BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2018. Vol. **15**(4), p. 937-947

Evaluation of Serum Status of Biochemical Indices of Liver Injury and Oxidative Stress in Rats Exposed to Warri River Levels of Pb and Other Identified Metallic Co Pollutants

E. A. Fadairo [1] and F.O. Obi[2]*

[1]Industrial Safety and Environmental Technology Department,
Petroleum Training Institute, PMB 20 Effurun, Delta State, Nigeria.
[2]Department of Biochemistry, Faculty of Life Sciences,
University of Benin, PMB 1154 Benin-City, Nigeria.

http://dx.doi.org/10.13005/bbra/2705

(Received: 03 November 2018; accepted: 14 December 2018)

This study investigatedserum status of biochemical indicators of liver injury and oxidative stress in rats exposed to Warri River level of lead(Pb) alone and in the presence of metallic co-pollutants. 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 water containing laboratory reconstituted Warri River Pb level on one hand and in the presence of laboratory reconstituted identified metallic co-pollutants including Fe, Ca, Cu, Mn, Mg, Zn via water on the other hand. The serum 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 + Cu, Pb + Fe and Pb + Zn, Pb + All metallic co-pollutants, 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 + Ca, Pb + Mn, Pb + 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 some identified metallic co pollutants like Cu, Fe and Zn. This study also revealed the possibility of significant (P<0.05) decrease in the activities of superoxide dismutase (SOD), catalase, plasma total and direct bilirubin and lipid per oxidation products of rats exposed to Warri River level of Pb in the presence of Ca, Mg and Mn relative to the Pb only group.

Keywords: Biochemical Indices, Liver Injury Warri River Pb level, Identified Metallic Co-Pollutants and Oxidative Stress.

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

*Corresponding author E-mail: ekakpan10@yahoo.com

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°24'00''N and 5°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 the 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 by promulgating policies supporting the use of as unleaded gasoline and prohibiting processes like 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 exposure to this pollutant.

Albeit, lead toxicity is considered a widely explored area of research, studies on the evaluation of serum status of biochemical indices of liver injury and oxidative stressof rats exposed to lead in the presence of some selected metallic co-pollutants are far from being exhausted.

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 QuimicaClinicaAplicada. (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

Preparation of Laboratory Reconstituted Water (Obi *et al.,* 2017)

The test water samples administered to

the rats were prepared as follows

Pb and Fe contaminated water(Pb: 0.21mg/L; Fe: 1.60mg/L)

Aliquots (7.50ul) each of the respective stock solutions, Pb and Fe (0.033g/L and 0.03g/L of $PbNO_3$ and $FeSO_4.7H_2O$) were transferred to into 25litres of deionized water

Pb and Ca contaminated water (Pb: 0.21mg/L;Ca:111.11mg/L)

Aliquots (750ul) each of the respective stock solutions ($Pb(NO_3)_2$:0.033g/L and $CaSO_4.12H_2O$:4.13g/L) were transferred into 25 litres deionized water

Pb and Cu contaminated water (Pb:0.21mg/L; Cu:0.012mg/L)

Aliquots (750ul) each of the respective stock solutions ($Pb(NO_3)_2$:0.033g/L and $CuSO_4.5H_2O$:0.00070g/L) were transferred into 25 litres deionized water

Pb and Mn Contaminated Water(Pb:0.21mg/L; Mn:0.22mg/L)

Aliquots (750ul) each of the respective stock solutions ($Pb(NO_3)_2$:0.033g/L and $MnSO_4.H_2O$:0.0067g/L) were transferred into 25 litres deionized water

Pb and Mg Contaminated Water (Pb:0.21mg/L; Mg:9.79mg/L)

Aliquots (750ul) each of the respective stock solutions ($Pb(NO_3)_2$:0.033g/L and $MgSO_4.7H_2O$:0.10g/L) were transferred into 25 litres deionized water

