

# Effects of food-chain mediated metal exposures on phosphatases' profile in rats

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## Abstract

This study seeks at investigating the separate and combined toxicities of Arsenic (As) and Cadmium (Cd) administered along food-chain. The natural food-chain was mimicked by exposing rats to diet containing milled Cd and As-exposed (at a dose of 0.4mg/100ml each) catfish as source of protein. Control groups were fed with normal formulated rat feed with non-metal contaminated fish as source of protein. The effects of Cd and As exposures were sought by determining indices of plasma and lysosomal membrane integrity in plasma and organs of the rats after three months exposure time. The results depict that Cd and As exposures in the rats led to bioaccumulation of the metals in their plasma and organs. The results gotten revealed significance ( $p < 0.05$ ) in gain of body weight in both Cd and As exposed rats when control is compared to them. Phosphatases' activities (ALP and ACP) in the plasma and vital organs were also significant in the metal inoculated rats when the control is compared to them. These results explained that arsenic and cadmium forms free radicals which cause stress due to oxidation, kidney impairment and immunologic disorders.

**Keywords:** Arsenic; Cadmium; Exposures; Food-Chain; Rats.

## 1. Introduction

Food-chain contamination is part of the common routes for entry of metals into the animal system (Oscarson *et al.* 1992) and therefore, monitoring the bioavailability pools of metals in contaminated feed is of uttermost concern. Cadmium and Arsenic can be transported, dispersed to and accumulated in plants, to animals and passed across the food chain to humans, Hongyu *et al.* (2005). Since mineral concentrations may consistently biomagnify from one trophic level to another, animals higher along the chain of food may accumulate more toxins than the constituents intended to provide, Montero *et al.* (1996). It is known that exposure in diets is the major route for metal bioaccumulation of many marine and terrestrial animals (Talmage & Walton 1991, Shore 1995, Wang & Fisher 1999, Komarnicki 2000). Metalloids and metals that accumulate in biological tissue may be converted to other chemical forms and passed on via numerous pathways. Humans, as the final consumers in the chain of food, are thus the likely recipients of enormous amounts of minerals from infected food and may accumulate same in their organs (Oskarson *et al.* 1992). These metals affect every living organism and have possible toxic impact on all levels of food-chain (Sami & Druzykli 2003). The effects of the toxicity are acute, when large amounts are ingested or the element is particularly toxic or chronic, with toxicity only being noticed after a long time (Toens *et al.* 1998, Bahemuka & Mobufu 1999, Ikeda *et al.* 2000). The latter may be the result of the bioaccumulation process across the food chain (Lacatus *et al.* 1996, Turkdogan *et al.* 2003).

Cadmium (Cd), a metal common in native environments, is also extremely toxic (Wang & Fisher 1999). Cd pollution has been known to be a potential health problem to wild animals as found by Toens *et al.* in 1998. Its uses in technology have escalated to increasing amounts in the ecosystem and also in body of humans

(Mah & Jalilehvand 2010). From a worldly view, human activities have led to increase in pollution. Present studies indicate that Cd is highly toxic towards multiple organs and a potent immunotoxicant. Researches on humans and rodents have depicted influence on immune system function (Pathak & Khandelwal 2006). Several literatures have explained that Cd exposure has the tendency to result to stress due to oxidation, reduce lymphocytes function and may ensue apoptosis and necrosis on humans and also rodents (Pathak & Khandelwal 2006, Pathak & Khandelwal 2007).

Arsenic exists naturally in 3 allotropic states of alpha, beta and gamma. The popular numbers of oxidation are +5, +3 and -3, which enable the element to give compounds of organic and non-organic nature in the ecosystem and in humans (Orloff *et al.* 2009). When carbon and hydrogen is bonded to arsenic, it is called organic, whereas it is inorganic when chlorine, sulphur and oxygen are combined to it.

Examples of inorganic arsenic are arsenite (As (III)) and arsenate (As (V)) that a methyl group can attach to, to form monomethylarsonic acid (MMA (V)) or removed as seen with arsenic acid having 2 methyl groups. Inorganic arsenic metabolism occurs through two electron decrease of arsenic having valency of 5 to one having valency of 3, which require glutathione, then formation of arsenic of organic nature with valency of 5 through oxidative methyl group addition (Hughes 2002). Organic arsenic is found to be less toxic than inorganic (Shi *et al.* 2004, Valko *et al.* 2005). Arsenic is harmful to majority of organs, of which the kidney may be the primary target (Cohen *et al.* 2006). The extent of as poisoning depends on many variants like dose, individual susceptibility and age. While long-term exposures to arsenic will affect the vesicle system and induces hypertension with cardiovascular diseases, the short-term arsenic exposures can result to cardiomyopathy and hypotension. The most prevalent effect of long-term as toxicity on the neuron is peripheral neuropathy and mani-

festation of toxic hepatitis in the gastro-intestine followed by elevation of enzymes of the hepatocytes.

