Lead Levels in Large Scale Mullet Tissues from two Rivers in Nigeria

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''ABSTRACT'' Large scale mullet (*Liza grandisquamis* valenclennces 1836) samples were taken from Escravos and Forcados rivers. Detection of lead in tissue using atomic absorption spectrophotometer (AAS) varied depending on body weights, gender and the two rivers had different levels of lead in tissues. The detection percentage trend was muscle > kidney > gonad > brain > fin. In both rivers, the muscles consistently had the lowest concentration of lead, and there was no significant correlation between the body weight and concentration of lead in all tissues examined. The results suggests the possibility of using fish tissues such as the muscles, kidney, gonad, brain or fin of *Liza grandisquamis* between body weight of 28.00g and 39.00g and the muscle of female *Liza grandisquamis* between the body weight of 48.00g and 113.00g for *Liza grandisquamis,* as a good bioassay indicator for detecting and comparing the impact of lead aquatic pollution and long term biomonitoring of the freshwater and marine ecosystem.

KEYWORDS: Lead, *Liza grandisquamis,* tissues, pollution, atomic absorption spectrophotometer (AAS)

INTRODUCTION

The river systems may be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents [1].

Aquatic ecosystem pollution is a natural or induced change in water quality with detrimental substances including pesticides and its analogue, effluents, sewage and heavy metals which makes it unusable or unsafe for food, human, animal health, industry, fishing, agricultural or leisure purposes. The magnitude of aquatic ecosystem pollution in various regions of the world is dependent on urbanization, agricultural and industrial activities including climate change with both global and local effects. The determination of metal ions concentration in natural water system has received increasing attention for monitoring environmental pollution due to the fact that metals are not biodegradable and find their way into the food chain and may accumulate in different organ of humans and animals. Cadmium (Cd) and lead (Pb) are the most abundant heavy toxic metal in the environment[2].

Lead is a heavy toxic metal which has never ceased to play a role in human existence but it performs no essential function in tissues of animal or fish tissues. It is naturally present as a trace metal in sea water at very low concentration reflecting its low solubility either in dissolved or and is in particulate form, which is in equilibrium and long-term effects of their addition must be to increase precipitation. The natural concentration of lead in surface water has been estimated at 0.02µg.L^{-1} and it rarely exceeds a few microgram. L^{-1} [3]. However, the concentration may become very high by discharges from domestic, agricultural, industrial, mining and from sediments disturbed by dredging or changes in PH exponentially. Aquatic animals are capable of concentrating more pollutants within their bodies to levels higher than those in their surrounding water[4]. Most lead released into the environment find its way into the aquatic place as a result of direct input, atmospheric deposition and erosion due to rain water[5], and domestic sewage sludge[6]. Fish tissues uptake of lead is from aquatic plants, sediments and gasoline containing lead that leaks from fishery boats[7]. In Nigeria[8], reported lead pollution in aquatic system. The major source of aquatic lead pollution in Nigeria as leaded gasoline[9] while lead was detected in fish *Cyprinus carpio* and *Clarias gariepinus* tissues from an artificial pond[10]. In fish, absorption of chemicals including lead occur primarily through brachial (across the gills), oral (via ingestion or contaminated food or water) and dermal routes. In reality, chemicals are almost always absorbed by fish through a combination of these processes, although for some chemicals, a specific exposure pathway may dominate. The excretory routes of lead from fish are generally through bile, urine, gills and mucus. It was suggested that there are more excretory routes than uptakes[11]. But generally accumulation of lead is greater than lead excretion. The form of lead, the water body; lotic or lentic system and differences between species affect the toxicity. The toxic effect occurs when excretory, metabolic, storage and detoxification mechanism are no longer able to counter uptake.

Studies carried out with different fish species have revealed that lead can produce toxic effect in fish by disturbing physiological activities[12], biochemical processes[13], reproduction, and growth[14], mortality[15], stimulation of internal activities and plasma corticosteroid and glucose levels[16] and secondary stress responses[17], resulting in normally a reduction in diversity but not necessarily in number, a change in balance of such processes as predation, competition and materials.