Pb and Zn Contaminated Water (Pb:0.21mg/L; Zn:0.059mg/L)

Aliquots (750ul) each of the respective stock solutions ($Pb(NO_3)_2$:0.033g/L and $ZnSO_4.7H_2O$:0.003g/L) were transferred into 25 litres deionized water

Pb and Co Metallic Pollutant Contaminated Water (Pb:0.21mg/L;Fe:1.60mg/L;Ca:111.11mg/L; Cu: 0.012mg/L; Mn:0.22mg/L; Mg: 9.79mg/L and Zn:0.059mg/L)

Aliquots (750ul) each of the respective stock solutions were transferred into 25 litres deionized water.

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 metallic co- pollutants used were calculated on the basis of Warri River concentrations of the respective metals at the time of this 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 orally by gavage to Pb and the identified metallic co- pollutants reconstituted in deionized water as follows: Group 1 rats (control-I),received deionized water only (5ml H_2O/kg bdwt by gavage); Group II rats received Ptiborehole water only (5ml H_2O/kgbdwt by gavage); Group III rats received Pb only stock (0.21mg/kg bd.wt) (5ml H_2O/kg bdwt) ;Group IV rats received Pb and Fe stock solution(5ml H_2O/kg bd. Wt); Group V rats received Pb and Ca stock solution(5ml H_2O/kg bd. wt); Group VI rats received Pb and Cu stock solution(5ml H_2O/kg bd. Wt); Group VII rats received Pb and Mn stock solution (5ml H_2O/ kg bd.wt); Group VIII rats received Pb and Zn stock solution(5ml H_2O/ kg bd. wt); Group IX rats received Pb and Mg stock solution(5ml H_2O/ kg bd .wt); Group X rats received Pb and all identified co- metallic pollutants (5ml/kg bd. wt) while Group XI rats received Warri river water (5ml H_2O/kg bdwt). At the end of the study period (90d), the animals were sacrificed in accordance with the rules of the local ethics committee of the Faculty of Life Sciences, University of Benin. Blood samples were collected by cardiac puncture and allowed to stand for 10mins, the supernatant (serum) were collected and stored at -20^{o}C and assayed immediately. The liver was excised and stored at the same temperature until required for analysis. A 24hr urine sample was obtained 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 graphpadinstat statistical package. All data are expressed as mean ±SEM. Significant level was assigned at $P<0.05$

Presentation of Results

Influence of Pb in the presence of metallic co-pollutants on organ body wt ratios and MDA levels

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.

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 in the products of lipid Peroxidation evident as malondialdehyde levels, relatives to the controls (Groups I, deionized water only exposed group and Group II: Pti borehole water -exposed group). Pb in combination with Cu (Pb+Cu), Fe (Pb+Fe), Zn (Pb+ Zn) and (Pb+ all co-polluting metals) caused significant ($P<0.025$) decreases in liver/body wt ratios and increases ($P<0.025$) in MDA levels in the respective rat groups relative to the controls. Also, Pb in the presence of Ca, Mn and Mg showed significant ($P<0.05$) increases in liver body weight ratios and decreases in levels of lipid peroxidation products of their respective rat group relative to the Pb-only group. There was a difference in liver body weight ratio and malondialdehyde (MDA) levels between the Warri river water-exposed rat group and the laboratory reconstituted river water group, although, the difference was not significant ($P>0.05$).

Influence of Pb in the presence of Metallic co-pollutants on serum SOD and CAT Activities

The results of the effects of Pb and metallic co-pollutants on superoxide dismutase (SOD) and catalase (CAT) activities are presented in Table 2.

