Marine Pollution Bulletin 211 (2025) 117386

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Bioaccumulation and genotoxic effect of heavy metal pollution in marine sponges from the Niger Delta

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ARTICLE INFO

Keywords:
Comet assay
DNA damage
Biomonitoring
Metal uptake
Bioavailability

ABSTRACT

In this study, levels of Al, Cu, Ni, Cd, Cr and Pb were quantified in seawater, sediments, and sea sponges from six sites in the Niger Delta and one relatively clean site outside the Niger Delta area using Inductively Coupled Plasma Optical Emission Spectroscopy and Inductively Coupled Plasma Mass Spectrometry. The metal levels in sponge tissues in μg/mg ranged from 0.22 ± 0.03–0.70 ± 0.10 (Al), 0.002 ± 2.2 × 10^{-5} - 0.004 ± 5.6 × 10^{-5} (As), 2 × 10^{-5} ± 5.3 × 10^{-6} -1.5 × 10^{-3} ± 4.6 × 10^{-6} (Cd), 2.3 × 10–3 ± 1.4 × 10–5 -0.02 ± 2,2 × 10^{-4} (Cu), 2.5 × 10^{-4} ± 8.6 × 10^{-6}- 2.0 × 10^{-3} ± 1.4 × 10^{-5} (Pb). In Sediment samples in mg/kg, the ranges were (0.883 ± 0.114–73.33 ± 0.10 (Al), 0.0007 ± 0.026–0.304 ± 0.009 (As),0.0086 ± 0.0045–0.198 ± 0.010 (Cr); 0.005 ± 0.001–0.063 ± 0.001 (Cu), 0.039 ± 0.004–0.0783 ± 0.0024(Ni), 0.0017 ± 0.002–0.056 ± 0.0046 (Pb). In the water sample, the metal levels in mg/L 0.06–0.92 (Al), 0.001–0.007 (Cd), 0.001–0.001 (Cr), 0.01–0.02 (Cu), 0.003–0.01 (Ni), 0.001–0.01(Pb). Metal levels in all sampling sites occurred in the order of decreasing concentration as Al > Cu > Ni > Cd > Cr > Pb (in seawater), Al > Cr > Ni > Pb > Cu > Cd (in sediment) and Al > As>Cu > Pb > Cd (in the sponge). The study further assessed DNA strand breaks in sea sponges as a biomarker of genotoxicity using the comet assay. There was a strong correlation between % DNA strand breaks in sponge cells from all sample locations and aluminium levels in sponge tissues from all sample locations. The highest metal levels were recorded in Sea sponges, followed by Sediment and then Sea water, with aluminium significantly higher than other metals in all three matrices studied. We, therefore, conclude that sea sponges are excellent sentinel species for toxic metal bioaccumulation, and DNA strand breaks are an efficient biomarker for aquatic pollutants biomonitoring.

1. Introduction

The Niger Delta is a coastal region in Nigeria which has been contaminated with toxic chemical cocktails and is still exposed to aquatic pollution since the advent of oil and gas exploration activities over 5 decades ago. The major cause of aquatic pollution in the Niger Delta has been linked to the discovery of petroleum and its associated products. The drilling for oil and/or its extraction processes has resulted in considerable environmental pollution from toxic chemical cocktails in the Niger Delta region. More disturbing is the fact that these toxic chemicals bioaccumulate in aquatic media including seafood, benthic fauna and underground seepages and eventually leach into underground water. This can lead to very serious health complications to both humans and animals (Raimi et al., 2019; Okoyen et al., 2020; Numbere, 2018; Sakib, 2021).

Since the advent of industrialisation and the accompanying boom in urban migration, there has been an increasing report of heavy metal pollution in coastal regions (Cebrian and Urizx, 2007; De Mestre et al., 2012; Iwegbue et al., 2018). Effluents from both domestic and clinical sewers have been identified as the major source of heavy metal contribution in coastal waters (Wogu and Okaka, 2011; Iwegbue et al., 2018). Pollution sources in the Niger Delta region are mainly associated with oil and gas exploration, agriculture and metal smelting activities, with most contributions being attributed to the oil and gas industries. Commonly reported pollutants in the region are Metals, Polycyclic Aromatic hydrocarbons (PAHs) and organochlorine pesticides (Ajao and Anurigwo, 2002; Iwegbue et al., 2018). Toxic metals degrade aquatic systems and are potentially toxic to marine organisms and humans (Javed and

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https://doi.org/10.1016/j.marpolbul.2024.117386
Received 27 August 2024; Received in revised form 30 November 2024; Accepted 30 November 2024
Available online 13 December 2024

Usmani, 2019; Fu and Xi, 2020; Hong et al., 2020; Sonone et al., 2020; Cui et al., 2022). The environmental persistence of potentially toxic metals can result in their bioaccumulation in lower invertebrates within the aquatic ecosystem (Mrozińska and Bąkowska, 2020; Jeong et al., 2023). This accumulation poses significant environmental risks, including disruptions to various abiotic parameters and water qualities such as dissolved oxygen concentration, temperature, pH, water hardness, and sediment organic matter (Jeong et al., 2023; Tang et al., 2023). Alterations in the balance of these essential parameters are known to enhance metal speciation in the aquatic ecosystem, their resultant bioavailability, and toxicity in biota commonly reported (Chormare and Kumar, 2022; Esteves-Aguilar et al., 2023). Furthermore, potentially toxic metals such as cadmium (Cd), lead (Pb), copper (Cu), Chromium (Cr), Nickel (Ni) and aluminium (Al) have genotoxic potentials and can induce DNA damage in marine organisms (Akpiri, 2018; Akpiri et al., 2019; Turan et al., 2020). Therefore, periodic monitoring and regular assessment of heavy metal levels are imperative.

Marine sponges are sessile invertebrates with significant ecological importance in the aquatic ecosystem. The prolific filter-feeding ability of sponges is a crucial characteristic contributing to their ecological importance (Roveta et al., 2021). Much of the physiology and biology of sponges rely on the continuous flow of water through their body, making this trait highly significant for the sponge. By filtering particles from the water column, sponges can absorb and retain xenobiotics, including potentially toxic metals ingested during feeding. This capacity to bioaccumulate xenobiotics in their tissues has positioned sponges as preferred candidates for monitoring heavy metal pollution in aquatic ecosystems, giving them an advantage over other aquatic organisms. (Gentric et al., 2016; Akpiri, 2018; Méndez et al., 2021; Krikech et al., 2022). However, heavy metal pollution in coastal systems has been reported as having significant adverse effects on sea sponges, thereby causing severe ecological damage to the aquatic ecosystem. For example, Kotelevtsev et al. (2009) reported that chronic exposure to potentially toxic metals resulted in inhibition and deactivation of aggregation factor, increased cell division, interference with sponge water filtration mechanism and decreased population growth. On the other hand, acute exposure causes a depletion of population and species diversity (Van der Oost et al., 2003). The correlation between exposure to pollutants and DNA damage highlights the inadequacy of solely monitoring pollutant levels in the environment. It becomes imperative to evaluate the biological impact using biomarkers of exposure, such as genome integrity (Martins and Costa, 2015; Zhang et al., 2020). Biomarkers are important tools in environmental risk assessment (ERA) since they provide information on biologically available contents of pollutants and their biological effects on living systems (Livingstone et al., 2000; Van der Oost et al., 2003; Martins and Costa, 2014). A good example of a reliable biomarker that is frequently employed in ERA is genotoxicity; it is measured using different endpoints such as DNA strand breaks, sister chromatid exchanges, micronuclei and chromosomal aberration; with DNA strand breaks being the mostly utilised due to its connection with the carcinogenic outcome (Reinecke and Reinecke, 2004). In addition to biomarkers as validation tools in the assessment of the impact of environmental toxicants in living systems, effect-based monitoring methods are deployed to complement chemical analysis in water quality assessment (Connon et al., 2012; Brack et al., 2019). Effect-based methods have been suggested as one of the measures to establish potential adverse effects from chemical pollution in the Water Framework Directive (WFD), 2000/60/EC. Considering the growing concern regarding increased pollution in the coastal marine environment and the need for the development of reliable biomarkers required as an early warning system in ERA, this study sets out to investigate the occurrence and levels of established metallic genotoxicants (Al, As, Cd, Cr, Cu, Hg, Ni and Pb) in Niger Delta coastal environment and to assess their genotoxic effects in terms of DNA strand breaks leading to loss of DNA integrity on the marine sponges (*Amorphinopsis kalibiama*) using the alkaline comet assay.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study are of analytical grade and were obtained from Merck Life Science UK Limited and Fisher Scientific.

