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*Full Length Research Paper*

# Sublethal effects of cadmium, manganese, lead, zinc and iron on the plasma electrolytes regulation of mice, *Mus Musculus*

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**The toxicological evaluations of cadmium, iron, manganese, lead and zinc were carried out against albino mice model, *Mus musculus.* On the basis of 96 hrLC50 value, cadmium (0.47 mM) was found to be the most toxic followed by zinc (2.40 mM), lead (2.42 mM), iron (4.25 mM) and manganese (5.70 mM) was least toxic. This study also evaluated the sublethal effects of cadmium, manganese, lead, zinc and iron in plasma samples utilising plasma electrolyte parameters as a biomarker using an albino mice model, *M. musculus.* Mice were subjected to sublethal concentrations of the selected heavy metals ($1/10^{th}$ of 96 hr$LC_{50}$). Blood plasma was collected after 7, 14, 21 and 28 days in long term experiment. Sodium ($Na^+$), potassium ($k^+$), chloride ($Cl^-$) ions bicarbonates ($HCO_3^-$), calcium ($Ca^{2+}$) and phosphates ($P0_4^{3-}$). Studies on the effect of heavy metals on plasma electrolytes revealed that Pb caused elevated level of sodium ($Na^+$), while Cd induced significant ($P < 0.05$) increase in potassium ($k^+$) and chloride ($Cl^-$) ions in treated mice groups. In addition, bicarbonates ($HCO_3^-$) and phosphates ($P0_4^{3-}$) levels increased significantly ($P < 0.05$) in treated mice exposed to Mn and Fe, respectively. The outcome of this study implied that heavy metals have toxic effects and plasma electrolyte is a useful tool for early detection and diagnosis of heavy metals pollution in the mammalian model.**

**Key words:** Electrolyte, blood plasma, heavy metals, *Mus musculus.*

## INTRODUCTION

The exploration and exploitation of natural resources using modern technology and the exponential growth of population have inadvertently resulted in the release of varied types and amounts of industrial wastes into the environment. These industrial wastes are complex admixtures of several classes of pollutant such as hydrocarbons and heavy metals (Oyewo and Don-Pedro, 2002). These have contributed immensely to the heavy metal load in the environment (Abdul, 2011; Chin et al., 2012). Heavy metals are metals with densities above 3.5 - 5 g/$cm^3$ and atomic number greater than 20 (Duffus, 2002). They are toxic at relatively low concentrations and persist in the environment long after the source of emission has been removed. They could therefore be classified as important sources of pollution (DeVagi and Arfiziah, 2009).

Heavy metals also bioaccumulate in one or several compartments across food webs as shown by several scientific observations (Chukwu, 1991; Otitoloju and Don-Pedro, 2002, 2004). Heavy metals are grouped into

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essential and non essential. The essential elements play important roles as prosthetic groups in enzymes and key metabolic activities in living organisms, for example, iron (Fe), copper (Cu), manganese (Mn), cobalt (Co), vanadium (V), molybdenum (Mo) and zinc (Zn). The non-essential metals, such as arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) are not needed in the physiological activities of living organisms hence they are usually toxic at relatively low concentrations (Falusi and Olanipekun, 2007; Raymond and Felix, 2011).

Human activities alter the natural geological and biological redistribution of heavy metals, via mining and other industrial processes which alter the chemical form of heavy metals released into the environment (Adriano, 2001). Presently, the amount of heavy metal exposure is hundred times higher (Howard, 2002) than in the past thus living forms have become a "warehouse" of heavy metals. This has led to a lot of concern about heavy metal pollution especially in industrialized and developing countries such as America, Asia Europe and African. Heavy metals have been found in air, water, land (soil/sediment) and all living organisms, including human samples (Öztürk et al., 2009). Thus living things are inadvertently exposed to different concentrations of heavy metals which accumulate in tissues, as such can be of public health concern. In addition, most of the information concerning the health effects of heavy metals largely stems from studies conducted on populations with relatively high exposure usually to individual metals in industry or in heavily polluted environments (Duruibe et al., 2007). Few studies have addressed the possible effects of chronic environmental exposure especially at short time intervals to these heavy metals. Heavy metals are discharged into the environment, either as constituents of industrial, domestic or agricultural wastes where they come in contact with air, water, soil and living forms.