The trend of accumulation of the metals in the organs in the Kidney were as follows - Zn > Cu > Pb > As > Cd; while the order of concentration of Lead in the organs were as follows - Liver > Kidney > Gills > Heart; also the levels of heavy metals ranged between 0.25- 8.96 ppm in the heart, 0.69- 19.05 ppm in the kidneys, 2.10-19.75 ppm in the liver and 1.95-20.35 ppm in the gills[18].The mean concentrations of lead in the muscle, kidney and liver were in the ranges of 0.00 – 0.004 mg/kg, 0.010 – 0.015 mg/kg, and 0.004 – 0.010 mg/kg respectively, the kidney had higher concentration of the metals compared to the liver, which is in turn higher than that in the muscle as a result suggested that the kidney of fish is a better bio-accumulator of heavy metals than the liver and the muscle[19].

The objective of the present investigation with large scale mullet *Liza grandisquamis* a catadromous fish that has a short maturation period of 3 – 4 months, involves the estimation of the concentration of lead in fish tissues from two different rivers in the Niger Delta Region of Nigeria, to detect probable gender differences in each river and the possibility of identifying the most appropriate fish tissue(s) as quantitative bio-indicator for detecting, comparing and long term biomonitoring of lead pollution in the freshwater and marine ecosystem.

STUDY AREA

The study area is between longitude 05^0 11' E and 06^0 12' E and latitude 05^0 02'N and 06^0 02'N.

Escravos river in addition to Domestic waste impaction have industrial waste generated from industrial activities by The Nigeria Ports Authority (NPA), Warri Port, Berger and Bilfiger B + B (Oil and gas company), Chevron – Texaco (oil and gas company) DBN (Oil Service Company), Nigeria National Petroleum Corporation (NNPC) Tank Farm, Shell Petroleum Development Company (SPDC) Escravos Beach Flow Station, Chevron Texaco Tank farm and Terminal Station, Hali Port (air strip), and other private oil servicing companies. On this river, oil spillages are common. Escravos river tributary studied transverses from Escravos through Otoriki, Ugbogoro, Ugbuye, Esungbo, Barira, Umamuden, Sugbo, Ugbotu to Omadino II towns and villages.

The Forcados river also in addition to domestic waste impaction, are industrial waste generated by activities of Timber and Saw milling Industries, Central Water Transport Company, Shell Petroleum Development Company (SPDC) Forcados, some oil servicing companies, the Warri Refinery and Petrochemical Company (WRPC) and Water transport activities and Delta Steel Company (DSC). Forcados river tributary studied transverses from Forcados through Aladja, Forcados, Jelejele, Ogbe-Ijoh, Adegbamu, Ibitomo, Penfold, Island, Forcados Flats, Dafalo gbene to Ovwian.

The Escravos river in addition to Domestic waste impaction have industrial waste generated from industrial activities by The Nigeria ports Authority (NPA) Warri port, Berger and Bilfiger B + B (Oil and gas company), Chevron – Texaco (oil and gas company) DBN (Oil Service Company), Nigeria National Petroleum Corporation (NNPC) Tank farm Shell Petroleum Development Company (SPDC) Escravos Beach Flow Station, Chevron Texaco Tank farm and terminal station, Hali Port (air strip), and other private oil servicing companies. On this axis, oil spillages are common.

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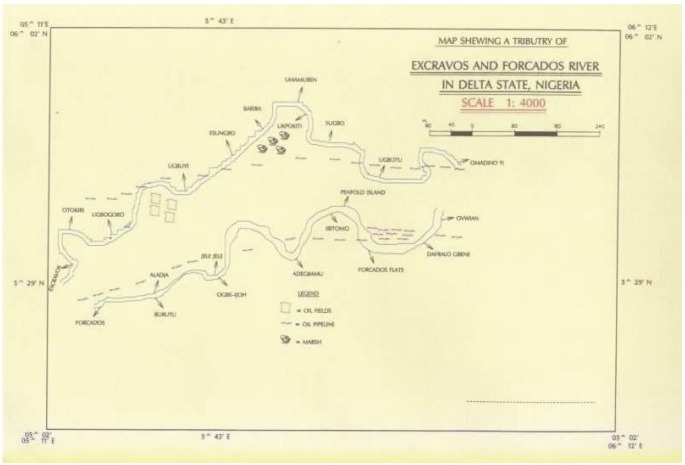

