Lagos Lagoon Sediment Organic Extracts Induce Developmental and Genotoxic Effects in *Danio rerio* (Zebrafish) Embryos

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INTRODUCTION

- **Sediments** are a sink for hydrophobic persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) in the aquatic environment (Lin, *et al.*, 1994).
- In the **Lagos lagoon**, PAHs-contaminated sediments and a decline in fish catch have been reported (Singh, *et al.*, 1995; Alani, *et al.*, 2012; Amaeze, *et al.*, 2014).
- **Early life stages of fish** such as *Danio rerio* (Zebrafish) are now being utilized for ecotoxcological studies and sediment risk assessment as part of the 3Rs (replacement, reduction and refinement) principle (OECD, 2013).
- **Genotoxic assessments** of sediments are crucial for evaluating their carcinogenicity.

- The **objectives of this study** were to investigate the developmental and genotoxic effects of Lagos lagoon sediment organic extracts on *D. rerio* embryos.

MATERIALS AND METHODS

- **Wild-type *Danio rerio*** (Chordata, Actinoterygii, Cyprinidae; weight: 1.13 ± 0.5g; length: 4.5 ± 0.2cm) were obtained from the breeding colony at the King's College London (KCL).
- **Sediments** were obtained from **four zones (Ilaje, Iddo, Atlas Cove and Apapa) of the Lagos lagoon (Table 1)** in July, 2013. They were freezed at -20^{0}C before transporting them to KCL.

- Extraction of the organic constituents of the sediments was according to Schnell, *et al.* (2013).
- **Sediment organic extract concentrations** utilized were;
  - 2.5, 6.25, 12.5 and 25 mg eQsed/ml for developmental toxicity studies;
  - 2.5 and 25mg eQsed/ml for genotoxicity studies. 0.25% DMSO served as the control.
- Embryos collection and exposure were according to Kumar, *et al.* (2013).
- 30 embryos (10 embryos in triplicates) per concentration were exposed from 0 to 72hours post fertilization (hpf) in 12-well plates containing 4ml of treatments and incubated at 29^{0}C.

- **Developmental endpoints** such as mortality, hatching success, number of heartbeats per minute (NHBpM) and abnormalities were assessed at 72hpf according to Kumar, *et al.* (2013).
- **Genotoxicity studies** were conducted using the unmodified and formamidopyrimidine DNA glycosylase (FPG)-modified alkaline single cell gel electrophoresis/Comet assay according to Landvik, *et al.* (2010) and Amaeze, *et al.* (2014). Single cells were obtained from whole *D. rerio* embryos (about 2 -10) by modifying a procedure described by Kosmehl, *et al.* (2008).

- **One way Analysis of Variance (ANOVA)** was used to test for significant difference between treatment means at $p<0.05$. Post-hoc tests were conducted using Duncan Multiple Range Test. Statistical analyses were carried out with SPSS 16.0.

Table 1: Location of Sampling Zones on the Lagos Lagoon and Description of Environment

Sampling Zone	Zone No.	Sampling Station	Location	Description
Ilaje	I	Ilaje 1	N06° 31' 28.2" E003° 24' 29.3"	Surroundings characterized by solid waste dumps and domestic sewage. Sand filling/dredging boats parked by the Fishermen. Several houses situated along the edge of the water.
		Ilaje 2	N06° 30' 56.8" E003° 24' 25.6"	
		Ilaje 3	N06° 30'.24.6" E003° 24' 15.2"	
Iddo	II	Iddo 1	N06° 28' 12" E003° 23' 0"	Dirt collection around the area. Stations near Iddo Lagos terminus bridge. Subsistence recycling of jute bags and chemical cans by handwashing.
		Iddo 2	N06° 28' 13.0" E003° 23' 05.1"	
		Iddo 3	N06° 28' 02.8" E003° 22' 54.1"	
Atlas Cove	III	Atlas Cove 1	N06° 24' 44" E003° 23' 43"	Sand filling of the area for construction projects. Petroleum piping and transfer to storage tanks in the Atlas cove area. Sandy substratum. Abandoned ship near the area.
		Atlas Cove 2	N06° 24' 57.4" E003° 23' 47.4"	
		Atlas Cove 3	N06° 25' 13.7" E003° 23' 50.2"	
Apapa	IV	Apapa 1	N06° 26' 15" E003° 19' 58"	Various tank farms for petroleum products storage positioned near the edge of the water, Ship wielding centres, shore-side auto-mechanic workshops.
		Apapa 2	N05° 26' 14.1" E003° 10' 40.4"	
		Apapa 3	N06° 26' 15.2" E003° 19' 59.4"	