Exposure of rats to Pb only and Pb in the presence of Cu, Fe, Zn and combined metallic co-pollutants caused a significant ($P<0.05$) increases in SOD and CAT activities of the rat relative to the controls (Groups I and II). Pb in the Presence Ca, Mn, and Mg caused significant ($P<0.05$) decreases in SOD and CAT activities relative to the Pb-only exposed rat group. There was also significant ($P<0.05$) elevations in the activities of SOD and CAT of the river water exposed rat group relative to the controls (Groups I and II), and a slight decrease in SOD and CAT activities of the river water- exposed rat group relative to the

laboratory reconstituted water. Although, it was not significant (P>0.05).

Influence of Pb in the presence of Metallic Co-Pollutant on serum AST and ALT Activities of the Rate

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 3.

Pb, Pb+ Fe, Pb+ Zn, Pb+Cu and Pb+ all metallic co-pollutants -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 (Pb+ Ca), Mn (Pb+ Mn) and Mg (Pb+ Mg) showed significant (P<0.05) decreases in the activities of AST and ALT relative to the Pb- only and Pb +Cu groups.

The influence of Pb in the presence of metallic co-pollutants on serum phosphatases activities of the rat

The effects of Pb in the presence of metallic co-pollutants on serum phosphatases activities of the rat are presented on Table 4.

The results of alkaline and acid phosphatase activities are presented in Table 4. ALT and AST activities were significantly(P<0.05) elevated in the Pb only, Pb + Cu; Pb+ Fe and Pb +Zn groups relative to the control (dH_2O GroupI) and (Pti borehole H_2O, Group II). Pb+ Ca, Pb+ Mg and Pb+Mg–exposed rat groups reversed the effects by Pb-only, Pb+Cu, and Pb+Zn.

Influence of Pb in the presence of metallic co-pollutants on serum total protein and bilirubin levels

The effects of Pb in the presence of metallic co-pollutants on serum protein and bilirubin levels are represented on Table 5

Table 5 shows the effects of Pb and metallic co-pollutants on serum total protein, urine protein and bilirubin levels of the rat. Pb-only and, Pb + Cu, Pb+ Fe, Pb+ Zn and Pb+ metallic co pollutants –exposed rat groups (Groups III, IV, VI $IX) showed significant (P<0.05) decreases in concentrations of serum protein of the respective groups relative to the controls (Groups I and II). There was a significant (P<0.05) increase in percentage(%) of urine protein of the

Table 1. Effect of Pb in the presence of Metallic Co-Pollutants on liver weight/100gm Body weight and serum MDA level of the rat

Treatment	Dose# (bd×3months)	Liver wt/ bd wt. ratio (mean±SEM)×10^{-3} n=5	Malondialdehyde level (µmole MDA/g tissue)
deionized water (Control-1) dH_2O	5ml/kg bd. wt	23.62±1.78	1.08±0.02
Pti borehole (Control-2), Pti H_2O	5mlH2O/kg bdwt	$21.10±1.26^{a}$	$1.44±0.12^{b}$
Pb only	0.21mg/kg bd.wt	$10.13±0.77^{bd}$	$5.05±0.06^{bd}$
Pb and Fe H_2O	0.21mg:1.60mg/kg bd.wt	$13.43±0.53^{bcf}$	$3.08±0.04^{bdf}$
Pb and Ca H_2O	0.2:111.1mg/kg bd. Wt	$14.55±0.92^{bdfg}$	$2.11±0.03^{bcfh}$
Pb and Cu H_2O	0.2:0.012mg/kg bd .wt	$6.67±0.92^{bdehj}$	$5.61±0.08^{bdfhj}$
Pb and Mn H_2O	0.2:0.22mg/kg bd. Wt	$13.38±0.91^{bcfhil}$	$3.30±0.03^{bdehjl}$
Pb and Mg H_2O	0.2:9.79mg/kg bd ,wt	$12.55±0.75^{bdegilm}$	$3.74±0.14^{bdfhjln}$
Pb and Zn H_2O	0.2:0.06mg/ kg bdwt	$18.29±0.70^{bcfhjlnp}$	$4.30±1.43^{bcegikmo}$
Pb and Co metals H_2O	0.21mg :mixed conc. co metals	$13.49±1.04^{bdegilmor}$	$2.98±0.07^{bdfgjlnpq}$
River water	5ml H2O/kg bdwt	$16.49±0.67^{bdfhjlnpqt}$	$2.21±0.07^{bdfhjlnpqt}$