Dermal absorption (skin), inhalation (lungs) and ingestion (mouth) are the route of entry into living forms which become detrimental to human health when they overwhelm the system. Conditions such as liver damage, cancer and other serious health conditions have been associated with metal exposure (Kan et al., 2011; Sham et al., 2011; Umme et al., 2011). Classical cases of heavy metal toxicities include *Minamata* caused by mercury, *Itai - Itai* caused by cadmium which has been known to contribute to any imaginable illness. For example, calcium when replaced by lead (Pb) in the bones can contribute to weakened bones and osteoporosis. Likewise, zinc (Zn) when displaced by cadmium (Cd) in the arteries cause inflammation and hardening of the arteries. Iron (Fe) that replaces zinc (Zn) and other minerals in the pancreas, adrenals and elsewhere can contribute to impaired blood sugar tolerance and diabetes. The available information on metal toxicity, as it relates to human beings, is derived mostly from health surveys among workers engaged in mining and processing especially in developing countries (Wang et al., 2001). This is a thing of concern especially in terms of biomonitoring where most of these effects become full blown before their detection in the biological system. However, heavy metals exert their toxic effect by generating reactive oxygen species (ROS) such as $O^{\cdot}{}_2^-$, $H_2O_2$, and $^{\cdot}OH$ causing oxidative stress. This could cause an imbalance of blood electrolyte, by administration of low initial levels of heavy metals thus illustrates its critical role in heavy metal toxicology and justifies its use in this study as a biomarker of heavy metal toxicity.

Ions of body fluids (electrolytes) have various functions such as contributing a majority of the osmotically active particles and providing buffer systems for the regulation of acid-base balance.

Electrolyte balance of ions (sodium, potassium, or calcium) in the blood is critical in regulating oxygen delivery to fluid imbalance within the cells. Electrolyte imbalance could cause cardiac arrhythmias, and complicate attempted resuscitation and postresuscitation care. Thus, an early warning signal of monitoring underlying electrolyte imbalances can prevent abnormalities such as cardiac arrest. Studies have incorporated electrolyte imbalance as a bioindicator of pollution and it is useful in identifying target organs of toxicity as well as the general health status of animals (Shaista et al., 2010; Chezhian et al., 2011; Mehmet et al., 2011). A lot of research has been done on the effects of heavy metal on electrolytes in fishes (Fernandes et al., 2007; Prem et al., 2008) and in humans especially in relation to disease and drugs activity (Oyewole and Malomo, 2009).

Despite the extensive study of heavy metals all over the world, there is paucity of data on some heavy metals toxicity such as Cd, Fe, Mn, Pb and Zn in relation to plasma electrolyte. Some of these selected heavy metals exhibit varying degrees of toxicity at different exposure periods and dose. There is therefore need to assess the impact of these metals that humans are inadvertedly exposed.

It was thus suggested that plasma ions levels may be employed for quantifying toxic effects of metals. Therefore, measurement of serum/plasma biochemical parameters in response to metal exposures can be especially useful to help identify target organs of toxicity as well as the general health status of animals. This study therefore aims at investigating and determining the effects of the selected heavy metals on plasma electrolyte using mice as a mammalian model.

## MATERIALS AND METHODS

A total of seventy two male albino mice (*M. musculus*) of similar sizes (19 - 24 g body weight and 10 - 15 weeks old) which served as test animals were purchased from the animal house in Nigerian Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria. Mice

were kept in ventilated plastic cages (20 x 12 x 9 cm) with wood shavings under conventional conditions of natural light-dark cycle in farm house located in NIMR. The room temperature was maintained at 30 ± 2°C (28 - 32°C) and the animals had free access to drinking fluid and a standard rodent laboratory chow purchased from Ladokun feeds Ltd, Ibadan. Acclimatization was done for 14 days during which they were handled with animal care in accordance with the Institute for Laboratory Animal Research (ILAR) guidelines prior to heavy metals treatment. Heavy metals investigated in this work were obtained as metallic salts from Fisons Laboratory Reagents, Analar grades in Nigeria of the following types. Zinc as $Zncl_2.3H_2O$, cadmium as $CdSO_4.8H_20$, lead as $PbSO_4$, manganese as $MnSO_4$ and ferric as $FeSO_4$ we used.

