



Genotoxic and Biochemica Effects of Consumption of Heavy Metal Contaminated Fish and Its Reversal Using Zinc Supplements in Rats

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Abstract: Introduction: Heavy metals such as lead, cadmium, chromium etc are environmental contaminants. They are also known today as human and animal carcinogens. Objectives: The aim of the study was to evaluate the genotoxic and biochemical effect of feeding rat with fish obtained from heavy metal contaminated water bodies in Lagos, Nigeria. Methods: DNA damage was investigated using DNA lading assay, while heavy metals and antioxidants were analyzed from fishes obtained from contaminated water bodies. Results: There was fragmentation of blood chromosomal DNA in rats fed with heavy metal contaminated fish, while in the control and zinc supplemented group, there was no fragmentation. Heavy metals were detected in the tissues and serum of exposed rat, while the activity of the serum catalase and reduced glutathione significantly decreased. Conclusion: The consumption of heavy metal contaminated fish resulted in DNA fragmentation and its deposition in the rat tissue. Zinc supplementation prevented the degradation of DNA and potentiated antioxidant activities.

Keywords: DNA; Catalase; Glutathione; Heavy metals; Genotoxicity.

1. Introduction

Heavy metals are amongst the most widely spread environmental contaminants in the world today. They are present in industrial wastes & they find their way into major water bodies. These metals are assimilated by aquatic creatures in the water bodies and they eventually bio accumulate upon long-term consumption in human beings [1]. Heavy metals are toxic elements, having the most adverse health effects on the public based on toxicity and exposure levels [2-5]. Therefore, they are important risk factors for human health [6]. They are also found to be carcinogenic and eventually lead to cancer and cardiovascular diseases [7, 8]. Some studies have reported genotoxic effects in people exposed to heavy metals [9]. A possible explanation for heavy metal induced carcinogenesis may involve the damage of DNA. Heavy metals have been shown to cause a number of DNA lesions, such as mutation and DNA steers breaks [10, 11]. Formations of free radicals seem to be the major cause of heavy metal genotoxicity. Studies have shown that heavy metal induced DNA damage in the result of free radical generation. Hence, antioxidants and free radical scavengers are useful in protecting against heavy metal toxicity [12].

The mutagenic potential of lead in human has been described in lead-exposed battery worker who showed increase in DNA breakage [13]. Calcium and chromium have also been shown to induce DNA damage [14]. Determination of the heavy metal composition in water bodies together with their genotoxic potentials are crucial for environmental protection and human health.

Lagos State is a major industrial area and effluents from many industries area being discharged into its water bodies without adequate treatment. Previous studies have shown that Carter Bridge and Makoko water bodies (part of the Lagos Lagoon) are heavily contaminated with heavy metals [15, 16]. Fishes obtained from these two water bodies contain high levels of heavy metal above the maximum contaminant level (MCL) of the WHO [16-18]

A variety of chemical and toxins may induce two different types of cell death such as necrosis and apoptosis. Apoptotic DNA fragmentation is often analyzed using Agarose gel electrophoresis to demonstrate a ladder pattern

The main goal of this study is to ascertain the genotoxic and bio chemical effects of feeding rat, with fishes from two heavily polluted water bodies of Lagos Lagoon, Nigeria.

2. Materials and Methods

2.1. Study Location

Lagos Lagoon in Nigeria is the largest lagoon system in the West African Coast, covering 208km². The Lagoon is an open tidal estuary and it is fed in the North by Ogun River, Majidun, Agoyi and Ogudu Creeks. In the South margin, we have Five Crowries, Badagry Creek while in the East, we have Lekki and Epe Lagoons [19].

2.2. Fish Sampling and Analysis

Two markets were selected for the purchase of the fish samples: Makoko market- close to Makoko water site of the Lagos Lagoon. Carter Bridge market- close to Carter Bridge site of the Lagos Lagoon. This is also known as Gbogbaniyi market, Lagos. The fish samples (*Sarotherodon melanotheron*) were bought and taken to the laboratory where they were cleaned, identified, weighed, oven dried and grounded to powdery form.

2.3. Animal Study

2.3.1. Experimental Animals

Twenty-one albino Sprague-Dawley rats (190g \pm 6.27) were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos State, Nigeria. They were randomly divided into four as follows: Group A, B, C and D.

Feed: The rats were fed with rat chow and water ad libitum. The commercial rat chow manufactured by Pfizer Livestock Feeds, NIG., PLC, Ikeja, Lagos State, Nigeria, was used for the study.

2.3.2. Experimental Design

The rats in group A were fed with fish obtained from Carter Bridge market (100g of powdered fish added to their feed on a daily basis). The rats in group B were fed with 100g of fish obtained from Makoko market, added to the rats pellets. The rats in group C were fed with 100g of fish from Carter Bridge market added to the rat pellets together with 0.013g of zinc supplement in the form of zinc sulphate. The rats in group D were fed with 100g of rat pellets alone which serves as the control group. After three months, the animals were anaesthetized and blood samples collected by cutting the jugular vein into heparin bottles. The rats were dissected and assay conducted on the liver, heart, ovary, kidney and lungs.

