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Cytotoxic and genotoxic responses of the RTgill-W1 fish cells in combination with the yeast oestrogen screen to determine the sediment quality of Lagos lagoon, Nigeria

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Abstract

Economic advancements in developing countries have seen an increase in urbanisation and industrialisation with a rise in the levels of discharge of effluents and municipal waste into aquatic ecosystems. Unfortunately, aquatic environmental regulations in these countries are often rudimentary and the development of environmental monitoring programmes will help identify ecological risks. As an example, the current study assesses the pollution status of 11 sampling sites in Lagos lagoon, Nigeria. The organic solvent sediment extracts were assessed for cytotoxicity and genotoxicity in rainbow trout gill-W1 cells. The induction of oestrogenic activities using the yeast oestrogen screen was also determined. The sediments were analysed for polycyclic aromatic hydrocarbons (PAHs) and other contaminants (polychlorinated biphenyls, organochlorine and organophosphate pesticides). Only sediments from three sites were cytotoxic at both 25 and 12.5 mg eQsed/ml using the Alamar Blue cell viability assay. The alkaline Comet assay showed that all sites caused significant DNA damage at 7 mg eQsed/ml; the extent of the damage was site specific. The measure of oxidative damage to DNA via the formamidopyrimidine DNA-glycosylase-modified Comet assay revealed similar results. Toxicity to yeast cells was observed in extracts from six sites; of the remaining sites, only two exhibited oestrogenic activity. There was no strong consistent relationship between sediment PAH concentrations and the cell toxicity endpoints. The dynamic nature of Lagos lagoon with its tides and freshwater inputs are suggested as factors that make it difficult to link the sources of pollution observed at each site with PAH levels and toxic endpoints. The study has demonstrated that the Comet assay is a sensitive endpoint to identify sediments that possess genotoxic contaminants, and this *in vitro* bioassay has the potential to be incorporated into an environmental monitoring framework for Lagos lagoon.

Introduction

Increasingly, environmental regulations and monitoring require testing of chemicals and environmental samples with fish to assess hazards and risks to the aquatic environment. The development of *in vitro* assays that reflect the effect on whole organism is an attractive alternative to whole-animal testing. Cytotoxicity has mainly been used as an end point with fish cell lines exposed to environmental samples (1,2), and results indicate a good correlation to cell viability and whole animal toxicity, but cells are generally less sensitive (3). The use of genotoxicity assays is of interest because they are more

sensitive than cytotoxicity assays (4) and alteration to DNA can identify potential chronic effects that influence population health (5).

The single cell gel electrophoresis (Comet) assay is a relatively quick and reliable method to detect DNA damage such as single- and double-strand breaks (6). The assay can be modified by the addition of endonucleases to assess oxidative damage to DNA (7). For example, formamidopyrimidine DNA-glycosylase (FPG) removes oxidised purines (8), but alkylated DNA lesions may also be detected by this enzyme (7). DNA repair mechanisms in fish cells have been described (9,10), and because DNA repair capacity has been reported to be low in some fish cell lines (11,12), they may provide a particular sensitive measure of the genotoxic effects of chemical contaminants (13–15). The Comet assay is not restricted to use for genotoxic hazard classification of chemicals and has been used successfully in determining the genotoxicity of complex environmental matrices such as sewage treatment plant effluent (16), marine coastal sediments (17,18), as well as sediment from the gypsum mining area (19) and the River Danube (20). The Comet assays applicability and relevance for ecogenotoxicology and use for environmental monitoring of the aquatic environment is becoming increasingly recognised (21,22).

It is estimated that over 2000 industries are situated close to Lagos lagoon and in the neighbouring Ogun States of Nigeria that discharge effluents into the lagoon (23). Lagos lagoon is also a major harbour for ships importing goods into Nigeria via the Tin Can Port, and this is another source of contaminants, such as oil and antifouling compounds (24,25). A number of studies have illustrated the rise in pollution within the brackish water lagoon based on chemical monitoring of surface waters (26–29). Further, biotic communities have been assessed for pathology and bioaccumulation of non-biodegradable compounds (30–32). These observations have been linked to diminishing health of its fin and shelf fish communities (25,33,34). As early as the 1990s, Osae-Addo and Abigail (35) reported a drop of almost 90% in fish catch from 1 000 000 kg/year in 1980 to ~100 000 kg/year in 1990 and attributed this to over fishing as well as a drop in fish populations as a consequence of health issues associated with pollutants. These dramatic effects on the health of biota often fail to draw the required attention from the general public and environmental regulators, and there is a need to identify environmental risks posed by pollution in the lagoon to advice on potential future health issues and strategies for pollutant mitigation and/or remediation.

Sediments are a sink for chemicals present in industrial and domestic effluents and reflect the historic contamination of water bodies (36). Sediments collected from the Lagos lagoon have been found to contain elevated concentrations of metals previously (37), in addition to marine sediments collected from sites worldwide, e.g. in Croatia (38), the Yellow Sea off China (39) as well as the rivers Danube, Elbe and Rhine in Europe (40). In addition, many of these studies also reported the presence of pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). The binding characteristic of chemicals to sediments often reduces contaminant bioavailability, but compounds may accumulate to concentrations that are many orders of magnitude greater than the overlying water. These contaminants are not locked into the sediment. For example, in Lagos lagoon, sand mining and harbour dredging are common (41). These practices inadvertently resuspend sediment into the water column, which is a major way in which these pollutants are reintroduced into the aquatic phase (42,43), leading to a continuous contaminant exposure route for biota living near the benthos (36,44). This pollution source is now recognised as a potential risk to environmental and human health (45). However, regulatory guidelines on allowable sediment toxicant loads are rarely derived or implemented. This is likely due to the complex nature of the mixtures of pollutants and geochemical dynamics governing contaminant bioavailability and toxicity. This lack of regulatory guidance for toxicant load in a key compartment of the aquatic environment prevents a holistic ecological risk assessment. Environmental biological monitoring is time consuming and costly. Therefore, the use of relevant *in vitro* models, such as reporter assays (46) or assays to measures cytotoxicity and genotoxicity in cell lines, can help to identify toxic environments (47,48). This is especially important in developing countries where the pressure on the aquatic environment from pollution is rapidly growing and there is an urgent need to develop simple monitoring tools to bridge the gap in the knowledge between contaminant load and impact.

This study follows a similar approach of Schnell *et al*. (49) that advocated the use of a suite of *in vitro* tests coupled with chemical analysis to identify potential toxic benthic sediments in the seas of northern Spain. Sediment from 11 sites along the west shore of Lagos lagoon, Nigeria, was evaluated in the rainbow trout gill (RTgill-W1) cell line. Cytotoxicity was assessed using the Alamar Blue assay measuring metabolic cell activity (50), and an unmodified and modified Comet assay was used to detect DNA damage (6). Furthermore, a yeast oestrogen screen was applied to determine the effect of endocrine disruption (46). The data obtained were used to rank sediments in terms of environmental risk and thus advise on toxic sites. Although all sites showed a degree of toxicity, interestingly the toxicity measures did not always coincide with the measured chemical contaminant load, indicating that restricted chemical analysis may not identify potential toxic sediments and an integrative approach that includes *in vitro* endpoints is necessary in sediment risk assessment.

Materials and methods

Study sites and sampling

This study was conducted in the Lagos lagoon, a tropical lagoon complex that stretches from Cotonu in the Republic of Benin and extends to the fringes of the Niger Delta in Nigeria along its 257 km course (Longitude 3°23′ and 3°53′E and latitude 6°26′ and 6°37′N). It is brackish water that is fed majorly in the north by the Ogun River and a host of other smaller rivers as well as tidal creeks. It discharges in the south into the South Atlantic Ocean through the Lagos harbour. Sediment was collected from 11 sampling sites (Figure 1) based on a previous study that evaluated metal loading (37). Sampling sites were divided into four zones: Zone I (Outer Lagos lagoon), 1. Victoria Island; 2. Apapa port and 3. Apapa tank farm; Zone II (mid/lower Lagos lagoon), 4. Iddo and 5. Makoko; Zone III (mid Lagos lagoon, University), 6. Unilag High rise; 7. Off third mainland bridge and 8. Off Unilag power station; and Zone IV (upper Lagos lagoon), 9. Ilaje; 10. Oworonshoki and 11. Odo Iyalaro. Descriptions of each sampling site along with characteristic sources of effluent contamination are given in Table 1. Approximately 1 kg of sediment was obtained from the surface at each sampling sites using 20 kg Van Veen grabs. Grabs were performed in triplicates per site and pooled together to ensure they are representative of each location. The samples were wrapped in aluminum foils *in situ* and transported chilled to the laboratory using ice packs.