Figure 1: Study Area showing a tributary of Escravos and Forcados river Nigeria

MATERIALS AND METHOD

Heavy metal lead (Pb) concentration in brain, fin, gonad, kidney and muscle of fish species *Liza grandisquamis* from one tributary of Escravos river and Forcados river in Delta State, Nigeria were estimated and the relationship between fish sex, and weight and metal concentrate (µg/g) evaluated. 50 samples and 30 samples were randomly collected during the month of April (beginning of the rainy season with the lowest dilution factor), from artesinal fishermen. Each fish sample was washed with distilled water to reduce the contamination and dried using blotting paper before been put into polythene bag. The polythene bags were put into a Colman cooler containing ice blocks to prevent spoilage and then taken and stored in a deep freezer to avoid post mortem, deterioration. At the end of the collection, the total fish samples were transported frozen in Colman cooler to the Veterinary Medicine Department Laboratory, University of Ibadan, Ibadan, where it was stored again still in its frozen form in a freezer, until it was ready to be analyzed. Gender was determined by the anatomy of the urogenital papillae which was confirmed by lethal method. Prior to dissection, each fish was allowed to thaw at room temperature and blotted dry with sterile blotting paper. Each fish sample for digestion was placed on a washable plastic surface. Dissection was done wearing a clean pair of non-chlorinated, non-powered latex examination glove.

During the dissection, care was taken to remove the tissues for lead analysis. The tissues taken from each fish sample were from the brains, fins, kidneys, muscles and gonads. The plastic surface has a plastic sheet placed on it, which was changed after each dissection. Special care was taken while isolating the various tissues from the fish to avoid contamination with the surrounding tissues, and also to avoid contact with the plastic. Knives, scissors, forceps after each tissue collection and the dirt and tissue contamination were removed by washing with distilled water. The washing of the beakers after digestion was done thoroughly with detergents solution rinsed with pipe born water (tap water) and soaked in 50% nitric acid for between 12-24 hours at room temperature, thereafter, it was rinsed with distilled water and put in the incubator at low heat to dry. The tissues isolated were weighed with metler balance and put into separate beakers and appropriately labeled. 15ml of freshly prepared 1:1 nitric acid and hydrogen peroxide solution was added to each sample. The reaction was allowed to subside thereafter the beakers were then properly covered with aluminum foil and incubated between 140 and 160^{o}C for 2 hours[20]. The digest was allowed to cool to room temperature and poured into 25ml volumetric flask, the beaker was rinsed with distilled water, poured into the same 25ml volumetric flask containing its digest. The volume in the 25ml volumetric flask was made up to 25ml in the volumetric flask using distilled water. It was then poured into a plastic bottle, labeled appropriately and kept, until it was analysed with Atomic Absorption Spectroscopy (AAS) analysis. 20 blanks of 15ml 1:1 freshly prepared nitric acid and hydrogen peroxide were treated as above, to check the reliability of the results from AAS reading, which has detection limit for lead as 0.05µg/ml. The total of 400 tissues from 80 fish samples (50 from Forcados and 30 from Escravos rivers) and 20 blanks were analysed for lead, using Atomic absorption spectrophotometer (AAS). All set of

results were analyzed using Excel and SPSS 15 software packages, Pearson correlation analysis for correlation coefficient and t-test.

Results

Table 1: Concentrations (μg/g) lead in fish tissues

					BRAIN	MUSCLE	KIDNEY	GONAD	FIN
Forcados river	Gender	Male	WEIGHT	28.00	.	0.33	52.70	21.10	11.70
				32.00	5.12	6.75	8.75	25.40	6.48
				35.50	15.00	12.25	9.05	11.04	9.01
				55.60	12.03	.	.	.	.
				67.20	.	.	.	8.10	.
				84.00	.	.	.	5.70	.
				87.48	.	.	.	5.28	.
				Total	**3**	**3**	**3**	**6**	**3**
		Female	WEIGHT	64.5	.	7.9	.	.	.
				67.90	.	8.50	13.55	.	.
				86.18	.	3.20	16.03	.	.
				91.00	.	0.36	15.00	.	.
				106.79	.	5.02	.	.	.
				121.92	.	4.89	27.03	.	.
				124.00	.	6.39	.	.	.
				131.55	.	6.62	.	.	.
				Total		**8**	**4**		
		Area Total			**3.00**	**11.00**	**7.00**	**6.00**	**3.00**
Escravos River	Gender	Male	WEIGHT	30.00	12.32	6.90	20.05	22.36	10.00
				39.00	22.10	23.40	10.40	12.10	14.60
				51.10	15.02	.	.	.	.
				58.00	12.40	.	.	.	.
				Total	**4**	**2**	**2**	**2**	**2**
		Female	WEIGHT	48.70	.	12.50	11.23	.	.
				76.21	.	5.20	15.26	.	.
				104.00	.	1.70	.	.	.
				110.00	.	5.11	27.30	.	.
				Total		4	3		
		Area Total			**4**	**6**	**5**	**2**	**2**
All Total					**7**	**17**	**12**	**8**	**5**

