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Evaluation of Biochemical Markers of Warri River Pb and Co-Polluting Metals Induced- Hepatotoxicity in The Rat

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Abstract: *This study investigated the hepatotoxic effects of Warri river environment lead in the presence of co-polluting metals using biochemical markers as indices of toxicity. A total of 55 albino rats (of Wistar strain) weighing an average of 150.00± 09.00g, divided into 11 groups were used for the study. Groups I and II represented the deionized and Pti borehole water controls, while groups III- XI represented the test rat groups orally treated with the river water – concentrations of laboratory formulated Pb salt solution either alone and in the presence of salt of Fe, Ca, Cu, Mn, Mg, Zn and all co-polluting metals via water. The biochemical –hepatotoxic indices investigated were liver/body wt. ratios, body wt. change, lipid per oxidation products, plasma ALT and AST, plasma and liver alkaline phosphatase activities, plasma catalase and superoxide dismutase activities, plasma total and conjugated bilirubin level, plasma and urine glucose concentration, and plasma and urine total protein concentration. Our findings revealed an overall significant ($P<0.05$) decrease in liver/body wt ratios and body wt change , significant ($P<0.05$) increase in plasma ALT and AST activities, induced ALP and ACP activities, increase in SOD and catalase activities, increased plasma and urine bilirubin concentrations, decreased plasma and increased urine total protein concentrations, increased Malondialdehyde (MDA) levels, while plasma and urine glucose levels were elevated in the groups of rats exposed to Pb only, Pb and Cu, Pb and Fe and Pb and Zn, Pb and co-polluting metals, and river water relative to their respective controls (deionized water and Pti tap water groups). There was a significant ($P<0.05$) reversal of the above parameters in the groups of rats exposed to Pb and Ca, Pb and Mn, Pb and Mg. There was also a difference in liver/weight ratio, body wt. change and all the other parameters evaluated in this study, between groups of rats treated with Warri river water relative to the laboratory reconstituted water, although the changes were not significant ($P>0.05$). Our findings revealed that, the presence of Ca, Mg and Mn in the river water significantly ($P<0.05$)) reversed the induced activities of ALT, AST, ACP and ALP by Pb and the synergizing co-pollutants of Warri River water. This study also revealed the possibility of a significant ($P<0.05$) decrease in the activities of superoxide dismutase (SOD), catalase , plasma total and direct bilirubin and decreased lipid per oxidation products of rats exposed to Pb in the presence of Ca, Mg and Mn relative to the Pb only group..*

Key Words: *Biochemical Markers, Warri River Lead and Co-Polluting Metals, Hepatotoxicity, the Rat.*

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I. INTRODUCTION

The liver is recognizably the largest internal organ in the human body. The contemporary prevalent incidences of liver diseases is bothersome because of its unique role in: detoxification of xenobiotics introduced to the human body through various sources, as a location for xenobiotics detoxifying enzymes and as an important site for series of biochemical reactions. Pb is reportedly one of the known hepatotoxic agents known to man. There are reports demonstrating the interference of lead with internal organs like the liver (Flora *et al.*, 2006). It is able to exhibit its toxicity because of its ability to affect virtually all organs or tissues through a mechanism that involves fundamental biochemical processes. Some of these mechanism s include the ability

of Pb to inhibit or mimic the action of calcium which affects all calcium dependent processes and interact with proteins(ASTDR, 2005) and the interference with the synthesis of heme, resulting in reduction in blood hemoglobin (ASTDR, 1999). More concern for lead toxicity stems from the fact that lead and lead products used in various industries and lost into the environment, eventually end up in the aquatic environment (Sandhir and Gill, 1995) and subsequently the human body through consumption of aquatic animals like fish and crayfish. Acute toxicity of Pb is workplace related and is quite uncommon, but chronic toxicity on the other hand is very common even at very low blood lead levels (Flora *et al*., 2006).

Warri River in the Niger Delta region of Nigeria is one of such aquatic environment, the river is located on $5^0$24'00''N and $5^0$28'00''E (USA, National Geospatial- intelligence Agency, 1994). Warri river is located in the Warri -South Local Government Area of Delta State and it is a harbor for major oil companies platform, a major sea port for the country. Its bank is a site for activities like storage of bunkered crude oil and its products, illegal modular refineries, welding and fabrication, auto-mechanic workshops. The river joins two major rivers, Forcados and Escravos through Jones creek in the lower Niger delta region. The major occupation of the indigents of the communities on shore the river is fishing and farming. Besides its use as a source of livelihood and aquatic food source, the still water end serves a source of drinking water to the communities on the river bank. Although, important measures have been adopted by regulatory authorities in the country to decrease or completely eliminate environmental lead exposure such as unleaded gasoline, lead smelting and coal combustion, there are speculations that the illegal make-shift refineries located on the bank of the river may not be in compliance.. Other measures adopted to reduce lead from the environment include: Removal of lead from paints, solder of canned foods and lead based solder in water system, battery recycling, grids and bearings, and glazed ceramics used for preparation and storage of foods. However, lead exposure is still a major environmental health problem in some specific communities. Currently, thousands of people obtaining sea foods and water from Warri River source may be at risk of organ dysfunction