RESULTS AND DISCUSSION

- The results of the **developmental toxicity studies (Figs. 1-4)** showed;
  - a dose-dependent increase in % mortality of embryos in Ilaje extract,
  - a dose-dependent decrease in % hatching of embryos in Ilaje & Iddo extracts,
  - a dose dependent decrease in NHBpM of embryos in Iddo & Apapa extracts
  - significantly higher **% abnormalities** of embryos in **Ilaje and Iddo sediment organic extracts** compared to the solvent control.
- The abnormalities observed in *D. rerio* embryos exposed to all the sediment organic extract treatments except Atlas Cove **(Figs. 5a-d)** were; tail curvature, scoliosis, tail tip curvature, elongated heart, haemorrhaging, severe yolk sac and pericardial oedemas **(Ilaje sediment),** tail tip curvature and yolk sac oedema **(Iddo sediment)** moderate yolk sac oedema and haemorrahaging **(Apapa sediment).**
- Similar teratogenic effects have been reported in *D. rerio* embryos exposed to sediment extracts of Qingdao coastal area, China. (Yang, *et al.,* 2010).

- The **genotoxicity studies** result **(Figs. 6-7)** revealed that % tail DNA in the FPG-modified Comet assay **(oxidative DNA damage)** was **significantly higher** in the **lowest Apapa** as well as **highest Ilaje** and **Iddo** sediment organic extract treated *D. rerio* embryo cells compared to the solvent control.
- This result agrees with Amaeze, *et al.* (2014) who reported high cyto- and genotoxic effects in the Lagos lagoon sites with high PAH content to rainbow trout gill primary cells (RTgill-W1).

- These results imply that **lipophilic organic contaminants** such as **carcinogenic PAHs, organochlorine pesticides, polychlorinated biphenyls** are present in these sediments in concentrations that are capable of eliciting **teratogenic / developmental** and **genotoxic / carcinogenic effects** in the organisms therein.

CONCLUSION AND RECOMMENDATION

- The study has established the significance of developmental and genotoxicity as **ecological risk indices** in the **Lagos lagoon.**

- **DNA damages and developmental abnormalities** may be **persistent over generations** leading to carcinogenesis and posing a **threat to the integrity of subsequent fish populations** (Kosmehl, *et al.*, 2007), other organisms in the food chain including **humans who consume them**.

- It is recommended that these indices should be **included in ecomonitoring programmes** for aquatic ecosystems in Nigeria.

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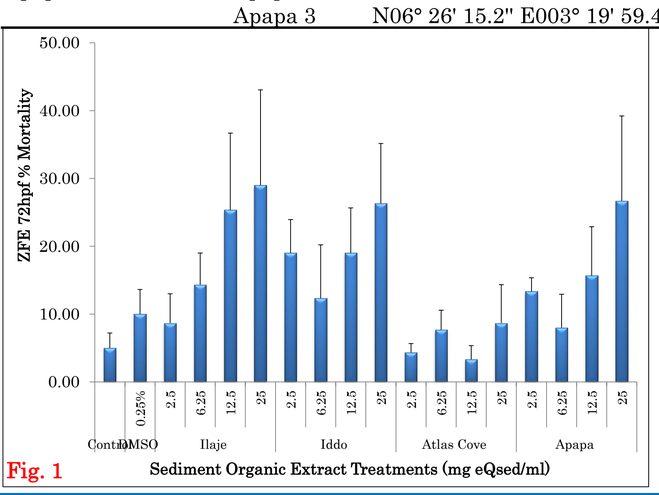