Number of tissues analyzed = 400
Total number of samples = 80
Number of samples from Forcados river = 50, total fish tissue analyzed = 250
Number of samples from Escravos river = 30, total fish tissue = 150
Below detection limit (<DL) 0.05/μg/ml for all 20 Blanks.

Table 2: Summary of lead concentration (μg/g) in fish tissues for both Forcados and Escravos rivers

AREA		FIN	BRAIN	MUSCLE	KIDNEY	GONAD
Forcados River	Detected	3	3	11	7	6
	Below detection limit or not detected	47	47	39	43	44
	Mean conc. detected	9.0633	10.7167	5.6555	20.3014	12.7700
	Std. Deviation of conc. Detected	2.61041	5.06924	3.50295	15.52902	8.48284
	Minimum conc. detected	6.48	5.12	.33	8.75	5.28
	Maximum conc. Detected	11.70	15.00	12.25	52.70	25.40
	Range of conc. detected	5.22	9.88	11.92	43.95	20.12
	% detected	6	6	22	14	12
	% not detected	94	94	78	86	88
Escravos River	Detected	2	4	6	5	2
	Below detection limit or not detected	28	26	24	25	28
	Mean conc. detected	12.3000	15.4600	9.1350	16.8480	17.2300
	Std. Deviation of conc. Detected	3.25269	4.60096	7.83291	6.98494	7.25492
	Minimum conc. detected	10.00	12.32	1.70	10.40	12.10
	Maximum conc. detected	14.60	22.10	23.40	27.30	22.36
	Range of conc. Detected	4.60	9.78	21.70	16.90	10.26
	% detected	6.66	13.33	20	16.66	6.66
	% not detected	93.34	86.67	80	83.34	93.34

Table 2 shows the summary of lead concentration in fish tissues in both the Forcados and Escravos rivers. The mean levels distribution was as follows: Kidney(20.30±15.52μg/g)>Gonad(12.77±8.48μg/g)>Brain(10.72±5.08μg/g)>Fin(9.06±2.61μg/g)>Muscle(5.66±3.50μg/g) in the forcados river, while the mean distribution was Gonad (17.22±7.25μg/g) > Kidney (16.85±6.98μg/g) > Brain(15.46±4.60μg/g) > Fin(12.30±3.25μg/g) > Muscle(9.14±7.83μg/g) for Escravos river. In both rivers, the muscle consistently had the lowest concentration of lead.

Table 3: Lead concentration correlation coefficients in fish tissues for male and female fishes in Forcados River

AREA	Gender			WEIGHT	LENGHT	FIN	BRAIN	MUSCLE	KIDNEY	GONAD
Forcados River	Male	WEIGHT	Pearson Correlation	1	.148	-.548	.356	1.000	-.882	-.858
			Sig. (2-tailed)	.	.752	.631	.768	.004	.313	.029
		LENGHT	Pearson Correlation	.148	1	-.420	-.975	-.524	.066	.289
			Sig. (2-tailed)	.752	.	.724	.144	.649	.958	.579
		FIN	Pearson Correlation	-.548	-.420	1	1.000	-.553	.878	-.275
			Sig. (2-tailed)	.631	.724	.	.	.627	.318	.823
		BRAIN	Pearson Correlation	.356	-.975	1.000	1	1.000	1.000	-1.000
			Sig. (2-tailed)	.768	.144	.	.	.	.	.
		MUSCLE	Pearson Correlation	1.000	-.524	-.553	1.000	1	-.885	-.649
			Sig. (2-tailed)	.004	.649	.627	.	.	.309	.550
		KIDNEY	Pearson Correlation	-.882	.066	.878	1.000	-.885	1	.220
			Sig. (2-tailed)	.313	.958	.318	.	.309	.	.859
		GONAD	Pearson Correlation	-.858	.289	-.275	-1.000	-.649	.220	1
			Sig. (2-tailed)	.029	.579	.823	.	.550	.859	.
	Female	WEIGHT	Pearson Correlation	1	.452	.	.	-.135	.943	.
			Sig. (2-tailed)	.	.261	.	.	.751	.057	.
		LENGHT	Pearson Correlation	.452	1	.	.	.781	.406	.
			Sig. (2-tailed)	.261	.	.	.	.022	.594	.
		FIN	Pearson Correlation	.	.	.	.	.	.	.
			Sig. (2-tailed)	.	.	.	.	.	.	.
		BRAIN	Pearson Correlation	.	.	.	.	.	.	.
			Sig. (2-tailed)	.	.	.	.	.	.	.
		MUSCLE	Pearson Correlation	-.135	.781	.	.	1	.010	.
			Sig. (2-tailed)	.751	.022	.	.	.	.990	.
		KIDNEY	Pearson Correlation	.943	.406	.	.	.010	1	.
			Sig. (2-tailed)	.057	.594	.	.	.990	.	.
		GONAD	Pearson Correlation	.	.	.	.	.	.	.
			Sig. (2-tailed)							