The consuming of exposed arsenic, cadmium diets along several metals can seriously reduce the body reserves of iron, ascorbic acid and other vital nutrients resulting to distortion of immune defense, retardation of growth of uterus, abnormal epidemics linked to malnutrition (Iyengar & Nair 2000).

## 2. Materials and methods

### 2.1. Animals

Male Wistar (no. 16) albino rats, mean weight  $129\pm 4$ g, were utilized for this study. The rats were obtained at the Delta State University's Animal House in Pharmacy Faculty, Abraka. Prior to commencement of the experiment, the rats were kept for 10-day period in cages to help them get use to the new climate.

### 2.2. Preparation of the diet

In order to simulate exposure to Cd and As through the food chain, four diets (1 control and 3 tests) that differed in terms of the nature of the protein were formulated. The test diets contained milled Cd, as and Cd+As exposed fish as a source of protein, while the control diet contained milled non-Cd and As exposed fish. Other components of the diets were corn starch (Livestock Feed depot, Warri), multivitamin/minerals mix (Vetindia Pharmaceuticals Limited, India), vegetable oil (obtained locally in Abraka, Nigeria), cellulose (analytical grade) and granulated refined sugar (obtained from Abraka market).

### 2.3. Experimental design

The rats were divided into four experimental groups of four rats each and housed singly in metabolic cages. Rats in the cages were maintained for 3 months on the control, Cd, As and Cd+As test diet respectively. They were allowed free access to water any time they are tasty.

### 2.4. Collection and treatment of samples

At the end of the specified period of exposures, the animals were fasted for three hours and weighed before sacrificed under chloroform anaesthesia. While under anaesthesia, each rat was slaughtered by heart puncture, using a hypodermic syringe and needle. The blood collected from the heart was transferred to heparinized tubes which were carefully swirled. Plasma was later obtained by centrifugation at 4000rpm for 10 minutes. The heart, liver and kidney were quickly excised, placed on ice and subsequently weighed. Portions of the liver, heart and kidney were homogenized to give 10% homogenates and centrifuged at 4000rpm for 10 minutes to obtain clear supernatants for biochemical analysis.

### 2.5. Determination of various biochemical parameters

The plasma and the supernatants obtained from the heart, kidney and liver were used for the determination of the activities of ALP (E.C. 3.1.3.1) and ACP (E.C. 3.1.3.2). ALP activity was assayed by the method of Roy (Roy 1970) based on the colorimetric endpoint method where the alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein monophosphate to produce a blue chromogen, which is measured photometrically using Teco Diagnostic test kits. The activity of the enzyme is expressed in international unit per litre. ACP activity was determined by the method of Hillman (Hillman 1971) as adapted in Teco Diagnostic test kits' kinetic method in which a coloured complex is produced when the  $\alpha$ -Naphthol released from the substrate  $\alpha$ -Naphthylphosphate by acid phosphatase is coupled with Fast Red TR. The activity of the enzyme is expressed in units per litre. One International Unit is defined as the amount of enzyme that cata-

lyzes the transformation of one micromole of substrate per minute under defined conditions.

## 2.6. Statistical analysis

The results were expressed as mean  $\pm$  SEM. Statistical analysis was done by one-way analysis of variance (ANOVA) using a computer software package (SPSS version 16.0, SPSS Inc. Cary, NC, USA). Least significant difference (LSD) test was used to check mean difference between the test and control groups. Statistical significance was considered at  $P < 0.05$ .

## 3. Results

The body weight gain and organ/body weight ratios in rats are presented in Table 1.

The body weight gain of rats administered Cd and As contaminated diet was significantly decreased ( $p < 0.05$ ) relative to control. The results indicate that there was a body weight loss of rats exposed to Cd and As in diet. Conversely, no significant difference ( $p > 0.05$ ) was observed in the body weight gain of rats fed a combination of Cd and As in diet as compared to control. The organ/body weight ratios for the liver and heart were not significantly different in all the experimental groups. Similarly, no significant difference was observed in the kidney/body weight of rats fed diet containing Cd and combination of Cd/As relative to control. On the other hand, this parameter was significantly increased in rats fed As via the food chain. Thus the study showed that the organ/body weight ratios of the rats were influenced by Cd and As exposures.