Fig. 1

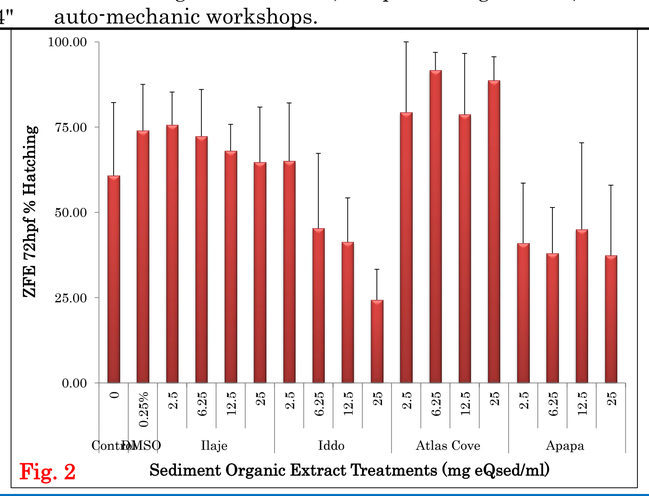

Fig. 2

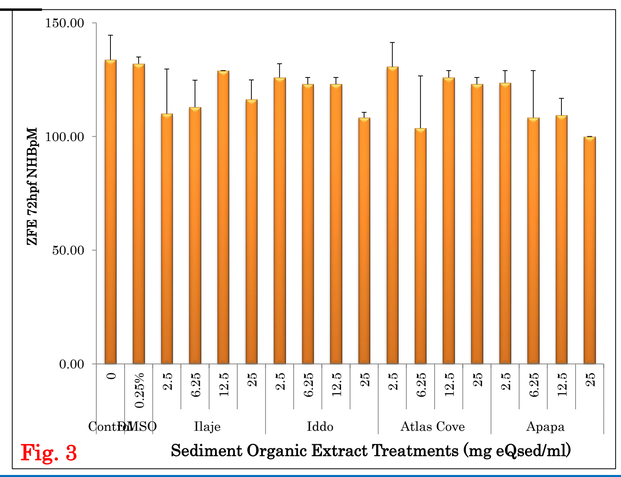

Fig. 3

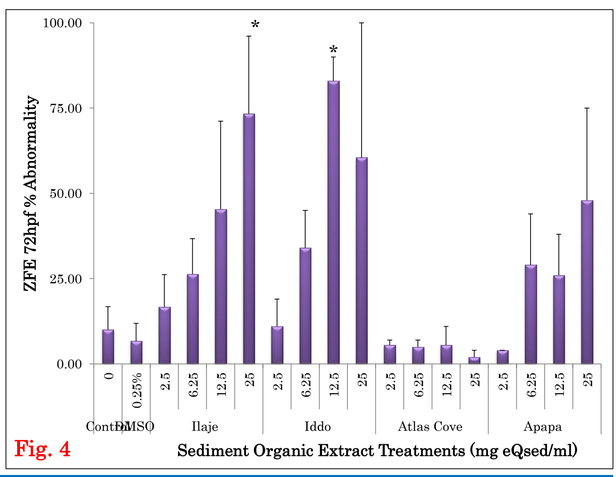

Fig. 4

Figs. 1-4: Results of Developmental Endpoints (% Mortality, % Hatching, Number of Heartbeats per Minute(NHBpM) and % Abnormality respectively) in 72hpf Zebrafish Embryo (ZFE) exposed to Lagos Lagoon Sediment Organic Extracts.
* represents significant difference at $p<0.05$

Fig. 5a: DMSO (0.25%) - treated 72hpf *Danio rerio* embryo without developmental abnormality (Mag.- 25x).

Fig. 5b: Ilaje sediment organic extract (25 mg eQsed/ml) - treated 72hpf *Danio rerio* embryo showing developmental abnormalities (tail curvature / scoliosis (arrow pointing), tail tip curvature TTC, severe yolk sac oedema YSE, severe pericardial oedema PE , elongated heart EH, (Mag.- 35x).

