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

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

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

This study investigated serum 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± 0.90g, 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...
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 mechanisms 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 indigenes 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 as 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 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 stress of 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°C-25.5°C; 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 PbNO₃ and FeSO₄·7H₂O) were transferred into 25 litres of deionized water.

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

Aliquots (7.50ul) each of the respective stock solutions (Pb(NO₃)₂: 0.033g/L and CaSO₄·12H₂O: 4.13g/L) were transferred into 25 litres of deionized water.

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

Aliquots (7.50ul) each of the respective stock solutions (Pb(NO₃)₂: 0.033g/L and CuSO₄·5H₂O: 0.00070g/L) were transferred into 25 litres of deionized water.

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

Aliquots (7.50ul) each of the respective stock solutions (Pb(NO₃)₂: 0.033g/L and MnSO₄·H₂O: 0.0067g/L) were transferred into 25 litres of deionized water.

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

Aliquots (7.50ul) each of the respective stock solutions (Pb(NO₃)₂: 0.033g/L and MgSO₄·7H₂O: 0.10g/L) were transferred into 25 litres of deionized water.

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

Aliquots (7.50ul) each of the respective stock solutions (Pb(NO₃)₂: 0.033g/L and ZnSO₄·7H₂O: 0.003g/L) were transferred into 25 litres of 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; Zn: 0.059mg/L)

Aliquots (7.50ul) each of the respective stock solutions were transferred into 25 litres of deionized water.

**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 10 minutes 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 were 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 was measured using 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).

**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...
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)×10³ n=5 | Malondialdehyde level (µmole MDA/g tissue) |
|------------------------------------|-------------------|-------------------------------------------|------------------------------------------|
| deionized water                    | 5ml/kg bd. wt     | 23.62±1.78                                | 1.08±0.02                                |
| Pti borehole                       | 5mlH2O/kg bdwt    | 21.10±1.26a                                | 1.44±0.12b                               |
| Pb only                            | 0.21mg/kg bd. wt  | 10.13±0.77bd                               | 5.05±0.06bd                              |
| Pb and Fe H₂O                      | 0.21mg:1.60mg/kg bd. wt | 13.43±0.53bfc                             | 3.08±0.04bdf                             |
| Pb and Ca H₂O                      | 0.2:111.1mg/kg bd. Wt | 14.55±0.92bcfg                           | 2.11±0.03bcf                            |
| Pb and Cu H₂O                      | 0.2:0.012mg/kg bd. wt | 6.67±0.92bcj                              | 5.61±0.08bcgh                           |
| Pb and Mn H₂O                      | 0.2:0.22mg/kg bd. Wt | 13.38±0.91bcj                              | 3.30±0.02blij                           |
| Pb and Mg H₂O                      | 0.2:9.79mg/kg bd ,Wt | 12.55±0.75bcgelm                          | 3.74±0.14blij                           |
| Pb and Co metals H₂O              | 0.2:0.06mg/ kg bdwt | 18.29±0.70blijpq                          | 4.30±1.43bijke                           |
| River water                        | 5ml H2O/kg bdwt   | 16.49±0.67blijpq                           | 2.21±0.07blijpq                          |

|                        |                   |                                      |                                      |
|------------------------|-------------------|---------------------------------------|---------------------------------------|
|                        |                   | (P>0.05); p<0.05 relative to their respective grp1 values; (P>0.05); p<0.05 relative to their respective grp 2 values |
|                        |                   | (P>0.05); p<0.05 relative to their respective grp 3 values; (P>0.05); p<0.05 relative to their respective grp 4 values |
|                        |                   | (P>0.05); p<0.05 relative to their respective grp5 values; (P>0.05); p<0.05 relative to their respective grp 6 values |
|                        |                   | (P>0.05); p<0.05 relative to their respective grp 7 values; (P>0.05); p<0.05 relative to their respective grp 8 values |
|                        |                   | (P>0.05); p<0.05 relative to their respective grp 9 values; (P>0.05); p<0.05 relative to their respective group10 values |
|                        |                   | (#= dose calculated based on Warri River concentrations of respective metals in a preliminary study). |