Sediment preparation and extraction

Sediment samples were air dried under a ceiling fan and packed in aluminum foil for transportation to King's College London, London, United Kingdom. Before extraction, samples were further

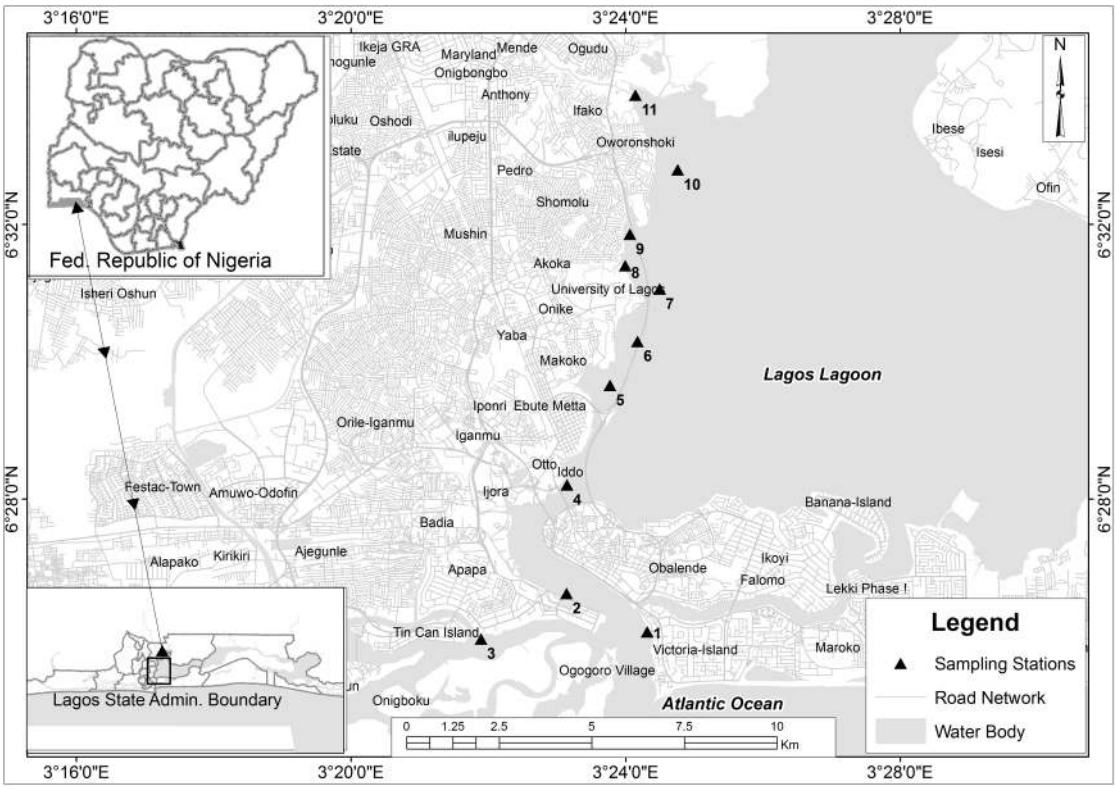

Figure 1. Map of sampling sites on the west coast of Lagos lagoon, Nigeria.

Table 1. Description of sampling sites in Lagos lagoon

Sampling sites	Location within Lagos lagoon	Sources of pollution/characteristics
1	Victoria Island	Solid waste dump, boat transportation. Relatively undisturbed.
2	Apapa port	Shipping activities, loading and offloading of cargo, cement and sugar plants, canals discharging municipal sewage.
3	Apapa tank farm	Petroleum products jetty, shore-side automechanic workshops, ship welding centres, solid waste dumps.
4	Iddo	Major centre for discharge of septic tank wastes, solid waste dump, subsistence recycling of jute bags and chemical cans by hand washing.
5	Makoko	Solid waste dumps, canals discharging municipal sewage, domestic sewage.
6	Off Unilag High Rise	Relatively undisturbed shallow coast line, receives wastes from other parts of the lagoon mainly by tidal action.
7	Off third mainland bridge (Unilag section)	Deep lagoon area receiving wastes from different parts of the lagoon at high tide. Off-road wastes from the third mainland bridge.
8	Off Unilag power station	Solid waste dumps, canals discharging municipal sewage, university campus sewage treatment plant effluents, electricity power station effluents.
9	Ilaje	Solid waste dumps, canals discharging municipal sewage, domestic sewage.
10	Oworonshoki	Solid waste dumps, domestic sewage, sand mining, boat construction and water transportation
11	Odo-Iya Alaro	Industrial effluents from Ikeja/Ogba Industrial Estate.

freeze dried to ensure that all moisture was removed. Extraction was similar to the protocol described in Schnell *et al.* (49) Samples were ground in clean ceramic crucibles and shaken through a 63-µm sieve, from which 2.5 g of sediment was placed into glass vials. After the addition of 10 ml of dichloromethane:hexane (1:1; v:v) mixture, the samples were sonicated for 10 min at 4°C and then centrifuged (2000 *g*) for 10 min at 4°C. The supernatant was placed in a fresh glass vial and the procedure repeated twice. Sediments were further extracted using a dichloromethane:acetone (1:1; v:v) mixture and the process repeated as described above. All supernatants were pooled and reduced to 5 ml over a stream of nitrogen gas. After the addition of 500 mg of activated copper to each extract, it was stored overnight at 4°C. Next day the supernatant was removed, placed into a fresh tube and evaporated as described earlier to complete dryness. Extracts were reconstituted with 500 µl of dimethylsulphoxide (DMSO) or methanol depending on the subsequent assay

performed. Extracts were stored at –20°C until analysis. The stock was equivalent to 5 g dry weight extract (eQsed)/ml.

RTgill-W1 cell culture and cell viability

RTgill-W1 cells (51) were routinely cultured in 75-cm^2 culture flasks at 18°C in Leibovitz's L-15 culture medium (Invitrogen) supplemented with 5% fetal bovine serum, 1% of penicillin–streptomycin solution (10 000 units/ml penicillin, 10 mg/ml streptomycin) and 1% of gentamycin solution (10 mg/ml). For exposure to sediment extracts, cells were seeded in 96-well tissue culture plates at a cell density of 50 000 cells per well in 100 µl medium and left overnight for attachment. Cultures ($n = 5$) were exposed to various concentrations of extract (dissolved in DMSO) and cells were incubated for 24 h at 18°C. Controls were treated with solvent, DMSO, only; the final concentration of DMSO was always kept at 0.5%. A serial dilution, 1:1–1:32, of sediment extract was made, and from each dilution, 5 µl was added to 1000 µl of media to give final concentrations ranging from 0.78 mg to 25 mg eQsed/ml. For cell viability assessment, 100 µl of the test solution was added to each well. From preliminary standard toxicity curves two extract concentrations, (1:1) 25 mg eQsed/ml and (1:2) 12.5 mg eQsed/ml were chosen for further in-depth analysis. Assays were performed in triplicate.

The metabolic cell activity as a measure of cell viability was monitored using Alamar Blue (resazurin; Invitrogen) (50). Just before starting the assay, 526 µl of Alamar Blue was added to 10 ml of L15/ex, a modified L15 culture medium, which contains salts, galactose and pyruvate, but no vitamins and amino acids. The test medium was replaced by 100 µl of the Alamar Blue solution and incubated for an hour. Fluorescence was measured using a fluorescence plate reader (Synergy HT from Bio-Tek) with excitation and emission wavelengths 530 and 590 nm, respectively. Results were reported as relative fluorescent units.

Comet assay

The alkaline version of the Comet assay was used to detect DNA damage, including single- and double-strand breaks and alkali-labile (e.g. apurinic) sites (52). The lesion-specific repair enzyme FPG was employed to characterise oxidative damage to DNA as described previously (53). RTgill-W1 cells were seeded into 25-cm^2 culture flasks (0.7×10^6 cells/4 ml media) and incubated for 24 h at 18°C. Each set of experiments included extracts from two sites as well as from site 11, Odo Iya-Alaro, a site of known to receive industrial effluent, which acted as an internal standard to account for inter-experimental variation. Cells grown in media containing 0.5% DMSO served as control. For each exposure, 22.5 µl of test extract was added to each flask. The concentration of extract used for treatment was based on the lowest dilution, where there was no loss of cell viability, this equated to 1:4 and 1:8 dilutions or 7 µg and 3.5 mg eQsed/ml.