**Preparation of test media for acute toxicity test**

A predetermined amount of Analar grade salt of each heavy metal compound was weighed (using an Oertling, 30TD top loading balance) and made up to a given volume to obtain a stock solution of known strength. The resulting solution was serially diluted to obtain solutions of required concentrations. The volume of the treated media (toxicant and distilled water) that was administered to the mice varied based on the results of preliminary experiments, to determine the volume of water that can be consumed at once without choking the animal via cannular feeding. In range finding experiments, the mice were exposed to a wide range of concentrations of each test compound to obtain an effective range of activity, initially relying on trial and error techniques.

**Differential acute toxicity of heavy metals against mice**

Active mice were treated to salts type of the heavy metals by cannular feeding. For series of bioassays, three animals in duplicates were exposed per treatment including untreated control. In these substantive bioassays after range finding preliminary trials, test animals were exposed to graded series of concentrations of each heavy metal compound in milligrams per body weight as follows: Cadmium as $CdSO_4.8H_20$ against mice at 0.28, 0.43, 0.57, 0.71 and 0.85 mM; Lead as $PbSO_4$ against mice at 0.66, 1.98, 3.30, 5.28 and 6.60 mM; Zinc as $Zncl_2.3H_2O$ against mice at 5.29, 6.35, 8.47, 10.58 and 12.70 mM; Manganese as $MnSO_4$ against mice at 1.32, 3.97, 6.62, 10.60 and13.25 mM; Ferric as $FeSO_4$ against mice at 1.64, 3.29, 4.93, 6.58, 8.22 and 9.87 mM

**Assessment of quantal response**

Mice were taken to be dead if they showed no movement of the body and appendage. Mortality assessments were carried out at definite time interval specified under appropriate bioassays for example 24 h over a 96 h period for the acute toxicity test.

**Heavy metals administration for chronic toxicity test**

Mice were divided into six experimental groups (n = 12 per group) for the sublethal experiment and a control group (n = 3) was also included. They were treated to 1/10 of $LC_{50}$ values of salts of the heavy metals as follows Pb = 0.24 mM, Fe = 0.45 mM, Cd = 0.05 mM, Zn = 0.24 mM and Mn = 0.57 mM (derived from experiments carried out in acute toxicity test) while control group was administered distilled water only. The metallic salts of the corresponding heavy metals of 1000 mg each of the salt was weighed out and diluted in 20 ml of distilled water. Solution of 0.1 ml was administered through cannular method to the mice over the experimental period. The control mice were fed the same volume of distilled water. Prior to sacrificing, 3 mice from each group were separated and fasted for 24 h before dissection. This procedure was maintained every 7 days for analysis under investigation.

**Collection and preparation of plasma samples from mice for electrolyte**

Mice were sacrificed at the end of each experimental period and their blood was collected into EDTA bottle immediately. Blood was then centrifuged at 4,000 g for 10 min using bench top centrifuge (MSE Minor, England) to remove red blood cells and recover plasma. Plasma samples were separated and collected using dry Pasteur pipette, labelled and stored in the refrigerator at -20°C for analyses. All samples were analysed within 24 h of sample collection.

**Measurement of biochemical parameters**

Plasma sodium, potassium and bicarbonate, calcium ions were measured by flame photometer as described by Tietz et al. (1994). Chloride ion estimation was measured by precipation titration by the method of Mohr and Volhard as described by Harris (2003). Inorganic phosphate was determined using the direct method of Fiske and Subbarow (1925).