Although , lead toxicity is considered a widely explored area of research, studies on evaluation of biochemical markers of Pb and Co polluting metals induced hepatotoxicity is far from being exhausted. It was therefore necessary to undertake this study in order to investigate some biochemical markers of the hepatotoxic nature of long term continuous exposure to Pb in the presence of co-polluting metals in the rat.

II. MATERIALS AND METHODS

A total of 55 albino rats (of Wistar strain) weighing an average of 150±10g were used for this study. The rats were maintained under controlled environmental conditions as follows: 24^0C-25.5^0C; 24hours lighting. They were fed commercial rations of growers mash and potable tap water *ad libitum* and allowed 7 d to acclimatize to the laboratory conditions, temperature and humidity, before commencement of the study. The animals were exposed to the test metallic pollutants twice daily for 90 d.

The test metallic pollutants used in this study were soluble salts of the respective heavy metal. All metallic salts and epinephrine used in the study were obtained from May and Baker (Dagenham, UK). 2-thiobarbituric acid (Koch light laboratories Ltd, UK). Alkaline and acid phosphatase, ALT and AST kit were produced by Quimica Clinica Aplicada. (QCA, Spain). Total protein and bilirubin and glucose reagents were products of Randox Laboratories LTD, United Kingdom. All other chemicals used in this study were of Analytical grade

Treatment of Animals

Treatment and management of animals were done according to the rules of local ethics committee of the faculty of life Sciences, University of Benin. The concentrations of Pb and co-polluting metals used were calculated on the basis of Warri River concentration of the respective metals at the time of the study. A total of 55 rats were used for this study. The rats were divided into eleven (11) groups of 5 rats each and exposed to Pb and co-polluting metals reconstituted in deionized water orally by gavage: Group 1 rats (control-I),received deionized water only (5ml H_2O/kg bd wt by gavage); Group II rats received Pti borehole water only (5ml H_2O/kg bd wt by gavage); Group III rats received Pb only (0.3mg/kg bd.wt) ;Group IV rats received Pb and Fe (0.3mg:0.20mg/kg bd. Wt); Group V rats received Pb and Ca (0.3mg/0.20mg/kg bd. wt); Group VI rats received Pb and Cu (0.3/0.19mg/kg bd. Wt); Group VII rats received Pb and Mn (0.3:0. 27mg/ kg bd.wt); Group VIII rats received Pb and Zn (0.3:0.2mg/ kg bd. wt); Group IX rats received Pb and Mg (0.3:0.2mg/ kg bd .wt); Group X rats received Pb and all co-polluting metals (5ml/kg bd. wt)while Group XI rats received river water (5ml H_2O/kg bd wt). At the end of the study period (90 d), the animals were sacrificed in accordance to the rules of the local ethics committee of the Faculty of Life Sciences, Universiy of Benin. Blood samples were collected by cardiac puncture and centrifuge at 3000rpm for 5mins, the supernatant (plasma) were collected and stored at -20^oC and assayed immediately. The liver was excised and stored at the same temperature until required for analysis. A 24hr urine sample was obyained and used for urine protein and glucose analysis.

Preparation of Tissue Homogenate

The liver was homogenized in ice-cold normal saline to obtain a 20% homogenate (1:4w/v). The homogenates were centrifuged at 4000rpm for 10minutes and the supernatant obtained were used for biochemical analysis**.**

