consistent characterization for each area. The two sediment samples from Mai-Ndombe River displayed different metal concentrations but both were at very low values.

Results showed much higher concentrations of all heavy metals in sediment samples from Maluku, Gombe, and Kinsuka areas in the Congo River and of one to three orders of magnitude higher when compared to the concentrations of heavy metals in sediments from the control area at the Mai-Ndombe River. Furthermore, in the Congo River sediments the contamination by metals displayed a minimum in the rural municipality of Maluku, located upstream of Kinshasa. Metal concentrations markedly increased in the capital city (Kinshasa) where industries and population are concentrated. At Kinsuka, downstream of the Kinshasa city area, the concentrations of metals in sediments slightly decreased because of the increasing distance to main waste discharges and dilution of pollutants in the Congo River.

This distribution pattern indicated a clear connotation of metal pollution to human activities and anthropogenic discharges from Kinshasa city into the Congo River. Among the 13 metals analyzed, 7 do have recommended Sediment Quality Guidelines (SQG) values for protection of aquatic life Table (2). In the Congo River, samples from the Maluku area showed concentrations above the SQG recommended values for two metals (2 out of 7), i.e., Cr (ranging from 57-74.2 mg kg^{-1}) and Hg (ranging from 0.2, 289-2.1 mg kg^{-1}). The concentrations of all other metals (5 out of 7) were below SQG values.

For the Gombe area, by the Kinshasa city, concentrations above the SQG values were recorded for all metals with available SQG recommended values (7 out of 7). These were: Cr (108-340 mg kg^{-1}), Cu (81.6-310 mg kg^{-1}), Zn (173-826 mg kg^{-1}), As (6.3-62.9 mg kg^{-1}), Cd (0.8-1.9 mg kg^{-1}), Pb (125-262 mg kg^{-1}) and Hg (0.7-2.9 mg kg^{-1}).

For sediments from the Kinsuka area, downstream of Kinshasa city, metal concentrations above the SQG values were observed for 5 out of the 7 metals with recommended SQG values. These were: Cr (63.3-74.8 mg kg^{-1}), Cu (63.6-6.7 mg kg^{-1}), Zn (112-380 mg kg^{-1}), Pb (87.9-89.7 mg kg^{-1}) and Hg (0.3-2.3 mg kg^{-1}).

In general, the heavy metal contents in the Congo River were much higher than in the control river for all the 13 metals analyzed, including those with no recommended (yet) SQG values. Among the three investigated areas of the Congo River, Gombe was the more polluted area, with higher metal concentrations and more metals. Gombe is also the more populated and more industrialized area.

Sediment samples from the control sites at Mai-Ndombe River (RMN1 and RMN2) displayed the lowest metal concentrations all below the values of Sediment Quality Guidelines (SGQ) adopted to protect aquatic fauna from the toxic effects of metals. The reduced contamination of this river by anthropogenic activities was, therefore, confirmed with the sediment analysis Table (2).

The metal concentrations in the investigated areas of the Congo River were in the range of values previously reported for the Congo River at the Ngamanzo, Kinkole, Kinsuka pécheur, and Demoulin sites by Mata *et al*. (2020a) and for the N'djili River by Lundemi *et al*. (2022). All these areas have been also impacted by intensive anthropogenic activities.

Regarding the potential toxicity of the Congo River sediments to aquatic biota, the sediment samples displayed concentration values above the Probable Effect Levels (PEL) for the elements Cr, Cu, Zn, As, Cd, Pb, and Hg in at least one area, while for the elements Cr and Hg the PEL values were exceeded in practically all samples Table (2). Therefore, the high concentrations of these metals in the Congo River sediments constitute a threat to aquatic life and, thus, may compromise the ecosystem health.

Table 2: Heavy metal concentrations in sediments (mg kg^{-1} dw) from Congo River and Mai-Ndombe River. results of determination of heavy metals in two sediment CRMs