After 24 h of incubation at 18°C, cells were trypsinised and resuspended in phosphate-buffered saline (PBS) to a final concentration of 2×10^6 cells/ml. Fifty microlitres of the cell suspension was mixed with 150 µl of 0.65% low melting point agarose (in PBS), pre-heated to 45°C and 75 µl aliquots were spread over agarose pre-treated microscope slides. Slides where placed on ice for 30 min until the agarose cell mixture was solidified and transferred into lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 250 mM NaOH pH 10) overnight at 5°C. Thirty microlitres of FPG enzyme (54 ng/µl) in enzyme buffer (0.1 M KCl, 40 mM HEPES, 0.5 mM EDTA, 0.2 mg/ml bovine serum albumin) or enzyme buffer alone as control were added and incubations were performed in a humidity chamber pre-heated to 37°C for 30 min. DNA unwinding was performed in denaturing/electrophoresis buffer (0.3 M NaOH, 1 mM EDTA) for a further 30 min at 4°C. Electrophoresis (22 V for 24 min at 0.3 A) was performed in a pre-cooled (4°C) horizontal electrophoresis tank. After electrophoresis, slides were placed in neutralisation buffer (0.4 M Tris-Cl pH 7.5), and subsequently fixed in methanol for 10 min. Dried slides were then stained with ethidium bromide (10 µg/ml in water) for 5 min. Comets where analysed using a Leica fluorescence microscope (Leica DMLB 020-519-010 LB30T). DNA damage was scored using the Comet IV capture system (version 4.11; Perceptive Instruments, UK). Fifty nucleoids were assessed per slide, and each sample was analysed in duplicate. All samples were measured blind. The results from both replicates were combined for further analysis. The tail intensity (% tail DNA), defined as the percentage of DNA migrated from the head of the comet into the tail, was used as a measure of DNA damage induced, because it has been shown to be a meaningful end point to assess genotoxicity (54).

Yeast oestrogen screen

The culture of yeast cells containing the human oestrogen receptor integrated into its genome and also carrying an expression plasmid with the oestrogen response element upstream of the *lacZ* reporter gene was performed as described (46). Yeast cells were incubated with chlorophenol red-b-*D*-galactopyranoside (CPRG) and in the presence of an oestrogenic compound the oestrogen receptor was activated, which induced the synthesis of beta-galactosidase, encoded for by *lacZ*. This enzyme breaks down CPRG changing the media from a yellow to red colour, the absorbance of which is measured at 540 nm.

Experiments were conducted in 96-well plates containing 200 µl of yeast culture (0.4×10^{-7} cell/50 ml). For each site a dilution of the stock was carried out generating a range of concentrations from 7.8 to 250 mg eQsed/ml (e.g. 1:32 to 1:1). Yeast grown in media containing 0.5% DMSO served as control. Each experiment included a blank (no DMSO), DMSO controls and a series of estradiol (E2) controls, with a range of 5×10^{-13}–5×10^{-8} M. E2 was dissolved in ethanol and 10 µl of the stock test solution was added to each well and allowed to dry before the addition of yeast cells. Cells were incubated at 32°C in a humidified atmosphere for 75 h, after which the absorbance at 540 and 630 nm was measured. The reading at 630 nm accounts for changes in growth and cell lysis. Samples where growth was less than the controls were not considered. Oestrogenic activity was calculated as follows: sample absorbance (540 nm) – [sample absorbance (630 nm) – control absorbance (630 nm)]. The experiment was performed three times.

Chemical analysis

A single sediment sample from each site was sent to the National Laboratory Services, Leeds, United Kingdom, a UKAS (Method accredited to International Organization for Standardization/International Electrotechnical Commission 17025) accredited laboratory for analysis of polyaromatic hydrocarbons, PCBs, organochlorine and organophosphate pesticides (see Appendix 1). For tetra- to octa-chlorinated dioxins and furans, PCB samples were extracted into toluene using a Dionex Accelerated Solvent Extraction (ASE)™ system and cleaned up to remove potentially interfering compounds such as fats. For quantification ^{13}C labelled isotopes of the compounds of interest are added to the sample before extraction and used as internal standards. For the other compounds sediment, samples were extract with dichloromethane between 12 and 14 h on a horizontal roller at room temperature. For some measurements, a fraction of the

dichloromethane extract was passed through a solid-phase extraction cartridge and extracted with hexane. All samples were analysed on a High-Resolution GC-MS (HR GC-MS) or GC-MS.

Statistical analysis

For the cytotoxicity assay, results are presented as the mean ± SD of replicate wells of three independent experiments and expressed as percentage of solvent controls. Significant difference between sites and the solvent control were calculated based on untransformed fluorescent units using the non-parametric Mann–Whitney rank sum test (SigmaStat v12.0).

For the assessment of strand breaks an one-way analysis of variance (ANOVA) revealed significant differences in % DNA tail intensity between the DMSO controls used during each set of experiments (one-way ANOVA failed the Shapiro–Wilkins normality test and Kruskal–Wallis rank test $P < 0.001$, SigmaStat v12.0), but there was no difference between the internal control site 11 (one-way ANOVA, failed homogeny of variance and passed Shapiro–Wilkins normality test, $P = 0.056$, SigmaStat v12.0). Because of the significant difference in the DMSO controls, a comparison between each site from different sets of experiments was not performed. Instead, for each experiment the difference in DNA tail intensity between exposure and the DMSO control, as well as between the two dilutions from each site, was assessed via a *t*-test or Mann–Whitney rank sum test depending on whether the data was normally distributed (SigmaStat v12.0). The extent of oxidative DNA purine damage was determined by subtracting the % DNA tail intensity from the FPG-treated cells from the buffer alone.

Results

Cytotoxicity in RTgill-W1 cells

Extracts from sites 1, 4, 5, 7 and 10 were not cytotoxic to RTgill-W1 cells. Extracts from sites 2, 3 and 11 were toxic at 25 mg eQsed/ml and sites 6, 8 and 11 toxic at both 25 and 12.5 mg eQsed/ml (Figure 2) compared to solvent controls (Mann–Whitney rank sum test, $P < 0.05$).

Genotoxicity in RTgill-W1 cells

All extracts at 7 mg eQsed/ml caused a significant increase in % DNA tail intensity and only site 1 at 3.5 mg eQsed/ml did not induce any DNA damage when compared with the DMSO control (Figure 3). A 2-fold dilution in the extracts from sites 3, 5, 7 and 8 caused a significant reduction in DNA damage, but this was not apparent at sites 1, 2, 4, 6, 9 or 11 (Figure 3). A ranking of site DNA damage based on subtracting the mean % DNA tail intensity from each site exposed to 7 mg eQsed/ml shows that site 7 induced the most DNA damage followed by site 8 > 10 > 4 > 3 > 11 > 9 > 2 > 1 > 5 > 6 (Table 2). A measure of oxidative damage to DNA determined using the FPG-modified Comet assay revealed a ranking of the greatest effect at 7 mg eQsed/ml of site 3 > 7 = 6 > 8 > 11 > 5 = 4 > 1 = 9 = 10 > 2 = DMSO control (Figure 4).

Yeast oestrogen screen

The DMSO and extracts from sites 1, 5, 6, 10 and 11 were not toxic to the yeast cells. However, sites 3 and 8 were toxic down to 7.8 mg eQsed/ml (1:32 dilutions) and sites 2, 4, 7 and 9 to 15.6 mg eQsed/ml (1:16 dilution). For the latter four sites, growth was seen at 7.8 mg eQsed/ml, but no oestrogenic activity was observed. Of the non-toxic extracts, sites 5 and 10 induced oestrogenic activity at the highest sediment extract load (250 mg eQsed/ml). However, the activity at site 5 was low but above the absorbance of the lowest observable concentration of E2 to induce activity, 0.5×10^{-11} M E2. Oestrogenic activity at site 10 was higher around the E2 EC50 (~0.2×10^{-10} M) value (Figure 5).