Gravimetry and Biochemical Assays

Body and liver organ weights were obtained using a Mettler electronic balance as outlined by Emmanuel *et al*., (2013). . Formulation of test and control water was done according to the method of Asagba and Obi (2005). Preservation of tissue homogenate was done according to the method of (Walter and Scutt,1974). Alkaline and acid phosphatase activities was estimated according to the method of Kind and King (1954) modified by Varley (1975). The principle of ALP was based on enzymatic end point, following formation of p-Nitro phenol, the rate of p-Nitro phenol formed was determined as ALP activity. While the activity of ACP was determined by the formation of a red color complex from the reaction of phenol and 4-Aminoantipyrine in an alkaline medium. Alanine and aspartate amino transferases activity were estimated according to the method of Reitman and Frankel (1957) outlined by Sood (2006). The level of lipid per oxidation in the liver homogenate supernatant was estimated using the method of Buege and Aust (1978). The procedure involves the determination of thiobarbituric acid reactive substances (TBARs), which are indicators of lipid per oxidation. Values of TBARs are reported as Malondialdehyde (MDA) quantified using a molar extinction coefficient of 1.5 ×105 M-1cm-1 and expressed as micromole MDA per gram wet of tissue. A superoxide dismutase (SOD; EC 1.15.1.1) activity in the liver homogenate supernatant was estimated according to the method of Misra and Fridovich (1972). SOD activity is expressed as units per gram of liver tissue (one unit is the amount of the enzyme necessary to cause 50% inhibition of epinephrine oxidation for 60secs).while catalase(CAT; EC 1.11.1.6) activity was measured using the method of Cohen (1970), where decomposed hydrogen peroxide is measured by reacting it with excess of potassium tetraoxomanganate (VII)(KMnO4) and residual KMnO4 is measured spectrophotometrically at 480nm.the result was expressed as units of enzyme activity/mg protein (U/mg protein) Protein was determined by method of Lowry *et al.,* 1951 while bilirubin was estimated according to the modified Jendrassik and Grof's method outlined by Sood (2006).

Statistical Analysis of Data

Statistical analysis was done using one way Analysis of Variance (ANOVA) and Fischer's protected least significant difference *post-hoc* testing or with unpaired student's *t* test when appropriate. The Turkey-Kramer multiple comparison test was used to evaluate the differences between means. All statistical calculations were done using the graphpad instat statistical package. All data are expressed as mean ±SEM. Significant level was assigned at $P<0.05$

III. PRESENTATION OF RESULTS

The results of the effects of Pb and selected co-polluting metals on organ body weight ratio and malondialdehyde levels of the rat are presented in Table I.

Table I: Effect of Pb and co-polluting metals on liver weight/100gm Body Weight and MDA Levels of the rat

S/No	Treatment	Dose# (bd×3months)	Liver wt/bd wt. ratio	Malondialdehyde level (µmole MDA/g tissue)
			(mean±SEM)×10^{-3} n=5	
I	deionized water (Control-1) dH_2O	5ml/kg bd. wt	23.62±1.78	1.08±0.02
II	Pti borehole (Control-2), Pti H_2O	5mlH2O/kg bd wt	$21.10±1.26^{a}$	$1.44±0.12^{b}$
III	Pb only	0.3mg/kg bd.wt	$10.13±0.77^{bd}$	$5.05±0.06^{bd}$
IV	Pb and Fe H_2O	0.3mg:0.21mg/kg bd.wt	$13.43±0.53^{bcf}$	$3.08±0.04^{bdf}$
V	Pb and Ca H_2O	0.3:0.2/kg bd. Wt	$14.55±0.92^{bdfg}$	$2.11±0.03^{bcfh}$
VI	Pb and Cu H_2O	0.3:0.19kg bd .wt	$6.67±0.92^{bdehj}$	$5.61±0.08^{bdfhj}$
VII	Pb and Mn H_2O	0.3:0.27/kg bd. Wt	$13.38±0.91^{bcfhil}$	$3.30±0.03^{bdehjl}$

VIII	Pb and Mg H_2O	0.3:0.2/kg bd ,wt	12.55 ± 0.75^{bdegilm}	3.74 ± 0.14^{bdfhjln}
IX	Pb and Zn H_2O	0.3:0.2/ kg bd wt	18.29 ± 0.70^{bcfhjlnp}	4.30 ± 1.43^{bcegikmo}
X	Pb and Co metals H_2O	0.3:mixed conc. co metals	13.49 ± 1.04^{bdegilmor}	2.98 ± 0.07^{bdfgjlnpq}
XI	River water	5ml H2O/kg bd wt	$16.49\pm0.67^{bdfhjlnpqt}$	$2,21\pm0.07^{bdfhjlnpqt}$

[a] (P>0.05); [b=] p(<0.05) relative to their respective grp1 value; [c] (P>0.05); [d=]p(<0.05) relative to their respective grp 2 values

[e] (P>0.05); [f=]P<0.05) relative to their respective grp 3 values; [g] (P>0.05); [h=]P<0.05)relative to their respective grp 4 values

[i] (P>0.05); [j]=P<0.05 relative to their respective grp5 values; [k] (P>0.05); [l]=P<0.05 relative to their respective grp 6 values

[m] (P>0.05); [n]=P<0.05, relative to their respective grp7 values; [o] (P>0.05); [p]=P<0.05 relative to their respective grp 8 values

[q] (P>0.05); [r]=P<0.05 relative to their respective grp9 values; [s] (P>0.05); [t]=P<0.05 relative to their respective group10 values

#= dose calculated based on Warri River concentrations of respective metals in a preliminary study.