2.2. Sample collection and processing

Sea water, sediment, and sea sponge (*A. kalibiama*) samples were collected in triplicates from six randomly selected Niger Delta sites and one relatively clean site outside the Niger Delta in Badagry, Lagos State, Nigeria (Figs. 1 and 2, Table 1) between October 2015 and January 2016. Sponge samples were collected from exposed mangrove stomp at low tide using a sterile scalpel. Sediment samples were collected using a hand trowel between 0.1 and 0.3 m depth. All samples were collected into transparent zip-lock bags and transported back to the Centre for Marine Pollution and Sea food Safety laboratory at the University of Port Harcourt, Nigeria within four hours of collection in seawater. *A. kalibiama* samples were immediately processed in the laboratory into single cells and cryopreserved in liquid nitrogen according to the method described by Akpiri et al. (2017).

2.3. Analysis of potentially toxic metals in samples of seawater, sediment and sponge structures

Samples of sponge, sediment and seawater collected from seven sites (Table 1) were independently analysed in three laboratories (Lab 1: Analytical Chemistry labs, Department of Chemistry Warwick University, UK; Lab 2: Environmental analysis labs, School of Earth Science University of Birmingham, UK; and Lab 3: Analytical chemistry labs, Kingston University, London). Concentrations of Al, Cu, Ni, Cd, Cr and Pb were analysed in each of the matrices using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Ultima (2) C) for water samples, and Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Agilent 7700 series, using Rhodium (Rh) as the internal standard) for sediment and sponge samples respectively. Sediment and sponge samples were digested using microwave-assisted acid digestion (CEM MARS 5 CORPORATION). Four millilitres of $HClO_4$ was added to digested samples and evaporated to near dryness under nitrogen. Subsequently, 5 M Aristar nitric acid (HNO_3) was added to the sample and digested until a clear solution was achieved. The digests were placed into 50 mL Falcon tubes and kept at 4 °C until use. Metal extracts in both samples were then measured against calibration curves generated with a multi-element standard (0 ppm, 0.1 ppm, 0.5 ppm, 2.5 ppm, 5 ppm and 10 ppm). Final metal concentrations in all analytes were reported in μg/l, mg/kg and μg/mg for metals in water, sediment and sponge samples, respectively.

2.4. Sponge cell culture

Cryopreserved sponge cells were thawed in a water bath at 37 °C for 1 min and resuspended in 5.5 mL of sponge media (16.5 g instant ocean sea salt in 500 mL Ultra High-Quality water, 0.2 % RPMI (Roswell Park Memorial Institute medium), 1 mg/mL PSG (penicillin, streptomycin, glutamine) solution, and 0.1 % *v*/v Pluronic® F-68). The sponge cell suspension was centrifuged at 300 ×*g* for 7 min. The supernatant was discarded while the sponge cell pellets were resuspended in 6 mL of fresh medium. The resuspended sponge cell (3 mL) was plated in a sterile T_{25} culture flask and made up to a final volume of 6 mL with sponge media. Plated sponge cells were left on a horizontal rotator shaker at 45 rpm at room temperature for up to 12 h. The culture media was changed by allowing aggregates to settle under gravity for about 3 min before carefully removing 3 mL of sponge media and replacing it with 3 mL of fresh media to form aggregates that maintained viability. It was observed that sponge cells rapidly formed aggregates that maintained

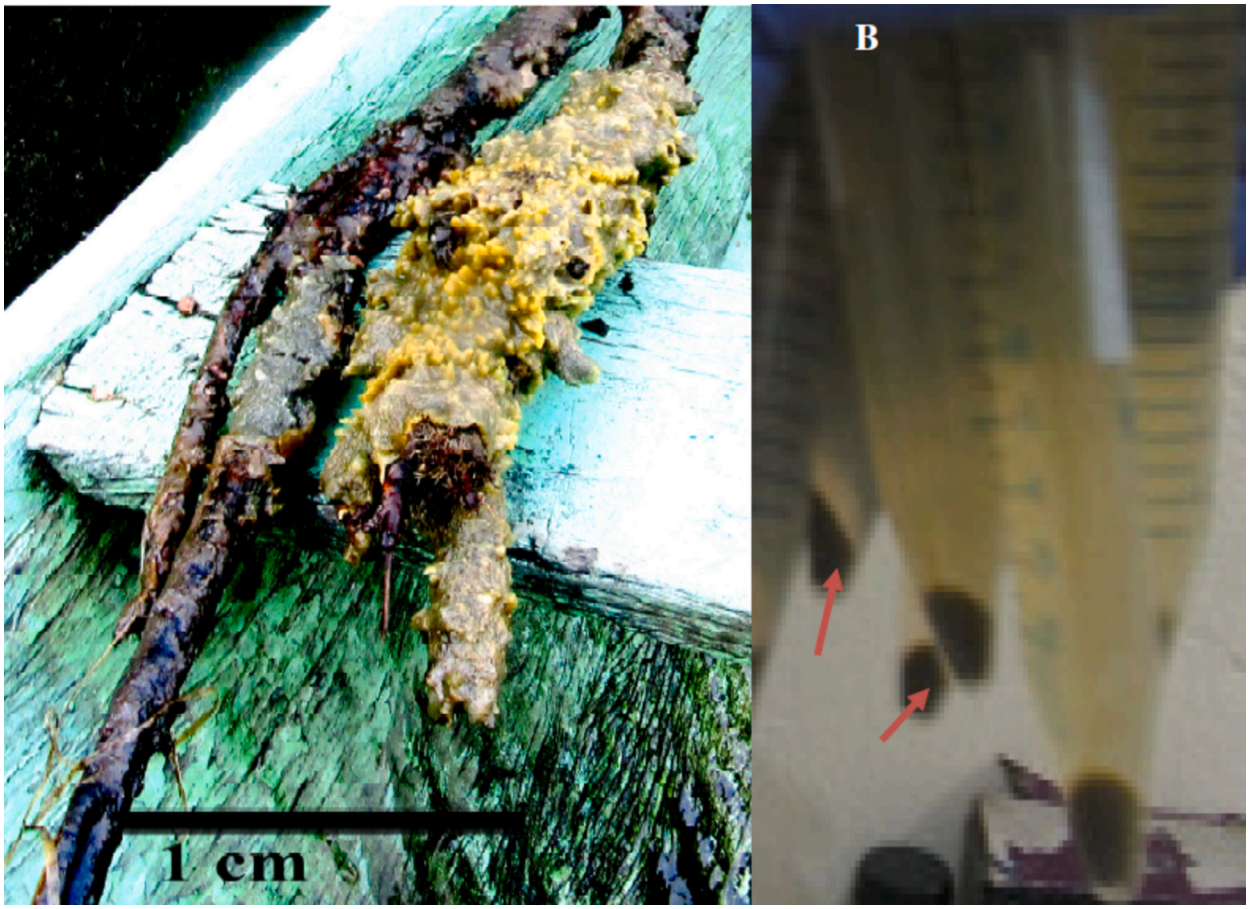

Fig. 1. Sponge cell collection and single cell prep a) Sample of *Amorphinopsis sp* attached to mangrove stomp b) Sponge cell pellet for onward processing for all bioassays.