In the forcados river there was no significant relationship between the male weight and the concentration of lead in all the tissues except the muscle with a correlation of one (Table 3). Such a correlation however can be very suspicious due to its level of perfection. A similar perfect relationship was noticed between concentration of lead in the brain and the kidney, the brain and

the muscle and the gonad and the brain but with a negative correlation. The female counterpart on the other hand showed no significant relationship between the weight of the fishes and the concentration of lead in any of the tissues analyzed.

Table 4: Lead concentration correlation coefficients in fish tissues for male and female fishes in Escravos River

AREA	Gender			WEIGHT	LENGHT	FIN	BRAIN	MUSCLE	KIDNEY	GONAD
Escravos Rivers	Male	WEIGHT	Pearson Correlation	1	.188	1.000	-.205	1.000	-1.000	-1.000
			Sig. (2-tailed)	.	.812	.	.795	.	.	.
		LENGHT	Pearson Correlation	.188	1	1.000	.922	1.000	-1.000	-1.000
			Sig. (2-tailed)	.812	.	.	.078	.	.	.
		FIN	Pearson Correlation	1.000	1.000	1	1.000	1.000	-1.000	-1.000
			Sig. (2-tailed)	.	.	.	.	.	.	.
		BRAIN	Pearson Correlation	-.205	.922	1.000	1	1.000	-1.000	-1.000
			Sig. (2-tailed)	.795	.078	.	.	.	.	.
		MUSCLE	Pearson Correlation	1.000	1.000	1.000	1.000	1	-1.000	-1.000
			Sig. (2-tailed)	.	.	.	.	.	.	.
		KIDNEY	Pearson Correlation	-1.000	-1.000	-1.000	-1.000	-1.000	1	1.000
			Sig. (2-tailed)	.	.	.	.	.	.	.
		GONAD	Pearson Correlation	-1.000	-1.000	-1.000	-1.000	-1.000	1.000	1
			Sig. (2-tailed)	.	.	.	.	.	.	.
	Female	WEIGHT	Pearson Correlation	1	1.000	.	.	-.865	.976	.
			Sig. (2-tailed)	.	.000	.	.	.135	.141	.
		LENGHT	Pearson Correlation	1.000	1	.	.	-.855	.982	.
			Sig. (2-tailed)	.000	.	.	.	.145	.120	.
		FIN	Pearson Correlation	.	.	.	.	.	.	.
			Sig. (2-tailed)	.	.	.	.	.	.	.
		BRAIN	Pearson Correlation	.	.	.	.	.	.	.
			Sig. (2-tailed)	.	.	.	.	.	.	.
		MUSCLE	Pearson Correlation	-.865	-.855	.	.	1	-.702	.
			Sig. (2-tailed)	.135	.145	.	.	.	.505	.
		KIDNEY	Pearson Correlation	.976	.982	.	.	-.702	1	.
			Sig. (2-tailed)	.141	.120	.	.	.505	.	.
		GONAD	Pearson Correlation	.	.	.	.	.	.	.
			Sig. (2-tailed)	.	.	.	.	.	.	.

In the Escravos river, the result of the analyses revealed that there is no relationship whatsoever between the weight of the fishes both male and female, and the concentration of lead in the tissues of the fishes (Table 4). Also there was no relationship between the concentrations of lead among the tissues.