Pb only exposed -rat group (Group III) showed a significant (P<0.05) decrease in liver organ/body weight ratio and a significant (P<0.05)increase im the products of lipid Peroxidation evident as malondialdehyde levels, relatives to their corresponding controls (Groups I: deionized water only exposed group and Group II: Pti borehole water -exposed group). Pb in combination with Cu, Fe, Zn and all co-polluting metals caused a significant (P<0.01)decrease in liver/body wt ratio and increase (P<0.01) in MDA levels in the respective rat groups relative to their controls. also, Pb in the Presence of Ca, Mn and Mg showed a significant (P<0.05) increase in liver body weight ratio and a decrease in MDA levels relative to the Pb-only group. There was a difference between the Warri river water and the laboratory reconstituted river water, although, the difference was not significant (P>0.05).

The results of the effects of Pb and co-polluting metals on superoxide dismutase and catalase activities are presented in Table II.

Table II: Effect of Pb and Co-polluting metals on Superoxide dismutase and Catalase activities

	Treatment	Dose (bd×3months	SOD Activity U/L	Catalase Activity U/L
			mean±SEM(n=5)×10^{-4}	
I	dH_20 only (control-1)	5ml H_2O/kg bd. wt	1.10±0.03	3.19±0.03
II	Pti H_2O (control-2)	5ml H_2Okg^{-1}bd wt	1.59±0.08^{b}	3.74±0.07^{b}
III	Pb only	0.3mg/kg bd.wt	2.59±0.09^{bc}	7.34±0.14^{bd}
IV	Pb and Fe H_2O	0.3mg:0.21mg/kg bd.wt	2.65±0.17^{bce}	4.53±0.17^{bdf}
V	Pb and Ca H_2O	0.3:0.2/kg bd. Wt	1.92±0.18^{bdeh}	4.00±0.04^{bdfh}
VI	Pb and Cu H_2O	0.3:0.19kg bd .wt	2.73±0.25^{bcegj}	9.28±0.09^{bdfhj}
VII	Pb and Mn H_2O	0.3:0.27/kg bd. Wt	1.98±0.20^{bcegil}	4.14±0.09^{bdfgil}
VIII	Pb and Mg H_2O	0.3:0.2/kg bd ,wt	2.12±0.16^{bcfgikm}	4.03±0.03^{bdfgilm}
IX	Pb and Zn H_2O	0.3:0.2/ kg bd wt	2.15±0.19^{bcegikmo}	4.56±0.28^{bdfgilmo}
X	Pb and Co metals H_2O	0.3:mixed conc. co metals	2.54±0.20^{bcegjkmoq}	7.06±0.74b^{dehjlnpr}
XI	River water	5ml H_2O/kg bd wt	2.26±0.10$^{bc\ fhjlmoqt}$	6.26±0.10^{bdfhjlnpqt}

*See Table I for interpretations of alphabetical nomenclature
**bdfhjlnprt= values significantly (P<0.05) different from corresponding evaluated groups
*** acegikmoqs= values not significantly (P>0.05) different from corresponding statistically evaluated groups

Exposure of rats to Pb only and Pb in the presence of Cu, Fe, Zn and combined co-polluting metals caused a significant (P<0.05) increase in SOD and Catalase activities of the rat relative to their respective controls (Groups I and II). Pb in the Presence Ca,Mn, and Mg caused significant (P<0.05) decreases in superoxide dismutase and catalase activities relative to the Pb-only exposed rat. There was also a significant (P<0.05) increase in the SOD and Catalase activities of the river water relative to their corresponding controls (Groups I and II). There was also a slight decrease in SOD and Catalase activities of River water exposed rat group relative to the laboratory reconstituted water, although, it was not significant (P>0.05).
The results of the effects of Pb and selected co-polluting metals of Warri River on plasma aspartate and alanine transaminases activities are represented on Table III.

Table III: Effect of Pb and Co-Polluting Metals s on Plasma AST and ALT Activities in the Rat