2.4. Determination of Heavy Metal Content

2.4.1. Digestion of Serum Samples

Serum was diluted 1:99 with deionized water for metallic estimation. One hundred mls of deionized water was transferred into a beaker covered with a watch glass. Boiling chips were added to aid boiling. The mixture was boiled and evaporated to the lowest volume on a hot plate (10-20ml) before precipitation occurs. Conc. HNO₃ was added until digestion was completed as shown by a light colored clear solution. The mixture was cooled and diluted to 100ml with deionized water. Portions of this solution were taken to the laboratory for metallic analysis with the Atomic Absorption Spectrophotometry.

2.5. Determination of Catalase Activity

Serum catalase activity was determined according to the method of Luck [20] and modified by AACC [21]. Serum (0.1ml) was added to the reaction mixture which contained 1ml of 0.01M phosphate buffer (pH7.0) and 0.4ml of 2MH₂O₂.

The reaction was stopped by the addition of 2ml of dichromate acetic reagents (5% potassium dichromate glacial acetic acid mixed in ratio 1:1). The activity of catalase was assayed calorimetrically at 620nm and expressed as μ moles of H₂O₂ consumed min per mg protein.

2.6. Determination of serum glutathione level

Glutathione level in serum was determined according to the method of Sedlak and Lindsay [22] and modified by AACC [21]. To the serum, 10% TCA was added. The supernatant (1ml) was treated with 0.5mls of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.27M, pH 8.0). The absorbance was read at 420nm.

2.7. DNA Extraction and Electrophoresis

DNA extraction and electrophoresis were performed according to the method described by Miller, *et al.* [23].

2.8. Reagents

Lysis buffer: 8.3g NH₄Cl, 1g of KHCO₃, 1.8ml of 5% EDTA (dissolved in 800ml distilled water, filtered and made up to 1000ml with distilled water).

Digestion buffer pH 8.0: 10mM NaCl, 10mM Tris (pH 8.0) 4 10mM EDTA (pH 8.0), 0.5% SDS, proteinase K (final concentration = 0.5 mg/ml).

Salting out: 0.6M NaCl.

Precipitation: Sodium acetate 3M (pH 5.2), 95% ethanol.

Washing: 70% ethanol

3M Sodium acetate buffer, pH 5.2 (50ml): 20.41g of NaAcetate was mixed with 32ml of distilled water and made up to 50ml with glacial acetic acid, sterilized by autoclaving (121°C, 15min) and stored at 4°C.

0.5M EDTA buffer, pH 8.0 (50ml): 9.31g of Na₂EDTA, 1g of NaOH and 30ml of distilled water was mixed together and the mixture made up to 50ml with distilled water, sterilized by autoclaving (121°C, 15min) and stored at 4°C.

2.9. TBE buffer (tris-borate EDTA)

2.9.1. 1 litres 10x TBE Preparation

108g Tris base, 55g boric acid, and 40mls 0.5M EDTA (pH 8.0) were mixed and autoclaved for 20 minutes.

2.9.2. 5x TBE Buffer Preparation

The following components were mixed to make 1 liter 5x TBE

6M Sodium chloride (50ml): 17.5g of NaCl and 30ml of distilled water mixture was mixed, made up to 50ml with distilled water and stored at 4°C.

1M Tris- HCl (50ml): 6.06g of Tris-base, 40ml of distilled water and 2.1 ml of conc. HCl was mixed together and made up to 50ml with distilled water, sterilized by autoclaving (121°C, 15 min) and stored at 4°C.

Ethidium bromide (10mg/ml): 10mg of Ethidium Bromide dye was mixed with 1ml of sterile water. The mixture was mixed gently in a foiled Eppendor tube and stored at room temperature.

Bromophenol blue (loading buffer) x4: 25g bromophenol blue and 4g of sucrose was mixed. 10ml of sterile water was added and the mixture mixed gently and stored at 4°C

3. Procedure

3.1. RBC Lysis

Nine mls of lysis buffer was added to 1ml of whole blood in a clean tube. The mixture was shaken vigorously and allowed to stand for 10 minutes. It was centrifuged at 2,000 rpm for 10 minutes. The centrifugation was repeated thrice. The resulting buffy coat was stored at -4°C for at least one hour.