Fig. 5c: Iddo sediment organic extract (6.25 mg eQsed/ml) - treated 72hpf *Danio rerio* embryo showing developmental abnormalities (tail tip curvature (arrow pointing), yolk sac oedema YSE) (Mag.- 25x).

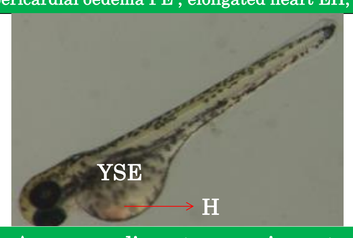

Fig. 5d: Apapa sediment organic extract (2.5 mg eQsed/ml) - treated 72hpf *Danio rerio* embryo with developmental abnormalities (moderate yolk sac oedema YSE, haemorrahaging H) (Mag.- 25x).

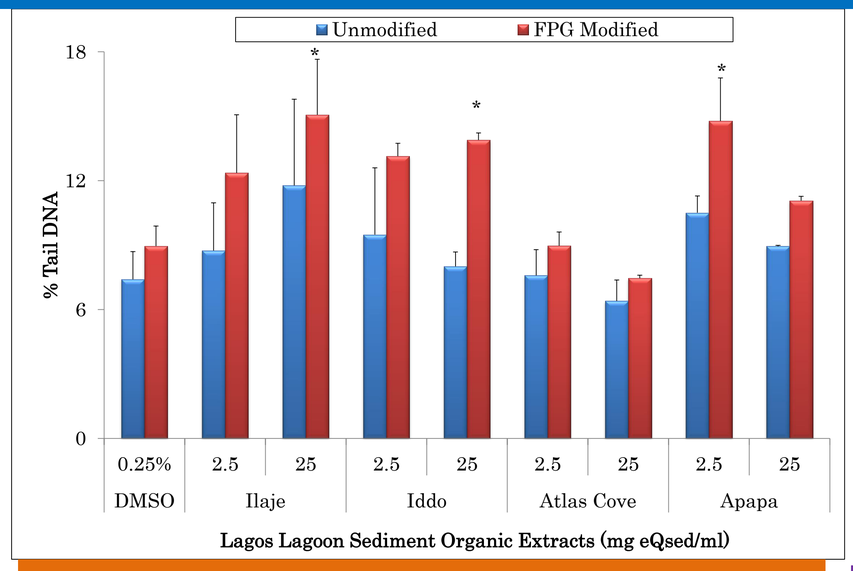

Fig. 6: Results of Unmodified and FPG-modified Comet Assay with 72hpf Zebrafish Embryo exposed to Lagos Lagoon Sediment Organic Extracts.
Key: * represents significant difference at $p<0.01$

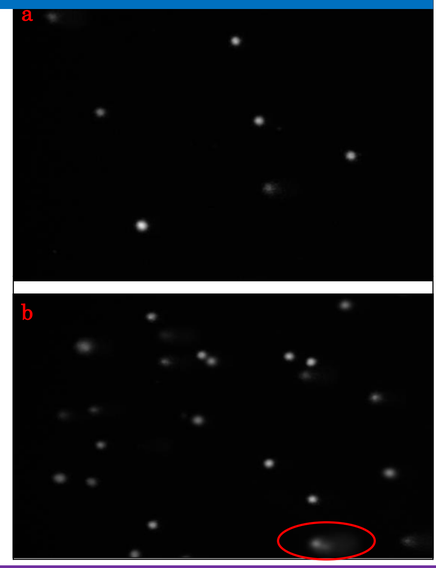

Fig. 7: a) 0.25% DMSO- & b) Apapa Sediment Organic Extract (25mg eQsed/ml) - treated *Danio rerio* Embryos Showing No Comets and Comets in Cells respectively

ACKNOWLEDGEMENTS: Sogbanmu, T. O. acknowledges the Association of Commonwealth Universities, L'Oreal-UNESCO and the University of Lagos for providing funding for this research, including six months of research at the Metals Metabolism Group of the King's College London.

Presented at the 10th University of Lagos Annual Research Conference and Fair, Lagos, Nigeria. 24th – 26th November, 2015.