S/No	**Treatment**	**Dose (bd×3months)**	**AST U/L**	**ALT U/L**
			mean±SEM (n=5)×10^{0}	
I	dH_20 only(Control-1)	5mlkg^{-1} bd. wt	38.00±2.00	23.60±0.92
II	Pti H_2O (Control-2)	5mlkg^{-1} bd. wt	39.20.00±0.86^{a}	45.00±1.70^{b}
III	Pb only	0.3mgkg^{-1} bd.wt	72.00±1.46^{bd}	60.00±1.14^{bd}
IV	Pb and Fe H_2O	0.3mg:0.21mgkg^{-1} bd.wt	74.00±0.71^{bdf}	69.00±1.00^{bcf}
V	Pb and Ca H_2O	0.3:0.2mgkg^{-1} bd. Wt	58.00±0.71^{bdfh}	37.00±1.41^{bcfh}
VI	Pb and Cu H_2O	0.3:0.19mgkg^{-1} bd .wt	91.40±1.02^{bdfhj}	67.80±1.50^{bdfhj}
VII	Pb and Mn H_2O	0.3:0.27mgkg^{-1} bd. Wt	62.00±0.71^{bcfhjl}	40.00±1.41^{bcfhjl}
VIII	Pb and Mg H_2O	0.3:0.2mgkg^{-1} bd ,wt	64.60±1.63^{bcfhjlm}	42.00±1.14^{bcfhilm}
IX	Pb and Zn H_2O	0.3:0.2mg kg^{-1} bd wt	52.40±1.08^{bdfgjlnp}	69.00±1.41^{bdfhjknp}
X	Pb and Co metals H_2O	0.3:Co pollutants mgkg^{-1} bd wt	69.20+0.86^{bdehjlnpr}	54.00±1.41^{bdfgjlnpr}
XI	River H_2O	5ml H_2Okg^{-1} bd wt	64.00±1.21^{bdfhjlnprt}	44.00±1.40^{bdehilnprt}

*See Table I for interpretations of alphabetical nomenclature
**bdfhjlnprt= values significantly (P<0.05) different from corresponding evaluated groups
*** acegikmoqs= values not significantly (P>0.05) different from corresponding statistically evaluated groups
Pb- exposed, and Pb and Fe, Zn, Cu and co-polluting metals -exposed rat groups caused significant increases in AST and ALT activities relative to their respective controls (Groups I and II). The groups of rats exposed to Pb in the presence of Ca, Mn and Mn showed a decrease in the activities of AST and ALT relative to the Pb- only group.

Table IV: Effects of Pb and Co Polluting Metals on Plasma Phosphatase Activities of the Rat.

S/No	Treatment	Dose (bd×3months	ALP KA units	total ACP KA units
			mean±SEM (n=5)×10^{-3}	
I	dH_20 only(control-1)	5ml/kg bd. wt	155.11±11.09	11.03±0.94
II	Pti H_20(control-2)	5ml/kg bd wt	202.76±14.73^{b}	18.98±0.84^{b}
III	River H_2O	5ml/kg bd. wt	293.10±10.91^{bd}	23.30±0.80^{bd}
IV	Pb only	0.3mg/kg bd.wt	375.27±1.01^{bde}	35.16±0.73^{bde}
V	Pb and Fe H_2O	0.3mg:0.21mg/kg bd.wt	401.59±10.66^{bcfh}	32.38±0.98^{bcfh}
VI	Pb and Ca H_2O	0.3:0.2/kg bd. Wt	229.43±0.59^{bdfhj}	18.87±0.37^{bdfhj}
VII	Pb and Cu H_2O	0.3:0.19kg bd .wt	454.24±0.77^{bdehjk}	41.19±0.55^{bdehjk}
VIII	Pb and Mn H_2O	0.3:0.27/kg bd. Wt	279.31±13.01^{bdfhjln}	39.40±1.11^{bdfhjln}
IX	Pb and Mg H_2O	0.3:0.2/kg bd ,wt	247.15±14.32^{bdehjlnp}	28.25±1.04^{bdehjlnp}
X	Pb and Zn H_2O	0.3:0.2/ kg bd wt	379.77±11.36^{bdfhjlnor}	37.86±1.25^{bdfhjlnor}
XI	Pb and Co metals H_2O	0.3:co polluting metals	315.27±18.15^{bdfhjlnprt}	30.35±1.07^{bdfhjlnprt}

*See Table I for interpretations of alphabetical nomenclature

**bdfhjlnprt= values significantly ($P<0.05$) different from corresponding statistically evaluated groups

*** acegikmoqs= values not significantly($P>0.05$) different from corresponding statistically evaluated groups

The results of alkaline and acid phosphatase activities are presented in Table IV.ALT and AST activities were elevation in the Pb only, Pb and Cu, Fe and Zn groups relative to the control (deionized water GroupI) and Pti borehole H_2O, Group II. Pb and Ca, Mg and Mn –exposed rat groups reversed the effects by Pb-only, Pb and, Cu, Zn.