**Statistical analysis**

Lethal concentration ($LC_{50}$) was calculated by log/probit regression line method (Finney, 1971). Data was analyzed with oneway analysis of variance (ANOVA). Differences at $P < 0.05$ were considered significant. This was used to compare several treatment means in appropriately designed experiments. Further analysis was carried out only when there is a significant difference at the 5% ($P < 0.05$) level of significance (taken as minimum requirement) based on Duncan multiple range test at 0.05 levels of significance using SPSS 10.0 computer software package (SPSS Inc., Chicago, U.S.A).

## RESULTS

### Acute toxicity of heavy metals on mice (*Mus musculus*)

On the basis of 96 $hrLC_{50}$ values, cadmium (Cd) with a 96 $hrLC_{50}$ value of 0.47 mM was the most toxic metal tested against *M. musculus* (mice) followed by Zn, Pb, Fe and Mn (96 $hrLC_{50}$ = 5.70 mM) as least toxic in a descending order of toxicity (Table 1) and there was an overlap in 95% confidence limit of 96 $hrLC_{50}$ value. There were similar magnitudes of toxicity for all the tested metals with overlaps in 95% confidence limit of 96 $hrLC_{50}$ value. Zinc (Zn) was also found to be significantly more toxic than Pb and each of the other metallic compounds. Manganese (Mn) was least toxic against the tested animal with no overlaps in 95% confidence limit of 96 $hrLC_{50}$ value. Computed toxicity factors (96 $hrLC_{50}$ ratios) showed that Cd was about 12.0x, 10.0x, 5.3x and 5.0x more toxic than Mn, Fe, Pb and Zn, respectively when

**Table 1.** Relative acute toxicity of selected heavy metals.

| | Time (h) | $LC_{50}$ (95% CL) mM $kg^{-1}$ | $LC_{95}$ (95% CL) mM $kg^{-1}$ | Regression equation | Slope ± SE | DF | TF |
|---|---|---|---|---|---|---|---|
| Cadmium | 24 | 1.07 (0.54 - 1.19) | 7.59 (3.79 - 8.93) | Y= 0.05+ 1.94x | 1.94 ± 1.20 | 3 | 1.00 |
| | 48 | 0.61 (0.45 - 0.89) | 1.82 (1.10 - 3.74) | Y= -2.95+ 3.44x | 3.44 ± 1.21 | 3 | 1.00 |
| | 72 | 0.54 (0.37 - 1.12) | 1.60 (1.01 - 3.55) | Y= -2.89+ 3.49x | 3.49 ± 1.19 | 3 | 1.00 |
| | 96 | 0.47 (0.37 - 0.56) | 0.94 (0.74 - 1.68) | Y= -7.22+ 5.55x | 5.55 ± 1.39 | 3 | 1.00 |
| Iron | 24 | 8.62 (5.57 - 17.50) | 71.90 (21.75 - 148.91) | Y= -0.57+ 1.79x | 1.79 ± 0.73 | 4 | 8.06 |
| | 48 | 6.13 (4.20 - 10.67) | 32.34 (15.29 - 67.64) | Y= -1.76+ 2.28x | 2.28 ± 0.74 | 4 | 10.05 |
| | 72 | 5.07 (3.58 - 6.96) | 19.07 (11.54 - 87.35) | Y= -3.25+ 2.86x | 2.86 ± 0.78 | 4 | 9.39 |
| | 96 | 4.52 (2.66 - 6.73) | 25.46 (12.82 - 102.04) | Y= -1.21+ 2.19x | 2.19 ± 0.70 | 4 | 9.62 |
| Manganese | 24 | 9.92 (6.51 - 18.46) | 66.94 (29.20 - 146.01) | Y= -1.30+ 1.98x | 1.98 ± 0.56 | 4 | 9.27 |
| | 48 | 6.86 (4.19 - 13.03) | 48.50 (22.94 - 105.84) | Y= -0.84+ 1.94x | 1.94 ± 0.52 | 4 | 11.25 |
| | 72 | 6.06 (3.54 - 9.52) | 43.43 (20.97 - 98.19) | Y= -0.69+ 1.92x | 1.92 ± 0.52 | 4 | 11.22 |
| | 96 | 5.70 (3.25 - 8.88) | 41.00 (20.05 - 94.19) | Y= -0.64+ 1.92x | 1.92 ± 0.52 | 4 | 12.13 |
| Lead | 24 | 11.63 (5.38 - 15.08) | 34.78 (20.98 - 43.09) | Y= -7.27+ 3.46x | 3.46 ± 2.34 | 2 | 10.87 |
| | 48 | 9.77 (3.24 - 14.22) | 32.06 (16.62 - 43.79) | Y= -6.06+ 3.19x | 3.19 ± 2.25 | 2 | 16.02 |
| | 72 | 13.02 (3.60 - 28.91) | 37.43 (25.91- 52.11) | Y= -5.03 + 3.09X | 3.09 ± 2.25 | 2 | 24.11 |
| | 96 | 2.42 (17.93 -43.09) | 34.78 (20.98 - 43.09) | Y= -7.27+ 3.46x | 3.15± 2.06 | 2 | 5.15 |
| Zinc | 24 | 3.83 (2.69 - 5.77) | 14.41 (8.16 - 110.39) | Y= -2.90+ 2.86x | 2.86 ± 0.86 | 3 | 3.58 |
| | 48 | 3.32 (2.69 - 5.77) | 20.99 (6.85 - 110.39) | Y= -2.16+ 2.86x | 2.81 ± 0.86 | 3 | 5.44 |
| | 72 | 2.84 (2.69 - 5.77) | 34.14 (14.74 - 110.39) | Y= -2.09+ 2.86x | 2.26 ± 0.86 | 3 | 5.26 |
| | 96 | 2.40 (1.29- 3.32) | 9.41 (5.81 - 49.35) | Y= -2.11+ 2.77x | 2.77 ± 0.84 | 3 | 5.11 |