Site/ material	Sample	Sc	Ti	V	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb	Hg
	M1	6.8	564.0	55.2	60.9	713.0	18.3	33.8	18.4	65.6	4.2	0.30	19.0	0.30
	M2	5.2	527.0	43.4	49.7	458.0	14.1	59.9	15.3	63.8	3.9	0.30	18.7	0.20
Maluku	M3	8.1	608.0	65.8	74.2	825.0	21.3	44.2	23.1	83.5	5.1	0.40	24.9	0.40
	M4	7.5	610.0	61.1	69.2	669.0	18.4	41.8	20.8	73.9	4.5	0.30	21.7	0.10
	M5	5.9	526.0	52.1	57.0	649.0	15.1	77.6	20.2	69.1	3.8	0.30	22.0	2.10
	Gb1	2.0	199.0	21.9	340.0	80.3	3.1	9.3	81.6	275.0	1.4	0.80	160.0	2.20
	Gb2	0.4	175.0	6.6	111.0	152.0	3.7	12.0	88.3	173.0	5.6	0.20	125.0	2.90
Gombe	Gb3	2.6	145.0	36.0	134.0	223.0	4.4	16.9	96.1	407.0	3.2	1.20	161.0	1.30
	Gb4	2.4	229.0	32.4	148.0	1837.0	65.2	212.0	310.0	311.0	62.9	0.60	167.0	0.70
	Gb5	6.5	332.0	72.3	108.0	218.0	9.4	35.4	112.0	826.0	6.3	1.90	262.0	2.20
	Ks1	0.8	193.0	12.2	68.1	716.0	4.7	2.4	63.6	119.0	0.6	0.00	89.7	2.30
	Ks2	1.3	70.0	11.1	72.4	20.0	7.6	3.1	68.5	112.0	0.7	0.10	87.9	2.10
Kinsuka	Ks3	2.6	194.0	22.7	63.3	285.0	4.8	10.1	72.6	380.0	1.5	0.20	88.2	1.20
	Ks4	1.4	172.0	14.9	74.8	63.4	3.3	7.0	76.7	377.0	0.6	0.10	89.7	0.40
	Ks5	1.2	50.3	14.8	14.2	43.5	0.6	1.9	8.2	252.0	1.6	0.10	7.6	0.30
Mai	RMN1	1.0	38.0	19.0	19.3	8.2	0.2	1.6	3.8	21.3	0.15	0.00	1.7	0.10
Ndombe	RMN2	0.0	27.0	2.8	4.3	2.5	0.0	0.0	1.6	7.7	0.0	0.00	0.1	0.08
LKSD4	Ref. values	-	-	32.0	21.0	430.0	11.0	32.0	30.0	189.0	12.0	1.90	93.0	Det.
values	-	-	31.2	19.6	426.9	11.2	31.6	29.5	179.3	11.4	1.60	92.7		
STSD4	Ref. values	-	-	51.0	30.0	1200.0	11.0	23.0	66.0	82.0	11.0	0.60	13.0	
	Det. values	-	-	47.8	27.4	1118.2	10.2	21.5	61.8	75.1	10.4	0.56	12.3	
	SQG	-	-	-	37.3	-	-	-	35.7	123.0	5.9	0.60	35.0	0.17
	PEL	-	-	-	90.0	-	-	-	197.0	315.0	17.0	3.50	91.3	0.48

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Evaluation of Sediment Pollution

The calculated values of the Geoaccumulation Index (Igeo) and Enrichment Factor (EF) are presented in Table (3). The results for these two parameters were interpreted according to a classification scale developed in previous studies and included in Table (3) (Atibu *et al*., 2018; Thevenon *et al*., 2013). The use of this classification scale renders it easier to interpret the overall results and to evaluate the contamination level.

The Mai-Ndombe River (RMN1 and RMN2 sediment samples) was selected as a control river because it did not have visible significant anthropogenic impacts. The calculated Igeo values for the RMN 1 and RMN2 samples ranged from 39.5-0.1 for Cr, Co, Cu, Zn, As, Cd, Pb, and Hg indicating that this site was “practically unpolluted” to “moderately polluted” by these heavy metals. Similarly, results for the EF parameter also indicated that sediments from the Mai-Ndombe River were “practically unpolluted” for most metals Table (3).

Contrasting with results for the controlled river, the sediments from the Congo River displayed Igeo values ranging from “practically unpolluted” to “extremely polluted”.

Furthermore, the EF values for sediments from the Congo River ranged also from “minor enrichment” to “extremely severe enrichment” depending on the metal under consideration, but globally they were markedly more contaminated than the controlled river.