[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.

above mentioned groups relative to the controls (deionized water and Pti potable water groups) and the Pb +Ca, Pb+Mn, and, Pb + Mg- exposed rat groups. Pb + Fe –exposed rat group revealed a 92.46%protein level while Pb and Ca –exposed rats showeda 34.70%.protein level. Total bilirubin concentration of Pb only –exposed rats and Pb + Cu, Pb+ Fe and Pb+ all metallic co pollutants was significantly ($P<0.05$) elevated while the reverse was the case for the rat groups exposed to Pb + Ca, Pb+ Mg and, Pb+ Mn 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

Table 2. Effects of Pb in the presence of metallic co-pollutants on SOD and CAT activities

Treatment	SOD ActivitiesU/L	CAT ActivitiesU/L mean±SEM(n=5)×10^{-4}
dH_20 only (control-1)	1.10±0.03	3.19±0.03
Pti H_2O (control-2)	$1.59±0.08^{b}$	$3.74±0.07^{b}$
Pb only	$2.59±0.09^{bc}$	$7.34±0.14^{bd}$
Pb and Fe H_2O	$2.65±0.17^{bce}$	$4.53±0.17^{bdf}$
Pb and Ca H_2O	$1.92±0.18^{bdeh}$	$4.00±0.04^{bdfh}$
Pb and Cu H_2O	$2.73±0.25^{bcegj}$	$9.28±0.09^{bdfhj}$
Pb and Mn H_2O	$1.98±0.20^{bcegil}$	$4.14±0.09^{bdfgil}$
Pb and Mg H_2O	$2.12±0.16^{bcfgikm}$	$4.03±0.03^{bdfgilm}$
Pb and Zn H_2O	$2.15±0.19^{bcegikmo}$	$4.56±0.28^{bdfgilmo}$
Pb and Co metals H_2O	$2.54±0.20^{bcegjkmoq}$	$7.06±0.74^{bdehjlnpr}$
River water	$2.26±0.10^{bc\ fhjlmoqt}$	$6.26±0.10^{bdfhjlnpqt}$

*See Table 1 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

Table 3. Effects of Pb in the presence of metallic co-pollutants on serum AST and ALT Activities of the Rat

Treatment	ASTU/L	ALTU/L
	mean±SEM (n=5)×10^{0}	
dH_20 only(Control-1)	38.00±2.00	23.60±0.92
Pti H_2O (Control-2)	$39.20.00±0.86^{a}$	$45.00±1.70^{b}$
Pb only	$72.00±1.46^{bd}$	$60.00±1.14^{bd}$
Pb and Fe H_2O	$74.00±0.71^{bdf}$	$69.00±1.00^{bcf}$
Pb and Ca H_2O	$58.00±0.71^{bdfh}$	$37.00±1.41^{bcfh}$
Pb and Cu H_2O	$91.40±1.02^{bdfhj}$	$67.80±1.50^{bdfhj}$
Pb and Mn H_2O	$62.00±0.71^{bcfhjl}$	$40.00±1.41^{bcfhjl}$
Pb and Mg H_2O	$64.60±1.63^{bcfhjlm}$	$42.00±1.14^{bcfhilm}$
Pb and Zn H_2O	$52.40±1.08^{bdfgjlnp}$	$69.00±1.41^{bdfhjknp}$
Pb and Co metals H_2O	$69.20+0.86^{bdehjlnpr}$	$54.00±1.41^{bdfgjlnpr}$
River H_2O	$64.00±1.21^{bdfhjlnprt}$	$44.00±1.40^{bdehilnprt}$

*See Table 1 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

+ Cu, Pb+ Fe, Pb+ Zn, Pb+ metallic co-pollutant and river water-exposed rat groups relative to their corresponding controls (Groups I and II).