C.L. = 95% confidence limit; S.E = standard error; D.F = degree of freedom.

$$\text{T.F (toxicity factor)} = \frac{\text{24/48/72/96 hr}LC_{50}\text{ value of other metals}}{\text{24/48/72/96 hr}LC_{50}\text{ value of the most toxic (Cd) metals}}$$

tested against mice (Table 1).

Toxicity indices including $LC_{50}$ values derived from 24, 48, or 72 h mortality response data showed similar trends of relative potency as was the case with 96 h data described below (Table 1).

The results (Table 2) showed that elevated levels of plasma electrolytes were induced significantly ($P < 0.05$) by the test heavy metals in varied levels following the heavy metal administration from 7, 14, 21 and 28 day in comparism with the control.

Cadmium (Cd) induced a consistent increase in $Na^+$ which was significantly ($P < 0.05$) different from the control group on the $28^{th}$ day in the treated group. The lowest and highest level of the $Na^+$ was recorded on the $7^{th}$ and $14^{th}$ (Table 2) in the treated group. Interestingly, $K^+$ significantly ($P < 0.05$) increased from the level in control group to that on the $14^{th}$ day in the treated group. There were significant ($P < 0.05$) differences in the level of $Cl^-$, $HCO_3^-$ and $PO_4^{3}$ which consistently increased from day 0 to the $28^{th}$ day.

Manganese (Mn) induced a consistent increase in the sodium ($Na^+$) which was significantly ($P < 0.05$) different from the levels in the control to that on the $21^{st}$ day but decreased on the $28^{th}$ day while $K^+$ significantly ($P <0.05$) increased from day 0 to $21^{st}$ day but decreased on the $28^{th}$ day. The $HCO_3^-$ increased consistently on the $28^{th}$ day while $Cl^-$ increased from day 0 to the $21^{st}$ day but also decreased on the $28^{th}$ day. $Ca^{2+}$ significantly ($P < 0.05$) increased from day 0 to the $14^{th}$ day but decreased on the $28^{th}$ day while $PO_4^{3-}$ consistently increased through out the exposure period.