Table 5: Lead concentration correlation coefficients in all fishes

AREA			WEIGHT	LENGHT	FIN	BRAIN	MUSCLE	KIDNEY	GONAD
Forcados River	WEIGHT	Pearson Correlation	1	.476	-.548	.356	-.134	-.105	-.858
		Sig. (2-tailed)	.	.073	.631	.768	.695	.823	.029
	LENGHT	Pearson Correlation	.476	1	-.420	-.975	-.120	.027	.289
		Sig. (2-tailed)	.073	.	.724	.144	.725	.954	.579
	FIN	Pearson Correlation	-.548	-.420	1	1.000	-.553	.878	-.275
		Sig. (2-tailed)	.631	.724	.	.	.627	.318	.823
	BRAIN	Pearson Correlation	.356	-.975	1.000	1	1.000	1.000	-1.000
		Sig. (2-tailed)	.768	.144	.	.	.	.	.
	MUSCLE	Pearson Correlation	-.134	-.120	-.553	1.000	1	-.603	-.649
		Sig. (2-tailed)	.695	.725	.627	.	.	.152	.550
	KIDNEY	Pearson Correlation	-.105	.027	.878	1.000	-.603	1	.220
		Sig. (2-tailed)	.823	.954	.318	.	.152	.	.859
	GONAD	Pearson Correlation	-.858	.289	-.275	-1.000	-.649	.220	1
		Sig. (2-tailed)	.029	.579	.823	.	.550	.859	.
Escravos River	WEIGHT	Pearson Correlation	1	.741	1.000	-.205	-.648	.660	-1.000
		Sig. (2-tailed)	.	.035	.	.795	.164	.225	.
	LENGTH	Pearson Correlation	.741	1	1.000	.922	.007	.178	-1.000
		Sig. (2-tailed)	.035	.	.	.078	.990	.774	.
	FIN	Pearson Correlation	1.000	1.000	1	1.000	1.000	-1.000	-1.000
		Sig. (2-tailed)	.	.	.	.	.	.	.
	BRAIN	Pearson Correlation	-.205	.922	1.000	1	1.000	-1.000	-1.000
		Sig. (2-tailed)	.795	.078	.	.	.	.	.
	MUSCLE	Pearson Correlation	-.648	.007	1.000	1.000	1	-.710	-1.000
		Sig. (2-tailed)	.164	.990	.	.	.	.179	.
	KIDNEY	Pearson Correlation	.660	.178	-1.000	-1.000	-.710	1	1.000
		Sig. (2-tailed)	.225	.774	.	.	.179	.	.

Table 5 shows the correlation coefficients irrespective of gender. In both rivers there seem not to be any significant relationship between the weight and concentration of lead in all the tissues. All other concentrations do not show a correlation with the weight of the fish or the concentrations among the different tissues.

Discussions

There is dearth of information on gender related tissues accumulation of lead by *L. grandisquamis* for discussion. This study shows that the levels of lead residue in *L. grandisquamis* are high but it appears that the fish may have adapted or rather developed tolerance for such levels. For public health practitioners, it has been documented that defrosting, cooking and sterilization by autoclave would reduce the content of lead and cadmium considerably[21], however, for the clinician it is a source of concern, knowing fully well that lead has special affinity for the brain and accumulates in high levels usually in the brain which is very important in the Brain-Pituitary-Gonadal axis and Thymus system in fish and can cause damage to the kidney, liver, brain and nerves and other organs expose to it. It could be inferred that despite the various adaptability processes available to fish and low percentage detection of the high lead levels in the fish populations, which could suggest recent lead pollution impacts, the *L. grandisquamis*, may not be performing optimally. Secondly, the anthropogenic activities should be regulated to reduce the impact of pollution in both water bodies. Thirdly, gender of *L. grandisquamis* differences exist as a factor in responses to accumulation of lead. Fourthly, the younger fishes (smaller sized) are more sensitive and could be used in lead detection using any of the tissues studied. This is because whenever it was present, in one tissue, it was found in other tissues that were analysed in this study. However for the adults above 100g body weight, the gonad of males and the muscle of the females are more sensitive and could be the preferred tissues to be used to for lead detection.

Conclusion

Gender has influence on the accumulation of lead, lead level in fish tissue could be used to compare and evaluate the level of impact of lead pollution of two or more rivers using the same fish species of known life cycle and Escravos river have higher burden of lead pollution than Forcados river.

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