viability, as shown by the 3-(4,5-dimethylethiazole-2-yl)-2,7-diphenyletetrazoliumbromide (MTT) assay in the following sections.

2.5. 3-(4,5-dimethylethiazole-2-yl)-2,7-diphenyl tetrazolium bromide (MTT) viability assay of sponge aggregates

Following sponge cell culture, the aggregates were removed from culture plates and washed thrice with 3 mL sponge media. The sponge aggregates were then dissociated into a single-cell suspension with 1 mL CMFSW+E. Dissociated sponge aggregates were moved into sterile Eppendorf tubes and centrifuged for 5 min at 4807 $\times g$ Single sponge cell pellets were washed three times with 3 mL CMFSW (to remove the EDTA) and resuspended in 1 mL 0.5 mg/mL MTT in sponge media. The sponge cell suspension was transferred into 12 well plates and incubated at 37 °C for three hours. After incubation, 100 μL DMSO was added to each well plate, and the absorbance was measured at 570 nm with an Infinite 200 Pro spectrophotometer against a 100 μL DMSO blank in Corning® 96 transparent flat bottom plate, supplied by Merck Life Science UK limited.

2.6. Comet assay for the measurement of DNA strand breaks

To assess DNA damage in *A. Kalibiama* caused by environmental pollutants, sponge cell aggregates were removed from culture plates after 12 h and washed thrice with 3 mL sponge media. The sponge aggregates were then dissociated into a single-cell suspension with 1 mL CMFSW+E. Dissociated sponge aggregates were moved into sterile Eppendorf tubes and centrifuged for 7 min at 859 $\times g$ (on a bench top Sanyo Gallen Kemp Micro Centaur), Supernatants were removed, and pellets were resuspended in 100 μL CMFSW containing no EDTA on ice. Aliquots of CMFSW (15 μL) suspended cells were combined with 150 μL of 0.5 % molten low melting point agarose (LMPA) in phosphate-buffered saline (PBS) and added on microscope slides coated with 0.5 % *w/v* normal melting point agarose in PBS. These were covered with coverslips and placed on ice for at least 20 min to set the agarose gel. After 20 min, coverslips were carefully removed from the slides (gently slid off horizontally), and slides were transferred into lysis buffer in Coplin jars for 1 h. Coplin jars with lids containing slides and buffer were placed on ice flakes in an ice box, covered with aluminium foil, and left in the cold room at 4 °C in the dark to ensure maximum cell lysis. The slides were then moved to a Fisher brand™ Horizontal Electrophoresis tank, including an electrophoresis buffer. Damaged DNA strands were allowed 45 min without power to unwind from the double super coil DNA helix; then, a 300-mA current at a voltage of 32 V was delivered to the electrophoresis apparatus for 30 min. The slides were washed with neutralisation buffer three times for 5 min before staining with 50 μL of SYBR gold. Slides were then placed in a new moist box wrapped in foil and left in the cold room overnight. Comet images were analysed afterwards using the Comet Assay IV software connected with a × 40 Nikon fluorescence microscope. Duplicate slides and at least three independent repeats were used in the experiments. The percentage-mean-tail-intensity of 50 Comet scores per slide was utilised as the genotoxicity endpoint (Duez et al., 2003; Cardoso et al., 2022).

2.7. Statistical analyses

Results were analysed with IBM SPSS Version 22.0 and Graph Pad Prism version 7.0. All data were normalised using Shapiro-Wilk's test