The influence of Pb in the presence of Metallic Co-Pollutants on serum protein and bilirubin levels

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

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

The influence of Pb in the presence of 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, V, 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
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 showed a 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 Activities U/L | CAT Activities U/L |
|-----------|-------------------|--------------------|
| dh2O only (control-1) | 1.10±0.03 | 3.19±0.03 |
| Pti H2O (control-2) | 1.59±0.08 | 3.74±0.07 |
| Pb only | 2.59±0.09 | 7.34±0.14 |
| Pb and Fe H2O | 2.65±0.17 | 4.53±0.17 |
| Pb and Ca H2O | 1.92±0.18 | 4.00±0.04 |
| Pb and Cu H2O | 2.73±0.25 | 9.28±0.09 |
| Pb and Mn H2O | 1.98±0.20 | 4.14±0.09 |
| Pb and Mg H2O | 2.12±0.16 | 4.03±0.03 |
| Pb and Zn H2O | 2.15±0.10 | 4.54±0.28 |
| Pb and Co metals H2O | 2.54±0.20 | 7.06±0.74 |
| River water | 2.26±0.10 | 6.26±0.10 |

*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 |
|-----------|--------|--------|
| dh2O only(Control-1) | 38.00±2.00 | 23.60±0.92 |
| Pti H2O (Control-2) | 39.20±0.86 | 45.00±1.70 |
| Pb only | 72.00±1.46 | 60.00±1.14 |
| Pb and Fe H2O | 74.00±0.71 | 69.00±1.00 |
| Pb and Ca H2O | 58.00±0.71 | 37.00±1.41 |
| Pb and Cu H2O | 91.40±1.02 | 67.80±1.50 |
| Pb and Mn H2O | 62.00±0.71 | 40.00±1.41 |
| Pb and Mg H2O | 64.60±1.63 | 42.00±1.14 |
| Pb and Zn H2O | 52.40±1.08 | 69.00±1.41 |
| Pb and Co metals H2O | 69.20±0.86 | 54.00±1.41 |
| River H2O | 64.00±1.21 | 44.00±1.40 |

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

| S/No | Treatment | ALP KA units total ACP KA units |
|------|-----------|----------------------------------|
|      |           | mean±SEM (n=5)×10⁻³               |
| i    | dH₂O only(control-1) | 155.11±11.09 11.03±0.94 |
| ii   | Pti H₂O(control-2)  | 202.76±14.73b 18.98±0.84b |
| iii  | Pb only     | 375.27±1.01bd 35.16±0.73bd |
| iv   | Pb and Fe H₂O | 401.59±10.66bde 32.38±0.98bde |
| v    | Pb and Ca H₂O | 229.43±0.59bdf 18.87±0.37bdf |
| vi   | Pb and Cu H₂O | 454.24±0.77bdfhi 41.19±0.55bdfhi |
| vii  | Pb and Mn H₂O | 279.31±13.01bdfhij 39.40±1.11bdfhij |
| viii | Pb and Mg H₂O | 247.15±14.32bdfhij 28.25±1.04bdfhij |
| ix   | Pb and Zn H₂O | 379.77±11.36bdfhjlp 37.86±1.25bdfhjlp |
| x    | Pb and Co metals H₂O | 315.27±18.15bdfhjlnp 30.35±1.07bdfhjlnp |
| xi   | River H₂O   | 293.10±10.91bdfhjlnprt 23.30±0.80bdfhjlnprt |