Zinc (Zn) induced a consistent increase in plasma $Na^+$, $K^+$which was significantly different ($P < 0.05$) from 0 - $21^{st}$ day but decreased on the $28^{th}$ day but $HCO_3^-$ and $Cl^-$ increased consistently from 0 - $14^{th}$ day but decreased on the $28^{th}$ day. $Ca^{2+}$ increased from 0 - $21^{st}$ day but decreased on the $28^{th}$ day while $PO_4^{3-}$ consistently and significantly ($P < 0.05$) increased from 0 - 28th day as in the case with Mn. Lead (Pb) induced a consistent increase in plasma sodium $K^+$ and ($Na^+$) which were signi-

**Table 2.** Plasma electrolyte parameters in mice treated with cadmium, manganese, zinc, lead and iron for 7, 14, 21 and 28 days.

| Parameter (mmol / L) | Control | Cadmium (Cd) | Manganese (Mn) | Zinc (Zn) | Lead (Pb) | Iron (Fe) |
|---|---|---|---|---|---|---|
| **7 days** | | | | | | |
| $Na^+$ | 23.67±0.27$^a$ | 41.33±0.33$^c$ | 40.44±0.51$^c$ | 36.67±0.33$^b$ | 41.00±0.88$^c$ | 36.59±0.19$^b$ |
| $K^+$ | 1.17±0.00$^a$ | 1.79±0.02$^c$ | 1.49±0.02$^b$ | 1.99±0.05$^d$ | 1.79±0.04$^c$ | 1.79±0.02$^c$ |
| $HCO_3^-$ | 4.09±0.17$^b$ | 6.68±0.02$^e$ | 4.50±0.17$^c$ | 5.44±0.19$^d$ | 7.67±0.00$^f$ | 1.79±0.17$^a$ |
| $Cl^-$ | 26.79±0.15$^a$ | 33.52±0.17$^d$ | 37.28±0.59$^e$ | 29.56±0.19$^b$ | 32.78±0.51$^c$ | 39.85±0.17$^f$ |
| $Ca^{2+}$ | 0.53±0.00$^a$ | 0.60±0.00$^b$ | 0.71±0.00$^d$ | 0.73±0.01$^e$ | 0.69±0.01$^c$ | 0.78±0.06$^f$ |
| $PO_4^{3-}$ | 0.42±0.01$^a$ | 0.55±0.01$^c$ | 0.45±0.00$^b$ | 0.46±0.00$^b$ | 0.63±0.03$^d$ | 0.67±0.00$^e$ |
| **14 days** | | | | | | |
| $Na^+$ | 23.67±0.27$^a$ | 74.67±0.29$^e$ | 74.00±0.50$^e$ | 72.67±0.76$^d$ | 71.00±0.87$^c$ | 69.33±0.29$^b$ |
| $K^+$ | 1.17±0.00$^a$ | 3.05±0.05$^b$ | 3.24±0.06$^c$ | 3.03±0.03$^b$ | 3.05±0.05$^b$ | 3.07±0.10$^b$ |