Buffy Coat lysis:

The buffy coat was thawed in ambient air. 400µL, of nucleic lysis buffer was added, the mixture was mixed with a vortex mixer and kept on ice for 30 minutes. 20µL of 10% SDS and 10µL of 20mg/ml proteinase K solution was added. The mixture was incubated overnight at 40°C. One-third volume of 6M NaCl was added and the mixture shaken vigorously, centrifuged at 3,000 rpm for 10 minutes while the supernatant was transferred to a new tube. 0.1ml of 3M Na-acetate (pH 5.2) together with 2ml of ice cold absolute ethanol was added to the supernatant. The mixture was kept at -4°C for 24 hours. It was centrifuged at 3,000 rpm for 10 minutes and the supernatant decanted. The DNA pellet was washed in 70% ethanol, centrifuged and the supernatant decanted. The resulting DNA pellet was air dried. 8µL of DNA per sample was loaded into 0.9% agarose gel pre-stained with ethidium bromide at 100mV for one hour. DNA was visualized using UV illumination.

3.2. Agarose gel electrophoresis

Materials: Agarose (SeaKem LE Agarose, Biozym Diagnostik, Germany).

TBE x10

Procedure: 0.94g of agarose gel was prepared by dissolving 0.9g of agarose powder 100ml of x1 TBE (prepared by 1:10 dilution of x10 TBE stock with distilled water). The mixture was boiled and the gel cooled to 50°C before the addition of 50µL of 1mg/ml ethidium bromide. The ethidium bromide-stained gel was poured into the gel casting tray with comb inserted for well creation. The DNA sample (8µL of DNA sample + 2µL of loading buffer) was loaded into each well of the gel submerged in x1 TBE (pH 8.3) buffer in the electrophoretic tank.

The circuit was closed and operated at 10v/cm for 45 minutes. The DNA bands were visualized under UV light using transilluminator and photographs of DNA taken using a digital camera.

3.3. Statistical Analysis of Result

Data entry and analysis were done using Graph pad prism software version 5.04. Data for the study were expressed as mean ± S.E.M. The Students t-test for independent samples and one way analysis of variance (ANOVA) were used to analyze the difference between the mean. Probability value less than 0.01 (p<0.01) was considered as highly significant.

Table-1. Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and Iron Levels in serum and tissues of rats for Makoko group.

Makoko Group	Pb mg/ml	Cd mg/ml	Cu mg/ml	Cr mg/ml	Mn mg/ml	Mg mg/ml	Fe mg/ml
SERUM	0.002±0.03*	0.029±0.024	0.049±0.074*	0.010±0.032 *	0.030±0.062	0.096±0.019	0.268±0.786
LIVER	0.006±0.039*	0.010±0.061	0.041±0.029*	0.002±0.058	0.086±0.073*	0.062±0.054*	0.2460.622
HEART	0.009±0.001 *	0.006±0.027	0.044±0.082*	0.006±0.037*	0.044±0.057	0.086±0.018*	0.469±0.790
OVARY	0.001±0.051	0.002±0.074	0.030 ±0.052	0.009 ±0.075*	0.046 ±0.059	0.0880.052*	0.586 ±0.654*
KIDNEY	0.001±0.063	0.009±0.076*	0.039±0.062*	0.001±0.079	0.074±0.069*	0.102±0.052	0.349±0.091
LUNG	0.001±0.004	0.001±0.065	0.019±0.084	0.007±0.077*	0.087±0.051*	0.054±0.069	0.597±0.085*
CONTROL	Pb mg/ml	Cd mg/ml	Cu mg/ml	Cr mg/ml	Mn mg/ml	Mg mg/ml	Fe mg/ml
SERUM	ND	ND	0.029±0.069	ND	0.027±0.028	0.146±0.368	0.391±0.582
LIVER	ND	ND	0.015±0.034	ND	0.019±0.043	0.050±0.371	0.397±0.490
HEART	ND	ND	0.027±0.073	ND	0.020±0.046	0.341±0.587	0.298±0.749
OVARY	ND	ND	0.028±0.092	ND	0.019±0.089	0.029±0.024	0.261±0.547
KIDNEY	ND	ND	0.029±0.045	ND	0.011±0.047	0.024±0.076	0.049±0.095
LUNG	ND	ND	0.025±0.096	ND	0.075±0.052	0.040±0.037	0.049±0.068

Values represent mean ± S.E.M of 5 rats

P<0.05, T-test for independent samples

ND = NOT DETECTED

Table-2. Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and Iron Levels in serum and tissues of rats for Carter Bridge group.