Influence of Pb in the presence of metallic co-pollutants on blood and urine glucose

The effects of Pb in the presence of some selected metallic co-pollutants on blood and urine glucose are represented in Table 6.

Table 6 shows the effects of Pb in the presence of metallic co-pollutants on blood and urine glucose concentration.

Rats exposed to Pb only, Pb + Cu, Pb+ Fe, and Pb+ Zn revealed a significant($P<0.05$) increase in blood glucose and a corresponding increase in urine glucose concentrations relative to the controls, deionized water and Pti borehole water

Table 4. Effects of Pb in the presence of metallic co pollutants on serum phosphatases activities of the rat

S/No	Treatment	ALP KA units mean±SEM (n=5)×10^{-3}	total ACP KA units mean±SEM (n=5)×10^{-3}
i	dH_20 only(control-1)	155.11±11.09	11.03±0.94
ii	Pti H_20(control-2)	202.76±14.73^{b}	18.98±0.84^{b}
iii	Pb only	375.27±1.01^{bd}	35.16±0.73^{bd}
iv	Pb and Fe H_2O	401.59±10.66^{bde}	32.38±0.98^{bde}
v	Pb and Ca H_2O	229.43±0.59^{bcfh}	18.87±0.37^{bcfh}
vi	Pb and Cu H_2O	454.24±0.77^{bdfhj}	41.19±0.55^{bdfhj}
vii	Pb and Mn H_2O	279.31±13.01^{bdehjl}	39.40±1.11^{bdehjk}
viii	Pb and Mg H_2O	247.15±14.32^{bdfhjln}	28.25±1.04^{bdfhjln}
ix	Pb and Zn H_2O	379.77±11.36^{bdehjlnp}	37.86±1.25^{bdehjlnp}
x	Pb and Co metals H_2O	315.27±18.15^{bdfhjlnor}	30.35±1.07^{bdfhjlnor}
xi	River H_2O	293.10±10.91^{bdfhjlnprt}	23.30±0.80^{bdfhjlnprt}

*See Table 1 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

Table 5. Pb in the presence of metallic co-pollutants on serum total protein and bilirubin levels

S/No	Treatment	Total Protein g/dl (mean ± SEM)×10^{0}		Bilirubin mg/dl (mean ± SEM) ×10^{-4} n=5	
		serum	Urine	Total	Direct (conjugated)
i	dH_20 only(control-1)	39.39±0.39	5.98±0.11	28.60±1.93	5.62±0.38
ii	Pti H_2O (control-2)	36.36±0.23^{b}	6.33±0.20^{a}	30.56±2.01^{b}	6.52±0.35^{a}
iii	Pb only	18.22±0.29^{bd}	13.18±0.39^{bd}	45.69±2.15^{bd}	10.03±0.32^{bd}
iv	Pb +Fe	12.47±0.16^{bdf}	11.53±0.23^{bde}	54.72±2.98^{bdf}	12.85±0.30^{bdf}
v	Pb + Ca	25.44±0.37^{bdfh}	8.69±0.28^{bdfh}	32.45±1.39^{bdfg}	7.37±0.40^{bcfh}
vi	Pb + Cu	8.04±0.16^{bdfhj}	6.66±0.34^{bdfhj}	59.08±2.54^{bcfhj}	13.84±0.65^{bdfgj}
vii	Pb +Mn	20.93±0.41^{bdfhjl}	9.04±0.21^{bdfhil}	40.55±2.50^{bdfhjl}	7.84±0.31^{bcfhil}
viii	Pb + Mg	29.99±0.36^{bdfhjlm}	8.87±0.26^{bdfhilm}	34.91±2.38^{bdfhjln}	8.56±0.40^{bdehiln}
ix	Pb + Zn	19.25±0.37^{bdfhjlnp}	14.06±0.28^{bdfgljnp}	49.39±3.17^{bdfhjlnp}	11.99±0.55^{bdegjlnp}
x	Pb + All metallic co -pollutants	20.17±0.32^{bdfhjlmor}	12.19±0.27^{bdehljnpr}	37.76±2.00^{bdfhilnpr}	8.87±0.36^{bdfhjlmor}
xi	River H_2O	19.39±0.61^{bdfhjlnors}	10.95±0.33^{bdfhjlnprt}	35.92±2.11^{bdfhjlnort}	7.87±0.33^{bcfhilmors}