*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)×10⁶ serum | Bilirubin mg/dl (mean ± SEM)×10⁻⁴ n=5 |
|------|-----------|-------------------------------------------|----------------------------------------|
| i    | dH₂O only(control-1) | 39.39±0.39 5.98±0.11 28.60±1.93 | 5.62±0.38 |
| ii   | Pti H₂O (control-2) | 36.36±0.23a 6.33±0.20a 30.56±2.01b | 6.52±0.35b |
| iii  | Pb only     | 18.22±0.29bd 13.18±0.39bd 45.69±2.15bd | 10.03±0.32bd |
| iv   | Pb +Fe      | 12.47±0.16bdf 11.53±0.23bdf 54.72±2.98bdf | 12.85±0.30bdf |
| v    | Pb + Ca     | 25.44±0.37bdfhi 8.69±0.28bdfhi 32.45±1.39bdfhi | 7.37±0.40bdfhi |
| vi   | Pb + Cu     | 8.04±0.16bdfhj 6.66±0.34bdfhj 59.08±2.54bdfhj | 13.84±0.65bdfhj |
| vii  | Pb + Mn     | 20.93±0.41bdfhj 9.04±0.21bdfhj 40.55±2.50bdfhj | 7.84±0.31bdfhj |
| viii | Pb + Mg     | 29.99±0.36bdfhjlp 8.87±0.26bdfhjlp 34.91±2.38bdfhjlp | 8.56±0.40bdfhjlp |
| ix   | Pb + Zn     | 19.25±0.37bdfhjlnp 14.06±0.28bdfhjlnp 49.39±3.17bdfhjlnp | 11.99±0.55bdfhjlnp |
| x    | Pb + All metallic co-pollutants | 20.17±0.32bdfhjlnpr 12.19±0.27bdfhjlnpr 37.76±2.00bdfhjlnpr | 8.87±0.36bdfhjlnpr |
| xi   | River H₂O   | 19.39±0.61bdfhjlnpr 10.95±0.33bdfhjlnpr 35.92±2.11bdfhjlnpr | 7.87±0.33bdfhjlnpr |

*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) | Urine glucose (mg/dl) mean±SEM (n=5) |
|------|-----------|-------------------|--------------------------------------|--------------------------------------|
| I    | dH₂O only(Control-1) | 5ml/kg bd. wt | 95.76±0.36 | 41.25±5.36 |
| II   | PbH₂O (Control-2) | 5mlH₂O/kg bdwt | 101.12±1.52* | 55.13±1.08* |
| III  | Pb only    | 0.21mg/kg bd. wt | 151.04±0.73bd | 80.78±2.95bd |
| IV   | Pb and Fe H₂O | 0.21mg:1.60mg/kg bd. wt | 163.09±0.95bdf | 96.03±0.62bdf |
| V    | Pb and Ca H₂O | 0.2:1.11mg/kg bd. Wt | 111.39±1.33dah | 45.08±0.67dah |
| VI   | Pb and Cu H₂O | 0.2:0.012mg/kg bd. Wt | 178.05±0.65dahij | 92.16±0.70dahij |
| VII  | Pb and Mn H₂O | 0.2:0.22mg/kg bd. Wt | 112.31±1.48dahili | 43.41±0.73dahili |
| VIII | Pb and Mg H₂O | 0.2:9.79mg/kg bd. Wt | 118.99±0.64dahijln | 59.11±0.60dahijln |
| IX   | Pb and Co metals H₂O | 0.2:0.06mg/kg bdwt | 159.27±1.02dahijlnp | 87.54±0.60dahijlnp |
| X    | Pb and Co metals H₂O | 0.21mg:mixed conc. co metals | 139.46±0.86dahijlnp | 80.29±0.70dahijlnp |
| XI   | River H₂O | 5ml H₂O/kg bdwt | 121.45±1.13dahijlnopt | 83.45±0.65dahijlnopt |

*See Table 1 for interpretations of alphabetical nomenclature
**bdahijlnp= values significantly (P<0.05) different from corresponding evaluated groups
***acegikmoqs= values not significantly (P>0.05) different from corresponding statistical evaluated groups

Discussion of Findings

The major consternation of this study was to evaluate some serum biochemical indices and the magnitude of oxidative stress occasioned by liver 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; Ikatsuet 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 hepatobiliary 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 peroxidation 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 lipid peroxidation. This implies that exposure of rats to Pb-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 Ahamedet 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 Ennnekuet 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 Shamal and Satyanaranyan (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.

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