Carter Bridge Group	Pb mg/ml	Cd mg/ml	Cu mg/ml	Cr mg/ml	Mn mg/ml	Mg mg/ml	Fe mg/ml
SERUM	ND	0.003±0.01*	0.049±0.074*	0.014±0.04 *	0.024±0.09	0.168±0.76	0.361±0.54
LIVER	ND	0.001±0.01*	0.021±0.60*	0.019±0.02	0.028±0.20*	0.101±0.30*	0.581±0.70
HEART	ND	0.001±0.07 *	0.029±0.40*	0.009±0.07*	0.036±0.73	0.089±0.43*	0.469±0.64
OVARY	ND	0.002±0.04	0.031±0.90*	0.006±0.08*	0.019±0.59	0.124±0.71*	0.360 ±0.74*
KIDNEY	ND	0.001±0.02	0.036±0.50*	0.003±0.05	0.025±0.07*	0.169±0.59	0.342±0.58
LUNG	ND	0.002±0.06*	0.029±0.08	0.006±0.04*	0.063±0.53*	0.047±0.27	0.493±0.38*
CONTROL	Pb mg/ml	Cd mg/ml	Cu mg/ml	Cr mg/ml	Mn mg/ml	Mg mg/ml	Fe mg/ml
SERUM	ND	ND	0.029±0.069	ND	0.027±0.028	0.146±0.468	0.391±0.582
LIVER	ND	ND	0.015±0.034	ND	0.019±0.043	0.050±0.371	0.397±0.490
HEART	ND	ND	0.027±0.073	ND	0.020±0.046	0.341±0.587	0.298±0.749
OVARY	ND	ND	0.028±0.092	ND	0.019±0.089	0.029±0.024	0.261±0.547
KIDNEY	ND	ND	0.029±0.045	ND	0.011±0.047	0.024±0.076	0.049±0.095
LUNG	ND	ND	0.025±0.096	ND	0.075±0.052	0.040±0.037	0.049±0.068

Values represent mean ± S.E.M of 5 rats

P<0.05, T-test for independent samples

ND = NOT DETECTED

Table-3. Lead, Cadmium, Copper, Chromium, Manganese, Magnesium and Iron Levels in serum and tissues of rats for Zinc group.

Zinc Group	Pb mg/ml	Cd mg/ml	Cu mg/ml	Cr mg/ml	Mn mg/ml	Mg mg/ml	Fe mg/ml
SERUM	ND	ND	0.021± 0.065*	0.011± 0.069*	0.019± 0.39	0.049± 0.073	0.281± 0.867
LIVER	ND	ND	0.021± 0.12*	0.010± 0.086	0.020± 0.063	0.051± 0.572	0.686± 0.978
HEART	ND	ND	0.027± 0.687	0.009± 0.043	0.016± 0.072	0.068± 0.063	0.681± 0.074
OVARY	ND	ND	0.029± 0.047*	0.006± 0.058	0.026± 0.073	0.068± 0.084	0.481± 0.642
KIDNEY	ND	ND	0.024± 0.62*	0.010± 0.071	0.021± 0.081*	0.046± 0.077	0.496± 0.094
LUNG	ND	ND	0.021± 0.053	0.004± 0.070*	0.053± 0.082	0.038± 0.074	0.612± 0.864*
CONTROL	Pb mg/ml	Cd mg/ml	Cu mg/ml	Cr mg/ml	Mn mg/ml	Mg mg/ml	Fe mg/ml
SERUM	ND	ND	0.029 ±0.069	ND	0.027± 0.028	0.146± 0.468	0.391± 0.582
LIVER	ND	ND	0.015 ±0.034	ND	0.019± 0.043	0.050± 0.371	0.397± 0.490
HEART	ND	ND	0.027± 0.073	ND	0.020± 0.046	0.341± 0.587	0.298± 0.749
OVARY	ND	ND	0.028± 0.092	ND	0.019± 0.089	0.029± 0.024	0.261± 0.547
KIDNEY	ND	ND	0.029 ±0.045	ND	0.011± 0.047	0.024± 0.076	0.049± 0.095
LUNG	ND	ND	0.025 ±0.096	ND	0.075± 0.052	0.040± 0.037	0.049± 0.068

Values represent mean ± S.E.M of 5 rats
P<0.05, T-test for independent samples
ND = NOT DETECTED

Table-4. Catalase activity and Glutathione level in rats fed contaminated Tilapia fish from Carter & Makoko Market, Zinc supplements & control.

Study location	Glutathione	Catalase
Control	1.27±0.49	0.48±0.09
Makoko	1.26±0.27	0.35±0.01
Carter	1.25±0.44	0.34±0.06
Zinc supplemented	1.28±0.28	0.49±0.04

Values represent mean ± S.E.M of 5 rats
P<0.05, T-test for independent samples

Table-5. Weights of organs of rats fed with fishes from Carter site and Makoko site

Groups	Heart (g)	Lung (g)	Kidney (g)	Ovary (g)	Liver (g)
Makoko group	0.67 ± 0.03	1.32 ± 0.6*	1.03 ± 0.3	0.22 ± 0.01*	5.80 ± 2.7*
Carter Bridge group	0.64± 0.07*	1.33 ± 0.5	1.04 ± 0.5**	0.21 ± 0.03*	6.35 ± 2.5
Zinc group	0.57 ± 0.07	1.34 ± 0.3	1.18 ± 0.6**	0.33 ± 0.05	6.81 ± 1.8*
Control	0.62 ± 0.04	1.59 ± 0.6	1.17 ± 0.6	0.32 ± 0.03	7.64 ± 2.7