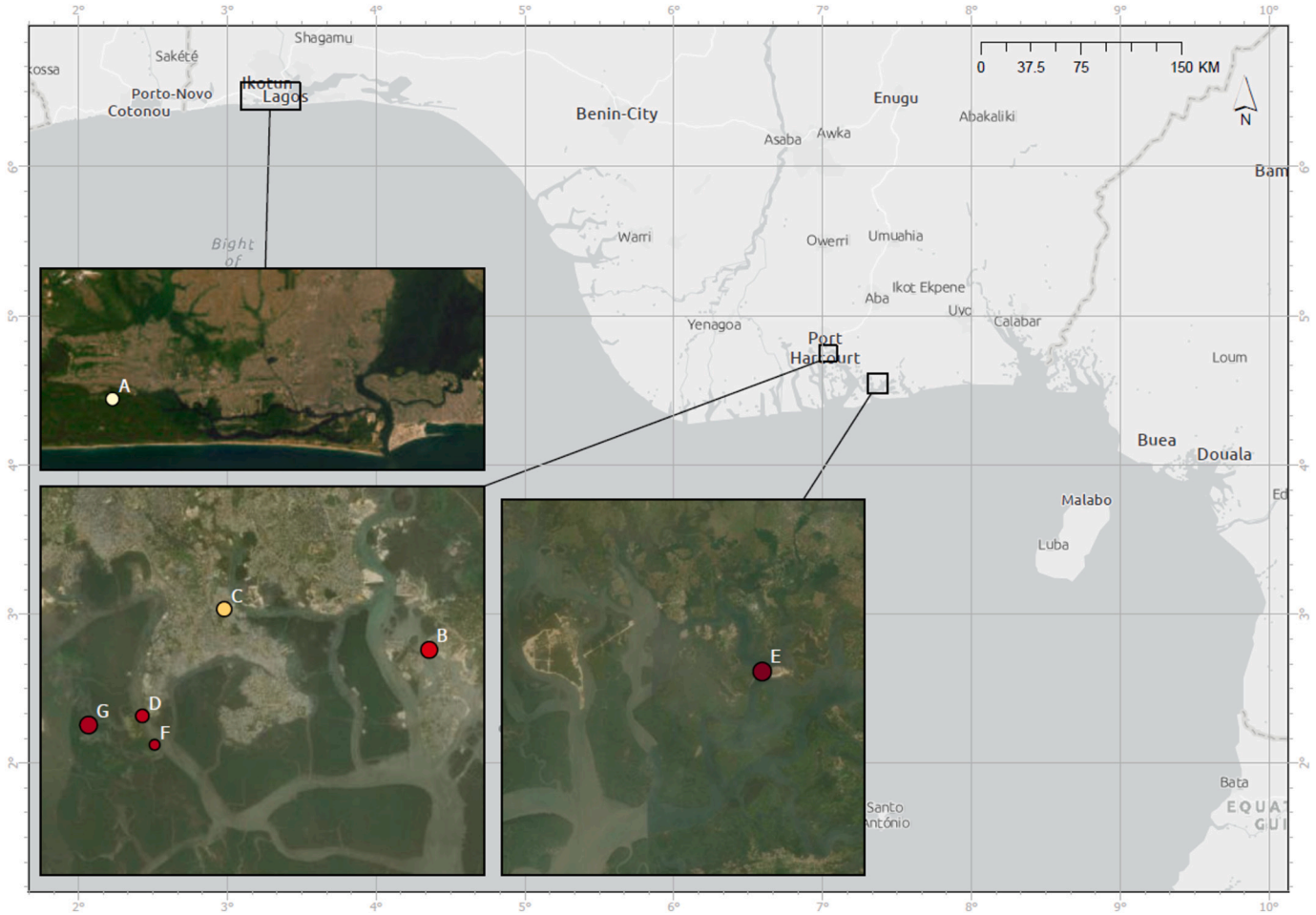

Fig. 2. A) Map of the study area showing sampling points. Symbology is point size = Al concentration, colour = % DNA strand break, and colour intensity/stronger red colour is equivalent to a higher amount of DNA damage. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Sampling sites in the Niger Delta area of Nigeria.

Sites	Site name	Geographical Coordinates
A	Topo-creek (Badagry)	6°26′18.5"N 3°09′06.8″E
B	Kalio-Ama Creek (Okrika)	4°45′32.7"N 7°04′45.0″E
C	All-Marine Creek, Ologbogbo (Marine Base Port Harcourt)	4°46′41.1″ - 4°46′18.5"N 7°01′20.5″ - 7°01′18.6″E
D	Kalibiama creek (Bonny Island)	4°44′26.8″N 7°00′10.9″E
E	Ebgomu River (Andoni Rivers state)	4°31′52.2"N 7°24′41.5″E
F	Issacka Creek (Eagle Island Port Harcourt)	4°44′01.7"N 7°00′28.1″E
G	Pokokri creek (Nembe/Brass Bayelsa state)	4°38′41.9"N 6°33′17.3″E

and homogeneity of variance was determined using Lavene's test. The mean difference between samples was measured using a two-way Analysis of Variance with Dunett's and Tukey multiple comparison test. Reported values are mean ± SEM (standard error of mean) at *P* values of ≤0.05.

2.8. Quality assurance

All chemicals and reagents used were of ultrapure analytical grade. For all measurements, reagent blanks (without the samples) was prepared and analysed in triplicate as the samples. Reported values are blank corrected mean ± SEM (standard error of mean). The following certified reference materials were analysed in triplicate following the same procedure for sample preparation for quality assurance and control; NIST 1646a, NIST 1566b, JLS, SGR, MAG, CCH, SCO and MON. Percentage recovery was between 90.5 % and 100 %.

3. Results

3.1. Concentrations of potentially toxic metals in sea water, sediment and sea sponge

Assessment of analysed metal levels in all three matrices: seawater, sediment and sea sponge structures consistently showed significant levels of aluminium above permissible levels as presented in Tables 2, 3 and 4 respectively. Metal levels in all sampling sites followed the order

Table 2
Semi-quantitative analysis of metal concentrations in the water column from all seven sample sites (mg/L water). Kindly refer to Table 1 for site codes.

Sites	Al	Cd	Cr	Cu	Ni	Pb
A	0.06	0.003	0.01	0.02	0.01	0.01
B	0.92	0.007	0.004	0.01	ND	ND
C	0.26	0.004	0.001	0.01	0.02	0.001
D	0.65	0.004	ND	0.01	ND	0.002
E	0.24	0.001	ND	0.013	0.003	ND
F	0.33	0.002	0.003	0.01	0.002	ND
G	0.25	0.002	ND	0.01	ND	0.003

ND: Not Detected.

Table 3
The concentration of metals in sediment from all sample sites. Data shown represent mean ± SEM; mg/kg, $n = 3$. Results are compared with metal concentrations (mg kg − 1) in the Forcados River sediments and sediment quality guidelines in Iwegbue et al. (2018), Table 4 and other sites within the Niger Delta, Table 5. Kindly refer to Table 1 for site codes.

Sites	Al	Cd	Cr	Cu	Ni	Pb
A	0.883 ± 0.114	−0.007 ± 0.0004	0.086 ± 0.0045	0.005 ± 0.001	0.0390 ± 0.004	0.0017 ± 0.0002
C	62.080 ± 7.875	−0.008 ± 8.8E-05	0.171 ± 0.015	0.048 ± 0.003	0.069 ± 0.0056	0.043 ± 0.0019
D	73.330 ± 2.242	−0.008 ± 6.15E-05	0.188 ± 0.005	0.048 ± 0.002	0.0783 ± 0.0024	0.042 ± 0.0015
E	33.451 ± 2.477	−0.008 ± 0.0002	0.123 ± 0.012	0.024 ± 0.002	0.050 ± 0.006	0.023 ± 0.0007
F	64.251 ± 4.737	−0.008 ± 5.94E-05	0.198 ± 0.010	0.063 ± 0.001	0.075 ± 0.0038	0.056 ± 0.0046
G	55.400 ± 13.393	−0.00804 ± 0.0002	0.185 ± 0.046	0.039 ± 0.006	0.072 ± 0.013	0.031 ± 0.004

Table 4
Metal concentrations (mg kg − 1) in the Forcados River sediments compared with sediment quality guidelines. Iwegbue et al. (2018).

Metals	TEL	PEL	ERL	ERM	<TEL	TEL-PEL	> PEL	< ERL	ERL -ERM	>ERM	LEL[a]	SEL[a]	USSQG-NP[b]	USSQG-MP[b]	USSQG-HP[b]
Cd	0.68	4.2	1.2	9.6	10 (19)	44 (81)	0	25 (46)	29 (54)	0	0.6	10	–	–	> 6
Pb	30.2	112.2	46.7	218	29 (54)	25 (46)	0	34 (63)	20 (37)	0	31	250	< 40	40–60	>60
Cr	52.3	160.4	81	370	43 (80)	11 (20)	0	52 (96)	2 (4)	0	26	110	< 25	25–75	>75
Ni	15.3	42.8	20.9	51.6	43 (80)	11 (20)	0	53 (100)	1 (0)	0	16	75	< 20	20–50	>50
Cu	18.7	108.2	34	270	48 (89)	6 (11)	0	54 (100)	0 (0)	0	16	110	< 25	25–50	>50
Co	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mn	–	–	–	–	–	–	–	–	–	–	460	1100	<300	300–500	>500
Zn	124	271	150	410	54 (100)	0 (0)	0	54 (100)	0 (0)	0	120	820	< 90	90–200	>200
Ba	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–
Fe	–	–	–	–	–	–	–	–	–	–	2×10^4	4×10^4	–	–	–
Al	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

TEL threshold effect level, PEL probable effect level, ERL effect range low, ERM effect range medium, SEL severe effect level, LEL lowest effect level, USSQG-NP US sediment quality guideline for non-polluted sites, USSQG-MP US sediment quality guideline for moderately polluted sites, USSQG-HP US sediment quality guideline for heavily polluted sites.

[a] Ontario Ministry of Environment and Energy, "Guidelines for the Protection and Management of Aquatic Sediment Quality in Ontario" August 1993, http://www.ene.gov.on.ca/envision/gp/

[b] Pradit et al. (2010).