| $HCO_3^-$ | 4.09±0.17$^a$ | 8.48±0.08$^b$ | 8.58±0.52$^b$ | 13.65±0.56$^d$ | 8.75±0.22$^b$ | 10.33±0.58$^c$ |
| $Cl^-$ | 26.79±0.15$^a$ | 52.50±2.50$^b$ | 54.67±4.07$^{bc}$ | 60.00±6.50$^{cd}$ | 62.17±1.04$^d$ | 51.17±1.04$^b$ |
| $Ca^{2+}$ | 0.53±0.00$^a$ | 1.12±0.01$^{bc}$ | 1.18±0.01$^{cd}$ | 1.18±0.00$^{cd}$ | 1.23±0.10$^d$ | 1.07±0.08$^b$ |
| $PO_4^{3-}$ | 0.42±0.01$^a$ | 0.80±0.06$^b$ | 0.82±0.05$^b$ | 0.81±0.06$^b$ | 1.25±0.00$^c$ | 1.23±0.00$^c$ |
| **21 days** | | | | | | |
| $Na^+$ | 23.67±0.27$^a$ | 65.50±0.50$^b$ | 75.67±0.29$^{de}$ | 72.67±0.29$^d$ | 71.50±0.50$^c$ | 75.17±0.58$^e$ |
| $K^+$ | 1.17±0.00$^a$ | 3.30±0.05$^b$ | 3.98±0.06$^c$ | 4.27±0.03$^d$ | 4.75±0.05$^f$ | 4.40±0.05$^e$ |
| $HCO_3^-$ | 4.09±0.17$^a$ | 8.50±0.50$^b$ | 9.50±0.87$^c$ | 11.33±0.29$^d$ | 8.43±0.12$^b$ | 8.80±0.26$^{bc}$ |
| $Cl^-$ | 26.79±0.15$^a$ | 61.67±1.44$^d$ | 57.28±0.58$^c$ | 59.50±0.50$^c$ | 56.50±1.50$^c$ | 53.83±0.29$^b$ |
| $Ca^{2+}$ | 0.53±0.00$^a$ | 1.13±0.02$^c$ | 1.12±0.00$^{bc}$ | 1.18±0.02$^{cd}$ | 1.23±0.05$^d$ | 1.07±01$^b$ |
| $PO_4^{3-}$ | 0.42±0.01$^a$ | 0.80±0.00$^b$ | 0.72±0.01$^b$ | 0.70±0.00$^b$ | 1.25±0.01$^c$ | 1.23±0.03$^c$ |
| **28 days** | | | | | | |
| $Na^+$ | 23.67±0.27$^a$ | 68.83±0.29$^{cd}$ | 69.33±5.11$^{cd}$ | 68.00±0.50$^c$ | 72.33±0.58$^d$ | 35.50±0.50$^b$ |
| $K^+$ | 1.17±0.00$^a$ | 3.42±0.03$^e$ | 2.58±0.08$^c$ | 2.70±0.01$^d$ | 2.62±0.08$^{cd}$ | 2.18±0.03$^b$ |
| $HCO_3^-$ | 4.09±0.17$^a$ | 8.83±0.29$^b$ | 10.83±0.29$^d$ | 9.33±0.29$^c$ | 9.45±0.05$^c$ | 9.48±0.03$^c$ |
| $Cl^-$ | 26.79±0.15$^a$ | 62.33±0.76$^d$ | 53.50±0.50$^b$ | 52.67±0.29$^b$ | 52.83±0.29$^b$ | 55.83±0.76$^c$ |
| $Ca^{2+}$ | 0.53±0.00$^a$ | 1.15±0.00$^d$ | 1.11±0.01$^c$ | 0.97±0.00$^b$ | 0.99±0.01$^b$ | 1.26±0.01$^e$ |
| $PO_4^{3-}$ | 0.42±0.01$^a$ | 0.86±0.01$^b$ | 0.86±0.04$^b$ | 0.86±0.00$^b$ | 0.85±0.01$^b$ | 0.88±0.03$^b$ |