Due to the standardization introduced with the use of Sc, the EF parameter provides more robust information than Igeo and renders it possible to better assess anthropogenic pollution. It has been proposed that when EF ≤0.5 this indicates the occurrence of bioturbation in the upper layers of sediments and dilution of contaminants in the top sediment layer with less contaminated sediment layers from underneath. For 0.5≤ EF ≤1.5, the metal concentration in the samples is considered to correspond acceptably to the natural heavy metal concentrations in crustal sources. However, when EF ≥1.5, metal enrichment has certainly occurred and can be attributed to the input of heavy metals from anthropogenic sources into the study area. Therefore, for a specific metal, the EF value can be used to identify whether site contamination has occurred (Feng *et al*., 2004; Zhang and Liu, 2002).

In general, the EF values were less than 1 in the controlled river (RMN 1) suggesting no enrichment or minor enrichment of heavy metals, except for Cr (5.5) and Ni (4) which displayed a “moderately severe enrichment”. In the investigated areas of the Congo River, the EF values of all samples varied between "minor enrichment" (EF <3) and "extremely severe enrichment" (EF >50) for Cr, Co, Cu, Zn, As, Cd, Pb and Hg Table (3). The EF values for heavy metals in the Congo River sediments followed the order Hg (1424) > As (192) > Pb (171) > Cu (97)> Cr (87) > Zn (67) > Cd (56) > Co (30). These values indicated a noticeable increase of concentrations for several metals (8 out of 12) in sediments, including very extreme pollution by toxic metals such as Hg, As, and Pb in the Congo River.

Table 3: Igeo et EF values for Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb, and Hg in sediment samples from the Congo River and Mai-Ndombe River

Igeo	Ti	V	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb	Hg	
M1	-3.0	-0.7	0.2	-0.3	0.3	0.2	-1	-0.7	0.9	1	-0.7	1.8	Igeo classification
M2	-3.1	-1.1	-0.1	-1	-0.1	1	-1.3	-0.7	0.8	1	-0.7	1.3	
M3	-2.9	-0.5	0.5	-0.1	0.5	0.6	-0.7	-0.4	1.2	1.4	-0.3	2.3	
M4	-2.9	-0.6	0.4	-0.4	0.3	0.5	-0.9	-0.5	1	1	-0.5	0.3	Igeo ≤ 0 Class 0: practically
M5	-3.1	-0.8	0.1	-0.5	0	1.4	-0.9	-0.6	0.8	1	-0.4	4.6	unpolluted
GB1	-4.5	-2.0	2.7	-3.5	-2.3	-1.7	1.1	1.4	-0.7	2.4	2.4	4.7	0<Igeo<1 Class 1: unpolluted
GB2	-4.7	-3.8	1.1	-2.6	-2	-1.3	1.2	0.7	1.3	0.4	2.1	5.1	to moderately polluted
GB3	-5.0	-1.3	1.3	-2	-1.8	-0.8	1.4	1.9	0.5	3	2.4	4	1<Igeo<2 Class 2: moderately
GB4	-4.3	-1.5	1.5	1	2.1	2.8	3	1.5	4.8	2	2.5	3.1	polluted
GB5	-3.8	-0.3	1	-2	-0.7	0.2	1.6	3	1.5	3.7	3.1	4.7	2<Igeo<3 Class 3: moderately
KS1	-4.5	-2.9	0.4	-0.3	-1.7	-3.6	0.8	0.2	-1.9	-	1.6	4.8	to heavily polluted
KS2	-6.0	-3.0	0.5	-5.5	-1	-3.3	0.9	0.1	-1.7	-0.6	1.6	4.6	3<Igeo<4 Class 4: heavily
KS3	-4.5	-2.0	0.3	-1.7	-1.6	-1.6	1	1.8	-0.6	0.4	1.6	3.8	polluted