Al > Cu > Ni > Cd > Cr > Pb (in seawater), Al > Ni > Cd > Cr > Cu > Pb (in sediment) and Cu > Cd > Pb > Al (in sponge). Overall, all the potentially toxic metals analysed were consistently detected in all sample locations except for Cr, Ni and Pb not detected in some sites in the water column (Table 2).

Results obtained from the semi-quantitatively analysed water column showed a relatively low metal concentration across all sample sites compared with metal concentrations in sediment and sponge structures.

The heavy metal concentrations obtained from the sediment from each of the sample sites were also compared with metal concentrations (mg kg − 1) in the Forcados River sediments, 8 other Niger Delta sites (Table 5) and with international sediment quality guidelines (Ontario Ministry of Environment and Energy and Pradit et al. (2010)) standards for metals in leachate (Table 4). Al value from all the sample sites is the highest of all the potentially toxic metals analysed. It is significantly higher than the permissible level while Cd, Cu, and Pb were lower than the allowable standard. Two-way ANOVA at $p < 0.05$ and Dunnett's multiple comparison tests showed a statistically significant difference between metal levels in sediment from sites C($p < 0.001$), Site D($p < 0.0001$), Site F ($p < 0.0001$) and Site E ($p < 0.01$).

The levels of metals in sponge tissues from all sampling sites are summarised in Table 6. Site E had the highest amount of Al, As, Cd and Cu measured, which was statistically significant compared to other sites at p* <0.05. Pb was highest in site F and significant at p* < 0.05.

3.2. Assessment of biological effects of heavy metal exposure on sea sponges

3.2.1. Sponge cell viability

The result showing the viability of the sponge cell is presented in Fig. 3. Site D revealed the highest metabolic activity of sponge cells followed by sites E and F respectively while site A has the lowest activity, with higher absorbance values indicative of greater cell viability.

3.3. Assessment of DNA strand breaks

The result of the Comet assay showed that Site E induced the highest genotoxic effects in the sea sponge closely followed by sites G, F and D respectively. All results showed that site A was the least polluted with the least genotoxic effect on the sea sponges. 1-way ANOVA with Bonferroni posthoc multiple comparison tests show a statistically significant difference between mean percentage DNA tail intensity of sponge cells from sites D, F, and G. Sample sites B and E were analysed using Kruskal-Wallis non-parametric test; $P = 0.049$ and 0.034 respectively.

Environmental pollutants with DNA damaging potentials are grouped into endogenous and exogenous sources; both sources mainly induce damage to the DNA through two main pathways, the production of reactive oxygen species (ROS) radicals and the introduction of error in DNA polymerase activities in normal metabolic processes (Griffin, 1996). Heavy metals such as chromium, vanadium, copper and iron are redox active metals, they act as catalysts for the oxidative damage of macromolecules. Their main mechanism of DNA Damage induction is through Fenton-like reactions within the cell membrane. Other ionic pollutants (cadmium, nickel, mercury, lead) induce oxidative damage to

Table 5
Metal concentrations (mg kg − 1) in different Niger Delta sites.

Matrix	Metals (mg kg − 1)													Authors
	Cd	Pb	Cr	Ni	Cu	Co	Ba	Mn	Zn	Fe	Al	As	Hg	
Sediment (Forcados River)	**0.78–2.16**	**11.5–72.0**	**21.9–49.6**	**4.75–11.7**	**4.48–13.7**	**5.03–11.7**	**2.04–3.34**	**153–545**	**11.7–35.1**	**9350–18,400**	**9560–25,000**	ND	ND	(Iwegbue et al., 2018)
Sediment														
	ND	4.9–6.1	4.9–11.2	0.45–3.9	ND	ND	ND	ND	23.6–69	ND	ND	ND	ND	
Kokori creek	ND	0.02–5.05	0.00–1.85	5.0–11.9	ND	ND	ND	ND	24.6–65	ND	ND	ND	ND	(Fatoba et al., 2016)
Kolo creek														
Moss (*Polytrichum juniperinum*)	0.001–0.092	0.001–17.380	0.004–8.793	1.425–21.730	2.350–110.760	0.989–1.950	ND	ND	23.5–130.6	ND	ND	ND	ND	(Ite et al., 2014)
Sediment (five sites in Benin river)	ND	0.00–0.29	0.03–0.47	0.03–0.19	0.01–0.47	ND	ND	ND	0.22–3.71	ND	ND	ND	ND	
	ND	0.001–0.31	0.04–0.47	0.05–0.25	0.01–0.50	ND	ND	ND	0.32–3.94	ND	ND	ND	ND	
• S1	ND	0.01–0.33	0.05–0.49	0.06–0.39	0.01–0.52	ND	ND	ND	0.32–4.14	ND	ND	ND	ND	
• S2	ND	0.01–1.91	0.05–2.87	0.13–1.23	0.01–3.06	ND	ND	ND	0.34–10.90	ND	ND	ND	ND	
	ND	0.02–2.05	0.11–3.07	0.13–1.23	0.00–3.27	ND	ND	ND	1.61–11.66	ND	ND	ND	ND	
• S3														(Ogbeibu et al., 2014)
• S4														
• S5														
Oron River (Akwa-ibom State														(Otitoju and Otitoju, 2013)
Tympanatus fuscastus														
	0.27	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	64.2	
Sediment														
	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM		
Water														
	NM	NM	NM	0.011	NM	NM	NM	NM	NM	NM	NM	NM		

Table 6
Metal levels in sponge samples (mean ± SEM, mg/kg; n = 3). Kindly refer to Table 1 for site codes.

Site ID	Al	Cr	Cu	As	Cd	Pb
A	1.322 ± 0.72	ND	0.002 ± 0.001	−0.044 ± 0.012	−0.00072 ± 0.001	0.0004 ± 0.0002
B	2.035 ± 1.21	ND	−0.0004 ± 0.0006	−0.068 ± 0.009	−0.00815 ± 0.007	−8.7E-05 ± 0.0003
C	1.640 ± 1.17	ND	0.0037 ± 0.001	−0.059 ± 0.004	−0.00143 ± 0.002	0.0002 ± 7.58E-05
D	1.294 ± 0.311	ND	0.0005 ± 0.0003	−0.063 ± 0.001	−0.003 ± 0.001	0.0001 ± 0.0002
E	2.447 ± 0.632	0.0057 ± 0.0099	0.0102 ± 0.013	−0.055 ± 0.0004	−0.00139 ± 0.0004	0.0004 ± 0.0002
F	0.821 ± 0.195	0.026 ± 0.046	0.002 ± 0.001	−0.06193 ± 0.002	−0.0016 ± 0.0013	0.0005 ± 0.0002
G	2.222 ± 0.933	0.005 ± 0.009	0.001 ± 0.00023	−0.0568 ± 0.001	−0.00155 ± 0.001	0.0005 ± 0.0001