Toxicity assessment

To assess the overall combined toxicity from the various *in vitro* tests, a ranking approach was used, where the sites were placed into

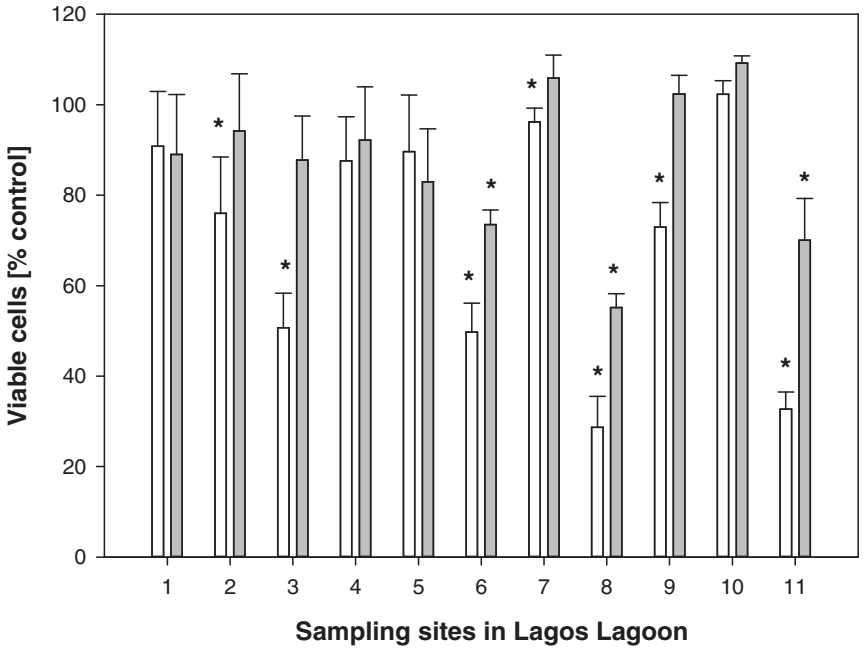

Figure 2. Cell viability in RTgill-W1 cells, as measured by the cells' ability to reduce Alamar Blue (resazurin). Results are expressed as viable cells (% control) and each bar represents an average of three independent experiments ± SD. The white bars represent a final exposure concentration of 25 mg eQsed/ml and the grey bars exposure to 12.5 mg eQsed/ml. Asterisks indicate significant difference (Mann–Whitney rank sum test, $P < 0.05$ performed on the untransformed fluorescent units) from controls.

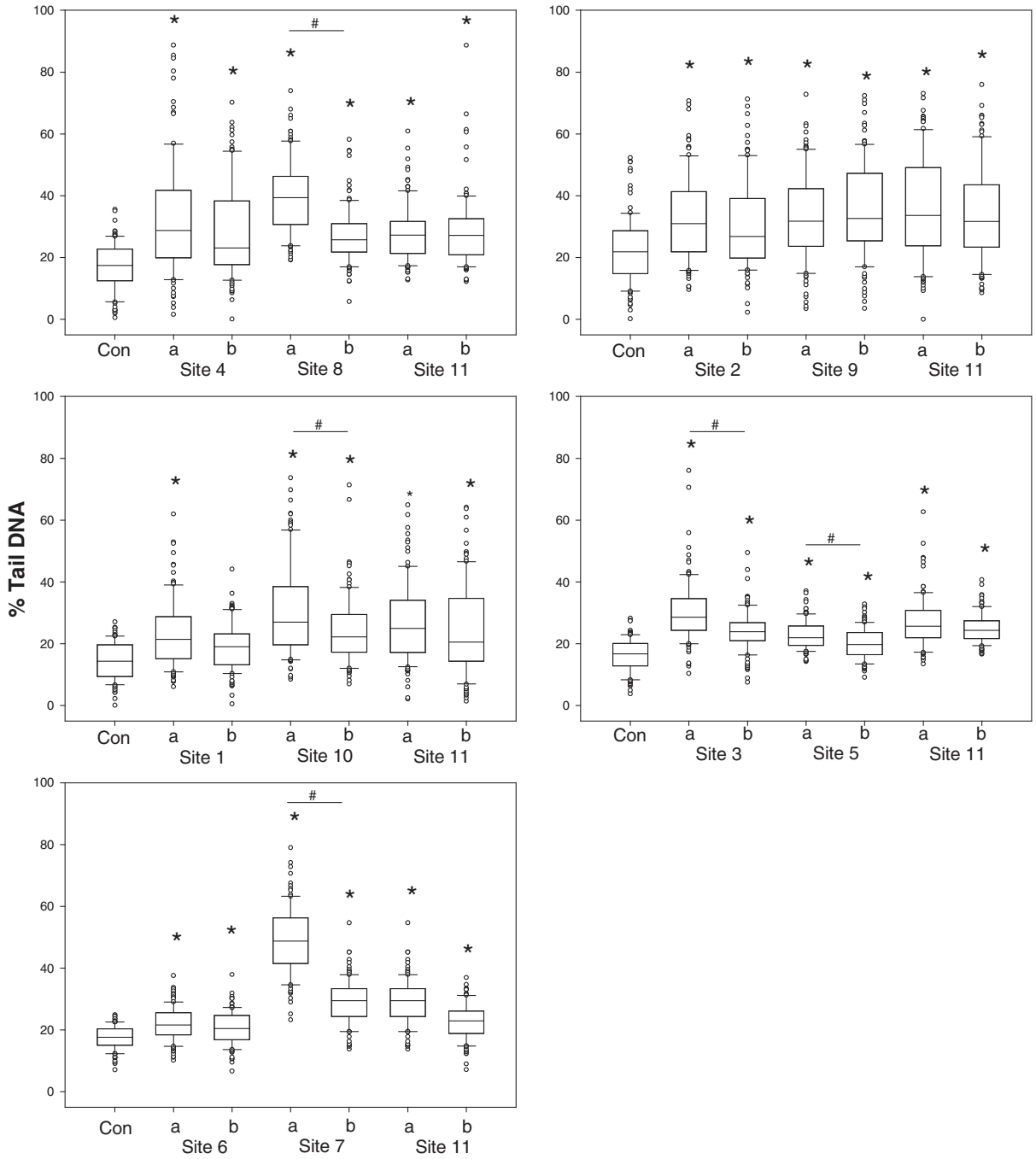

Figure 3. DNA damage as measured by the Comet assay in RT-gill W1 cells following 24-h exposure to (a) 7 and (b) 3.5 mg eQsed/ml. Boxes represent middle 50% range of data with the bar representing the median ($n = 100$). The whiskers represent the range and circles the outliers, which represent any value that lies more than one and a half times the length of the box from either end. Asterisks indicate significant differences to the solvent controls (Con), whereas # indicates significant difference between the two dilutions 7 and 3.5 mg eQsed/ml, if the data was normally distributed a Student's *t*-test was applied, and if not normal a Mann–Whitney *U*-test was used, $P < 0.05$ was considered significant.

four arbitrary ranges and the scores combined to give an overall toxic unit measure (Table 2). The toxicity rank for the cell viability assay (Figure 2) was as follows: rank 1, toxic at both concentrations and cell viability <40%; rank 2, toxic at both concentrations, with cell viability >50%; rank 3, toxic at one concentration; rank 4, non-toxic. Because there was significant difference between the controls for each set of experiments, the difference in the average % tail DNA intensity at an exposure concentration of 7 mg eQsed/ml from each site to the corresponding control (Figure 3) was used and ranked as follows: rank 1 (most toxic), Δ 16–32; rank 2, Δ 12–15; rank 3, Δ 10–12; and rank 4, Δ <10. For oxidative damage to DNA (Figure 4), ranking was as follows: rank 1, >22; rank 2, 20; rank 3, 10–20; and rank 4, <10. For the yeast assay, values were based on the lowest dilution to cause significant death of the cells: rank 1, 1:32 dilution; rank 2, 1:16; rank 3, 1:8; and rank 4, non-toxic. Overall toxicity based on the ranking revealed the hierarchy of toxicity as follows: 8 > 3 > 7 > 11 > 6 = 4 > 9 = 2 > 10 > 5 > 1. For comparative purposes sediment total PAH (tPAH) concentrations were also ranked: rank 1,

Table 2. Ranking of sites for total sediment PAH concentrations as well as cell viability, induced DNA damage and yeast toxicity

Sites in Lagos lagoon	PAH	Cell viability	Comet assay (unmodified)	FPG-modified Comet assay	Yeast toxicity	Total toxic unit
1	4	4	4	3	4	15 (11)
2	2	3	3	4	2	12 (7)
3	2	2	2	1	1	6 (2)
4	2	4	2	3	2	11 (5)
5	4	4	4	3	4	15 (10)
6	3	2	4	1	4	11 (5)
7	3	4	1	1	2	8 (3)
8	1	1	1	2	1	5 (1)
9	1	3	3	3	3	12 (7)
10	3	4	1	4	4	13 (8)
11	1	1	2	3	4	10 (4)

See text in Results for details of ranking ranges for Classes 1–4. Total toxic unit = sum of the rank for the cell viability, Comet assay, modified Comet assay and yeast toxicity. The number in parentheses equals rank of total toxicity.