*See Table 1 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 6. Effects of Pb and metallic co -pollutants on blood and Urine Glucose

S/No	Treatment	Dose(bd×3months)	blood glucose(mg/dl) mean±SEM (n=5)×10^{-3}	Urine glucose(mg/dl)
I	dH_20 only(Control-1)	5ml/kg bd. wt	95.76±0.36	41.25±5.36
II	Pti H_2O (Control-2)	5mlH2O/kg bdwt	$101.12±1.52^{b}$	$55.13±1.08^{b}$
III	Pb only	0.21mg/kg bd.wt	$151.04±0.73^{bd}$	$80.78±2.95^{bd}$
IV	Pb and Fe H_2O	0.21mg:1.60mg/kg bd.wt	$163.09±0.95^{bdf}$	$96.03±0.62^{bdf}$
V	Pb and Ca H_2O	0.2:111.1mg/kg bd. Wt	$111.39±1.33^{bdfh}$	$45.08±0.67^{adfh}$
VI	Pb and Cu H_2O	0.2:0.012mg/kg bd .wt	$178.05±0.65^{bdfhj}$	$92.16±0.70^{bdfhj}$
VII	Pb and Mn H_2O	0.2:0.22mg/kg bd. Wt	$112.31±1.48^{bdfhil}$	$43.41±0.73^{adfhil}$
VIII	Pb and Mg H_2O	0.2:9.79mg/kg bd ,wt	$118.99±0.64^{bdfhjln}$	$59.11±0.60^{bdfhjln}$
IX	Pb and Zn H_2O	0.2:0.06mg/ kg bdwt	$159.27±1.02^{bdfgjlnp}$	$87.54±0.60^{bdfhjlnp}$
X	Pb and Co metals H_2O	0.21mg :mixed conc. co metals	$139.46±0.86^{bdfhjlnpr}$	$80.29±0.70^{bdehjlnpr}$
XI	River H_2O	5ml H2O/kg bdwt	$121.45±1.13^{bdfhjlnort}$	$83.45±0.65^{bdehjlnprt}$

*See Table 1 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

groups. Pb + Ca, Pb + Mg and, Pb + Mn exposed rat groups showed, significant (P<0.05) decreases in plasma and urine glucose concentrations relative to the Pb only, Pb + Cu, Pb+ Fe and Pb+ all metallic co-pollutants. There was also a difference in the blood and urine glucose concentrations of river water and laboratory reconstituted Pb +metallic co-pollutants- exposed rats, albeit, it was not significant (P=0.065)

Discussion of Findings

The major consternation of this study was to evaluate some serum biochemical indices and the magnitude of oxidative stress occasioned byliver injury in rats exposed to Warri River levels of Pb in the presence of some selected metallic co-pollutants.

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 3) 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 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 membrane damaging effect of Pb. 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 metallic co pollutants of Warri river could result in elevation of some enzymes, non-enzymatic molecules and lipid peroxidation products (MDA), usually use as biochemical markers of liver toxicity and oxidative stress in order, to give a molecular rationale to 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.

Findings from this study shows that Pb only and, Pb +Cu, Pb+Fe - exposed rats 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 +Cu,Pb+ Fe 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 P-only, Pb+Cu, and Pb+ 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-only and in the presence of Cu and Fe metallic co-pollutants, 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 observed in this study.