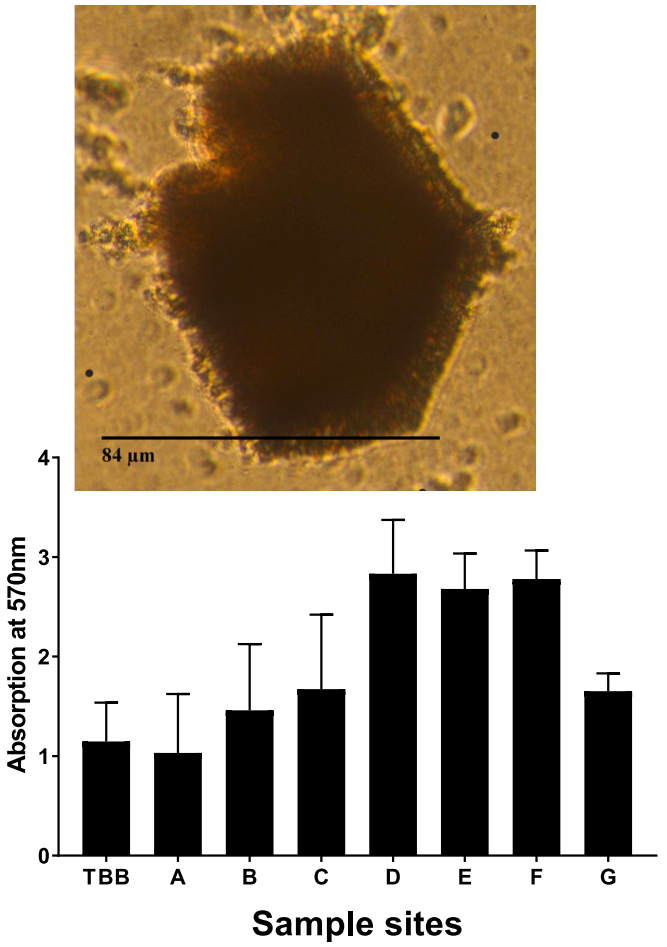

Fig. 3. Cryopreserved sponge cells: a) Sponge aggregate with well-defined feature showing viability b) MTT viability assay showed no statistically significant difference between cryopreserved sponge cells from the Niger Delta sites (TBB: Tenby Bay sample; A: Topo Creek Badagry in Lagos state; B: Kalio-Ama river Okrika in Rivers State; C: Al-marine Ologbogbo, Marine Base Port Harcourt in Rivers state; D: Kalibiama Creek, Bonny Island in Rivers state; E: Egbomu River, Andoni in Rivers state; F: Isaka Creek Eagle Island Port Harcourt in Rivers state; G: Pokokri creek Nembe/Brass Bayelsa state. Data displayed are mean ± SEM, $n = 4$.

cellular molecules via redox cycling and depletion of cellular sulfhydryl antioxidants (Stohs and Bagchi, 1995). Both mechanistic processes produce large amounts of ROS which undermine intrinsic cellular defences, resulting in molecular aberrations like; single and double-strand DNA damages, lipid peroxidations, cytotoxicity, genotoxicity, mutagenicity, neurotoxicity and alteration of calcium homeostatic pathways and are the main precursors to various disease conditions (Ercal et al., 2001; Stohs and Bagchi, 1995).

3.4. Relationships between metal accumulation and DNA damage

High concentrations of aluminium were consistently detected in all environmental samples. Therefore, Pearson's correlation was used to determine whether the DNA damage reported could be attributed to the high accumulation of aluminium in the environmental matrices. The relationship between aluminium accumulation in sponge tissues and DNA strand break is presented in Fig. 5. There was, however, no correlation between DNA strand breaks and other metals (Fig. 5b).

4. Discussion

In this study, we analysed environmental samples of seawater, sea sponges, and sediments obtained from six polluted sites in the Niger Delta and a comparatively clean site as the control site, for metal concentration and bioaccumulation. DNA strand breaks were measured in sea sponges as biomarkers of genotoxicity to establish the adverse effects of exposure to environmental pollutants. All the results reaffirmed the increasing pollution status of the Niger Delta, as previously reported by other studies (Ekpete et al., 2019; Udoh and Amadi, 2020; Chris and Anyanwu, 2022). The choice of metal pollutants over organic pollutants in this study is due to the main difference in the mechanism of toxicity of both groups of xenobiotics. Heavy metals although readily bioaccumulated in aquatic biota, body load unlike bioaccumulation of organic pollutants does not necessarily imply toxicity in biological systems (Phillips and Rainbow, 1993); Rainbow and Luoma (2011). This is because bioaccumulated metals are either removed by excretory processes or stored up in nontoxic forms, hence biologically unavailable to the organism and would not illicit any adverse effect (Phillips and Rainbow, 1993; Rainbow, 2002). Metals, however, become biologically available in living organisms by passive adsorption through active sites on the semi-permeable membrane. Within the cell, they metabolically interact with cellular biomolecules and metabolic processes by interfering with cellular macromolecules and biological pathways. Metal toxicity therefore results when bioavailable metal contents reach a threshold concentration within an organism. Because bioavailable contents can only be measured with bioassays, it is imperative to regularly monitor metal levels in the environment to detect when contamination or presence becomes a pollutant.

All metals (Al, Cd, Cr, Cu, Ni, Pb) analysed in each of the environmental samples in this study are part of the top list of the International Agency for Research on Cancer (IARC) priority carcinogenic metals (Fay and Mumtaz, 1996). In the aquatic ecosystem, toxic metals are mostly taken up by sessile epifaunae, such as sea sponges. The uptake of potentially toxic metals involves two mechanistic pathways in determining the fate of the chemicals within benthic biota (Fulke et al., 2020). Bioaccumulated toxic metals are either detoxified/biotransformed into inactive concretions that bind to lysosomes and are subsequently excreted or become biologically available and biomagnified along the ecological food chain. As prominent members of the coral reef community and benthic aquatic ecosystem, sea sponges can bioaccumulate extensive varieties of aquatic pollutants, including toxic metals, within their tissues for an extended period (Rodríguez and Morales, 2020).

Significant levels of Al were consistently detected in water from all

sampling sites. This can be an implication of leaching and dissolution processes from the exploration activities in the Niger Delta. Likewise, corrosion of aluminium-based infrastructure used for the exploration activities and rapid urbanization occurring the in Niger Delta could be the possible explanation for the elevated concentration of Al in the water samples from all sites. The major oil companies operational in the region include; Shell Petroleum Development Company of Nigeria (SPDC), ExxonMobil, Total Nigeria, Chevron, Elf, Agip Texaco, Phillip, PAN Ocean and Statoil. The Nigeria Liquefied Natural Gas (NLNG) also plays a major role in the region. The Nigerian National Petroleum Corporation (NNPC) now NNPC Limited, has over the years, developed a series of joint venture activities with the oil majors. Other Indigenous oil firms that operate in the region are; Dubri Oil, Consolidated Oil, and AMNI International Petroleum Company. The levels of aluminium reported herein in all samples are above permissible guidelines; WHO/FDA in WHO (2013) 1 mg/kg and ATSDR (2008) 0.05–0.2 mg/L. All other metal levels reported herein were within the permissible limits (Ighalo and Adeniyi, 2020). A similar trend was reported in sediments and the sea sponge. Elevated aluminium levels were consistently detected in sediments from all sampling sites. In the sea sponge, aluminium was also the most accumulated toxic metals compared to other analysed metals. The distribution of Al, As, Cd, Cu and Pb in sponge tissues from the sampling sites were in the order Site E > Site B > Site G > Site C > Site D > Site A > Site F. The result of this study agrees with the work done by Cebrian and Urizx (2007), Venkateswara Rao et al. (2009) and Rodríguez and Morales (2020), who also reported that sea sponges could accumulate toxic metals in their body tissues due to their feeding strategy and lifestyle. Hence, sea sponges could be employed as reliable biomonitors in coastal environments.