Table V: Effects of Pb and Co-Polluting Metals on Plasma Total Protein and Bilirubin Concentration of the Rat

S/No	Treatment	Dose (bd×3months	Total Protein g/dl		Plasma Bilirubin mg/dl	
			(mean±SEM)×10^{-2}		(mean ± SEM) ×10^{-2} n=5	
			Plasma	Urine	Total	Direct (conjugated)
I	dH_20 only (control-1)	5ml H_2O kg^{-1} bd. wt	39.39±0.39	5.98±0.11	28.60	5.62±0.38
II	Pti H_2O (control-2)	5ml H_2O Kg^{-1} bd.wt	36.36±0.23^{a}	6.33±0.20^{a}	30.56^{a}	6.52±0.35^{a}
III	Pb only	0.3mgkg^{-1} bd.wt	18.22±0.29**	13.18±0.39**	45.69**	10.03±0.32**
IV	Pb and Fe H_2O	0.3mg:0.21mgkg^{-1} bd.wt	12.47±0.16***	11.53±0.23***	54.72***	12.85±0.30***
V	Pb and Ca H_2O	0.3:0.2kg bd^{-1}. Wt	25.44±0.37***	8.69±0..28***	32.45^{***g}	7.37±0.40***
VI	Pb and Cu H_2O	0.3:0.19kg bd^{-1} .wt	8.04±0.16^{***j}	6.66±0.34^{***j}	59.08^{***j}	13.84±0.65^{***g}
VII	Pb and Mn H_2O	0.3:0.27kg bd^{-1}. Wt	20.93±0.41^{***jp}	9.04±0.21^{***n}	40.55^{***jp}	7.84±0.31^{***n}
VIII	Pb and Mg H_2O	0.3:0.2kg $^{-1}$bd ,wt	29.99±0.36^{***jm}	8.87±0.26^{***nm}	34.91^{***jm}	8.56±0.40^{***nm}
IX	Pb and Zn H_2O	0.3:0.2kg^{-1} bd wt	19.25±0.37^{***jmp}	14.06±0.28^{***gjmp}	49.39^{***jmp}	11.99±0.55^{***gjp}
X	Pb and Co metals H_2O	0.3:Co polluting metals	20.17±0.32^{***jmn}	12.19±0.27^{***gjmp}	37.76^{***jmp}	8.87±0.36^{***jmnr}
XI	River H2O	5ml H_2Okg^{-1} bd wt	19.39±0.61^{***jmn}	10.95±0.33^{***gjmp}	35.92^{***jmn}	7.87±0.33^{***mnr}

*See Table I for interpretations of alphabetical nomenclature

**bdfhjlnprt= values significantly (P<0.05) different from corresponding evaluated groups

*** acegikmoqs= values not significantly (P>0.05) different from corresponding statistical evaluated groups.

Table V shows the effects of Pb and co-polluting metals on plasma total protein, urine protein and bilirubin levels of the rat. Pb-only and, Pb and Cu, Fe, Zn and co-polluting metals –exposed rat group (GroupIII) showed decrease(P<0.05) concentration of plasma protein relative to the controls (Groups I and II) while there was a significant (P<0.05) increase in percentage(%) of urine protein of the above mentioned group relative to their controls (deionized water and Pti potable water groups) and the Pb and Ca, Pb and Mg, and, Pb and Mg groups. Pb and Fe –exposed rat group revealed 92.46% while Pb and Ca –exposed revealed 34.70%. the total bilirubin concentration of Pb –exposed rats and Pb and, Cu, Fe, Zn and combined co-polluting metals was significantly (P<0.05) elevated while the reverse was the case for the rat groups exposed to Pb and Ca, Pb amd Mg and, Pb and Mn. Although the level of bilirubin in the group of rats exposed to Pb and Mn was not significantly (P>0.05) reduced relative to the controls (deionized water and Pti borehole water groups). There was also a significant increase in direct bilirubin concentration of Pb only and, Pb and Cu, Fe, Zn, co-polluting metals, and river water-exposed rat groups relative to their corresponding controls (Groups I and II).

Table VI: Effects of Pb and Co-Polluting Metals on Plasma and Urine Glucose

S/No	**Treatment**	**Dose (bd×3months)**	**plasma glucose (mg/dl)**	**Urine glucose (mg/dl)**
			mean±SEM (n=5)×10^{-3}	
I	dH_20 only(Control-1)	5mlkg^{-1} bd. wt	95.76±0.36	41.25±5.36
II	Pti H_2O (Control-2)	5mlkg^{-1} bd. wt	101.12±1.52^{b}	55.13±1.08^{b}
III	Pb only	0.3mgkg^{-1} bd.wt	151.04±0.73^{bd}	80.78±2.95^{bd}
IV	Pb and Fe H_2O	0.3mg:0.21mgkg^{-1} bd.wt	163.09±0.95^{bdf}	96.03±0.62^{bdf}
V	Pb and Ca H_2O	0.3:0.2mgkg^{-1} bd. Wt	111.39±1.33^{bdfh}	45.08±0.67^{adfh}
VI	Pb and Cu H_2O	0.3:0.19mgkg^{-1} bd .wt	178.05±0.65^{bdfhj}	92.16±0.70^{bdfhj}
VII	Pb and Mn H_2O	0.3:0.27mgkg^{-1} bd. Wt	112.31±1.48^{bdfhil}	43.41±0.73^{adfhil}
VIII	Pb and Mg H_2O	0.3:0.2mgkg^{-1} bd ,wt	118.99±0.64^{bdfhjln}	59.11±0.60^{bdfhjln}
IX	Pb and Zn H_2O	0.3:0.2mg kg^{-1} bd wt	159.27±1.02^{bdfgjlnp}	87.54±0.60^{bdfhjlnp}
X	Pb and Co metals H_2O	0.3:Co pollutants mgkg^{-1} bd wt	139.46±0.86^{bdfhjlnpr}	80.29±0.70^{bdehjlnpr}
XI	River H_2O	5ml H_2Okg^{-1} bd wt	121.45±1.13^{bdfhjlnort}	83.45±0.65^{bdehjlnprt}