Values represent mean ± S.E.M of 5 rabbits.
*P<0.05, **P<0.01, ANOVA

Figure-1. Agarose gel electrophoresis of chromosomal DNAs from the blood samples of rats fed with fish diet and control. Lane 1. 100 bp DNA ladder (Bioline); Lanes 2 – 3 & 10 = Makoko; Lanes 4 – 5, 7 = Carter; Lanes 6, 8, 9 = Control; Lanes 12 & 13 = Zinc

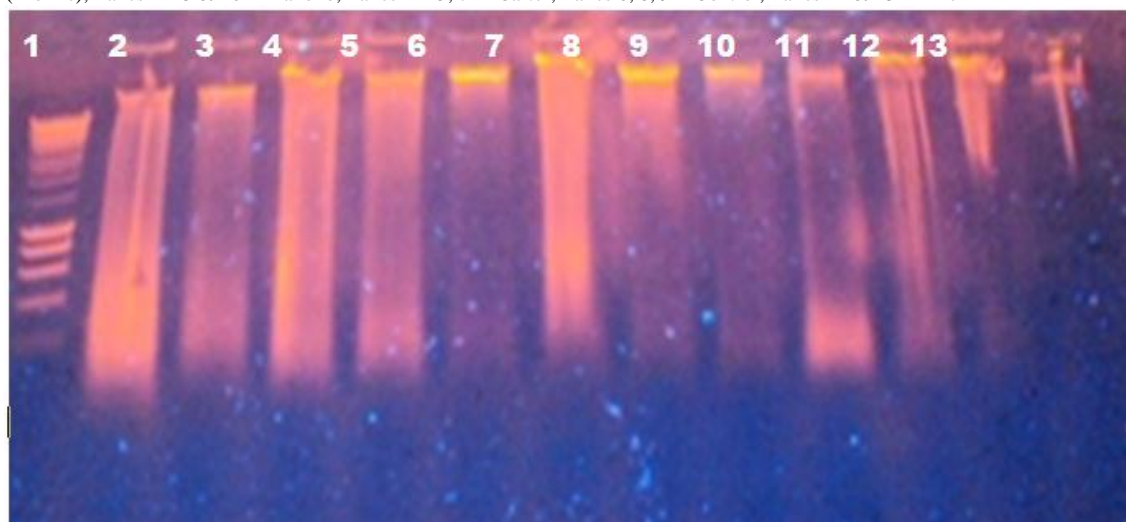


Table-6. Purity and yields of DNA from the blood samples.

Sample	Yield ng/uL	Purity A260/A280
Makoko -2	528.5	1.78
Makoko -3	428.1	1.71
Makoko -10	392.8	1.84
Carter – 4	503.7	1.75
Carter – 5	429.7	1.68
Carter – 7	405.8	1.70
Zinc – 12	458.2	1.70
Zinc – 13	527.1	1.80
Control – 6	388.5	1.78
Control - 8	582.6	1.81
Control - 9	427.3	1.75

4. Discussion

In the Western part of the country, especially Lagos State, there are numerous industries. Effluent from these industries greatly distress the geochemistry of the soil and also introduce heavy metals into the soil & water bodies [24, 25]. According to the United State Environmental Protection Agency USEPA [3], the Maximum Contaminant Levels (MCL) in fishes is 0.01mg/g for Cadmium, 0.05mg/g for lead and 0.01mg/g for chromium.

Previous studies have found lead concentration in Tilapia fish to range from 0.31 mg/g to 0.65mg/g [26].

Results showed that the administration of the fish diet produced a marked decrease of liver, heart, lung, kidney and ovary weights. This is in accordance with previous data indicating weight loss in testes, seminal vesicles and epididymis accompanied by a loss of reproductive capacity in rats exposed to cadmium [27].

Agarose gel electrophoresis of cellular DNA is the usual method for demonstrating apoptosis, with the appearance of a characteristic ladder pattern [28]. Invitro, cells exposed to cadmium result in a DNA ladder similar to that observed in Dexa-treated cells confirming the induction of apoptosis by heavy metals [29]. The same result has been observed for mouse cells [28] human T cell line, human histiocytic lymphoma cell line [29], human promycotic cells [30] and normal human mononuclear cells [31]. Apoptosis is rare in health rodents but it can be enhanced after exposure to carbon tetrachloride, ethanol, thioacetamide anti dioxin [10]. In the present study, apoptosis as demonstrated by DNA fragmentation was noted in the rats fed the fish diet.

Many possible cellular mechanisms have been hypothesized to explain heavy metal toxicity, but oxidative stress is a major process responsible for triggering cytotoxicity pathways that lead to the generation of reactive oxygen species ROS [32]. Khan, *et al.* [33] found that lead induced oxidative stress in exposed human populations. A similar finding was also reported for cadmium [34]. Interactions between metal ions such as iron, cadmium chromium or copper could affect the generation of reactive oxygen species and the formation of DNA strand breaks. Nagy, *et al.* [35] have reported that increasing oxidative stress caused DNA damage.