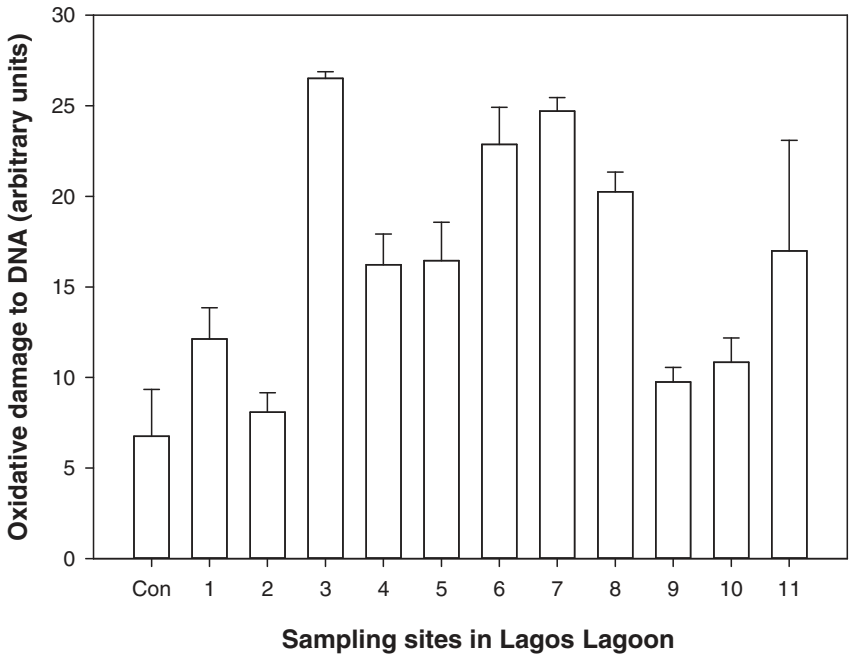

Figure 4. Oxidative damage to DNA assessed by the FPG-modified Comet assay in RT-gill W1 cells following 24-h exposure to 7 mg eQsed/ml. Values represent the difference in % DNA tail intensity recorded in the presence or absence of FPG. The values represent the average of repeats and the average % DNA tail intensity measured in 50 cells from each repeat ($n = 100$).

tPAH > 3000 µg/kg; rank 2, tPAH 1000–2000 µg/kg; rank 3, tPAH 40–300 µg/kg and rank 4, <1 µg/kg.

Discussion

This study, for the first time, used the RTgill-W1 cell line and a suite of *in vitro* bioassays to assess the environmental quality of sediments at various sites along the southern and western shore of Lagos lagoon, Nigeria. The bioassays included the cells' ability to reduce Alamar Blue, a measure of energy metabolism that correlates with cell viability as well as the induction of DNA damage (including oxidative damage to DNA) via the Comet assay, a measure of genotoxicity. In addition, a yeast oestrogen screen was used as a measure of the presence of endocrine disrupting compounds. DNA damage proved the most sensitive end point and was induced at each site. In the other two assays, some sites were not toxic whilst others proved toxic, particularly to the yeast cells, which hindered the assessment of oestrogenic activity. However, sediment oestrogenic activity was detected at two sites (5 and 10). Interestingly, there was one site, 8, that ranked high on toxicity where sediment PAH measurements were also extremely high (Table 2); in other instances, this relationship was not always apparent suggesting that other cytotoxic and genotoxic contaminants were present.

The sites chosen have different pollutant inputs and hydrological characteristics (Table 1). The organic solvent extraction method released the majority of organic pollutants from the sediment. The chemical analysis was targeted towards a range of polyaromatic hydrocarbons, PCBs, organochlorine and organophosphate pesticides and revealed the presence of at least one compound at each site. The sediment contamination was dominated by PAHs; no PCBs were identified and only cypermethrin (308 µg/kg) at site 3 and dichlorodiphenyltrichloroethane (24 µg/kg) at site 2 was additionally detected. Based on tPAH, the contamination could be grouped into high: sites 8, 9 and 11 (tPAH > 3000 µg/kg); medium: sites 2,

3 and 4 (tPAH 1000–2000 µg/kg); medium/low: sites 6, 7 and 10 (tPAH 40–300 µg/kg); and low, sites 1 and 5 (<1 µg/kg) (Table 3). These values are similar to those measured in the Yellow Sea, China (range 408–4032 µg/kg from 3 sites (39). It is difficult to relate the description of the surrounding industry and potential effluent inputs at each site to the tPAH load, the exception being site 1 where the description is 'relatively undisturbed' and there is minimal PAH contamination (Table 3). Site 6 is also described as relatively undisturbed shallow site, but is tidally active receiving waters from other regions and this may in part explain the PAH contamination (tPAHs 263 µg/kg). In contrast, site 5 receives municipal and domestic sewage as well as being located close to a waste dump, similar to the description of site 9, but the tPAHs at both sites are in marked contrast, site 5, 0.8 µg tPAH/kg and site 9, 3510 µg tPAH/kg. The site descriptions give an indication of the pollutant inputs, but discrepancies are likely because of additional petrogenic sources of PAHs associated with the main port for Nigeria being situated in Lagos. In addition, hydrogeomorphological and hydrological processes within the lagoon are complex and will cause widespread dispersal of sediments and their associated contaminants.

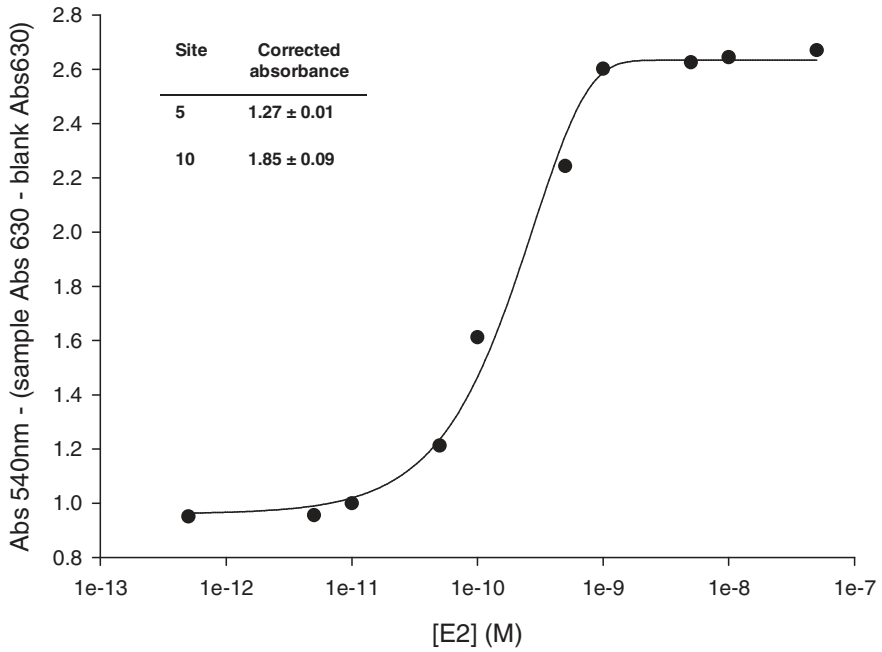

Figure 5. An example of the standard curve for estradiol (E2) stimulated oestrogen receptor activity in the yeast oestrogen screen (YES) assay. Inset indicates those sites (5 and 10) that induced oestrogen receptor activity and values in the table represent the average ± SEM ($n = 3$) of corrected absorbance [e.g. Abs. 540 nm – (sample Abs. 630 nm – blank abs. 630 nm)]. Sites 2, 3, 4, 7, and 9 proved toxic to the yeast cells whereas 1, 6 and 11 were non-toxic but did not induce any oestrogenic activity.

Table 3. PAH content of sediment samples in Lagos lagoon

Compounds	1	2	3	4	5	6	7	8	9	10	11
Acenaphthene	0.4	14.8	10.7	5.8	0.8	1.6	ND	ND	29.7	1.8	73.8
Acenaphthylene	ND	15.5	ND	4.8	ND	ND	1.18	ND	ND	ND	ND
Anthracene	ND	20.8	ND	287	ND	ND	ND	69	104	ND	103
Benzo[*b*+*k*]fluoranthene	ND	ND	ND	80	ND	40	ND	ND	ND	ND	ND
Benz[*a*]anthracene	ND	58.2	62.4	70.1	ND	27.6	ND	311	374	ND	128
Benzo[*a*]pyrene	ND	76.4	73.1	ND	ND	28.4	ND	253	393	ND	98.9
Benzo[*b*]fluoranthene	ND	ND	ND	78.1	ND	38.1	ND	ND	ND	ND	ND
Benzo[*e*]pyrene	ND	110	108	ND	ND	21.3	ND	227	462	ND	146
Benzo[*ghi*]perylene	ND	115	108	48.4	ND	22.8	29.1	261	674	14.4	0
Chrysene	ND	63.1	82.7	115	ND	ND	ND	314	394	ND	107
Coronene	ND	58	56.5	21.5	ND	ND	ND	109	285	ND	97.9
Cyclopenta[*cd*]pyrene	ND	16.1	15.7	ND	ND	ND	ND	ND	ND	ND	0
Dibenz[*a*,*h*]anthracene	ND	14.2	16.7	9.7	ND	4.9	3.3	39.8	59.8	ND	16.2
Fluoranthene	ND	183	161	71	ND	39.3	31.4	715	843	ND	370
Fluorene	ND	46.5	30.5	42.8	ND	ND	11.3	44.5	126	ND	210
Indeno[1,2,3-*cd*]pyrene	ND	96.8	92.6	ND	ND	ND	ND	ND	525	ND	ND
Perylene	ND	238	73.3	153	ND	ND	66.8	154	164	ND	280
Phenanthrene	ND	101	116	88.6	ND	ND	31.7	390	514	ND	847
Pyrene	ND	348	290	88	ND	39.1	40.9	623	1040	24.6	610
Total PAHs	0.4	1575	1297	1164	0.8	263	216	3510	5988	41	3088

All values expressed as µg/kg dry weight sediment. ND = not detected.