Table 3: Continue

KS4	-4.7	-2.6	0.5	-3.8	-2.2	-2.1	1	1.8	-1.9	-0.6	1.6	2.3	4<Igeo<5 Class 5: heavily to
KS5	-6.5	-2.6	-1.9	-4.4	-4.6	-4	-2.2	1.2	-0.5	-0.6	-2	1.8	extremely polluted
RMN1	-6.9	-2.2	0.1	0	-0.2	0	0.1	0	-1.2	-	0	-39.5	5<Igeo Class 6: extremely polluted
RMN2	-6.9	-2.2	-1.4	-6.8	-6.2	-4.2	-3.3	-2.3	-3.9	-	-4.1	0.3	
EF	Ti	V	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb	Hg	
M1	0.30	1.49	2.8	1.9	3	2.7	1.2	1.5	4.5	5	1.5	8.7	
M2	0.37	1.53	3	1.6	3	6.3	1.3	1.9	5.5	6.5	2	7.6	
M3	0.28	1.49	2.9	1.9	2.9	3	1.3	1.6	4.6	5.5	1.7	9.7	
M4	0.30	1.49	2.9	1.6	2.7	3.1	1.2	1.5	4.4	4.5	1.6	2.6	EF interpretation values
M5	0.33	1.62	3	2	2.8	7.2	1.5	1.8	4.7	5.7	2.1	69.9	EF < 1: no enrichment
GB1	0.36	2.01	53.4	0.7	1.7	2.6	18	21.3	5.1	44.9	43.9	216.1	EF < 3: minor enrichment
GB2	1.60	3.0	87.2	7	10.2	16.5	97.1	66.9	102.7	56.1	171.2	1424.1	EF 3-5: moderate enrichment
GB3	0.20	2.54	16.1	1.6	1.9	3.6	16.3	24.3	9	51.8	34.1	98.2	EF 5-10: moderately severe
GB4	0.35	2.48	19.4	14	29.9	48.7	56.8	20.1	192.2	28.1	38.4	57.3	enrichment
GB5	0.19	2.04	5.2	0.6	1.6	3	7.6	19.7	7.1	32.8	22.2	66.5	EF 10-25: severe enrichment
KS1	0.88	2.80	26.8	16.4	6.5	1.7	35	23	5.5	0	61.7	564.7	EF 25-50: very severe
KS2	0.20	1.57	17.5	0.3	6.4	1.3	23.2	13.4	3.9	8.6	37.2	317.3	enrichment
KS3	0.27	1.60	7.7	2	2	2.1	12.3	22.6	4.2	8.6	18.7	90.7	EF > 50: extremely severe
KS4	0.45	1.95	16.8	0.8	2.6	2.8	24.1	41.7	3.1	8	35.2	56.1	enrichment
KS5	0.15	2.26	3.7	0.7	0.6	0.9	3	32.5	9.8	9.4	3.5	49.1	
RMN1	0.14	3.5	5.5	0	0	4	1	0.8	0	-	-	0	
RMN2	-	-	-	-	-	-	-	-	-	-	-	-	

Experimental Assessment of Sediment Ecotoxicity

The growth inhibition percentage and mortality rate of the ostracods (*Heterocypris incongruens*) exposed to Congo River sediments are shown in Table (4).

The Maluku sediments, from upstream Kinshasa city, generally contained lower concentrations of heavy metals than the other areas investigated in the Congo River and caused mortality rates ranging from 15.9-72.6% and growth inhibitions of 76.4, 28.1 and 24.8% for samples M1, M2 and M5. These sediments displayed heavy metal concentrations generally below the SQG and PEL values in Table (2).

Results from ecotoxicity tests showed that all sediment samples from the Gombe and Kinsuka areas caused a mortality rate of 100%, except for Ks3 sediments whose mortality rate was 85.9% Table (4). This high mortality was caused by the toxicity of heavy metals and possibly also enhanced by other pollutants eventually present in the sediments (Devarajan *et al*., 2015). It must be noted that the sediments from the Gombe and Kinsuka areas, which caused the highest mortality rates, have also the highest heavy metal concentrations and higher than recommended values for the SQG and the PEL Table (2).

Table 4: Mortality percentages and growth inhibition of ostracods (*Heterocypris incongruens*) exposed to sediment samples from the Congo River

Site	Sample	Number of test performed	Mortality (%)	Growth inhibition (%)
Maluku	M1	3	26.7±1.2	76.4±8.3
	M2	3	17.9±5.4	28.1±3.7
	M3	2	72.6±5.2	n/d
	M4	3	49.3±8.5	n/d
	M5	3	15.9±4.7	24.8±4.2
Gombe	Gb1	4	100	n/d
	Gb2	4	100	n/d
	Gb3	3	100	n/d
	Gb4	2	100	n/d
	Gb5	2	100	n/d
Kinsuka	Ks1	4	100	n/d
	Ks2	3	100	n/d
	Ks3	3	85.9±3.7	n/d
	Ks4	4	100	n/d
	Ks5	4	100	n/d

Globally, these results place both approaches used in ecotoxicological risk assessment (the experimental toxicity tests with ostracods and the comparison of metal concentrations in sediments against SQG and PEL recommended values) in good agreement. Furthermore, the results of experimental sediment toxicity tests using ostracods matched very well with the evaluation of sediment pollution made through the calculated EF parameter.