The heavy metal content of wastewater samples were analyzed in *Allium cepa* genotoxicity test and found to induce significant chromosome aberrations [36]. The application of prokaryotic tests stems with biomarkers such as DNA fragmentation in different tissues of test organisms seemed to be a useful combination for the assessment of cytotoxic and genotoxic potential in surface waters and secondary effluents [37]. Krishnamurthi, *et al.* [38] used four genotoxicity assays namely chromosomal aberration, DNA strand break, DNA laddering and P53 accumulation tests in human peripheral mononuclear blood cells and showed genotoxic potentials of contaminated waste water

samples. Free radicals generated by heavy metals could bind to the purine and pyrimidine of nucleic acids to cause base substitution and breakage of DNA and eventually induce mutation [1]. They could also interact with proteins in a way that will affect their structure and functions.

Cadmium, copper and iron have been reported to induce the production of reactive oxygen species in eukaryotic systems [39, 40]. The administration of zinc tablets to the rats prevented the degradation of DNA induced by the fish diet. Zinc is an antioxidant and may prevent the formation of reactive oxygen species and peroxidation in the blood samples. Thus it may be concluded that an oxidative pathway is involved in heavy metals induced DNA fragmentation. To the best of my knowledge, this is the first report on induced DNA fragmentation by the consumption of fish diet in rats. Other reports on heavy metal induced genotoxicity in organisms (by exposure to the heavy metals itself) are in concert with this study [14, 41-44].

DNA fragmentation is a major genetic damage and it is associated with cancer predisposition. It has been established that there exists a frequent occurrence of chromosomal aberrations in cancer cells.

Recent data indicate that adverse health effects of heavy metal exposure may occur at lower exposure levels than previously anticipated [45]. Many individuals in Europe already exceed these exposure levels and the margin is very narrow for large groups [45].

5. Conclusion

Measures should be taken everywhere in Nigeria to reduce heavy metals exposure in the general population in order to minimize the risk of adverse health effects.

The sources of aquatic protein (Tilapia fish) in the Carter Bridge and Makoko water bodies are highly contaminated with heavy metals from industrial effluents.

Consumption of this fish produces negative metabolic changes. Dietary Zinc is effective in ameliorating the negative effects seen following long term administration. It is recommended that dietary supplement of zinc be taken daily as prophylactic and the therapeutic management of effects of consumption of heavy metal contaminated fish.

References

- [1] Sang, N. and Li, G., 2004. "Chromosomal aberrations induced in mouse bone marrow cells by municipal land fill leachate." *Environ. Toxicol. Pharmacol.*, vol. 20, pp. 291-224.
- [2] United State Environmental Protection Agency USEPA, 2001. "Factsheet update on natural listing of fish and wildlife advisories. Washington Office of water, US Environmental Protection agency."
- [3] United State Environmental Protection Agency USEPA, 2010. "Our Nation's air." Status and trends through 2008. U.S. Environmental Protection Agency, Office of Air Planning and Standards, Washington.
- [4] World Health Organization, 1992. *Cadmium* vol. 134. Geneva, WHO: Environmental Health Criteria.
- [5] World Health Organization, 1995. *Lead* vol. 165. Geneva, WHO: Environmental Health Criteria.
- [6] Bakare, A. A., Pandey, A. K., Bajpayee, M., Bhargav, D., Chowdhuri, D. K., Singh, K. P., Murthy, R. C., and Dhawan, A., 2007. "DNA damage induced in human peripheral blood lymphocytes by industrial wastes and municipal sludge leachates." *Environmental and Molecular Mutagenesis*, vol. 48, pp. 30-37.
- [7] Abel Sotin, A., Gibson, B. L., and Sanborn, M. D., 2002. "Identifying and managing adverse environmental health effects." *Canadian Medical Association Journal*, vol. 166, pp. 372-374.
- [8] Roggli, V. L. and Sanders, L. L., 2000. "Asbestos content of lung tissue and carcinoma of the lung." *Ann. Occup. Hyg.*, vol. 44, pp. 109-117.
- [9] Paulus, J., Rydzynski, K., Dziubaltowska, E., Wyszynska, K., Natarajan, A. T., and Nilsson, R., 2003. "Genotoxic effects of occupational exposure to lead and cadmium." *Mutation Research*, vol. 540, pp. 19-28.
- [10] Krichah, R., Rhouma, B. K., Hallegue, D., Tebourbi, O., Joulin, V., Couton, D., and Sakly, M., 2003. "Acute cadmium administration induces apoptosis in rat thymus and testicle, but not liver." *Polish Journal of Environmental Studies*, vol. 12, pp. 589-594.
- [11] Turkez, H., Sisman, T., Incekara, U., Geyikoglu, F., Tatar, A., and Keles, M. S., 2009. "The genotoxic and biochemical effects of wastewater samples from a fat plant in Erzurum." *Clit Sayi*, vol. 2, pp. 55-63.
- [12] Forrester, L. W., Latinwo, L. M., Fasanya-Odewumi, C., Ikediobi, C., Mbuya, O., and Nworga, J., 2000. "Comparative studies of cadmium-induced single strand breaks in female and male rats and ameliorative effects of selenium." *Int. J. of Molecular Medicine*, vol. 6, pp. 449-452.
- [13] Fracasso, M. E., Perbellini, L., Solda, S., Talamini, G., and Franceschetti, P., 2002. "Lead induced DNA strand breaks in lymphocytes of exposed workers: role of reactive oxygen species and protein kinase C." *Mutat. Res.*, vol. 515, pp. 159-169.
- [14] Depault, F., Cojocar, M., Fortin, F., Chakrabarti, S., and Lemieux, N., 2006. "Genotoxic effects of chromium (VI) and cadmium (II) in human blood lymphocytes using the electron microscopy in-situ end labelling (EM-ISEL) assay." *Toxicology in Vitro*, vol. 20, pp. 513-518.
- [15] Bolawa, O. E. and Gbenle, G. O., 2012. "Physiochemical parameters and metallic constituents of Makoko, Maroko and Carter Bridge rivers in Lagos, Nigeria." *Instasci Journal of Chemistry*, vol. 2, pp. 56-63.
- [16] Bolawa, O. E., Gbenle, G. O., Adewusi, O. R., and Mosuro, A. O., 2012. "Metal concentration in tissues of *Pseudolithus senegalensis* (Croaker fish) and *Ethmalosa fimbriata* (Bonga fish) obtained from Carter Bridge river and Makoko river, Lagos State, Nigeria." *Marine Science*, vol. 2, pp. 101-104.