The highest cytotoxicity in RTgill-W1 cells at 25 mg eQsed/ml was observed at site 8 (28.6% viable cells), with similar values at site 11 (32.7% viable cells); three other sites 3, 6 and 9 were cytotoxic, the others non-toxic (Figure 2). Similar levels of cytoxicity, using the same assay but using PLHC-1 cells derived from topminnow (*Peociliopsis lucida*) were observed at a number of sites from Northern Spain (49). However, this level of cytotoxicity was seen at sediment extract concentrations of 100 mg eQsed/ml, four times that in the current study. The tPAH content of the Spanish sediments also greatly exceeded, by an order of magnitude (~55 000 tPAHs µg/kg) (55) those measured in Lagos lagoon. The discrepancy in cytotoxicity may be because of the presence of unidentified cytotoxic compounds or because the cell line (RTgill-W1) used in the current study is more sensitive than PLHC-1. RTgill-W1 cell line was chosen for the study because it is derived from the gills, an organ of fish that is constantly bathed in water and thus the main site of waterborne toxicant uptake and toxic action. RTgill-W1 cells lack CYP1A enzyme activity, a major phase 1 enzyme involved in biotransformation of many organic contaminants including PAHs (56). Thus, this cell line may be more susceptible if toxicity is due to the parent compound and not a metabolite.

All sites proved to be genotoxic at a sediment concentration of 7 mg eQsed/ml. A 2-fold dilution of the extract significantly reduced toxicity at sites 3, 5, 7, 8 and 10, but the testing of further concentrations would be necessary to determine a dose–response curve and derivation of low- or no-effect concentrations. Of the three sites ranked most genotoxic, only site 8 is also ranked in the sites with the highest PAH content and PAHs are known to induce DNA damage (57). In other studies assessing genotoxic action via the Comet assay or other bioassays (e.g. micronuclei assay) in cell lines or zebrafish embryos, DNA damage has been observed on exposure to moderately PAH contaminated sediments (4.2–4.8 mg/kg) (56). The other two sites with elevated PAHs would also fit the moderately contaminated category as defined by the US Environment Protection Agency; site 9 (5988 tPAH µg/kg) and site 11 (3088 tPAH µg/kg) (Table 3) are ranked in the third and second category for DNA damage. The genotoxicity of some PAHs may be attributed to their metabolites, e.g. benzo[*a*]pyrene (B[*a*]P) genotoxicity is due to the formation of B[*a*]P-7,8-dihydrodiol-9-10-epoxide that can bind to DNA-forming adducts, which, however, may not be efficiently detected by the Comet assay. In addition, the lack of CYP1A enzyme activity in RTgill-W1 cells (56) may mean that PAH like B[*a*]P that require metabolic activation to cause genotoxicity may not be well detected. The other two sites, 7 and 10, showing the most DNA damage are ranked in the third category, and the PAH content was 216 and 41 tPAHµg/kg, respectively. Site 7 is hydrologically dynamic (Table 1) and receives wastes from various sources in the lagoon, whereas site 10 is one of the main regions in the lagoon for sand-mining activity, and thus these sites may receive other classes of compounds that were not measured but are genotoxic. Taken together, this suggests that within a complex contaminant matrix there may be unknown interactions between compounds that may influence bioavailability and mode of action, as well as other compounds that have not been measured that are potent genotoxins. Similarly, chemical analysis did not correlate to Comet assay results in zebrafish embryos exposed to sediments from the Rhine River (58).

Recently, Kienzler *et al*. (10) have shown that RTgill-W1 and RTL-W1 cells have both functioning nucleotide excision repair and photoactivation repair when exposed to UV light. This observation has implications for the current study and others assessing environmental samples. This is because first, there may well be differences in the DNA repair capabilities between cell lines and second, within the environmental matrix, there may be factors that stimulate or inhibit the DNA repair machinery resulting in an under- or overestimation of the maximum DNA damage potential, making comparisons between sites difficult.

To our knowledge, the FPG-modified Comet assay has, until now, not been used to measure DNA damage associated with oxidative stress in fish cell lines exposed to environmental samples. Two studies used the FPG-modified Comet assay to show that ultraviolet A in combination with titanium oxide nanoparticle induces oxidation of purine DNA bases in goldfish skin cell line (59) and the RT gonad cell Line (RTG2) (60). Kienzler *et al*. (4) also used the FPG-modified Comet assay to test the sensitivity of three cell lines, RTgill-W1, RTL-W1 and PLHC-1, to diuron, cadmium, methyl methane sulphonate, hydrogen peroxide, 3-4-dichloroalinie and B[*a*]P. FPG treatment increased the sensitivity between 10- and 1000-fold compared with the normal Comet assay, but this was dependent on cell line and chemical (4). FPG treatment identifies additional endogenous DNA damage and Keinzler *et al*. (4) reported a 2-fold increase in % DNA tail intensity in each cell line under control conditions. The current results show a higher level of DNA damage detected following FPG treatment at sites 3, 6, 7 and sites 8, compared with the DMSO controls (Figure 4). Sites 7 and 8 were also amongst the sites with greatest DNA damage in the absence of FPG and site 3 in the second grouping (Table 2). Of interest is site 6, where only minor DNA damage was observed with the standard Comet assay, but significant damage was present in cells treated with FPG. Currently, we have no explanation for this observation. Metals are known to induce reactive oxygen species (ROS) production, but the organic solvent extraction used in the study would not remove metals form the sediment, and the sediments of site 6 have the lowest metal contents (Amaeze, Bury and Otitoloju, unpublished data). There are no untoward effluent inputs known at this site, but it would appear that there are compounds undetected that induce ROS resulting in DNA damage that the FPG treatment, an assay known to amplify the effects of agents in addition to oxidative stress (61), is capable of detecting.

Sediments from site 2, 4, 5, 6 10 and 11 were not toxic to the yeast cells, and of these, only sites 5 and 10 induced oestrogenic activity in the YES screen with site 10 being more potent than site 5 (Figure 5). At all other sites, yeast cell cytotoxicity was severe and prevented oestrogenic activity measurements. Oestrogenic properties of sediments have been observed previously (e.g. see recent articles, refs. (49,62)). Sites 5 and 10 have municipal and domestic waste effluent discharges that may explain the presence of oestrogenic compounds in the sediments. However, in contrast, sites 2 and 4 also received sewage effluent discharge.

Conclusion

Our study showed that at certain location in Lagos lagoon the sediments possess contaminants that are cytotoxic, genotoxic and endocrine disrupting. Chemical analysis of PAHs showed a gradient of contamination, but the concentrations did not always reflect site descriptions of known effluent discharge and sources of pollution. This suggests that the hydrologically dynamic lagoon widely distributes sediments and contaminants. The lagoon is a vital source of fish protein and the potential for these contaminants to accumulate in the food chain poses a health risk and requires further attention.

Often regulation requires the measurement of a set of priority pollutants, such as certain PAHs, PCBs and organophosphate pesticides.

This study analysed these groups of compounds, but found that the sediment PAH load did not correlate to toxicity in a number of cases, indicating the presence of other toxic compounds. Similar conclusions were drawn from a survey of Northern Spain marine sediments (49). It is not feasible, due to cost and time, to perform a chemical analysis of all known contaminants and thus a targeted approach based on bioassay-directed analysis may be an alternative. This is of particular interest to developing countries where there is a rapid rise in industry, intensive agriculture, population and standard of living resulting in greater domestic effluent discharge into the aquatic environment. Rapid cost-effective environmental bioassays for different classes of toxicants will help target areas of concern. The challenge is to demonstrate the ecological significance of these *in vitro* assay end points.