MTT viability assay using cryopreserved single field sponge cells showed no statistically significant difference compared to cryopreserved single sponge cells from *Hymeniacidon perlevis*. *H. perlevis* was adopted as a negative control because the originating environment is near pristine regarding metal pollution. *H. perlevis,* was obtained from Tenby Bay in Pembrokeshire. The study further assessed metal-induced DNA strand breaks in single sponge cells as a biomarker of genotoxicity using the alkaline Comet assay. The Comet assay is widely used for assessing genotoxicity because of its high sensitivity (Dhawan and Anderson, 2016; Cayir et al., 2019; Møller, 2022) and has been adopted in the assessment of pollutant-induced DNA damage in aquatic invertebrates, including sea sponges (Akpiri et al., 2017; Sunday et al., 2022).

DNA damage in sponge samples from all sites in the Niger Delta was statistically significant at $p < 0.05$ compared to the sponge cell sample from Topo Creek, Badagry/Lagos (Fig. 4). Damaged single sponge cell DNA (Fig. 4A) were recorded from all Niger Delta sites, with strand breaks differing from site to site. The highest level of DNA damage was observed with sponge samples from site E, Egbomu, in Andoni. This site also had the highest concentration of all metals analysed. The highest concentration of potentially toxic metals in this site may be due to several anthropogenic and geogenic factors such as geological composition, urbanization, oil exploration activities, oil pollution, agriculture and metal smelting activities occurring near the site. The percentage of DNA strand breaks was in the mean ± SEM range of 6.38 ± 1.56 in the control site A, 10.33 ± 3.27 for site B; 7.82 ± 1.64 for site C; 10.83 ± 2.40 for site D; 11.63 ± 3.68 in samples from site E; 10.97 ± 1.42 for site F; and 11.13 ± 3.11 site G. Following the significant level of strand breaks and metal concentrations in the untreated field samples of sea sponges from the Niger Delta, it is therefore safe to imply that there is a significant level of metal pollution in the Niger Delta aquatic ecosystem to elicit deleterious impact on living systems. Hence, the DNA strand break induction recorded must have been substantially contributed by both toxic metals and other toxic chemical cocktails in the environment. This hypothesis agrees with the EPA report on the synergy of aquatic pollutants in the induction of toxic effects, rather than on individual pollutant levels (EPA, 2018). Thus, bioassays and biomarkers are useful for easy identification of contaminated sites or environments (Steinert et al., 1998). The result of this study agrees with previous in vitro studies confirming that sea sponges are excellent biomarkers of genotoxicity (Akpiri et al., 2017; Bartolotta et al., 2009).

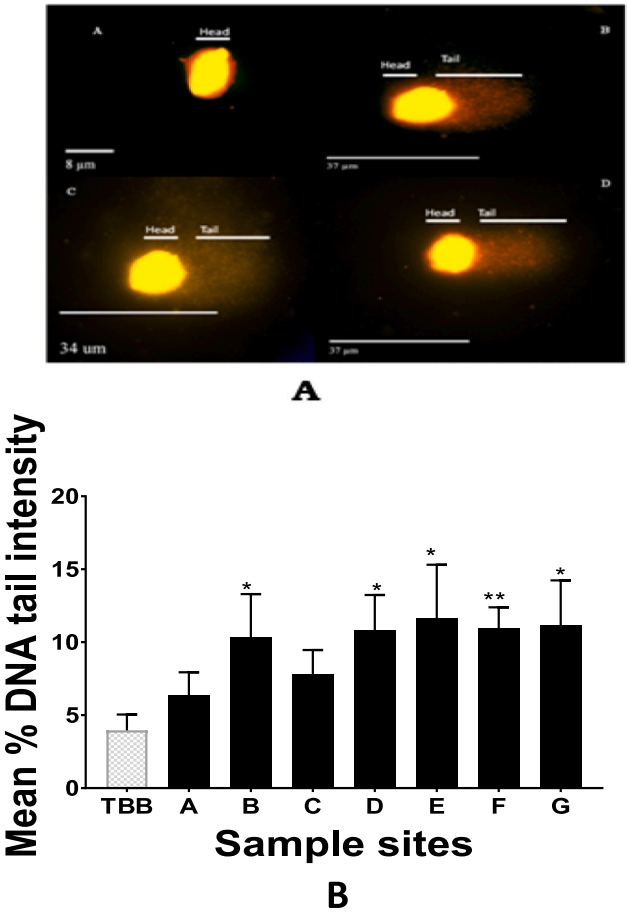

Fig. 4. Comet assay assessment of DNA strand breaks in samples obtained from the Niger Delta A) Representative Comet a) undamaged control image from site A: Topo Creek Badagry in Lagos state (b) Image from site F: Isaka Creek Eagle Island Port Harcourt in Rivers state (c) Image from site E: Egbomu River, Andoni in Rivers state(d) Image from sample site G: Pokokri creek (Nembe/Brass Bayelsa state). B) % DNA tail intensity for each site compared to sponge cells obtained from site A: Topo Creek Badagry in Lagos state and Tenby Bay Castle beach in Pembrokshire. Data shows mean values of the percentage median tail intensity ± SEM P* < 0.05, $n = 6$.

Toxic metal pollution in the Niger Delta aquatic system due to anthropogenic activities has been well documented in the literature (Chinedu and Chukwuemeka, 2018; Aigberua et al., 2020; Ibezim-Ezeani et al., 2022). The observed relationship between metal levels in sponge and DNA strand breaks could indicate an important relationship between bioaccumulation, bioavailability, and subsequent toxicity.

High concentrations of aluminium were consistently detected in all environmental samples. Fig. 5 shows a strong positive correlation between aluminium levels in sponge tissues vs % DNA strand breaks which was statistically significant at $P > 0.005$. This result further agrees with the work done by Akpiri (2018), Pannetier et al. (2019) and Benson et al. (2022), who reported that DNA damage in aquatic organisms is due to environmental pollutants. Furthermore, a previous study by Akpiri et al., 2019 has shown that Aluminium is genotoxic even at non-cytotoxic concentrations. The study reported a strong correlation between Aluminium-induced oxidative DNA damage and reactive oxygen species (ROS) formation, suggesting the active involvement of reactive oxygen radicals in the mechanism of aluminium-mediated toxicity. The strong correlation between Aluminium level and oxidative DNA damage