Statistical Correlations

Spearman's order correlation coefficients were calculated to investigate possible sources and pathways of heavy metals in sediments, as well as to identify heavy metals that were the main causes of ostracod mortality. Results are presented in Table (5).

A positive and significant correlation ($p<0.05$) was recorded between sediment OM and metal concentrations, namely for Cr (r = 0.62), Ni (r = 0.626), Cu (r = 0.599), As (r = 0.739), Ag (r = 0.87), Cd (r = 0.786), Pb (r = 0.564); $CaCO_3$ and Cr (r = 0.678), Cu (r = 0.519), As (r = 0.553), Ag (r = 0.834), Cd (r = 0.785), Pb (r = 0.509). These positive correlations confirmed the ability of sediment organic matter to concentrate heavy metals from river water.

Significant negative correlations ($p<0.05$) were observed between certain metals and sediment grain size, indicating that at least for some metals, their concentration was controlled by surface adsorption on sediment grains and, therefore, concentrations were enhanced in the fine grain sediments.

The percentage of ostracods mortality was positively and significantly correlated ($p<0.05$) with the concentrations of Cr (r = 0.612), Cu (r = 0.653), Zn (r = 0.733), Pb (r = 0.661), and Hg (r = 0.522), indicating that these metals were significantly associated to the high mortality rate of ostracods. However, a negative correlation was observed between ostracod mortality and metal concentrations for Co (r = 0.588), Ni (r = 0.596), and Se (r = 0.548) indicating no association of these metals with enhanced mortality and, possibly, even indicating an action opposite to the previous group of metals.

Correlations of concentrations were tested for pairs of heavy metals looking for their potential association. In general, concentrations of paired metals were positively correlated, but there were also several negative correlations: Cr/Cu (r = 0.896), Cr/Se (r = 0.718), Co/Ni (r = 0.811), Ag/Cd (r = 0.887), Se/Hg (r = 0.654), Pb/Hg (r = 0.674). The positive correlations observed between these paired metals suggested that metals could have the same sources and similar transport pathways (e.g., Cr/Cu were significantly associated in sediments and it is known that both are used together in common metal alloys). On the other hand, the negative and significant correlations ($p<0.05$) observed for some metal pairs (e.g., Se/Hg) suggested that they do not come from the same source and might have followed different environmental transport pathways (Lundemi *et al*., 2022; Haller *et al*., 2009; Poté *et al*., 2008).

The main findings of this research may be summarized as follows:

- The Enrichment Factor (EF) parameter values indicated that sediments in the Maluku area, displayed “minor enrichment”, “moderate enrichment” or moderately severe enrichment‖ for Cr, Co, Ni, As, Cd, and Hg; sediments from the Gombe area suffered severe “to extremely severe” enrichment in Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb 414 and Hg; and sediments from the Kinsuka area displayed “moderate enrichment” to “extremely severe enrichment” in Cr, Mn, Co, Cu, Zn, As, Cd, Pb, and Hg
- The Geoaccumulation Index (Igeo) values underlined that the more contaminated sediments in the Congo River corresponded to the more populated and industrial areas
- Globally, both EF and Igeo values were much higher for sediments from the Congo 419 River in comparison to a control river with minor anthropogenic activities
- In the Congo River sediments, high concentrations of heavy metals were observed for Cr and Hg in almost all the samples and exceeding the values set by the Sediment Quality Guidelines (SQG) and the Probable Effect Levels on biota (PEL); values for Cu, Zn and Pb exceeding the SQG values were observed in samples from Gombe and Kinsuka areas; sediments from Gombe area showed also high concentrations for As and Cd and above the SQG values
- In sediment toxicity tests carried out with ostracods (*Heterocypris incongruens*), the Maluku samples induced mortality percentages varying between 15.9 and 72.6%, with growth inhibition percentages varying from 24.8-76.4% for the samples having shown mortality lower than 30%. However, ostracod mortality was 100% with almost all the sediment samples from the Gombe and Kinsuka areas. The mortality of ostracods was attributed to the toxic effects of Cr, Cu, Zn, Pb, and Hg present in high levels in the sediments
- Based on the correlation matrix analysis for the parameters measured on sediments, there were several potential sources of contamination and transport routes of the studied heavy metals, including urban wastewater, sewers, and septic tanks discharged without treatment into the Congo River