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References

1. Fent, K. (2001) Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. *Toxicol. In Vitro*, 15, 477–488.
2. Schirmer, K. (2006) Proposal to improve vertebrate cell cultures to establish them as substitutes for the regulatory testing of chemicals and effluents using fish. *Toxicology*, 224, 163–183.
3. Segner, H. (2004) Cytotoxicity assay with fish cells as an alternative to the acute lethality assay with fish. *Altern. Lab. Anim.*, 32, 375–382.
4. Kienzler, A., Tronchère, X., Devaux, A. and Bony, S. (2012) Assessment of RTG-W1, RTL-W1, and PLHC-1 fish cell lines for genotoxicity testing of environmental pollutants by means of a FPG-modified comet assay. *Toxicol. In Vitro*, 26, 500–510.
5. Devaux, A., Fiat, L., Gillet, C. and Bony, S. (2011) Reproduction impairment following paternal genotoxin exposure in brown trout (*Salmo trutta*) and Arctic charr (*Salvelinus alpinus*). *Aquat. Toxicol.*, 101, 405–411.
6. Tice, R. R., Agurell, E., Anderson, D. *et al.* (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ. Mol. Mutagen.*, 35, 206–221.
7. Azqueta, A. and Collins, A. R. (2013) The essential comet assay: a comprehensive guide to measuring DNA damage and repair. *Arch. Toxicol.*, 87, 949–968.
8. Albertini, R. J., Anderson, D., Douglas, G. R. *et al.* (2000) IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutat. Res.*, 463, 111–172.
9. Kienzler, A., Bony, S. and Devaux, A. (2013) DNA repair in fish and interest in ecotoxicology: a review. *Aquat. Toxicol.*, 134–135, 47–56.
10. Kienzler, A., Tronchère, X., Devaux, A. and Bony, S. (2013) UV-induced nucleotide excision repair (NER) and photoreactivation repair (PER) in two trout fish cell lines used in ecotoxicological assessment studies. *J. Photochem. Photobiol. B*, 125, 51–55.
11. Bols, N. C., Barlain, A., Chirino-Trejo, M., Caldwell, S. J., Goegan, P. and Lee, L. E. J. (1994) Development of a cell line from primary culture of rainbow trout *Oncorhynchus mykiss* (Walbaum) gills. *J. Fish Dis.*, 17, 601–611.
12. Notch, E. G. and Mayer, G. D. (2009) 17α-Ethinylestradiol hinders nucleotide excision repair in zebrafish liver cells. *Aquat. Toxicol.*, 95, 273–278.
13. Papis, E., Davies, S. J. and Jha, A. N. (2011) Relative sensitivity of fish and mammalian cells to the antibiotic, trimethoprim: cytotoxic and genotoxic responses as determined by neutral red retention, Comet and micronucleus assays. *Ecotoxicology*, 20, 208–217.
14. Babich, H. and Borenfreund, E. (1991) Cytotoxicity and genotoxicity assays with cultured fish cells: a review. *Toxic. In Vitro*, 5, 91–100.
15. Kocan, R. M., Sabo, K. M. and Landolt, M. L. (1985) Cytotoxicity/genotoxicity: the application of cell culture techniques to the measurement of marine sediment pollution. *Aquat. Toxicol.*, 6, 165–177.
16. Llorente, M. T., Parra, J. M., Sánchez-Fortún, S. and Castaño, A. (2012) Cytotoxicity and genotoxicity of sewage effluent plant effluent in rainbow trout cells (RTG-2). *Water Res.*, 46, 6351–6358.
17. Davoren, M., Ni Shuilleabhain, S., Hartl, M. G. J., Sheehan, D., O'Brien, N. M., O'Halloran, J., Van Belkt, F. N. and Mothersill, C. (2005) Assessing the potential of fish cell lines as tools for the cytotoxicity testing of estuarine sediment aqueous extracts. *Toxicol. In Vitro*, 19, 421–431
18. Šrut, M., Traven, L., Štambuk, A., Kralj, S., Žaja, R., Mićović, V. and Klobučar, G. I. V. (2011) Genotoxicity of marine sediments in the fish hepatoma cell line PLHC-1 as assessed by Comet assay. *Toxicol In Vitro*, 25, 308–314.
19. Ternjej, I., Srček, V. G., Mihaljević, Z. and Kopjar, N. (2013) Cytotoxic and genotoxic effects of water and sediment samples from gypsum mining are in channel catfish ovary (CCO) cells. *Ecotoxicol. Environ. Saf.*, 98, 119–127.
20. Boettcher, M., Grund, S., Keiter, S., Kosmehl, T., Reifferscheid, G., Seitz, N., Rocha, P. S., Hollert, H. and Braunbeck, T. (2010) Comparison of in vitro and in situ genotoxicity in the Danube River by means of the comet assay and the micronucleus test. *Mutat. Res.*, 700, 11–17.
21. Kleinjans, J. C. S. and van Schooten, F. J. (2002) Ecogenotoxicity: the evolving field. *Environ. Toxicol. Pharmacol.*, 11 (SI), 173–179.
22. Jha, A. N. (2008) Ecotoxicology applications and significance of the comet assay. *Mutagenesis*, 23, 207–221.
23. Odiete, W. O. (1999) *Environmental Physiology of Animals and Pollution*. Diversified Resources Ltd, Lagos, Nigeria.
24. Cundy, A. B., Croudace, I. W., Cearreta, A. and Irabien, M. J. (2003) Reconstructing historical trends in metal input in heavily-disturbed, contaminated estuaries: studies from Bilbao, Southampton Water and Sicily. *Appl. Geochem.*, 18, 311–325.
25. Singh, J., Hewawassam, H. and Moffat, D. (1995) *Nigeria: Strategic Options for Redressing Industrial Pollution*, Vol. 1. Industry and Energy Division, West Central Africa Department, West and Central Africa Dept. World Bank, Washington, DC.
26. Amaeze, N. H., Egonmwan, R. I, Jolaoso, A. F. and Otitoloju, A. A. (2012) Coastal environmental pollution and fish species diversity in Lagos Lagoon, Nigeria. *Int. J. Environ. Protect.*, 2, 8–16.
27. Akpata, T. V. I. and Ekundayo, J. A. (1978) Faecal pollution of the Lagos lagoon. *Niger. J. Sci.*, 12, 39–49.
28. Ajao, E. A. (1996) *Review of the State of Pollution of the Lagos Lagoon. Technical Paper No. 106*. Nigerian Institute of Oceanography and Maritime Research (NIOMR).
29. Oyewo, E. O. (1998) Industrial sources and distribution of heavy metals in Lagos lagoon and their biological effects on estuarine animals. PhD Thesis. University of Lagos.
30. Otitoloju, A. A. and Don-Pedro, K. N. (2004) Integrated laboratory and field assessments of heavy metals accumulation in edible periwinkle, *Tympanotonus fuscatus var radula* (L.). *Ecotoxicol. Environ. Saf.*, 57, 354–362.
31. Otitoloju, A. A., Don-Pedro, K. N. and Oyewo, E. O. (2007) Assessment of potential ecological disruption based on heavy metal toxicity, accumulation and distribution in media of the Lagos lagoon. *Afric. J. Ecol.*, 45, 454–463.
32. Ajagbe, F. E., Osibona, O. A. and Otitoloju, A. A. (2012) Diversity of the edible fishes of the Lagos Lagoon, Nigeria and the public health concerns based on their lead (Pb) content. *Int. J. Fish. Aquacult.*, 2, 55–62.
33. Nubi, O. A., Ajao, E. A. and Nubi, A. T. (2008) Pollution assessment of the impact of coastal activities on Lagos Lagoon, *Nigeria. Sci. World J.*, 3, 83–88.
34. Emmanuel, B. E. and Samuel, O. B. (2009) Comparative study of mercury accumulation in some water fishes in a tropical lagoon and its adjacent creek in south western Nigeria. *Afric. J. Environ. Sci. Technol.*, 37, 180–185.
35. Osae-Addo, A. (1992) *Nigeria: Industrial Pollution Control Program*.