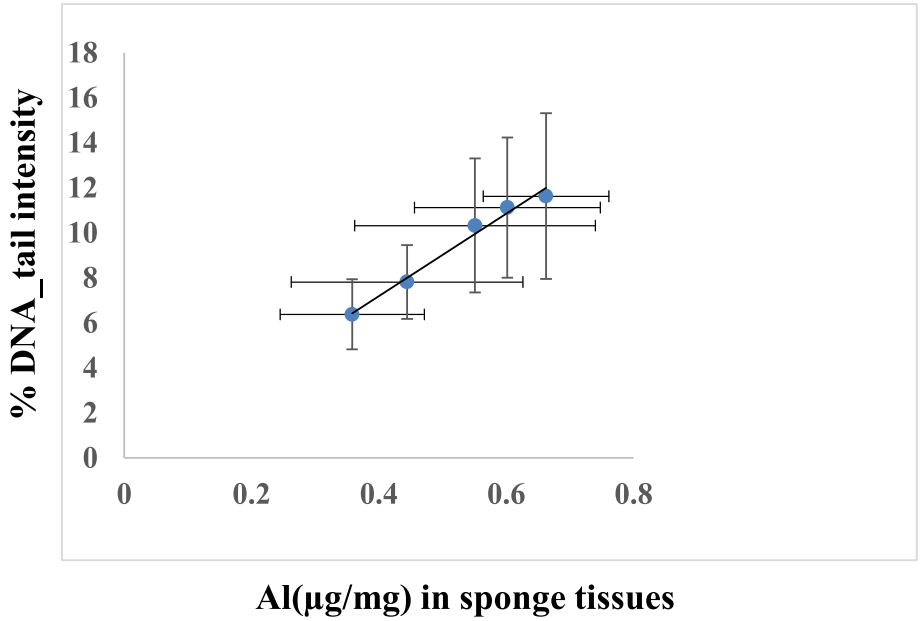

Fig. 5. 98 % Correlation between % DNA strand break in sponge cells from all sample locations and aluminium levels in sponge tissues from the sample location. R^2 = 0.9823; Displayed data is mean ± SEM, n = 3 P = 0.05.

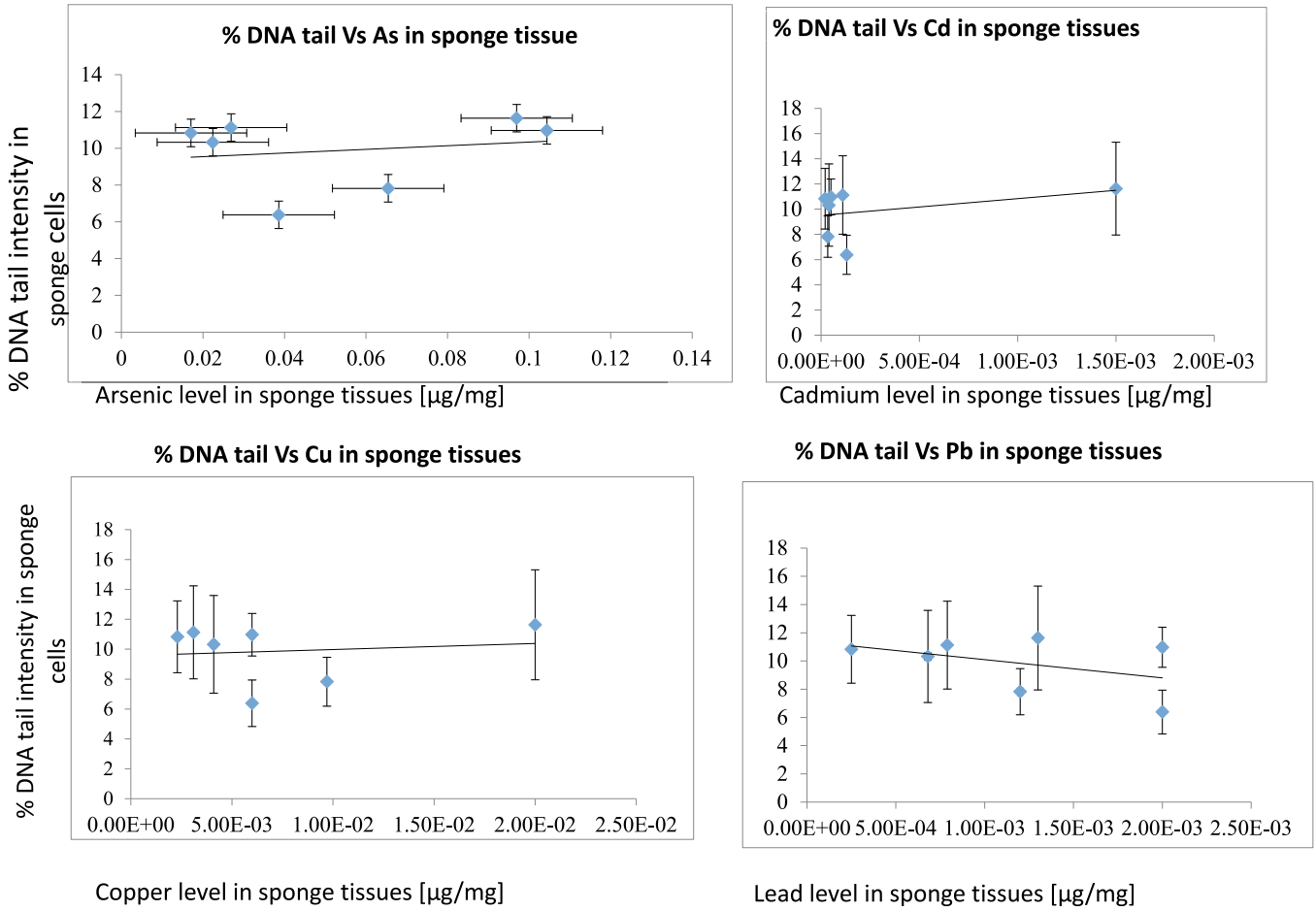

Fig. 5b. Pearson's correlation between DNA damage and levels of As, Cd, Cu, and Pb. Results showed little or no correlation between levels of As, Cd, Cu and Pb and DNA damage in sponge cells. Displayed data is mean ± SEM, n = 3, R^2 Values As = 0.0329; Cd = 0.133; Cu = 0.0157; Pb = 0.1879.

in the untreated field samples in the present study further confirms that Aluminium is genotoxic and DNA strand break is a reliable biomarker of genotoxicity.

Biomarkers provide information on biologically available contents of pollutants, needed for early warning signals; therefore, they are an important factor in Environmental Risk Assessment (ERA) and biomonitoring studies. (Livingstone et al., 2000; Martins and Costa, 2014; Van der Oost et al., 2003).

DNA damage in sponges measured with the comet assay technique in this study is an important biomarker of genotoxic effects of environmental pollutants and can be used to determine sample locations with the highest expression of biological effects from metal pollution, which would save time and money involved in chemical monitoring of individual pollutants. This information is also vital for pollution biomonitoring, Environmental risk assessment and policy making.

In conclusion, our study provides compelling evidence of significant heavy metal pollution in the aquatic ecosystems of the Niger Delta. The bioaccumulation of potentially toxic metals, in sea sponges underscores the pervasive nature of pollution resulting from anthropogenic activities, including oil and gas exploration. The observed genotoxic effects highlight the potential health risks posed by heavy metal contamination to aquatic biota. Our findings also suggest a strong positive correlation between the metal levels as the key contributor to DNA damage and genotoxicity in the studied environments. These results underscore the urgent need for comprehensive environmental monitoring, regulatory interventions, and pollution mitigation strategies to safeguard the health and integrity of aquatic ecosystems in the Niger Delta. Therefore, it is important to consider other possible pollutants to adequately predict this complex mixture's overall toxic effect in humans and animals.

CRediT authorship contribution statement

Rachael U. Chidugu-Ogborigbo Rachael: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **U. Sunday Nkopuyo:** Writing – original draft, Software. **J. Hodges Nikolas:** Writing – review & editing, Supervision, Project administration, Conceptualization. **James Barker:** Resources, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Dr Rachael Ununuma Chidugu-Ogborigbo reports financial support was provided by the Commonwealth Scholarship Commission. Grant ID: NGCS-2014-430.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.117386.

Data availability

Data will be made available on request.

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