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 of liver toxicity evaluated. Fe and Zn were synergistic in their respective combined effects with Pb. Our finding is not in consonance with an earlier report referenced by Enunekue*t 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 some of our findings may not be in alignment with our hypothesis and some study reports, but there are reports 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 metallic co-pollutants to the levels of Pb and re-evaluate them on some of the biochemical markers evaluated in this study.

ACKNOWLEDGEMENT

The authors wish to appreciate the following laboratories: Department of Biochemistry Laboratory, University of Benin; Chemical Pathology Laboratory, University of Benin; Benin-City and Analtrace Laboratory, Okwokwoko, Delta state, Nigeria.

REFERENCES

1. Flora,S.J.S. Flora, G.J.S Saxena. G. Environmental occurrence, health effects and management of lead poisoning. In: Cascas SB, Sordo J, editors. Lead: Chemistry, Analytical Aspects, Environmental Impacts and Health Effects. Netherlands: Elsevier Publication 2006 pp. 158–228.
2. .Beuge, J.A and D.S. Aust .Microsomal Lipid Peroxidation. In: Colewick, S.P and Kaplan, N.O (eds). Methods in Enzymology vol.52.Academic Press, New York, 1978, PP302-310.
3. Misra, H.P and I Fridovich.The role of superoxide anion in auto oxidation of epinephrine and a Simple assay for superoxide dismutase.*J. Biol. Chem*, 1972; **247**(10): 3170-3175
4. Cohen, G. Dembiee, C and Marcus, J. Measurement of catalase activity in tissue extracts. *Anal Biochem,* 1970; **34**: 30-38.
5. Lowry, OH. NJ. Rosebrough AL..Farr, RJ Randall RJ. Protein measurement with Folinciocalteau reagent. *J.BiolChem*, 1951; **193**: 265- 275
6. Der., R. Z. Fahim,Y. Mohammed and M. Fahim. Environmental interaction of Lead and Cadmium on reproduction and metabolism of male rats. *Res Commum ChemPathol Pharmacol*, 1976; **14**: 689-714.
7. Obi, F.O., Adaikpoh, M.A., Ugheighele, S.E and Edokpolor, O. Evaluation of Cadnium toxicity in the African catfish (Clariagariepinus)in the presence of calcium and magnesium based on selected blood parameters.*Journal of Food and Agricultural Sciences,* 2017.
8. Horiguchi, H. M. Sato,N. Konno and M .Fukushima. Long Term Cadmium Exposure induces Ammonia in rats through hypo induction of erythropoietin in the kidneys. *Arch. Toxicol*. 1996; **71**: 1996, 11-19.
9. Ikatsu,, H. S. Shinoda, S.,T Nakajima. CYP2EI levels in rat liver injured by the interaction between carbon tetrachloride and chloroform. *J. Occup Health,* 1998; **40**: 223-229.
10. Obi, F.O., and E.A.Fadairo. Influence of Anthocyanin-Free Aqueous Extract of *Hibiscus sabdariffa L* Petal on Cadmium Toxicity in Male Rats. *Nig. J. Life Sc*. 2013; **3**(1): 78-92
11. Oboh, H.A., F.E. Olumese, N.B Aguebor-Ogie, and O. E., Nuagbe. Effects of *Hoslundiaopposita* leafy vegetable on some antioxidant enzymes in organs of normal Wistar rats. *Nig. J. Life Sc*. 2013; **3**(1): 93-100.
12. AhamedM.S Behari, J.R A. Kumar, and M K. Siddiqui, Interaction of Pb with some essential trace metals in the blood of anemic Children. *ClinicaChimicaActa*; 2007; **377**(1-2): 92-97.
13. Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Copper. Atlanta, GA: Centers for Disease Control; 2005.
14. Allissa, E. M , and G.AFerns, GA. Heavy Metal Poisoning and Cardiovascular Diseases.*J. Toxicol*: 2011: 870125.doi; 10.1155
15. Henderson, A.R. and D.W.Moss. Enzymes in Burtis CA and Ashwood ER.(eds).Fundamental of Clinical Chemistry 4th ed. W.B. Sanders. Philadelphia, 2001; PP 352-89.
16. Brinkman, K., .M Hofstede, H.J and DM. Burger. Adverse Effects of Reverse Transcriptase Inhibitors: Mitochondria toxicity as common pathway. *.AIDS*, 1998; **12**: 1735-1744