Values are mean ± SD, n = 3. Values with different alphabetical superscripts along a row are significantly different at P < 0.05.

significantly (P < 0.05) different from that in the control to the 21st and 28th day while $HCO_3^-$ increased consistently from day 0 - 28th$^{th}$. $Cl^-$ increased from 0 - 14$^{th}$ day but decreased on the 28$^{th}$ day while $Ca^{2+}$ and $PO_4^3$ significantly (P < 0.05) increased from day 0 - 21$^{st}$ day but decreased on the 28$^{th}$ day.

Iron (Fe) induced a consistent increase in plasma sodium ($Na^+$) and $K^+$ which were significantly (P < 0.05) different from the control to the 21$^{st}$ day but decreased on the 28$^{th}$ day. $HCO_3^-$ increased consistently from day 0 - 14$^{th}$ day but decreased (P < 0.05) significantly on the 28$^{th}$ day while $Cl^-$ and $Ca^{2+}$increased significantly (P < 0.05) from 0 - 28$^{th}$ day while $PO_4^{3-}$ consistently increased from 0 - 21$^{st}$ day but decreased on the 28$^{th}$ day. At the end of the 28$^{th}$ day, the descending orders of plasma electrolyte with respect to the selected heavy metals were as follows: Sodium ($Na^+$): Pb > Mn > Cd > Zn > Fe; Potasium ($k^+$): Cd > Zn > Pb > Mn > Fe; Bicarbonate ($HCO_3^-$): Mn > Fe > Pb > Zn > Cd; Chloride ion (Cl): Cd > Fe > Mn > Pb > Zn; Calcium ($Ca^{2+}$): Fe > Cd > Mn > Pb > Zn; Phosphate ($P0_4^{3-}$): Fe > Cd, Mn, Zn > Pb

## DISCUSSION

In the single action toxicity studies which showed that Cd was the most toxic of the heavy metals tested followed by Zn, Pb, Fe and Mn, in descending order of toxicity against albino mice, *M. musculus* could be explained based on the fact that the relatively high toxicity of Cd could have been as a result of its high electronegativity and the resultant affinity to sulphydryl (SH) groups which lead to denaturing of many enzyme systems with the SH group. The lowest toxicity which was established as man-

ganese (Mn) could be due to the free inorganic $Mn^{2+}$ which is usually dominant in manganese solutions but which is not lipid soluble hence their transference across membranes would be inhibited. The implications of the low toxicity of Mn if coupled to low excretability in exposed mice is that the mammal could accumulate high concentrations without ill effects or lethal action and thus become a potential danger to the systems in the event of an amplification along the food chain.

The established induction of a significant increase of sodium ($Na^+$) ions by Pb followed by Mn, Cd, Zn and Fe in descending order is an indication that Pb has an activation action on the monovalent cation transport in the plasma of the mice. Sodium is the major positive ion (cation) in fluid outside the cells. The present finding was supported by Sheikh et al. (2011). They observed increase in $Na^+$ and $Cl^-$ in Wistar rats exposed to 14 and 28 days treatment with mercuric chloride. The elevation in plasma $Na^+$ might not be beneficial to exposed living systems for example humans because it has been demonstrated that it induces hypernatrema. This could be used as a biomarker of Pb exposure. The increase in plasma $K^+$ level (hyperkalemia) by Cd observed in the exposed group might occur due to erythrocyte destruction caused by the Cd exposures which probably lead to the release of $K^+$. Hyperkalemia has been reported to arise in condition characterized by excess destruction of cells with redistribution of $K^+$ from the intracellular to the extracellular compartment as in massive haemolysis (Guyton and Hall, 2000). Furthermore, the significant increase in plasma $K^+$ level may also be attributed to cation exchange of $H^+$ and $K^+$ between intracellular and extracellular spaces or erythrocyte swelling. This probably led to hemolysis and contamination of plasma with extracellular $K^+$ or a reduction in the extracellular spaces and/or an increase in the efflux of plasma $K^+$ from intracellular compartment. Potassium is the dominant intracellular cation and plasma ionic dilution would favour efflux into extracellular fluid. On the other hand, it is also possible that the alterations observed on the plasma ions may simply result from non-specific stress of the mice.

Generally, iron induction of the higest level of calcium ions ($Ca^{2+}$) and phosphates ions ($PO_4^{3-}$) initiated the entrance into the plasma. $Ca^{2+}$ influx activates a number of enzymes, including phospholipases, endonucleases and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane and DNA. Thus, an increase in $Ca^{2+}$ induced by Fe could be damaging to the cells and therefore should be monitored.

## Conclusion

The administration of selected heavy metals over the experimental period has established the fact in this study that Cd, Fe, Mn, Pb and Zn have the ability to induce and synergise significant imbalance in plasma electrolyte in mice in a short period of time and therefore can be used as a biomarker of heavy metal pollution as well as an early warning signal of cell deterioration.

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