Table 5: Spearman's correlation matrix for selected parameters analyzed in sediment samples

Variables	Cr	Co	Ni	Cu	Zn	As	Se	Ag	Cd	Pb	Hg
WC	0.495	-0.374	-0.531	0.538	0.481	-0.299	-0.508	-0.119	-0.136	0.542	0.658
OM	0.62	0.266	0.626	0.599	0.245	0.739	-0.402	0.870	0.786	0.564	0.310
$CaCO_3$	0.678	0.054	0.483	0.519	0.202	0.553	-0.424	0.834	0.785	0.509	0.256
Grain size	0.045	-0.77	-0.788	0.239	0.572	-0.639	-0.374	-0.604	-0.571	0.234	0.381
Mortality	0.612	-0.588	-0.596	0.653	0.733	-0.222	-0.548	-0.065	-0.092	0.661	0.522
Cr		-0.086	0.068	0.896	0.582	0.204	-0.718	0.529	0.512	0.872	0.475
Co			0.811	-0.089	-0.439	0.620	0.289	0.423	0.357	-0.130	-0.362
Ni				0.061	-0.296	0.751	0.079	0.698	0.637	0.025	-0.272
Cu					0.771	0.213	-0.746	0.436	0.433	0.974	0.579
Zn						-0.073	-0.411	0.081	0.241	0.763	0.394
As							0.116	0.745	0.605	0.148	-0.130
Se								-0.185	-0.052	-0.731	-0.654
Ag									0.887	0.452	0.109
Cd										0.444	0.015
Pb											0.674

Conclusion

The determination of metal concentrations in Congo River sediments revealed that there is severe contamination of this river, especially in the river segment by Gombe and Kinsuka in the Kinshasa province. This was supported by a comparison with metal concentrations in sediments from a control river in the same region. The contamination of the Congo River was originated by uncontrolled anthropogenic (urban and industrial) discharges which increased the heavy metal concentrations in sediments noticeably. The calculation of metal contamination parameters, namely the Igeo and EF, confirmed the strong enhancement of metal concentrations in Congo River sediments much above the natural geogenic background.

The evaluation of sediment toxicity was performed in two ways. One, through comparing metal concentrations in sediments against the Sediment Quality Guidelines (SGQ) and Probable Effects Level (PEL) adopted by Canada for the protection of aquatic biota. The other is through experimental determination of the sediment toxicity to aquatic organisms using ostracods as bioindicators. Results from both approaches were in agreement and indicated very high toxicity of Congo River sediments to aquatic biota.

The Congo River is used by the population as a source of irrigation water and drinking water. Although the river water has not been analyzed, the very high metal concentrations detected in sediments indicate that metal concentrations in river water may exceed the internationally recommended limits for metals in drinking water for the protection of human health. The prospects for future work include analysis of heavy metals in drinking water.

Globally, the results from this research showed that the degradation of the Congo River by pollution is of high concern and the aquatic ecosystem of the second major river of Africa is at risk. Measures to prevent further pollution and revert the current situation are urgently needed.

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Author's Contributions

Didier Makonko Mabidi: Involved in the conceptualization and validation of the methodology, conducted fieldwork and sample collection, involved in the data analysis, in writing some parts of the manuscript, read, and approved the final manuscript.

Honoré Manyingu Mutwejile: Involved in the calculation of parameters and in writing some parts of the manuscript, read, and approved the final manuscript.

Georgette Ngakiama Beni Ngweme, Alexis Bwabitulu Nienie, John Mputu Kayembe and Séraphin Ntumba Lusamba: Commented on previous versions of the manuscript, read and approved the final manuscript.

Emmanuel Kazinguvu Atibu: Conceptualization and validation of the methodology, make the calculation of parameters, analyzed the data and wrote the first manuscript draft.

Fernando Piedade Carvalho: Conceptualization and validation of the methodology, data interpretation and was involved in writing the first manuscript, read and approved the final manuscript.

John Poté: Designed the project, conceptualization and validation of the methodology, analyzed data and was involved in writing the first manuscript, read and approved the final manuscript.

Ethics

We claim to have followed and respected ethical standards throughout this research and publication. The consent of the parties was obtained and the confidentiality of the data, if any, was ensured to ensure the integrity of the research. We are willing to address any potential ethical issues that may arise after publication.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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