17. Devasagayam.TPA.,Boloor KK and T. Ramasarma. Methods of Estimating Lipid Peroxidation: An Analysis of merits and Demits. *Indian Journal of Biochemistry and Biophysics*, 2003; **10**: 300-308
18. Enuneku, AA.,Ezermonye L.I, and P. Agbure, Hispathological Effects and Biological Accumulation of Copper in the Perinwinkle (*Tympanotonusfuscatus* var. radula). *Nig J. Life. Sc*. 2013: **3**(1)*,* 34-35.
19. GutteridgeM., and S.Wilkins. Copper dependent hydroxyl radical damage to ascorbic acid, formation of thiobarbituric acid reactive products. *FEBS Letts,* 1982; 137-330,
20. Reitman, S and Frankel.S Alanine and Aspartate Transaminases Procedures*. Amer. J Clin. Path*, **28**: 56.
21. Sharma, V.J.,and S. Satyanarayan, Effects of Selected Heavy Metals on Histopathology of different Tissues of Earthworm, *Eudrilluseugeniae*. *Journal of Environmental Monitoring and Assessment*, 2011; **180** *(1-4),* 257-267.
22. Maritim, AC., RA, Sanders, and JB.Natkins III, Diabetes Oxidative Stress and Antioxidant Enzyme: A review. *J. Biochem Mol. Toxicology,* 2003; **17**((1), 24-38.
23. Halliwell, B. Biochemistry of Oxidative Stress. Biochem Soc*Trans*, 2007; **35**(5), 1147-50
24. Agency for Toxic Substances and Disease Registry (ATSDR, 1999) Public Health Service. Atlanta: U.S. Department of Health and Human Services Toxicological Profile for Lead
25. A.A Humid,O.O. Aiyelaagbe, L.A. Usman,O.M. Ameen, and A. Lawal. Antioxidants: its Medicinal and Pharmacological Applications. *African Journal of pure and Applied Chemistry.* 2010; **4**(8), 142-151
26. Sandhir, R and K.D. Gill. Effect of Pb on Lipid Peroxidation in Liver of Rats. *Biol Trace Elem. Res,* 1995; **48**(1), 1957-1967
27. United State Geo Spatial Agency (USGSA) 1994. Fort Belvoir North Area, Springfield, Virginia
28. Sood. R (2006) Textbook of Medical Laboratory Technology. First Edition, Jaypee Brothers Medical publishers Ltd., New-Delhi.
29. Emmanuel, I.N. , Nat, C.E., E. Anthony, C.E Paul,, V.E. Jude, R.E Tochukwu, E.E. Joseph. 2013. Effects on *Hibiiscussabdariffa* calyces on Serum cholesterol, Body weight and liver biomarkers of RattusNoverjieus*. Inter.J. IMP*, **46**: 4, ISSN: 2051-2062
30. Asagba. S.O and F.O.Obi. A comparative evaluation of the biological effects of environmental cadmium-contaminated control diet and laboratory cadmium supplemented test diet. *Biometals,* 2005; **18***:* 153-161
31. Varley, H. 1975. Practical Clinical Biochemistry, 4th Ed. Textbook of Medical Laboratory Technology. First Edition, Jaypee Brothers Medical publishers Ltd., New-Delhi, 2006.