*See Table I for interpretations of alphabetical nomenclature

**bdfhjlnprt= values significantly (P<0.05) different from corresponding evaluated groups

*** acegikmoqs= values not significantly (P>0.05) different from corresponding statistical evaluated groups.

Table VI shows the effects of Pb and Co-polluting metals on blood and urine glucose concentration.
Rats exposed to Pb only, Pn and Cu, Fe, and Zn revealed a significant(P<0.05) increase in plasma glucose and a corresponding increase in urine glucose concentrations of the rat relative to the controls, deionized water and Pti borehole water groups. Pb and Ca, Pb and Mg and, Pb and Mn exposed rat groups showed, a significant decrease in plasma and urine glucose relative to the Pb only, Pb and Cu, Fe, Zn and combined co-polluting metals groups. There was also a significant difference between river water exposed rats and laboratory reconstituted Pb and co-polluting metals exposed rats.

IV. DISCUSSION OF FINDINGS

The major consternation of this study was to evaluate the hepatotoxic effects of Pb in the presence of some selected co-polluting metals of Warri River using selected biochemical markers.
Alterations in body weight change and organ/body weight ratio are frequently used as indicators of chemical toxicity (Der *et al*., 1976; Horiguchi *et al.,* 1996; Ikatsu *et al.,* 1998). The significant decrease in liver organ /body weight (Table I), of Pb only, and, Pb and Fe, Cu and Zn -exposed rat groups gives credence to the reports by Der et al., 1976; Horiguchi and his team (1996) and Ikatsu et al., 1998).This finding is in agreement with a previous work done by Obi and Fadairo, 2013, where Cd- exposed rats showed significant decline in organ/body weight ratio.

The body is known to protect itself from oxygen free radical toxicity by enzymatic antioxidant mechanisms (glutathione peroxidase , superoxide dismutase and catalase) and by non enzymatic antioxidant mechanisms like increase in certain proteins like albumin and bilirubin (Oboh *et al.,* 2013), the significant (P<0.05) increases in catalase and superoxide dismutase activities, bilirubin levels of Pb-only rat group and Pb and Cu-exposed rats could be a strategy by the enzymatic and non enzymatic antioxidant proteins to protect the liver organ against the possible free radical stimulating effect of Pb only and Pb in synergy with Cu, Fe and Zn.. Our finding is in agreement with the previous work by Oboh *et al.,* 2013. Our finding also showed a significant (P<0.05) statistical correlation between bilirubin levels (Table III) and the levels of MDA (Table I), as bilirubin level was observed to increase along side with MDA levels in correspondence with the group of rats exposed to Pb-only and, Pb, in the presence of co-polluting metals like Fe, Cu and Zn.. This agrees with the previous works by Ahmed *et al*., 2005 and Oboh *et al.,* 2013. Lipid Peroxidation is initiated by free radicals like superoxide and hydrogen peroxide. The significant (P<0.05) increase in the products of lipid Peroxidation in the plasma of rats exposed to Pb only and Pb and Fe, Cu and Zn may not be surprising because of report suggesting that transition metals catalyze highly reactive free radical form(Allisa and Fern, 2011)

ALT and AST activities are usually considered strong indicators of optimum liver function. Increased levels of ALT is found mainly in liver diseases like hepatitis and other hepatic diseases and a slight l ALT elevation is also seen in myocardial infarction. ALT is found in variety of tissues but it is mainly found in the liver (Sood, 2006). AST is found mainly in the heart muscle, liver cells, skeletal muscle and kidneys (Sood, 2006). In this study, the significant (P<0.05) increases in the activities of the liver function enzymes (ALT and AST) in the

plasma of rats exposed to Pb only, could suggest a leak in the membrane of hepatic cells of rats following the damaging effect of Pb on cells. This finding is consistent with the report of Sood, 2006.