- [17] Bolawa, O. E., Gbenle, G. O., and Ebuehi, O. A. T., 2014. "Endocrine disruption by the consumption of fish (Tilapia) and its reversal using zinc." *International Journal of Aquaculture*, vol. 4, pp. 85-88.
- [18] World Health Organization, 2004. "Recommendation health based limits in occupational exposure to heavy metals." WHO, Geneva. Techn. Report Series. No. 647.
- [19] Lawson, E. O., 2011. "Physiochemical parameters and heavy metals content of water from the mangrove swamps of Lagos Lagoon, Lagos Nigeria." *Advances in Biological Research*, vol. 5, pp. 8-21.
- [20] Luck, H., 1971. *Catalase in: Methods of enzyme analysis. Bergmeyer H. Editor* vol. 885. New York: Academic Press.
- [21] AACC, 1984. "American association for clinical chemistry." In: Selected methods for the small clinical chemistry laboratory, Willard R and Meites FS (eds). WB Saunders Co. Philadelphia.
- [22] Sedlak, J. and Lindsay, R. H., 1968. "Estimation of total, protein bound and non protein sulfhydryl groups in tissues with Ellmans reagent." *Analytical Biochemistry*, vol. 25, pp. 192-206.
- [23] Miller, S. A., Dykes, D. D., and Polesky, H. F., 1988. "A simple salting out procedure for extracting DNA from human nucleated cells." *Nucleic Acids Res.*, vol. 16, pp. 12-15.
- [24] Saeed, A., Sohail, M., Rashi, N., and Igbam, 2013. "Effects of heavy metal toxicity on the biochemical response in tomato plants grown in contaminated soil." *Bangladesh J. Sci. Ind. Res.*, vol. 48, pp. 229-236.
- [25] Nayek, S., Gupta, S., and Saha, R. N., 2010. "Metal accumulation and its effects in relation to biochemical response of vegetables irrigated with metal contaminated water and waste water." *L. Hazard Mat.*, vol. 178, pp. 588-595.
- [26] Bolawa, O. E., 2015. "Biochemical responses of sprague dawley rats and new zealand rabbits following long-term dietary exposure to heavy metal contaminated fish." Ph.D thesis, Department of Biochemistry, University of Lagos, Lagos State, Nigeria, West Africa.
- [27] Saygi, S., Deniz, C., Kutsal, O., and Viral, N., 1991. "Chronic effects of cadmium on kidney, liver, testes and fertility of male rats." *Biol. Tr. Elem. Res.*, vol. 31, pp. 209-211.
- [28] Fujimaki, H., Ishido, M., and Nohara, K., 2000. "Induction of apoptosis in mouse thymocytes by cadmium." *Toxicol Lett.*, vol. 115, pp. 99-101.
- [29] Li, M., Kondo, T., Zhao, Q. L., Li, F. J., Tanabe, K., Arai, Y., Zhou, Z. C., and Kasura, M., 2000. "Apoptosis induced by cadmium in human lymphoma U937 cells through calcium-calpain and caspase-mitochondra dependent pathways." *J. Biol. Chem.*, vol. 275, pp. 39702-39703.
- [30] Galan, A., Garcia-Bermejo, L., Troyano, A., Vilaboa, N. E., Fernandez, C., De-Blase, E., and Aller, P., 2001. "The role of intracellular oxidation in death induction (apoptosis and necrosis) in human promyocytic cells treated with stress inducers (cadmium, heat, X-rays)." *Eur. J. Cell Biol.*, vol. 80, pp. 312-314.
- [31] de la Fuente, H., Portales-Perez, D., Baranda, I., Diaz -Barriga, F., Saavedra-Alanis, V., Layse-Ca, E., and Gonzalez-Amaro, R., 2002. "Effect of arsenic, cadmium and lead on the induction of apoptosis of normal human mononuclear cells." *Clin. Exp. Immunol.*, vol. 129, pp. 69-72.