**Table 1:** Effects of Food-Chain Mediated Metal Exposures on Body Weight Gain and Organ/Body Weight Ratios in Rats

Parameter	Control	Cd	As	Cd+As
Body weight gain (g)	77.50 $\pm$ 7.60 <sup>a</sup>	27.10 $\pm$ 7.91 <sup>b</sup>	39.28 $\pm$ 11.66 <sup>b</sup>	80.08 $\pm$ 5.40 <sup>a</sup>
Liver/body weight ratio	0.03 $\pm$ 0.00 <sup>a</sup>	0.03 $\pm$ 0.00 <sup>a</sup>	0.04 $\pm$ 0.00 <sup>a</sup>	0.03 $\pm$ 0.00 <sup>a</sup>
Heart/body weight ratio	0.00 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.01 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
Kidney/body weight ratio	0.01 $\pm$ 0.00 <sup>a</sup>	0.01 $\pm$ 0.00 <sup>a</sup>	0.01 $\pm$ 0.00 <sup>b</sup>	0.01 $\pm$ 0.00 <sup>a</sup>

Values are given as mean  $\pm$  SEM, n=4. Values not sharing a common superscript letter in the same column differ significantly at ( $p < 0.05$ ).

The effects of Cd, As and a combination of both metals on plasma and organ ALP and ACP activity in rats is presented in Table 2. The plasma, liver, heart and kidney ALP and ACP activity in rats given Cd, As and a combination of Cd and As contaminated diet was significantly increased ( $p < 0.05$ ) relative to control. Similarly, multiple comparisons of organ ALP activity in Cd, As and Cd+As contaminated rats showed significant increase relative to control. Plasma ALP and tissue ACP activity in Cd, As and a combination of Cd and As contaminated rats showed no significant difference ( $p > 0.05$ ) when compared to one another, while plasma ACP activity in the rats administered a combination of Cd and As significantly increased when compared to those given Cd and As separately. However, the hepatic ALP in rats fed As contaminated diet was significantly decreased as compared to those fed either Cd or combination of Cd and As. Similarly, the renal ALP was significantly decreased in rats fed a combination of Cd and As compared to those fed either Cd or As diet. This study reveals that Cd and As exposures via the food-chain for three months altered phosphatases' activities in rats.

**Table 2:** Effects of Food-Chain Mediated Metal Exposures on Plasma And Organ ALP and ACP Activities in Rats.

Parameter	Control	Cd	As	Cd + As
Plasma				
ALP	30.87±0.57 <sup>a</sup>	43.16±3.60 <sup>b</sup> (39.81%)	42.64±3.11 <sup>b</sup> (38.13%)	42.64±1.52 <sup>b</sup> (38.13%)
ACP	7.46±1.49 <sup>a</sup>	13.43±2.86 <sup>b</sup> (80.03%)	10.45±2.86 <sup>b</sup> (40.08%)	20.90±3.86 <sup>c</sup> (180.16%)
Liver				
ALP	19.81±5.16 <sup>a</sup>	53.06±2.24 <sup>b</sup> (167.84%)	35.87±1.73 <sup>c</sup> (81.07%)	73.25±4.96 <sup>d</sup> (269.76%)
ACP	34.12±0.00 <sup>a</sup>	51.18±9.85 <sup>b</sup> (50.00%)	51.18±7.06 <sup>b</sup> (50.00%)	51.18±9.85 <sup>b</sup> (50.00%)
Heart				
ALP	22.43±4.65 <sup>a</sup>	47.83±5.32 <sup>b</sup> (113.24%)	60.54±0.75 <sup>c</sup> (169.91%)	65.02±1.43 <sup>c</sup> (189.88%)
ACP	34.12±0.00 <sup>a</sup>	34.12±0.00 <sup>a</sup> (00.00%)	42.65±8.53 <sup>b</sup> (25.00%)	42.65±8.53 <sup>b</sup> (25.00%)
Kidney				
ALP	14.95±1.22 <sup>a</sup>	50.82±4.05 <sup>b</sup> (234.93%)	66.52±6.73 <sup>c</sup> (344.95%)	35.12±0.75 <sup>d</sup> (134.92%)
ACP	34.12±0.00 <sup>a</sup>	51.18±9.85 <sup>b</sup> (50.00%)	51.18±9.85 <sup>b</sup> (50.00%)	51.18±9.85 <sup>b</sup> (50.00%)

Abbreviations as in Table 1.