Alkaline and acid phosphatases activities have been reported to be elevated when the liver all integrity is affected by either a disease process or a toxic substance (Emede and Igben, 2013). In this study, there was also an increased plasma ALP and ACP activities of the Pb only rat group. Our findings also agrees with the report of Henderson and Moss (2001) who reported that serum or plasma ALP activities are of particular interest in the investigation of two groups of conditions, namely bone disease associated with increased osteoblastic activities and hepatobilliary disease. Group III rats (Pb only-exposed) relative to control I (deionized water) and control II (Pti borehole water). The significant ($P<0.05$) increase in plasma ALP could be an indication of the onset of osteoblastic activities in bones of the Pb exposed rat group or, could also be due to disruption of the liver parenchyma cells by Pb. This finding is also in agreement with the work of Brinkman *et al.,* 1998.

Our present study was based on the hypothesis that the Pb and some selected co polluting metals of Warri river could result in elevation of some enzymes and non enzymatic molecules usually use as biochemical markers of liver toxicity in order , to give a molecular rationale on the speculated increased incidences of liver diseases and liver related problems in the Niger delta region of Nigeria. Our findings revealed that Pb only and Pb in the presence of Cu, Fe, Zn, all co-polluting metals significantly induced the activities of ALT, AST, ALP, ACP, SOD, catalase, and increased the levels of bilirubin and glucose in Plasma and urine, caused a negative decrease in liver /body weight ratio, increased concentration urine protein, decrease Protein concentration of plasma, and a significant increase in the products of lipid per oxidation in the rat, relative to their respective controls, deionized water and Pti borehole water.

In our report. Pb only and, Pb and Cu, Fe and Zn- exposed rats groups also caused a significant ($P<0.05$) increase in plasma glucose and urine glucose concentration. The significant ($P<0.05$) increases in Plasma and Urine glucose could be as a result of other factors like impairment of kidney tubular transport mechanism and morphology by these metals. Furthermore, malondialdehyde is a biomarker for measuring oxidative stress, and (Devasagayam *et al.,*2003; Maritime *et al*., 2003), demonstrated the role of oxidative stress occasioned by high MDA levels in the pathology of diseases like diabetes and other related conditions, the significant($P<0.05$) increases in plasma glucose and urine glucose of Pb exposed and Pb and Cu, Fe and Zn-exposed rat groups could be due to the inability of the induced antioxidant enzymes and molecules (catalase, SOD and bilirubin) of same groups of rats to inhibit the production of reactive oxygen species (ROS), which then resulted in significant increase in the formation of products of lipid peroxidation(MDA) in these groups of rats. Our findings is not in agreement with the previous reports of Halliwell, 2007; Hamid *et al*., 2010, who demonstrated that antioxidant molecules and enzymes are produce to counter the consequences of ROS generated from products of lipd peroxidation. This implies that exposure of rats to Pb and, Pb and Cu, Zn and Fe may have resulted in cellular responses leading to induction of antioxidant enzymes but it appears that the antioxidant enzymes and molecules were unable to antagonize the effect of Pb and, Pb and Cu. Fe, Zn from breaking down polyunsaturated fatty acids thereby resulting in the significant ($P<0.05$) high MDA of same groups of rats with high SOD, catalase and bilirubin.

V. CONCLUSION

Overall, the overall effects of Pb only, and, Pb and Fe, Pb and Zn in the reduction of organ body weight ratio, increased ALP activities and increase plasma bilirubin concentration were synergistic as against our hypothesis of a remedial interaction. This does not align with a previous work of Ahamed *et al.,* 2007, who demonstrated that essential elements like Ca, Zn, Fe and Selenium counteracted the negative effects of Pb. With the exemption of Ca, which, antagonized the effect of Pb in most biochemical markers evaluated, Fe and Zn were synergistic in their combined effects with Pb. Our finding is not in consonance with an earlier report referenced by Enuneku *et al.,* 2013, who showed that binary mixture of Cd and Zn on mortality of biological specie was antagonistic instead of synergistic but agrees with the report of Shamar and Satyanaryan (2011), who demonstrated that co administration of Pb and Cu to vital tissues of the earth worm, caused more deleterious effect relative to when Pb was administered alone. Although our findings may not be in alignment with our hypothesis and some study reports, but there are report demonstrating that transition metals, particularly the divalent ions such as Fe and Cu, are known to further catalyze highly, reactive free radicals forms (Allisa and Fern, 2011). Tagging along inconsistency in some of our findings especially as regards the reports on the antagonistic effects of some transition metals like zinc on Pb exposed rats, there is therefore, a need for a further study to equate the doses of these co-polluting metals with that of Pb and re-evaluate them on some of the biochemical markers evaluated in this present study.

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