- [32] Pinheiro, M. C., Macchi, B. M., Vieira, J. L., Oikawa, T., and Amoras, W. W., 2008. "Mercury exposure and antioxidant defenses in women: a comparative study in the Amazon." *Environmental Research*, vol. 107, pp. 53-59.
- [33] Khan, D. A., Qayyum, S., Saleem, S., and Khan, F., 2008. "Lead induced oxidative stress adversely affects health of workers." *Toxicology and Industrial Health*, vol. 24, pp. 611-618.
- [34] Chater, S., Douki, T., Garrel, C., Favier, A., Sakly, M., and Abdelmelek, H. C. R., 2008. "Cadmium-induced oxidative stress and DNA damage in kidney of pregnant female rats." *Comptes Rendus Biologies*, vol. 331, pp. 426-432.
- [35] Nagy, E., Johansson, C., Zeisig, M., and Moller, M., 2005. "Oxidative stress and DNA damage by the urban air pollutant 3-NBA and its isomer 2-NBA in human lung cells analyzed with three independent methods." *Journal of Chromatography. B: Analytical Technologies in the Biomedical and Life Sciences*, vol. 827, pp. 94-103.
- [36] Rank, J. and Nielsen, M. H., 1998. "Genotoxicity testing of wastewater sludge using the Allium cepa anaphase-telophase chromosome aberration assay." *Mutation Research*, vol. 418, pp. 113-119.
- [37] Dizer, H., Wittekindt, E., Fischer, B., and Hansen, P. D., 2002. "The cytotoxic and genotoxic potential of surface water and wastewater effluents as determined by bioluminescence and selected biomarkers." *Chemosphere*, vol. 46, pp. 225-233.
- [38] Krishnamurthi, K., Saravana, D., Hengstler, J. G., Hermes, M., Kumar, K., Dutta, D., Muhil, V. S., Subin, T. S., Yadav, R. R., et al., 2008. "Genotoxicity of sludges, waste water and effluents from three different industries." *Archives of Toxicology*, vol. 82, pp. 965-971.
- [39] Ghio, A. J., Sibajoris, R., Carson, J. L., and Samet, J. M., 2002. "Biologic effects of oily fly ash." *Environ. Health Perspect*, vol. 110, pp. 89-94.
- [40] Radetski, C. M., Ferrari, B., Cotelle, S., Masfaraud, J. F., and Ferard, J. F., 2004. "Evaluation of the genotoxic, mutagenic and oxidant stress potentials of the municipal solid waste incinerator bottom ash leachates." *Sci. of the Total Environment*, vol. 333, pp. 209-216.
- [41] Celik, A., Comelekoglu, U., and Yalin, S., 2005. "A study in the investigation of cadmium chloride genotoxicity in rat bone marrow using micronucleus test and chromosome aberration analysis." *Toxicology and Industrial Health*, vol. 21, pp. 243-248.

- [42] Rozgaj, R., Kasuba, V., and Blanusa, M., 2005. "Mercury chloride genotoxicity in rats following oral exposure, evaluated by comet assay and micronucleus test." *Arh Hig Tok Sikol*, vol. 56, pp. 9-15.
- [43] Virag, P., Brie, I., Postescu, I. D., Prede-Schrepler, M., Fischer-Fodor, E., and Soritau, O., 2009. "Comparative study of two evaluation methods for the genotoxic effects of environmental heavy metals on normal cells." *Toxicology and Industrial Health*, vol. 25, pp. 253-258.
- [44] Kasuba, V., Rozgaj, R., Saric, M. M., and Blanusa, M., 2002. "Evaluation of genotoxic damage of cadmium chloride in peripheral blood of suckling Wistar rats." *Journal of Applied Toxicology*, vol. 22, pp. 271-272.
- [45] Jarup, L., 2003. "Hazards of heavy metal contamination." *British Medical Bulletin*, vol. 68, pp. 167-182.