36. Knott, N. A., Aulbury, J. P., Brown, T. H. and Johnston, E. L. (2009) Contemporary ecological threats from historical pollution sources: impacts of large-scale resuspension of contaminated sediments on sessile invertebrate recruitment. *J. Appl. Ecol.*, 46, 770–781.
37. Don-Pedro, K. N., Oyewo, E. O. and Otitoloju, A. A. (2004) Trend of heavy metal concentrations in Lagos lagoon ecosystem, Nigeria. *West Afric. J. Ecol.*, 5, 103–114.
38. Traven, L., Žaja, R., Lončar, J., Smital, T. and Mićović, V. (2008) CYP1A induction potential and the concentration of priority pollutants in marine sediment samples—in vitro evaluation using PLHC-1 fish hepatoma cell line. *Toxicol. In Vitro*, 22, 1648–1656.
39. Yang, F., Zhang, Q., Guo, H. and Zhang, S. (2010) Evaluation of cytotoxicity, genotoxicity and teratogenicity of marine sediments from Qingdao coastal areas using in vitro fish cell assay, comet assay and zebrafish embryo test. *Toxicol. In Vitro*, 24, 2003–2011.
40. Menzel, R., Swain, S. C., Hoess, S., Claus, E., Menzel, S., Steinberg, C. E., Reifferscheid, G. and Stürzenbaum, S. R. (2009) Gene expression profiling to characterize sediment toxicity—a pilot study using Caenorhabditis elegans whole genome microarrays. *BMC Genomics*, 10, 160.
41. Omogoriola, H. O., Williams, A. B., Ukaonu, S. C., Adegbile, O. M., Olakolu, F. C., Mbawuike, B. C., Akinnigbagbe, A. E. and Ajulo. A. A. (2012) Survey, biodiversity and impacts of economic activities on mangroves ecosystem in eastern part of Lagos Lagoon, Nigeria. *Nat. Sci.*, 10, 30–34.
42. Bradshaw, C., Tjensvoll, I., Sköld, M., Allan, I. J., Molvaer, J., Magnusson, J., Naes, K. and Nilsson, H. C. (2012) Bottom trawling resuspends sediment and releases bioavailable contaminants in a polluted Fjord. *Environ. Pollut.*, 170, 232–241.
43. Eggleton, J. and Thomas, K. V. (2004) A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environ. Int.*, 30, 973–980.
44. Hedman, J. E., Tocca, J. S. and Gunnarsson, J. S. (2009) Remobilization of polychlorinated biphenyl from Baltic Sea sediment: comparing the roles of bioturbation and physical resuspension. *Environ. Toxicol. Chem.*, 28, 2241–2249.
45. Hollert, H., Keiter, S., König, N., Rudolf, M., Ulrich, M. and Braunbeck, T. (2003) A new sediment contact assay to assess particle bound pollutants using zebrafish (*Danio rerio*) embryos. *J. Solid Sediments*, 3, 197–207.
46. Routledge, E. J. and Sumpter, J. P. (1997) Structural features of alkylphenolic chemicals associated with estrogenic activity. *J. Biol. Chem.*, 272, 3280–3288.
47. Ames, B. N. (1983) Dietary carcinogens and anticarcinogens. *Science*, 221, 1256–1264.
48. Kamer, I. and Rinkevich, B. (2002) In vitro application of the comet assay for aquatic genotoxicity: considering a primary culture versus a cell line. *Toxicol. In Vitro*, 16, 177–184.
49. Schnell, S., Olivares, A., Piña, B., Echavarri-Erasun, B., Lacorte, S. and Porte, C. (2013) The combined use of the PLHC-1 cell line and the recombinant yeast assay to assess the environmental quality of estuarine and coastal sediments. *Mar. Pollut. Bull.*, 77, 282–289.
50. Dayeh, V. R., Schirmer, K., Lee, L. E. J. and Bols, N. C. (2003) The use of fish-derived cell lines for investigation of environmental contaminants. In Hodgson, E. (ed.), *Current Protocols in Toxicology*. Wiley, New York, pp. 1–17.
51. Bols. N. C., Dayeh, V. R., Lee, L. E. J. and Schirmer, K. (2005) Use of fish cell lines in the toxicology and ecotoxicology of fish. In Mommensen, T. P., Moon, T. W. (eds), *Biochemistry and Molecular Biology of Fishes*. Elsevier, B.V., Amsterdam, pp. 43–84.
52. Arlt, V. M., Cole, K. J. and Phillips, D. H. (2004) Activation of 3-nitrobenzanthrone and its metabolites to DNA-damaging species in human B lymphoblastoid MCL-5 cells. *Mutagenesis*, 19, 149–156.
53. Landvik, N. E., Arlt, V. M., Nagy, E. *et al.* (2010) 3-Nitrobenzanthrone and 3-aminobenzanthrone induce DNA damage and cell signaling in Hepa1c1c7 cells. *Mutat. Res.*, 684, 11–23.
54. Kumaravel, T. S. and Jha, A. N. (2006) Reliable Comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. *Mutat. Res.*, 605, 7–16.
55. Sánchez-Avíla, J., Vicente, J., Eschavarri-Erasun, B., Porter, C., Tauler, R., Lacorte, S. (2013) Sources, fluxes and risk of organic micropollutants to the Cantabrian Sea (Spain). *Mar. Pollut. Bull.*, 72, 119–132.
56. Bury, N. R., Schnell, S. and Hogstrand, C. (2014) Gill cell culture systems as models for aquatic environmental monitoring. *J. Exp. Biol.*, 217, 639–650.
57. Brinkmann, M., Blenkle, H., Salowsky, H., Bluhm, K., Schiwy, S., Tiehm, A. and Hollert, H. (2014) Genotoxicity of heterocyclic PAHs in the micronucleus assay with the fish liver cell line RTL-W1. *PLoS One*, 9, e85692.
58. Kosmehl, T., Krebs, F., Manz, W., Braunbeck, T. and Hollert, H. (2007) Differentiation between bioavailable and total hazard potential of sediment-induced DNA fragmentation as measured by the comet assay with zebrafish embryos. *J. Soils Sediments*, 7, 377–387.
59. Vevers, W. F. and Jha, A. N. (2008) Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro. *Ecotoxicology*, 17, 410–420.
60. Reeves, J. F., Davies, S. J., Dodd, N. J. F. and Jha, A. N. (2008) Hydroxyl radicals ((OH)-O-center dot) are associated with titanium dioxide (TiO_2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. *Mutat. Res.*, 640, 113–122.
61. Azqueta, A., Arbillaga, L., López de Cerain, A. and Collins, A. (2013) Enhancing the sensitivity of the comet assay as a genotoxicity test, by combining it with bacterial repair enzyme FPG. *Mutagenesis*, 28, 271–277.
62. Creusot, N., Budzinski, H., Balaguer, P., Kinani, S., Porcher, J.-M., Aït-Aïssa, S. (2013) Effect-directed analysis of endocrine-disrupting compounds in multi-contaminated sediment: identification of novel ligands of estrogen and prename X receptors. *Anal. Bioanal. Chem.*, 405, 2553–2566.

Appendix 1: The List of Polyaromatic Hydrocarbons, PCBs, Organochlorine and Organophosphate Pesticides Analysed for by the National Laboratory Service

1,2,3-Trichlorobenzene; 1,2,4-Trichlorobenzene; 1,3,5-Trichlorobenzene; 2,3,5,6-Tetrachloroaniline; 2,3,5,6-Tetrachlorothioanisole; Acenaphthene; Acenaphthylene; Aldrin; Anthanthrene; Anthracene; Atrazine; Azinphos-ethyl; Azinphos-methyl; Bendiocarb; Benzo[*b*+*k*]fluoranthene; Benz[*a*]anthracene; Benzo[*a*]pyrene; Benzo[*b*]fluoranthene; Benzo[*e*]yrene; Benzo[*ghi*]perylene; Benzo[*k*]fluoranthene; Bifenthrin; Carbophenothion; Chlordane-*cis*; Chlordane-*trans*; Chlorfenvinphos; Chlorpropham; Chlorpyrifos; Chlorpyrifos-ethyl; Chlorpyrifos-methyl; Chrysene; Coronene; Coumaphos; Cyanazine; Cyclopenta[*cd*]pyrene; Cyfluthrin; Cyhalothrin-Lambda; Cypermethrin; DDD-op; DDD-pp; DDE-op; DDE-pp; DDT-op + DDD pp; DDT-op; DDT-pp; Deltamethrin; Desethylatrazine; Desisopropylatrazine; Desmetryn; Diazinon; Dibenz[*a,h*]anthracene; Dichlobenil; Dichlorvos; Dieldrin; Dimethoate; Endosulfan A; Endosulfan B; Endrin; Ethion; Ethofumesate; Fenchlorphos; Fenitrothion; Fenthion; Fluoranthene; Fluorene; Fonofos; HCH-*alpha*; HCH-*beta*; HCH-*delta*; HCH-*epsilon*; HCH-*gamma*; Heptachlor; Heptachlor Epoxide-*cis*; Heptachlor Epoxide-*trans*; Heptachlor epoxide; Hexachlorobenzene; Hexachlorobutadiene; Indeno[*1,2,3-cd*] pyrene; Iodofenphos; Isodrin; Malathion; Metazachlor; Methoxychlor; Methyl Parathion; Mevinphos; Napropamide; Parathion-ethyl; Pendimethalin; Permethrin-*cis*; Permethrin-*trans*; Permethrin; Perylene; Phenanthrene; Pirimiphos-ethyl; Pirimiphos-methyl; Prometryn; Propachlor; Propazine; Propetamphos; Pyrene; Simazine; Tecnazene; Terbutryn; Triazophos; Trietazine; Trifluralin; Vinclozolin.