The activities of the phosphatases are in IU/L.

Figures in brackets represent percentage inhibition relative to control.

## 4. Discussion

Heavy metals are not biodegradable and persist in the environment for long periods causing serious ecological problems. In addition, some toxic metals may mimic essential metals and thereby gain access to important molecular targets. These toxic metals enter into organisms via food, drinking water and air and are bio-persistent pollutants that accumulate at the top of the food-chain (Scheifer *et al.* 2006). Thus the food-chain is one of the natural routes of entry of heavy metals into humans. However, in most animal studies on heavy metal toxicity, exposures to metals is not often through the food-chain, but by direct addition to the food offered to the experimental animals.

The objective of the present study was to determine the effects of food-chain mediated metal exposures on phosphatases' profile in rats. The findings of the study revealed a decrease body weight gain of rats administered Cd and As via the food-chain (Table 1). An increased kidney/body weight ratio was also observed in rats fed As contaminated diet. The decreased body weight gain and increased kidney/body weight ratio are indications of toxicity of these metals. Changes in body weight gain and organ/body weight ratio have been used as indices of toxicity of chemicals (Timbrell 1991). The changes observed in the body weight gain of the rats exposed to these metals correlates with the findings of Prabu and Nabi (Nabi *et al.* 2005, Prabu *et al.* 2013).

Quantitative assessment of enzyme is a reliable indicator of stress imposed on the organism by environmental pollutants such as heavy metals (Cheng 1988). Many physiological processes including activity of many lysosomal hydrolytic enzymes are inhibited by heavy metals even though these metals may also activate certain enzymes. Two important enzymes that can be influenced by heavy metals are ALP and ACP both differing in their subcellular distribution. These enzymes were also monitored in the plasma and organs of rats exposed to Cd, As and combination of both metals in diet. The findings of the study revealed that plasma and organ levels of ALP and ACP were influenced by separate treatment with these metals and treatment with both metals (Table 2). The increased activity of ALP in the liver and other organs of the exposed rats and the corresponding increase in the plasma may be due to *de novo* synthesis of the enzyme molecule. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum (Wright & Plummer 1974). It is often employed to assess the integrity of the liver plasma membrane (Akanji *et al.* 1993), since it is localized predominantly in the microvilli in the bile canaliculi. Since ALP hydrolyzes phosphate monoesters, the enzymes hyper-production observed in the metal exposed rats could constitute a

threat to the life of the cells that are dependent on a variety of phosphate esters for their vital processes (Butterworth & Moss 1966) as it may lead to indiscriminate hydrolysis of phosphate ester metabolites of the liver. Consequently, this may adversely affect the facilitation of the transfer of metabolites across the cell membrane. It follows therefore that such hyper-production of ALP may also have severe consequences on the architecture of the cells of the affected organs.

Lysosomal response was considered as the most reliable effect observed during stress (Grundy *et al.* 1996) and ACP is a lysosomal marker enzyme that exists in the tissue and serum (Braun *et al.* 1989). Metals may disrupt lysosomal membrane integrity and cause its destabilization followed by release of stored lysosomal hydrolases into the serum thereby increasing the activity of the enzyme in the serum. The above explanation justifies the hyperactivity of ACP in the plasma and organs of rats after separate and combined treatment with Cd and As in food (Table 2). The higher activity of ACP in the plasma and organs of metal exposed rats can also be due to the hyper-synthesis of ACP and its subsequent release into the blood occasioned by metal insult. This may be a necessary defensive mechanism in response to metal exposures. Previous studies show that ACP activity in serum can reflect the immune state of the two species of Scallop, *A. Irradians* and *C. Farreri* (Mu *et al.* 1999, Liu *et al.* 2004, Zhang *et al.* 2005). Interactions between the heavy metals tested can lead to synergism or to antagonism or rather to an addition of the effects. Thus, the decrease in the combined effect of Cd and As on kidney ALP activity (Table 2) as compared to the effect on the same parameter by Cd and As alone suggest antagonism between these metals.

## 5. Conclusion

In conclusion, this study indicates that Cd and As are toxic metals that affects plasma phosphatases and causes degenerative changes in the organs of rats. The inhibition of these enzymes may disturb immune function, cause myocardial damage and generates reactive oxygen species (ROS).

## Conflicts of interest

The authors declare that there are none.

## Acknowledgement

Special thanks to the Staff of the Department of Biochemistry, Delta State University, Abraka for their support.

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